THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society of Public Analysts and Other Analytical Chemists

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7-8, IDOL LANE, LONDON, E.C.3
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Published for the Society by
W. HEFFER & SONS, LTD., CAMBRIDGE, ENGLAND

Volume 77 Price 6s. 6d.
Subscription Rate, inclusive of Abstracts C, 80 - per annum, Post Free
No. 916, Pages 333-388 July, 1952

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, May 7th, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. R. Nicholls, C.B.E., F.R.I.C.

The following papers were presented and discussed: "A Routine Method for the Analysis of Table Jellies," by Miss E. M. Chatt, B.Sc., F.R.I.C.; "The Determination of Oxalates in Fresh Plant Material," by C. J. L. Baker, A.R.I.C.; "The Determination of Small Quantities of Ammonium Di- or Tri-ethanolamine Alginate in Rayon-Finishing Solutions and on Rayon Yarn," by E. G. Brown, A.M.C.T., F.R.I.C., and T. J. Hayes.

NEW MEMBERS

Mary Andross, B.Sc., F.R.I.C.; Arthur Derek Campbell, M.Sc. (N.Z.), A.N.Z.I.C.; George Valentine Francis, B.Sc., Ph.D. (Liv.); Karl Gunnar Mautitz Gran; Paul Geoffrey Jeffery, B.Sc. (Lond.), A.R.C.S.; Alfred Henry Moore; Edwin William Stanley Press, B.Sc. (Lond.), F.R.I.C.; John Jeffrey Reid; Neville Stuart Thom, B.A. (Cantab.).

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held on Friday, May 2nd, 1952, at 7 p.m. in the North British Hotel, Edinburgh. Mr. H. C. Moir presided and forty-two members and friends were present.

A lecture entitled "Chemistry and the Law" was given by J. K. McLellan, M.A., B.Sc., A.R.I.C., and was illustrated by lantern slides and exhibits. A discussion followed.

MICROCHEMISTRY GROUP

A JOINT Meeting of the Group with the Bristol and District Sections of the Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry was held in the Lecture Theatre of the Chemical Department of Bristol University on Wednesday, April 23rd, 1952, at 7 p.m.

The following paper was presented and discussed: "The Use of Cylinder Oxygen in the Organic Micro-determination of Nitrogen," by H. Swift and E. S. Morton. This was followed by an open discussion on "Standard Substances for Organic Micro-analysis."

During the afternoon, visits were made to the chocolate and cocoa works of J. S. Fry & Sons Ltd., Somerdale, and to the University of Bristol Agricultural and Horticultural Research Station at Long Ashton.

PHYSICAL METHODS GROUP

THE Thirty-seventh Ordinary Meeting of the Group was held at 6 p.m. on Friday, May 16th, 1952, at the University College, Swansea. This was a joint meeting with the South Wales Section of the Royal Institute of Chemistry, and was preceded by a visit to the B.I.S.R.A. Research Laboratories, Swansea. Dr. J. Haslam was in the Chair and fifty-two members and visitors were present.

The following papers on Ion Exchange Resins were presented and discussed: "The Theory of Ion Exchange," by Professor C. W. Davies, D.Sc., F.R.I.C.; "Some Newer Applications and Techniques of Cation and Anion Exchange Resins in Chemical Analysis," by

G. H. Osborn, F.R.I.C., A.M.Inst.M.M.; "The Determination of Individual Rare Earths by Radioactivation using Ion Exchange Separation," by F. W. Cornish, Ph.D., A.R.I.C.

ANALYTICAL METHODS COMMITTEE

SUB-COMMITTEE ON METHODS OF ANALYSIS OF ICE-CREAM

A SUB-COMMITTEE has been appointed to formulate methods of analysis of ice-cream in respect of the Ministry of Food Ice-Cream Order.

The Sub-Committee consists of J. H. Hamence, M.Sc., Ph.D., F.R.I.C. (Chairman); J. G. Davis, Ph.D., D.Sc., F.R.I.C.; G. E. Forstner, M.Sc., F.R.I.C.; J. King, O.B.E., F.R.I.C.; K. A. Williams, B.Sc., Ph.D., A.Inst.P., F.R.I.C.; M. G. Read, B.Sc., F.R.I.C. (Honorary Secretary).

Obituary

JOHN FRANCIS HUTCHINS GILBARD

JOHN FRANCIS HUTCHINS GILBARD died in London at his home on April 24th, 1952, in his eighty-third year, sixty-two years after his election to membership of the Society. He was born in London and educated at Vermont College, Clapton, and at Finsbury Technical College, under Meldola and Streatfeild. He joined Bernard Dyer in 1888 and was his assistant during the remainder of his working life. From early in this period his chemical knowledge and great analytical skill were of immense assistance to Dyer in the many original investigations that Dyer undertook. It is now nearly sixty years since the first contribution from Dyer and Gilbard appeared in *The Analyst* in 1893—dealing with the composition of genuine and spent ginger—and this was followed in 1895 by a paper on cinnamon and a review of the examination of over 1000 samples of linseed and other oil-cakes and feeding stuffs with respect to their natural content of free fatty acids. This latter was a detailed account of work of such a specialised character bearing on the question of deterioration of feeding quality due to enzyme and mould activity that it is still commonly consulted when such questions arise in commercial practice. In 1896 a paper on the detection and determination of drawn or exhausted caraways was published. Finally, in 1911, Gilbard gave a short account of his own work on a colour reaction for the active principle (resins) of caulophyllin, formerly used as an abortifacient.

To those of us who knew Gilbard personally he was a strong character with firm and often unpredictable views on all sorts of subjects. So to Bernard Dyer he was occasionally very provocative by his obstinacy in holding views on chemical matters that were more or less diametrically opposed to Dyer's. This led occasionally to somewhat stormy scenes, but as we, the weaker members of the staff, knew quite well that these would inevitably end happily in reconciliations of a frankly human nature, we always enjoyed such occasions. But, in fact, he was a pioneer both in food analysis and in bacteriology. In these days of intense activity in the field of antibiotics it is a memory of great interest that Gilbard in his earlier bacteriological work was occasionally bothered by contamination of his plates and then speculated, with what we now recognise as a prophetic foresight, on the odd effects of mould growth.

With the outbreak of the second world war, Gilbard's strength began to give way. Yet he was extraordinarily resilient; for not only was he an active air-raid warden during the war, but in the years that immediately followed he underwent two major operations from which he recovered apparently almost unaffected. To the end he retained his mental faculties, and, on the last occasion I saw him, only a short time ago, he was quietly humorous about his resemblance to Einstein; a likeness that had been noted some years ago and had slowly become more and more marked.

Gilbard was elected to the Society in 1890, he became a Fellow of the Chemical Society in 1895, and a Fellow of the Institute of Chemistry—as it then was—in 1899. Sometime about this latter period he was appointed a Gas Examiner to the London County Council and later, additionally, to West Ham.

He married twice, first Thirza Hawke in 1902, and secondly Rita Johnson.

GEORGE TAYLOR

A Routine Method for the Analysis of Table Jellies

By E. M. CHATT

(*Presented at the meeting of the Society on Wednesday, May 7th*, 1952)

A speedy routine method for the analysis of table jellies is presented, together with analytical data that form the basis of equations used to calculate the percentages of sucrose, invert sugar and glucose from the optical rotation and reducing power of clarified solutions.

Although no new principles are involved in the following description of a routine method for the analysis of table jellies, data are presented that form a basis for calculating the percentages of the individual sugar components.

General procedure

The direct refractometer reading should be determined on several sections taken from the interior of the tablet. The sugar equivalent of such a reading is sometimes about 1·5 per cent. too high, owing to the interference of gelatin, citric acid and non-sucrose sugars. The appropriate corrections to be applied for the calculation of the true soluble solids are presented in Table I. Only the uncorrected reading is required by the Statutory Instrument.

Table I

Corrections to be applied to apparent soluble solids by direct refractometer reading where certain percentages of constituents other than sucrose are present

Constituent	Amount of constituent in sample, %	Correction when amount of soluble solids is approximately 60%	70%	75%	85%
Invert sugar	20	+0·4	+0·45	+0·5	+0·6
	40	+0·75	+0·9	+1·00	+1·2
	50	+0·95	+1·1	+1·25	+1·5
	60	+1·15	+1·35	+1·5	+1·8
	70		+1·55	+1·75	+2·1
	75				+2·55
Glucose dry solids	20	−0·3	−0·25	−0·25	−0·25
	40	−0·55	−0·5	−0·5	−0·45
Citric acid	1	+0·1	+0·1	+0·1	+0·1
	2	+0·2	+0·2	+0·2	+0·2
Tartaric acid	1	+0·1	+0·1	+0·1	+0·1
	2	+0·25	+0·25	+0·25	+0·25
Gelatin	5	−0·85	−0·8	−0·75	−0·7
	10	−1·7	−1·6	−1·5	−1·4

The sample is prepared for analysis by cutting it into small pieces weighing not more than 0·5 g.

Gelatin is determined by the Kjeldahl method on about 2·5 g of sample and the factor 5·55 is used to convert nitrogen to gelatin protein.

For the determination of the percentages of soluble solids by specific gravity measurement and of acid and sweetening components, a 10 per cent. w/v solution is prepared by weighing 25 g of the sample into a beaker and dissolving by warming it to between 40° and 50° C with 75 ml of distilled water in as short a time as possible to prevent inversion of the sucrose. The solution is transferred to a 250-ml flask with cold distilled water, cooled to 20° C and then diluted to the graduation mark with distilled water at 20° C and mixed.

If required, the approximate concentration of soluble solids in the 10 per cent. solution can be calculated from the specific gravity at 20° C by subtracting 1 from the specific gravity and dividing by a common solution factor, 0·00386. As this factor is exact for sucrose at 15·5° C only and within a limited range of concentration, the appropriate corrections to be

applied for temperature and concentration are shown in Table II. Further corrections for the respective concentrations of acid, gelatin, glucose dry solids and invert sugar in the 10 per cent. solution of the sample are shown in Table III.

TABLE II

CORRECTIONS TO BE APPLIED TO SOLUBLE SOLIDS WHEN SOLUTIONS ARE MADE AT 20° C

Approximate specific gravity 20° C/20° C	Correction, grams per 100 ml of 10% solution
1·023	+0·01
1·031	+0·02
1·038	+0·03

TABLE III

CORRECTIONS TO BE MADE FOR SUBSTANCES OTHER THAN SUCROSE IN 10 PER CENT. SOLUTIONS OF TABLE JELLIES

Constituent	Approximate amount of constituent in original sample, %	Correction, grams per 100 ml of 10% solution
Invert sugar	20	0·0
	40	−0·01
	60	−0·01
Glucose dry solids	20	−0·04
	40	−0·07
Citric acid	0·5	0·0
	1·0	−0·01
Tartaric acid	0·5	−0·01
	1·0	−0·02
Gelatin	5	+0·09
	10	+0·17

The acid content of the sample can be calculated from the volume of 0·1 *N* sodium hydroxide solution required to neutralise 50 ml of the 10 per cent. solution, with phenolphthalein as indicator. As a rule, there is no difficulty in seeing the end-point in coloured solutions.

METHOD OF DETERMINING THE SUGAR COMPONENTS

REAGENTS—

Phosphotungstic acid reagent—Mix equal volumes of a 10 per cent. solution of sodium chloride and of a 10 per cent. solution of phosphotungstic acid.

Hydrochloric acid, 6·34 N.

Fehling's solution—Prepare and accurately standardise as described in Lane and Eynon's method.[1] The addition of 1 ml of *N* sulphuric acid to each litre of the original copper sulphate solution retards the formation of a deposit during storage.

Methylene blue—A 1 per cent. aqueous solution.

PROCEDURE—

Prepare a 7·5 per cent. clarified solution of the sample by placing exactly 150 ml of the 10 per cent. solution into a 200-ml flask. Add 40 ml of the phosphotungstic acid reagent (50 ml may be required if the percentage of gelatin exceeds 10) and dilute the solution with water to 200 ml at 20° C. Shake thoroughly and filter through a fluted 15-cm Whatman No. 4 or No. 1 filter-paper. Filtration should be rapid and the filtrate bright and clear. By measuring the optical rotation, at 20° C, of the filtrate so prepared from a commercial sample of table jelly at intervals over a period of time, it has been established (Table IV) that no appreciable inversion of sucrose occurs within 3 hours of filtering.

Determine the optical rotation of the filtrate at 20° C and calculate therefrom the specific rotation, D, of the sample.

Determine the optical rotation after inversion as follows. Place a 100-ml flask containing 80 ml of the filtrate and 10 ml of 6·34 *N* hydrochloric acid in a water-bath at 62° to 63° C. Bring the solution to 60° C in 2 minutes and maintain it at that temperature for 10 minutes.[2]

Then cool the solution to 20° C, dilute it to 100 ml and set it aside for 1 hour before taking polarimeter readings at 20° C. If the acid solution becomes opalescent on dilution to 100 ml, it should be cleared by shaking it with 0·1 g of Filtercel and filtered before polarimeter readings are taken. Finally, calculate the specific rotation of the sample.

TABLE IV

POLARIMETER READINGS AT 20° C ON THE FILTRATE FROM A 7·5 PER CENT. CLARIFIED SOLUTION OF A SAMPLE OF TABLE JELLY

Time after filtration, hours	Mean of readings in 2-dm tube	Mean of readings in 4-dm tube
0	+6·08°	—
1	+6·07°	+12·15°
2	+6·08°	+12·16°
3	+6·09°	+12·16°

Prepare a 1·5 per cent. solution of the sample, by neutralising 40 ml of the uninverted filtrate with 2 *N* sodium hydroxide solution and diluting with water to 200 ml, for the purpose of determining the percentage of reducing sugars in terms of invert sugar by the method of Lane and Eynon.[1] As the percentages of sucrose and reducing sugar in table jellies vary over a wide range, it is not always appropriate to use 25 ml of Fehling's solution and in some determinations where 10 ml are used it may be necessary to read from the column headed "1 g of sucrose per 100 ml." Some operators may prefer to use the constant volume titration method[3] and not the tables.

CALCULATION—

The percentages of the sugar components can be calculated from the following equations—

$$\text{Sucrose, S, } = \frac{D - 1}{0{\cdot}884},$$

where 0·884 is the inversion divisor factor applicable to sodium illumination and angular degrees.

The part of D, the specific rotation before inversion, that is contributed by sucrose can be calculated by multiplying S by 0·665. The algebraic sum, A, of the rotation due to invert sugar and glucose is represented by D − 0·665S.

If B = the percentage of reducing sugars in the sample, expressed as invert sugar,
X = the percentage of glucose dry solids in the sample,
Y = the percentage of invert sugar in the sample,
G = the specific rotation of glucose dry solids,
R = the reducing power of glucose dry solids
and −20 = the specific rotation of invert sugar under the above conditions of preparation of solution,
it follows that:—

$$GX - 20Y = 100A \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$RX + Y = B \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

If the glucose used is available for analysis, the factors for G and R can be substituted in the above equations. Otherwise, the mean values G = +144° and R = 46 per cent., calculated to dry solids, for recent samples delivered over a period of 18 months for analysis at the B.F.M.I.R.A. laboratories (see Table V) can be substituted in equations (1) and (2). These mean values are in fairly close agreement with the corresponding values, +143·4° and 43·9 per cent., for a range of glucose samples received in 1924.

Hence

$$144X - 20Y = 100A \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)'$$

$$0{\cdot}46X + Y = B \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)'$$

Equation (2)′ can be expressed as—

$$144X + 317Y = 317B$$

and by subtracting equation (1)′ from it and solving for Y,

$$337Y = 317B - 100A \quad .. \quad .. \quad .. \quad .. \quad (3)$$

$$X = 2{\cdot}2(B - Y) \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Equations (3) and (4) can be applied to the calculation of invert sugar and glucose dry solids in the sample.

Finally, apply the factor 0·994 for the volume of material precipitated in clarifying. This factor was derived by comparing the angular rotation of the filtrate from a clarified solution, A, containing initially 6·6 of sucrose and 0·8 g of gelatin per 100 ml, with that of another solution, B, prepared by diluting a 13·2 per cent. solution of sucrose to twice its volume with the filtrate from a 1·6 per cent. solution of gelatin alone, which had

TABLE V

POLARISATION AND REDUCING POWER OF SAMPLES OF CONFECTIONERS' GLUCOSE (DRY BASIS)

Brand	Polarisation, $[\alpha]_D^{20}$	Reducing power as percentage of invert sugar	Brand	Polarisation, $[\alpha]_D^{20}$	Reducing power as percentage of invert sugar
1	+146·3°	45·6	3	+144·0°	46·6
	140·5	47·4		143·4	46·8
	143·0	46·8		147·5	42·4
	143·9	47·2		146·2	43·7
	142·0	46·0		138·2	48·7
	141·5	47·7		143·2	47·6
	141·6	44·7		143·1	48·3
	143·2	46·9			
	144·5	45·9	Mean	+143·7	46·2
	140·7	48·0			
			4	+146·6°	45·5
Mean	+142·7	46·6		143·0	45·2
				145·3	45·0
2	+146·8°	47·2		149·5	42·8
	142·2	46·3		144·1	45·8
	143·6	45·7		149·4	42·5
	144·6	45·3		144·0	44·8
	143·4	46·5			
	143·2	46·5	Mean	+146·1°	44·5
	140·0	47·4			
			5	+145·0°	44·3
Mean	+143·4	46·4			
		Mean of all samples		+143·8°	45·9

been clarified with phosphotungstic acid. Hence it was possible to study the change in concentration under entirely similar conditions. Two similarly treated 10 per cent. solutions of commercial glucose solutions were compared for the purpose of observing the deviation over a relatively large angle. The readings are shown in Table VI. From the values obtained for B/A, the correction factor, by which all results for the sugar determinations should be multiplied, can be taken as 0·994.

TABLE VI

DERIVATION OF CORRECTION FACTOR

Solution	Readings in 2-dm tube		Factor, B/A
	Clarified, A	Unclarified, B	
Sucrose, 6·6%	+ 8·78°	+ 8·73°	0·9943
Glucose, 10% (commercial)	+22·98°	+22·84°	0·9939

RAPID DETERMINATION ON A GLUCOSE-FREE SAMPLE—

When it is known that a sample contains no glucose, the following rapid routine analysis will provide results that are within approximately 0·6 per cent. of the sum of the sucrose and invert sugar contents.

A 1 or 2 per cent. solution of the sample is prepared by diluting a 10 per cent. solution, and the percentage of invert sugar is determined without preliminary clarification. The percentage of invert sugar after inversion is determined on a 0·8 per cent. solution by inverting 40 ml of the 10 per cent. solution with 5 ml of 6·34 *N* hydrochloric acid solution, in the

manner described above, and diluting it to 50 ml with distilled water. Twenty millilitres of the 8 per cent. solution so prepared are neutralised with sodium hydroxide solution and made up to 200 ml.

Results obtained by this method are compared with those for clarified solutions in Table VII, from which it will be seen that the error on reducing sugars before inversion is comparatively small, whereas the effect of non-removal of gelatin from the inverted solution gives results which are 0·6 to 0·7 per cent. in excess of the total sugar present and about 0·5 per cent. in excess of the sucrose content of the one sample in which it was determined.

TABLE VII

COMPARISON OF THE REDUCING POWER OF CLARIFIED AND UNCLARIFIED SOLUTIONS CONTAINING GELATIN

Sample	Clarified solution		Unclarified solution	
	Invert sugar, %	Sucrose, %	Invert sugar, %	Sucrose, %
Table jelly "A," before inversion	40·23	28·80	40·36	29·30
Table jelly "A," after inversion	70·55		71·20	
Table jelly "B," before inversion	32·06		32·22	
Calves' feet jelly "C," after inversion ..	19·09		19·69	
Calves' feet jelly "D," after inversion ..	11·97		12·66	

My thanks are due to Miss J. G. Holliwell and Miss E. M. Johnson for their assistance in the experimental work and to the Council of the British Food Manufacturing Industries Research Association for permission to publish this communication.

REFERENCES

1. Lane, J. H., and Eynon, L., *J. Soc. Chem. Ind.*, 1923, **42**, 32T.
2. Jackson, R. F., and Gillis, C. L., U.S. Bureau of Standards, *Scientific Paper No.* 375, 1920.
3. Proceedings of the Tenth Session of the International Commission for Uniform Methods of Sugar Analysis 1949, Subject 6; *Int. Sug. J.*, 1950, **52**, 184.

THE BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION
RANDALLS ROAD
LEATHERHEAD, SURREY

DISCUSSION

THE PRESIDENT said that difficulties were frequently encountered with preparations containing liquid glucose together with other sugars. To the confectionery manufacturer, liquid glucose was a sweetening ingredient that could replace sucrose on an equivalent weight basis as far as soluble solids were concerned; but to the chemist it was a sweetening material of which only about one half was sugar. It was a variable product but, as made to-day, the variations were relatively small. The usual methods of analysis were straightforward, but the interpretation of the results in terms of ingredients was difficult. For certain legal purposes a standard had been laid down based on the sum of the sucrose plus the reducing sugars. This figure bore little relation to the sum of the sweetening ingredients used.

The paper was likely to be useful to manufacturers and others who required to find the amount of glucose added to table jellies, and it did not seem to necessitate much more analytical work.

MR. T. MCLACHLAN asked if the factor 5·55 used for converting nitrogen content to gelatin protein gave the amount of gelatin itself; if not, would the author give the factor she used to convert nitrogen to gelatin.

MISS CHATT said that the ash from a table jelly containing about 10 per cent. of gelatin would not be likely to exceed 0·2 per cent. of the sample. The factor 5·55 was therefore sufficiently close to the true figure for dry gelatin content to apply a correction for gelatin to the observed percentage of soluble solids, as determined by refractometer.

MR. K. A. SARGENT asked if there was any reason for choosing the hydrolysis conditions quoted by Jackson and Gillis in preference to others such as hydrolysis with *N* hydrochloric acid for 5 minutes at 155° F or with approximately 0·5 *N* hydrochloric acid for 1 minute at 212° F.

MISS CHATT said that the Jackson and Gillis' method of inversion was the most reliable one for the polarimetric determination of sucrose. Comparable results for reducing sugars had been obtained by other methods of inversion.

MR. D. D. MOIR asked whether the author was fully satisfied that the phosphotungstic clearing agent completely precipitated the gelatin. It was not generally realised that gelatin had a negative specific rotation of more than 300°, and that unless the constituent that gave rise to that rotation could be completely precipitated serious error would result.

MISS CHATT pointed out that several trials had been made with various concentrations of salt and phosphotungstic acid in the clarifying reagent, but the recommended procedure had been found to give the optimum conditions for completely removing gelatin.

MR. J. G. MALTBY said, with regard to methods for the inversion of sucrose, that if strong acid were used, care must be taken to avoid a local excess of alkali when neutralising, in order to prevent loss of fructose before estimating total sugars. He made this point although that particular determination was not made in the proposed scheme.

When dealing with old dried jelly, he said, there might be migration of some of the constituents either towards or away from the skin (efflorescence, crystallisation and so on). To get an average, it was necessary to dissolve the whole sample.

MISS CHATT said that in order to avoid disturbances due to an excess of alkali, the angular rotation after inversion was determined in acid solution. When a solution was being prepared for the determination of reducing sugars, an aliquot of this solution should be considerably diluted before neutralisation.

Special precautions should of course be taken to ensure an average sample when deterioration had occurred.

MR. V. H. PARKS said that the equation for the calculation of sugars quoted by Dr. H. E. Cox on page 32 of his book "The Chemical Analysis of Foods" had been found very reliable.

MISS CHATT explained that the equation referred to differed mainly in that the optical rotation was expressed in Ventzke units. It appeared from the text of the book that the formula was used to calculate the percentage of glucose syrup and not glucose solids, in which event the reducing power, as invert sugar, was somewhat above the average for present-day syrups.

THE PRESIDENT, in thanking the author for presenting the paper, expressed his satisfaction that she had adopted the Jackson and Gillis conditions for inversion. This method had been very carefully worked out as the one most generally suitable for the inversion of sucrose, and it was the standard procedure adopted by the Condensed Milk Committee of the Society many years ago. All who had had experience of the determination of sucrose recognised the advantage of a single standard method of inversion.

The Determination of Oxalates in Fresh Plant Material

BY C. J. L. BAKER

(*Presented at the meeting of the Society on Wednesday, May 7th,* 1952)

A method is described for determining total oxalates in plants, by extraction with hydrochloric acid, precipitation as calcium oxalate from the deproteinised extract and subsequent titration with potassium permanganate. Soluble oxalates are determined in a similar manner, in aqueous extract. The later stages of the determination are carried out on a semi-micro scale, so that a number of determinations can be made simultaneously. The method is designed for fresh green plants only, as oxalate is lost on drying the material.

OXALIC acid is found in various quantities throughout the plant world, where it occurs mainly as the calcium or potassium salt of the acid. The amount present, although generally small, is considerable in some plants, *viz.*, docks, sorrels, spinach, rhubarb, certain fungi and the leaves of beet and related succulents. The poisonous properties of the free acid and its soluble salts are well known, and Hickenbotham and Bennett,[1] working in Australia on the poisoning of sheep by Soursob (*Oxalis cernua*), suggest that the calcium salt undergoes decomposition and absorption during the later stages of digestion. Talapatra, Ray and Sen[2] have studied calcium metabolism in ruminants fed on fodder rich in oxalate.

In this country the feeding of sugar beet tops to farm stock is usually practised with caution, scour and other undesirable effects being attributed to the oxalates present.

In view of the significance of the oxalate content of plants normally used as foods, it is surprising that so little detailed information about its determination is to be found

in the literature, although Talapatra, Ray, Kehar and Sen[3] describe a method for paddy straw and similar materials. The increasing use of fodder beet as feed for cattle and pigs in this country, together with the arrival of many new varieties, gave rise to enquiries about the advisability of using the tops as feed, and for that reason the method described here was devised.

Experimental

The method of Talapatra *et al.* was primarily designed for the analysis of paddy straw and similar coarse fodders. Total oxalates are extracted with sodium carbonate solution, and in this respect the method resembles the one used in toxicological investigations. The method was tried with several samples of beet leaves, but without success. The alkaline extracts were very dark in colour, acidification produced little improvement and the calcium oxalate precipitates were so heavily contaminated that no determinations could be made.

It was found that the oxalates could be extracted from finely divided material of this nature with hot *N* hydrochloric acid solution and that oxalic acid remains stable under these conditions. This confirms the observation of Hoover and Karunairatnam[4] that oxalic acid in plant materials remains stable during digestion with hot 1·5 *N* hydrochloric acid for at least 12 hours. The extracts were light brown, and the oxalate precipitates produced by adding a calcium salt at pH 4·5, although much cleaner than those formed in Talapatra's method, were somewhat contaminated by protein material. Contamination was almost completely eliminated by adding a suitable deproteinising agent before precipitating the oxalate. The deproteinising agent must be effective at a pH low enough to retain calcium oxalate in solution and must not form a calcium salt that subsequently co-precipitates with oxalate. A modification of the phosphoric - tungstate reagent of Sendroy and Van Slyke, mentioned by Hawk, Oser and Summerson,[5] was found to be ideal for the purpose. Oxalate was precipitated from the deproteinised extract with calcium chloride in acetic acid buffer solution of pH 4·5. The precipitate was washed with 5 per cent. acetic acid saturated with calcium oxalate to prevent loss of the salt by solution in the acetic acid. Precipitates were clean and dissolved readily in dilute sulphuric acid, leaving no residue; titration with 0·02 *N* potassium permanganate gave a sharp and persistent end-point to within one drop.

A similar technique was applied to aqueous extracts of the leaves in the determination of water-soluble oxalates; again it was necessary to remove the protein although this was present in comparatively small amounts.

By comparing the amount of oxalate found in samples of fresh leaves with that in leaves dried in an oven at 100° C for 24 hours, it was found that drying caused appreciable loss of oxalate, so fresh material was used for routine analysis. Qualitatively, the method is as satisfactory for dry material as for fresh and could probably be adapted to suit the requirements of dry substances.

Method

Apparatus—

A disintegrating machine—a Waring blendor is suitable—and 50-ml tapered centrifuge tubes of resistance glass.

Reagents—

Diluted hydrochloric acid (1 + 1).

Ammonium hydroxide solution—Sp.gr., 0·880.

Phosphoric - tungstate reagent—Dissolve 24 g of sodium tungstate in water, add 40 ml of syrupy phosphoric acid, sp.gr. 1·75, and dilute to 1 litre.

Calcium chloride buffer—Dissolve 25 g of anhydrous calcium chloride in 500 ml of 50 per cent. v/v glacial acetic acid and add this solution to a solution of 330 g of sodium acetate in water, diluted to 500 ml.

Wash solution—A 5 per cent. v/v solution of acetic acid kept over calcium oxalate at room temperature. Shake the solution periodically and filter before use.

Sulphuric acid—A 10 per cent. v/v solution.

Potassium permanganate—A 0·02 *N* solution, prepared as required by diluting a 0·1 *N* solution.

PROCEDURE—

For total oxalate—Homogenise 60 g of chopped green material with about 100 ml of water in the blendor and transfer the mixture to a 600-ml beaker with the minimum number of washings. Add 2 volumes of diluted hydrochloric acid (1 + 1) to each 10 volumes of liquid (to give an approximately normal concentration) and one or two drops of capryl alcohol and boil for 15 minutes. Allow to cool, transfer to a 500-ml volumetric flash, dilute to the mark and after occasional shaking set it aside overnight. Mix and filter through a dry paper. Transfer, by means of a pipette, 25 ml of filtrate into a tube fitted with a stopper, add 5 ml of phosphoric - tungstate reagent, mix by inverting once or twice and set the mixture aside for 5 hours. Centrifuge for 10 minutes at 3000 r.p.m. and radius 6 inches, transfer exactly 20 ml of the clear solution to a 50-ml centrifuge tube and add ammonium hydroxide dropwise from a burette until the solution is alkaline, as indicated by the formation of a slight precipitate of phosphotungstate. Add 5 ml of the calcium chloride reagent, stir with a fine glass rod and leave the tube overnight in a refrigerator at 5° to 7° C. Centrifuge for 10 minutes, carefully remove the supernatant liquid and wash the precipitate with 20 ml of wash solution, stirring vigorously with a fine rod until the precipitate is broken up and the impurities dissolve. Centrifuge for 10 minutes, carefully remove the washings, dissolve the precipitate in 5 ml of 10 per cent. sulphuric acid, place the tube in a water-bath at 100° C for 2 minutes and titrate the oxalic acid with 0·02 *N* potassium permanganate.

$$1 \text{ ml of } 0{\cdot}02\ N\ KMnO_4 \equiv 0{\cdot}00090 \text{ g of } (COOH)_2.$$

Twenty millilitres of deproteinised extract are equivalent to 2·0 g of sample.

For water-soluble oxalate—Homogenise another 60 g of sample and transfer it to a beaker with water, as before. Boil for 15 minutes, cool, dilute to 500 ml, mix, set it aside overnight and filter it through a paper capable of retaining calcium oxalate. Transfer 25 ml of the filtered extract to a stoppered tube by means of a pipette, add 2·5 ml of diluted hydrochloric acid (1 + 1) and then 2·5 ml of phosphoric - tungstate reagent; complete the determination by the procedure described for total oxalate.

RESULTS

Blank determinations on oxalate-free material such as cabbage and kale indicated the specificity of the method. Quantities of pure sodium oxalate ranging from 0·25 to 1·00 per cent. were added to freshly chopped cabbage, to give concentrations like those found in beet leaves. Recoveries with the acid extraction technique are shown in Table I.

TABLE I

RECOVERIES OF OXALATE ADDED TO FRESHLY CHOPPED CABBAGE

Oxalic acid equivalent of sodium oxalate added, theoretical, %	Oxalic acid found, %	Recovery, %
nil	nil	—
0·168	0·166	98·8
0·336	0·334	99·4
0·504	0·504	100·0
0·672	0·670	99·7

Mean recovery = 99·5 per cent.

Recovery tests were also carried out with similar quantities of oxalate added to chopped fodder beet leaves with acid and water extraction techniques, and gave the results shown in Table II. Care was taken to ensure a reasonably homogeneous sample of chopped leaves, because all errors from sample variation would be chargeable to the added oxalate.

These results show recoveries to be excellent under the prescribed conditions, amounts of oxalic acid as low as 3 mg being recovered in some experiments. Further tests showed rates of precipitation to be related to concentration of oxalate; 10 mg were almost completely recovered after 3 hours, but smaller amounts required more time. Precipitation conditions were finally standardised, a period of 16 hours or more in a refrigerator at 5° to 7° C being normally used.

TABLE II

RECOVERIES OF OXALATE ADDED TO FRESHLY CHOPPED FODDER BEET LEAVES

Oxalic acid equivalent of sodium oxalate added, theoretical, %	Oxalic acid found, %	Recovery of added oxalic acid, %	Recovery of added oxalic acid, %
Total oxalate method—			
nil	0·380	—	—
0·168	0·549	0·169	100·1
0·336	0·720	0·340	101·0
0·504	0·877	0·497	98·5
0·672	1·044	0·664	99·0
			Mean = 99·6
Soluble oxalate method—			
nil	0·092	—	—
0·168	0·263	0·171	102·0
0·336	0·430	0·338	100·6
0·504	0·589	0·497	98·6
0·672	0·765	0·673	100·2
			Mean = 100·4

The efficiency of the extraction method was examined by studying the variation in oxalate content of the extract with length of time in contact with the solid material. The results of eight tests are shown in Table III.

TABLE III

EQUILIBRIUM OF OXALATE CONTENT OF EXTRACTS REMAINING IN CONTACT WITH INSOLUBLE MATERIAL (BEET LEAVES)

Sample No.	Oxalic acid found in the material		
	After 16 hours, %	After 48 hours, %	After 96 hours, %
1	0·437	0·435	0·436
2	0.643	0·661	0·661
3	0·680	0·682	0·684
4	0·527	0·540	0·533
5	0·581	0·580	0·583
6	0·441	0·441	0·441
7	0·549	0·554	0·554
8	0·583	0·589	0·585

It is evident from these results that the oxalate present in the sample soon becomes uniformly distributed through the liquid phase and that determination need not be delayed.

The filtered extracts from acid and water treatments were retained and examined at intervals up to 14 days after their preparation. The acid extracts remained clear, but

TABLE IV

QUADRUPLICATE DETERMINATIONS OF TOTAL OXALIC ACID IN TWO SAMPLES OF BEET LEAVES

	Oxalic acid, %	Mean and its standard error, %
Variety A	0·624, 0·620, 0·626, 0·632	0·626 ± 0·005
Variety B	0·472, 0·464, 0·464, 0·469	0·467 ± 0·004

moulds quickly developed in the aqueous solutions. However, the oxalate content remained unchanged throughout, indicating that extracts can be safely kept for several days before carrying out determinations.

Finally, the reproducibility of the method was tried with two samples of beet leaves, each sample being analysed in quadruplicate. Results are shown in Table IV.

As an over-all test of the reproducibility of the method, these results show remarkably close agreement, particularly with material in which sampling errors might well be expected.

The author is indebted to Dr. A. Eden for his interest and encouragement during this investigation.

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Ministry of Agriculture and Fisheries
Anstey Hall, Trumpington, Cambridge

January, 1952

Discussion

The President thanked the author for presenting a paper that had given evidence of the excellent manipulative technique of agriculturalists working with great accuracy on small amounts of material. He asked if it could be proved that oxalate only was precipitated and not the salt of any other acid.

Mr. Baker said that proof of specificity had been mainly by inference, based upon the negative results obtained when the method was applied to oxalate-free plants, but he mentioned that oxalic acid was unique in producing a calcium salt that was insoluble at pH 4·5.

Dr. J. Haslam enquired if it was possible to prove that the calcium salts of other organic acids were not present, by weighing the precipitate as $CaC_2O_4.H_2O$ before dissolving it in acid.

Mr. Baker said the oxalate precipitates were too small to permit critical gravimetric checks with the facilities at his disposal.

Dr. H. Amphlett-Williams said that he had been rather concerned for some years past about the toxicological aspect of the high proportions of oxalic acid that occured in certain vegetable purées and canned baby foods. He had found 0·24 per cent. of water-soluble oxalate in strained spinach purée; this was equivalent to 0·3 g of oxalic acid in a 4½-ounce tin, the whole of which was recommended on the label for daily consumption by a baby of 7 months. The minimum fatal adult dose of oxalic acid was generally given as 4 g and, by Gaubins' or Young's method of calculation, the corresponding dose for a child under 1 year would be one twelfth of this, *viz.*, 0·3 g; this quantity would be capable of combining with the whole daily requirement of calcium.

Dr. J. H. Hamence said that he was surprised to hear the author placed his solutions in the refrigerator in order to precipitate the oxalate, as in his experience, calcium oxalate came down far more readily if the solution was heated in a water-bath. He asked Mr. Baker if he had tried using acetone to assist precipitation. This method had recently been advocated for the determination of calcium in soils and had been used with promising results. It was also used in the wash liquors, particularly when the calcium oxalate was removed by centrifuging.

Mr. Baker agreed that the water-bath technique had advantages when precipitating calcium in macro quantities with oxalate, the main effect being to encourage "granulation." In his experience, however, small amounts of oxalic acid were not readily precipitated from solutions at high temperatures, and the best results were obtained under the cool conditions indicated in the paper. He thanked Dr. Hamence for drawing his attention to the use of acetone.

Changes in Potential of the Dropping-Mercury Electrode during Drop-Formation, and Measurement of Potential in Polarographic Analysis

By W. FURNESS

The electromotive force applied across the terminals of a polarographic cell is influenced slightly by the magnitude and direction of the polarographic current. The change in diffusion current due to the growth and fall of mercury drops is, likewise, accompanied by a pulsation in the applied electromotive force. The amplitude of this pulsation varies partly as the value of the diffusion current at maximum drop size and partly as a function of the position occupied by the sliding contact on the polarograph potentiometer. With a Tinsley polarograph reasonable agreement has been found between calculated and observed effects.

Because of the pulsation it is preferable, when plotting polarograms manually, to determine the diffusion current and the potential of the dropping-mercury electrode when the rate of change of applied electromotive force is least, that is, at the instant of maximum drop size. This potential, with respect to that of a reference electrode, can be measured to the nearest tenth of a millivolt by the Poggendorff compensation method.

Polarographic cells incorporating a quiet mercury-pool to conduct the diffusion current are not always satisfactory; in contact with certain oxy-acids of sulphur, for example, such electrodes may acquire insoluble films in which event their potential and electrical resistance become erratic. These complications are avoidable by means of cells having a pair of permanently attached calomel electrodes. The use of such cells in conjunction with the Tinsley polarograph has been tested by reference to the well known polarograms of cadmium and thallium.

Whilst accuracy in the measurement of diffusion current is the foremost requirement in quantitative polarographic analysis, references to the potential of a polarised electrode are fundamental to the use of polarographic data in qualitative analysis. For many practical purposes it often suffices to record polarograms automatically, in which event the electromotive force applied to the cell circuit can be found approximately from the scale of the abscissa. For other purposes, which justify manual plotting of a polarogram as described by Müller,[1] readings of electromotive force applied by the polarograph potentiometer must be corrected for the iR drop or iR gain throughout the cell circuit in order to find the potential of the dropping-mercury electrode with reference to that of the quiet electrode of the polarographic cell. In the most accurate work, however, the potential of the dropping-mercury electrode at every point on the manual polarogram should be referred to the potential of a standard half-cell that does not conduct the polarographic current, the potential difference being measured potentiometrically. A circuit for this purpose has been described by Lingane and Kolthoff.[2]

In the application of this last-mentioned procedure two problems may arise. First, it may be noticed that the potential of the dropping-mercury electrode is unsteady at every point upon a polarographic wave except where the diffusion current is very small. Secondly, in investigations of the polarographic behaviour of certain sulphur-containing compounds, the surface of a quiet, internal mercury-pool electrode may become covered by a film of sulphide whose electrical resistance is high enough to prevent the dropping-mercury electrode being maintained, even approximately, at a definite potential. In the present work, therefore, the factors that influence the value of electromotive force applied to the cell circuit have been examined and a technique for measuring the potential of the dropping-mercury electrode has been developed. Throughout this investigation a Tinsley polarograph, model V3211, was used, but the conclusions will be applicable, although perhaps to a varying degree, to work with other pen-recording instruments.

Influence of the diffusion current on the electromotive force applied to the cell circuit

In the basic circuit illustrated in Fig. 1, DB is the uniform potentiometer wire across which the battery maintains a steady current when the switch, S, is closed. The fall in potential per unit length of the potentiometer wire is adjusted to some convenient value by means of the resistance, R_1, in series with the battery. The quiet electrode of the polarograph cell is connected through a galvanometer and its shunts, G, to the fixed point, A. The dropping-mercury electrode, whose capillary is immersed in a supporting electrolyte containing, for instance, a reducible ion, is connected to the sliding contact, C, which can occupy any position along DB. Let C move to a point between A and B such that the process of reduction occurs at the dropping-mercury electrode. A current equal to the sum of the diffusion and residual currents then flows from A through G to the quiet electrode, thence through the cell and dropping-mercury electrode back to the potentiometer wire at C.

Kolthoff and Lingane[3] treated this network as a parallel circuit of ohmic resistances and so arrived at an expression for the electromotive force, E_a, applied to the cell circuit, which can be written as—

$$E_a = \frac{R_{AC}R_{c.c.}}{(R_{AC} + R_{c.c.})R_{AB}} \times E_{Bridge},$$

where E_{Bridge} denotes the e.m.f. between A and B,
R_{AB} denotes the effective resistance between A and B,
R_{AC} denotes the resistance of the potentiometer wire between A and C
and $R_{c.c.}$ denotes the resistance of the polarographic cell circuit.

But, as the dropping-mercury electrode is subject to concentration polarisation, the current flowing through the cell cannot be proportional to the electromotive force applied externally across its poles, and for this reason the above expression does not at all times strictly define the value of the electromotive force applied across the polarographic cell circuit. The difference in potential between the points A and C is given strictly in accordance with Ohm's

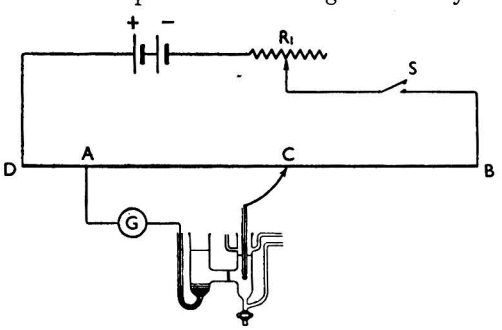

Fig. 1. Basic circuit of the polarograph, with polarographic cell having one external half-cell such as the saturated calomel electrode

law by the product of the resistance of that portion of the potentiometer wire between A and C and the current flowing at any instant along AC. However, the current flowing from A to C along the potentiometer wire cannot remain constant whilst the current in the polarographic cell circuit is continually changing throughout the life of each mercury drop at the capillary, and so it follows that the potential between A and C must also vary throughout the life of each drop.

Theoretical—

Case I—Considering the reduction of a metal ion at the dropping-mercury electrode, let us specify that reduction occurs only when the potential of the dropping-mercury electrode is made negative with respect to that of the quiet electrode of the reference half-cell. As the sliding contact C moves from A in the direction of B no appreciable current is taken from the potentiometer wire until the potential of C with respect to A approaches the polarographic reduction potential of the metal ion. Afterwards, the current taken from the

potentiometer wire follows the current - voltage curve, which is the polarogram of the metal ion. The current flowing in the potentiometer wire from A to C is less than that flowing from D to A or from C to B by a quantity equal, at any given instant, to the current flowing in the polarographic cell circuit.

If contact between the dropping-mercury electrode and the point, C, is temporarily broken the current throughout the whole length of the potentiometer wire becomes steady and uniform at all points. Let its value be denoted by I. If the potential difference across the terminals of the battery is V, the resistance per unit length of the potentiometer wire is r and its length l, then—

$$V = I(rl + \mathrm{R}_1).$$

Let contact again be made between the dropping-mercury electrode and the point C, and let the current flowing from A to C in the polarographic cell circuit at any given instant be denoted by i_1. If the current flowing at the same instant through the battery is denoted by I_1, and the distance between A and C is d, then—

$$V = I_1r(l - d) + (I_1 - i_1)rd + I_1\mathrm{R}_1.$$

Hence,

$$I(rl + \mathrm{R}_1) = I_1rl - i_1rd + I_1\mathrm{R}_1,$$

from which

$$I = I_1 - i_1rd/(rl + \mathrm{R}_1).$$

Let the potential of C on the potentiometer wire against the potential of A be denoted by E whilst the dropping-mercury electrode is temporarily disconnected, and by E_1 when the instantaneous value of the diffusion current is i_1, after the connection at C has been re-made. Then,

$$E = -Ird$$

and

$$E_1 = -(I_1 - i_1)rd.$$

If it is assumed that the diffusion current is negligibly small at the instant following detachment of the previous drop, the change in potential of C with respect to A up to any instant during the life of a single drop is given by—

$$\begin{aligned}\Delta E = E_1 - E &= [I - I_1 + i_1]rd \\ &= [i_1 - i_1rd/(rl + \mathrm{R}_1)]rd \\ &= i_1rd[1 - rd/(rl + \mathrm{R}_1)] \quad .. \quad .. \quad .. \quad (1)\end{aligned}$$

Therefore, whilst the diffusion current is increasing on account of the growth of the drop, the potential of the dropping-mercury electrode must become more positive with respect to A, and, at any given instant, the extent of the change must be proportional to the instantaneous value of the diffusion current. The extent of the change is dependent also upon the position of the sliding contact C, but is independent of the resistance of the polarographic cell circuit between A and C.

Case II—If polarographic reduction occurs whilst the potential of the dropping-mercury electrode is positive with respect to that of the quiet electrode, that is, whilst contact C lies between D and A, no current is taken from the potentiometer wire. Instead, the current I_2 flowing through the battery is supplemented between C and A by the diffusion current i_2. The potential of C, therefore, which is already more positive than that of A at the beginning of the formation of each drop, becomes still more positive as the diffusion current increases with increasing drop size. By use of the previous system of notation and method of derivation,

$$I(rl + \mathrm{R}_1) = I_2r(l - d) + (I_2 + i_2)rd + I_2\mathrm{R}_1,$$

from which

$$I = I_2 + i_2rd/(rl + \mathrm{R}_1).$$

Now

$$E = Ird$$

and

$$E_2 = (I_2 + i_2)rd,$$

therefore,

$$\begin{aligned}\Delta E = E_2 - E &= [I_2 - I + i_2]rd \\ &= [i_2 - i_2rd/(rl + \mathrm{R}_1)]rd \\ &= i_2rd[1 - rd/(rl + \mathrm{R}_1)] \quad .. \quad .. \quad .. \quad (2)\end{aligned}$$

Cases III and IV—There are two other cases to be considered in which the diffusion current is anodic. If C lies between A and B, the anodic diffusion current supplements the current from the battery in that part of the potentiometer wire between A and C. Finally, when C lies between D and A, current is taken from the potentiometer wire if an anodic current flows in the cell circuit. If the anodic currents flowing from the dropping-mercury electrode towards the quiet electrode within the polarographic cell are given the values i_3 and i_4 the changes in the potential of the point C with respect to A during the life of each drop are calculated to be—

$$\Delta E = E_3 - E = -i_3 rd[1 - rd/(rl + R_1)] \quad .. \quad .. \quad .. \quad (3)$$

when C is a point between A and B, and

$$\Delta E = E_4 - E = -i_4 rd[1 - rd/(rl + R_1)] \quad .. \quad .. \quad .. \quad (4)$$

when C is a point between D and A.

The general case—If the convention be now adopted that a reduction of the substance responsible for concentration polarisation at the dropping-mercury electrode gives rise to a positive diffusion current, whilst oxidation is accompanied by a negative diffusion current, the symbol i can be substituted in the above equations for i_1, i_2, $-i_3$ and $-i_4$. Further, if the resistance of that part of the potentiometer wire between A and C be denoted by R_{AC} and that of the whole potentiometer wire by R_{DB}, the general expression for ΔE becomes—

$$\Delta E = iR_{AC}\left(1 - \frac{R_{AC}}{R_{DB} + R_1}\right) \quad .. \quad .. \quad .. \quad .. \quad (5)$$

If ΔE be taken to denote the change in potential of C with respect to that at A during the complete life-period of a single drop, the corresponding value of i to be substituted in equation (5) is the instantaneous value of the diffusion current at maximum drop size.

The inferences to be drawn from equation (5) can be stated as follows.

(*a*) When the position of contact C coincides with the point A, ΔE will be zero irrespective of the value of the diffusion current.

(*b*) For any other given position of C, ΔE should be directly proportional to i as long as the resistances R_{DB} and R_1 remain constant.

(*c*) As the position of contact C changes along the potentiometer wire, the ratio $\Delta E/i$ should attain a maximum value equal to $\frac{1}{4}(R_{DB} + R_1)$ when $R_{AC} = \frac{1}{2}(R_{DB} + R_1)$.

(*d*) The value of the ratio $\Delta E/i$ should be independent of the resistance of the polarographic cell circuit and also independent of the potential gradient along the potentiometer wire.

(*e*) Since, in the practice of polarography, it is desirable that $\Delta E/i$ should be as small as possible, polarographs should be designed so that the resistance $(R_{DB} + R_1)$ is as small as is compatible with the maintenance of a very steady potential gradient along the potentiometer wire.

EXPERIMENTAL—

Equation (5) and the inferences (*a*), (*b*), (*c*) and (*d*) have been tested by experiment.

The potentiometer wire of the Tinsley polarograph, type S10600, model V3211, has the high resistance of 118·5 ohms per volt length and this instrument was used in conjunction with a polarographic cell having an external saturated calomel electrode of the type previously described.[4,5] Solutions containing $Pb^{\cdot\cdot}$, $Fe^{\cdot\cdot\cdot}$, S_2O_4'' or S_2O_3'' in appropriate supporting electrolytes were selected as depolarisers to provide data appropriate to Cases I, II, III and IV above. Throughout the tests the resistances of the potentiometer wire R_{DB} and of R_1 remained constant.

A Tinsley vernier potentiometer, type 3126B, standardised frequently against a Weston cell, was connected across the points of the Tinsley polarograph, *viz.*, terminal "E" and terminal "electrode —," which correspond to points A and C, respectively, of Fig. 1. Contact C was adjusted to the desired position on the polarograph potentiometer wire, without having the dropping-mercury electrode connected to it, and the steady potential difference between C and A was measured on the vernier potentiometer. Next, the dropping-mercury electrode was connected to C. The oscillations of the galvanometer in the Poggendorff compensation circuit then coincided with the growth and fall of each drop at the capillary, and the vernier potentiometer was so adjusted that these oscillations showed a balance of

electromotive force at the instant of maximum drop size. The sum of the diffusion and residual currents recorded at the same instant on the Tinsley pen-recorder was also noted. The two readings on the vernier potentiometer gave, respectively, the values of E and $E_{1,2,3,\text{ or }4}$, and hence the algebraic difference ΔE.

RESULTS—

The experimental data are presented in Tables I and II and also in Fig. 2, where comparisons are made between the observed values of $\Delta E/i$ and values calculated from equation (5).

TABLE I

DETERMINATION OF THE RATIO $\Delta E/i$ FOR VARIOUS POSITIVE VALUES OF i, WHILST R_{AC} HAS SEVERAL DISCRETE VALUES

Polarographic reduction of ferric ion in 0·2 N potassium nitrate, with 0·01 per cent. of gelatin

Polarograph potentiometer, nominal setting, volts	R_{AC}, ohms	Normality of ferric ion, $\times 10^3$	i, μA	Potential of C with respect to A: Dropping-mercury electrode connected to C, mV	Potential of C with respect to A: Dropping-mercury electrode disconnected from C, mV	ΔE, mV	$\Delta E/i$: Observed, mV per μA	$\Delta E/i$: Calculated, mV per μA
+0·1	11·85	2	6·4	85·76	85·69	0·07	0·011	0·012
		4	12·8	85·12	85·98	0·14	0·011	
		10	32·3	85·9	85·54	0·4	0·012	
		20	64·5	85·3	84·50	0·8	0·012	
0	0	2	6·4	−12·30	−12·30	0·00	0·000	0·000
		4	12·9	−11·80	−11·81	0·01	0·001	
		10	32·6	−12·43	−12·47	0·04	0·001	
		20	65·0	−12·58	−12·65	0·07	0·001	
−0·5	59·25	2	6·5	−520·47	−520·82	0·35	0·054	0·052
		4	13·0	−520·93	−521·65	0·72	0·055	
		10	32·7	−518·5	−520·21	1·7	0·052	
		20	65·5	−516·1	−519·63	3·5	0·053	
−1·0	118·5	2	6·4	−1012·80	−1013·40	0·60	0·094	0·089
		4	12·9	−1011·46	−1012·65	1·19	0·092	
		10	31·9	−1010·4	−1013·28	2·9	0·091	
		20	63·5	−1005·8	−1011·67	5·9	0·093	

When R_{AC} is zero A and C are coincident so that ΔE should always be zero irrespective of the value of i. On the Tinsley polarograph the precision with which C can be set to coincide with A is not high, nevertheless, Table I shows the values of $\Delta E/i$ to be negligibly small. For any other point on the cathodic wave of ferric ion, where according to convention the diffusion current is positive, the potential of the dropping-mercury electrode always becomes more positive with respect to that of the calomel electrode whenever the diffusion current is increasing. For values of i within the range 6 to 65 μA the ratio $\Delta E/i$ was found to remain constant at each of the specified values of R_{AC}. On the other hand, when the diffusion current is negative, as illustrated by the examples of the dithionite (S_2O_4'') and thiosulphate waves reported in Table II, the change in potential of C with respect to A is in a negative direction as each drop grows at the dropping-mercury electrode. Here again, the observed values of ΔE were found to be directly proportional to the corresponding values of i, the ratio varying only according to the chosen value of R_{AC}.

The values given in Tables I and II for i are those recorded directly by the pen-recorder at the instant of maximum drop size. These are known to be lower than the real instantaneous values at maximum drop size by approximately 3 per cent.[6] For this reason the data presented as observed values of $\Delta E/i$ are subject to correction and, with this allowance, the observed values are in satisfactory agreement with those calculated from equation (5).

By the use of one or two accumulators to provide the potential gradient along the potentiometer wire it was possible, with a single solution of lead in a potassium nitrate supporting electrolyte, to observe the variation of the ratio $\Delta E/i$ over a range of values of

R_{AC} from 59·3 to 308·1 ohms. The observed and calculated values of $\Delta E/i$ are not tabulated here but have been plotted, along with other data from Tables I and II, in Fig. 2. Subject to the previously mentioned correction of 3 per cent., the agreement is regarded as satisfactory. The observed maximum of 0·123 mV per μA (0·119 mV per μA after correction) compares with the calculated value of $\frac{1}{4}(R_{DB} + R_1)$ which is equal to 0·117 mV per μA, and this observed maximum occurs at a point whose abscissa is 235 ohms, a value which is coincident with the calculated value of $\frac{1}{2}(R_{DB} + R_1)$. In the same experiment, for values of R_{AC} from 106·7 to 154·1 ohms, values of $\Delta E/i$ were obtained both with one and with two accumulators

TABLE II

DETERMINATION OF THE RATIO $\Delta E/i$ FOR VARIOUS NEGATIVE VALUES OF i, WHILST R_{AC} HAS TWO DISCRETE VALUES

(*a*) Polarographic oxidation of dithionite ion (S_2O_4'') in 0·5 *M* di-ammonium hydrogen phosphate, *M* ammonium hydroxide, with 0·01 per cent. of gelatin
(*b*) Polarographic oxidation of mercury on the wave due to thiosulphate ion in 0·2 *N* potassium nitrate

Polarograph potentiometer, nominal setting, volts	R_{AC}, ohms	i, μA	Potential of C with respect to A: Dropping-mercury electrode connected to C, mV	Dropping-mercury electrode disconnected from C, mV	ΔE, mV	$\Delta E/i$, Observed, mV per μA	$\Delta E/i$, Calculated, mV per μA
(*a*) *For various concentrations of the dithionite ion—*							
−0·2	23·70	−88·0	−204·7	−202·60	−2·1	0·024	0·023
		−62·0	−203·9	−202·48	−1·4	0·023	
		−39·5	−203·38	−202·46	−0·92	0·023	
		−21·2	−202·94	−202·46	−0·48	0·023	
(*b*) *For various concentrations of the thiosulphate ion—*							
+0·1	11·85	−85·5	85·37	86·37	−1·00	0·012	0·012
		−64·0	85·58	86·30	−0·72	0·011	
		−43·0	85·83	86·30	−0·47	0·011	
		−22·0	86·05	86·29	−0·24	0·011	

across the polarograph potentiometer. Such a two-fold change in the potential gradient along the potentiometer wire had no effect on the values found for $\Delta E/i$. Nor did the internal resistance of the polarographic cell have any influence, for similar results were obtained with several types of cells whose internal resistances varied from a few hundred to several thousand ohms.

INFLUENCE OF THE DIFFUSION CURRENT ON THE POTENTIAL OF THE DROPPING-MERCURY ELECTRODE

Let us suppose a polarographic cell to be constructed so that two identical half-cells, X and Y, are connected through liquid junctions with the supporting electrolyte in which the tip of the dropping-mercury electrode, Z, is immersed (Fig. 3). Also, let the quiet electrode of the half-cell, X, be connected through a galvanometer G to point A, and let the dropping-mercury electrode, Z, be connected to the sliding contact, C.

As long as no current flows through the polarographic cell the potentials of the electrodes of the two half-cells, X and Y, must be equal to one another and to that of point A, whilst the potential of the dropping-mercury electrode is equal to that of point C. In this instance, the potential of the dropping-mercury electrode against that of either of the half-cells is given strictly by the position of contact C and is equal to $-Ird$. This value can be measured with great accuracy by the Poggendorff compensation method, a potentiometer being connected across C and A, or across C and the half-cell, X, or across C and the half-cell, Y.

Now let a reducible ion be introduced into the supporting electrolyte so that a diffusion current i_1 flows from A to C via G, X and Z. The potential difference between C and A now changes to the value $-(I_1 - i_1)rd$, with the previous notation, but this is numerically larger than the potential difference between C and the half-cell, X, by the quantity i_1R_{AX}, where

R_{AX} denotes the resistance of the direct path taken by the diffusion current between A and X. The potential of C with respect to X is, therefore, given by—

$$-(I_1 - i_1)rd + i_1R_{AX}.$$

As the half-cell, Y, does not conduct any portion of the polarographic current its electrode must be at a more negative potential than that of the half-cell, X. Provided that its liquid junction with the supporting electrolyte does not lie along the path followed by the polarographic current between half-cell X and the tip of the dropping-mercury electrode, Z, half-cell Y can be used as a reference electrode against which the potential of the supporting

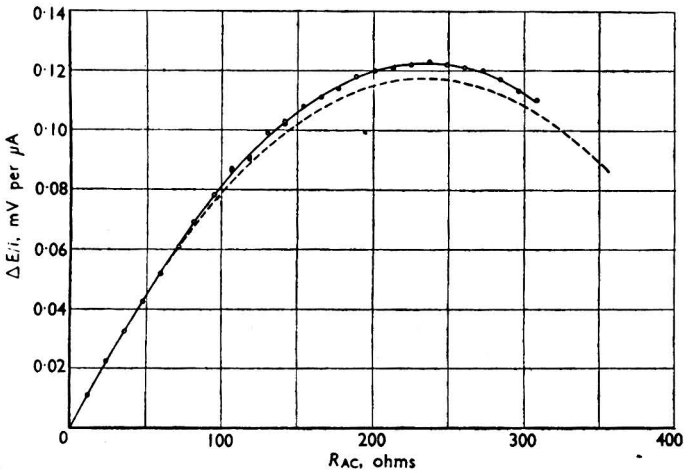

Fig. 2. Relationship between $\Delta E/i$ and the position of the contact along the potentiometer wire. The plot of $\Delta E/i = R_{AC}[1 - R_{AC}/(R_{DB}+R_1)]$ is shown by the broken line. Observed values of $\Delta E/i$ are plotted against selected values of R_{AC}, thus —o—o—o—o—

electrolyte in immediate contact with the dropping-mercury has a standard value. In these circumstances, the potential of C with reference to the electrode of half-cell Y is given by—

$$-(I_1 - i_1)rd + i_1(R_{AX} + R_{cell}) \quad .. \quad .. \quad .. \quad .. \quad (6)$$

where R_{cell} is the resistance of the path within the polarographic cell between the electrode of half-cell X and the supporting electrolyte in immediate contact with the dropping-mercury electrode. Because of the resistance of the thread of mercury, which will be denoted by R_{ZC}, the potential $E_{d.m.e.}$ of the mercury drop with reference to the electrode of half-cell Y is given strictly by the equation—

$$E_{d.m.e.} = -(I_1 - i_1)rd + i_1(R_{AX} + R_{cell} + R_{ZC}) \quad .. \quad (7)$$

If the total change in potential of the dropping-mercury electrode, against the reference electrode, Y, throughout the life of the drop is denoted by $\Delta E_{d.m.e.}$, and if i_1 represents the diffusion current at maximum drop size, we can write—

$$\begin{aligned}\Delta E_{d.m.e.} &= -(I_1 - i_1)rd + i_1(R_{AX} + R_{cell} + R_{ZC}) - (-Ird) \\ &= i_1rd[1 - rd/(rl + R_1)] + i_1(R_{AX} + R_{cell} + R_{ZC}).\end{aligned}$$

A little further consideration will show that, if the instantaneous value of a cathodic or anodic diffusion current at maximum drop size be denoted by i, having regard to the convention relating to its sign, the general expression for $\Delta E_{d.m.e.}$ can be written as—

$$\Delta E_{d.m.e.} = iR_{AC}\left(1 - \frac{R_{AC}}{R_{DB} + R_1}\right) + i(R_{AX} + R_{cell} + R_{ZC}) \quad .. \quad (8)$$

Measurement of the Potential of the Dropping-Mercury Electrode

Choice of Method—

In all polarographs incorporating the circuit shown in Fig. 3 the potential of the dropping-mercury electrode against a reference half-cell can only be strictly proportional to the distance

AC if the polarographic current is zero. Otherwise, for any given position of C, the potential of the dropping-mercury electrode pulsates with a frequency equal to the drop rate and with an amplitude proportional to the instantaneous value of the diffusion current at maximum drop size. The maximum value of the first term on the right-hand side of equation (8) is $\frac{1}{4}i(R_{DB} + R_1)$ but, since the resistance of the potentiometer wire in many polarographs is low, this term can be negligible in comparison with the second. In the Tinsley polarograph, however, the value of $\frac{1}{4}(R_{DB} + R_1)$ is approximately 117 ohms and, since R_{AX} is very small the value of $(R_{AX} + R_{cell} + R_{ZC})$ is chiefly dependent on the resistance of the polarographic

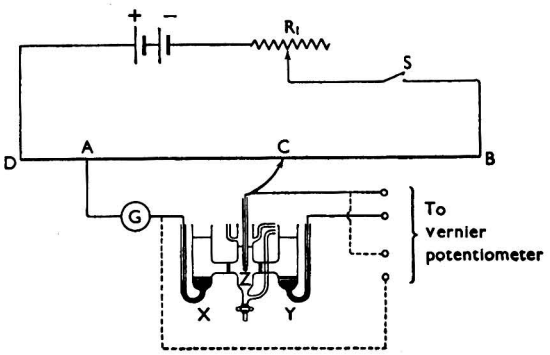

Fig. 3. Basic circuit of the polarograph, but with polarographic cell having two external half-cells and connections to vernier potentiometer. The half-cell on the right is the reference electrode

cell, which may be only a few hundred ohms. For this reason, if accuracy in potential measurements to the nearest millivolt or better is desired when plotting manual polarograms with the Tinsley polarograph, the effect of both terms on the right-hand side of equation (8) must be taken into account, especially if the diffusion current approaches or exceeds 10 μA. Some investigators, following Lingane,[7] have preferred to work with very dilute solutions of the depolariser in order that the iR correction should be negligibly small, but when half-wave potentials are to be measured this procedure suffers from the disadvantage that the residual current (which is not always accurately known) forms an appreciable part of the total current at the dropping-mercury electrode.

In order to find accurately the potential of the dropping-mercury electrode, $E_{d.m.e.}$, when the diffusion current is large, it would be possible first to measure the steady potential difference between C and A (Fig. 1) whilst the dropping-mercury electrode is temporarily disconnected from C, secondly to measure the diffusion current i when connection at C is re-made, thirdly to calculate $\Delta E_{d.m.e.}$ from equation (8) and consequently to find arithmetically the value of $E_{d.m.e.}$. Such a procedure would be tedious and would involve a knowledge of the internal resistance of the polarographic cell.[1,8] It is in every respect preferable to measure the potential of point C (Fig. 3) against a reference half-cell such as Y, which does not conduct the polarographic current. The instantaneous value of this potential difference has been defined above, equation (6), and its value should be measured at the instant when its rate of change is least, that is, at the instant of maximum drop size when the rate of change of diffusion current is least. The only correction then needed is for the potential difference iR_{ZC} (with due regard to the sign of i) across the capillary of the dropping-mercury electrode and also across any tungsten contacts that may have been incorporated in the dropping-mercury electrode assembly.[9,10]

DESIGN OF POLAROGRAPHIC CELL—

Polarographic cells with a dropping-mercury electrode, a quiet "working" mercury-pool electrode and a reference half-cell were used by Lingane and Kolthoff[2] and by later workers. Occasionally, the quiet working mercury electrode acquires a surface film whose electrical resistance is so high that the potential of the dropping-mercury electrode becomes erratic. This happens, for example, in the polarographic examination of certain sulphur compounds. The difficulty can be avoided by the use of an external half-cell as the quiet working electrode

of the polarographic cell, and if a second half-cell is incorporated this can serve as the reference electrode against which the potential of the dropping-mercury electrode may be measured. Thus, the two half-cells would function as X and Y, respectively, of Fig. 3. Such a polarographic cell has been constructed, having two saturated calomel half-cells disposed symmetrically about the central dropping-mercury electrode compartment. In other respects its features and principal dimensions are similar to those of the H-type cell previously described.[5] This cell is intended for use only in the circuit shown diagrammatically in Fig. 3 when polarograms are plotted manually; it does not supplant the simpler H-type of polarographic cell when polarograms are being automatically recorded.

PROCEDURE FOR MEASUREMENT OF DROPPING-MERCURY ELECTRODE POTENTIAL—

During the plotting of a polarogram, the cell is partially immersed in a thermostat at 25·0° C. The dropping-mercury electrode and one of the saturated calomel electrodes (hereinafter called the working calomel electrode) are connected to the polarising unit of the polarograph, whilst the dropping-mercury electrode and the other saturated calomel electrode (the reference electrode) are connected directly to the terminals of a Tinsley vernier potentiometer, type 3126B, as indicated in Fig. 3. These are the only connections to the polarographic

TABLE III

DATA FOR MANUAL POLAROGRAM OF 1·82 MILLIMOLAR CADMIUM SULPHATE IN 0·1 *N* POTASSIUM CHLORIDE, WITH 0·01 PER CENT. OF GELATIN

Temperature, 25·0° C $m^{\frac{2}{3}}t^{\frac{1}{6}} = 1{\cdot}90$ mg$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$

Values of i recorded at instant of maximum drop size and corrected for residual current

E_I *vs.* S.C.E., volts	$E_I + iR_{ZC}$ *vs.* S.C.E., volts	E_{II} *vs.* S.C.E., volts	$E_I + iR_{ZC} - E_{II}$, volts	i, μA	$(i_d - i)$, μA	$\log_{10} i/(i_d - i)$
−0·41454	−0·41454	−0·41456	0·00002	0	—	—
−0·52337	−0·52337	−0·52362	0·00025	0·16	13·60	$\bar{2}$·07
−0·5721	−0·5720	−0·5743	0·0023	1·64	12·12	$\bar{1}$·131
−0·5810	−0·5809	−0·5843	0·0034	2·80	10·96	$\bar{1}$·407
−0·5894	−0·5893	−0·5949	0·0056	4·40	9·36	$\bar{1}$·672
−0·5967	−0·5965	−0·6049	0·0084	6·04	7·72	$\bar{1}$·893
−0·6035	−0·6033	−0·6141	0·0108	7·88	5·88	0·127
−0·6094	−0·6091	−0·6224	0·0133	9·20	4·56	0·305
−0·6200	−0·6197	−0·6358	0·0161	11·24	2·52	0·65
−0·6310	−0·6307	−0·6487	0·0180	12·42	1·34	0·97
−0·7942	−0·7938	−0·8138	0·0200	13·76	—	—

cell that are required for plotting the polarographic waves manually. In some experiments, however, connections were also taken from the dropping-mercury electrode and working calomel electrode to a second pair of terminals on the vernier potentiometer; these connections are indicated in Fig. 3 by broken lines.

For each point to be plotted on the polarographic wave it is only necessary (*i*) to adjust the polarising unit of the polarograph to apply an appropriate electromotive force between the dropping-mercury electrode and the working calomel electrode, (*ii*) to observe the value recorded for the diffusion current i at maximum drop size and (*iii*) to measure E_I, the potential of C (Fig. 3) against the reference electrode, Y, at the instant of maximum drop size. A little further information, relating only to the internal resistance of the polarographic cell, can be derived by reporting also for each point on the polarographic wave the potential E_{II} of point C against the working calomel electrode.

TREATMENT OF DATA—

Values of i plotted against those of E_{II} would give the curve called by Müller[1] the current - voltage curve, but a plot of i against values of E_I (corrected for potential difference across the capillary of the dropping-mercury electrode) would give the current - potential curve from which the half-wave potential could be found by inspection.

To illustrate the treatment of the data, some results relating to a manual polarogram of a solution of cadmium sulphate (1·82 millimolar in 0·1 *N* potassium chloride, with 0·01 per cent. of gelatin) are presented in Table III. This polarogram is not reproduced here, but

in Fig. 4 the function $\log_{10} i/(i_d - i)$ is plotted against the corresponding values of $(E_I + iR_{ZC})$ and of E_{II}. With values of $(E_I + iR_{ZC})$ as abscissae the plot is linear and leads directly to the results—

$$2{\cdot}303RT/nF = 0{\cdot}031 \text{ volt}$$

and $$E_{\frac{1}{2}} = -0{\cdot}600 \text{ volt against the saturated calomel electrode.}$$

With values of E_{II} as abscissae the plot deviates from the straight line; the displacement measured along the abscissa is proportional to the diffusion current at that point, as shown more clearly inset in Fig. 4. The difference between $(E_I + iR_{ZC})$ and E_{II} for any given value of i is equal to the electromotive force required to drive the diffusion and residual currents through the polarographic cell and dropping-mercury electrode. The slope of the

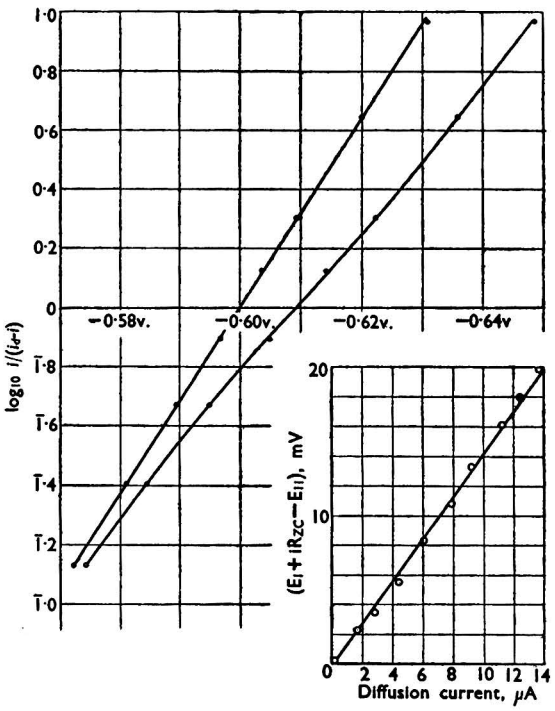

Fig. 4. Graphs of the data from Table III relating to the polarogram of 1·82 millimolar cadmium sulphate, 0·1 *N* potassium chloride, with 0·01 per cent. of gelatin. The function $\log_{10} i/(i_d - i)$ is plotted against $(E_I + iR_{ZC})$ (linear), and against E_{II} (non-linear). The inset shows the linear relationship between $(E_I + iR_{ZC} - E_{II})$ and i. The slope, equivalent to 1430 ohms, gives the internal resistance of the polarographic cell and dropping-mercury electrode

linear plot in Fig. 4 (inset), therefore, gives the ohmic resistance of the path followed by the diffusion current through the polarographic cell and the capillary of the dropping-mercury electrode.

The precision with which values of E_I and E_{II} can be balanced on the vernier potentiometer at the instant of maximum drop size depends upon (*i*) the value of $\Delta E_{d.m.e.}$, (*ii*) the drop rate of the dropping-mercury electrode and (*iii*) the period of the galvanometer in the Poggendorff circuit. Throughout the above work a Tinsley galvanometer, type S.S.2. 45, of period 2·0 seconds was used to detect compensation; with a drop time at the capillary of 3·4 seconds, the point of balance at maximum drop size could be found to the nearest tenth of a millivolt or better so long as $\Delta E_{d.m.e.}$ did not exceed 25 mV. This degree of

precision is adequate, for in plotting polarograms manually the possible errors in measurement of diffusion current with the present equipment do not permit half-wave potentials to be reported with an accuracy better than one millivolt.

In order to test the procedure outlined above further, the half-wave potentials of the thallous and cadmium ions, at various concentrations, were determined in potassium nitrate and potassium chloride supporting electrolytes. The values so obtained, recorded in Table IV, are in close agreement with other published values.[7]

TABLE IV

HALF-WAVE POTENTIALS, $E_{\frac{1}{2}}$, REFERRED TO THE SATURATED CALOMEL ELECTRODE AT 25·0° C

Ion	Concentration, millimolar	Supporting electrolyte	$E_{\frac{1}{2}}$ *vs.* S.C.E., volts
Thallous	1·20	0·1 *N* KNO_3, 0·01% gelatin	−0·455
"	2·00	" "	−0·455
"	4·01	" "	−0·457, −0·458
"	7·61	" "	−0·459
"	10·02	" "	−0·460
Cadmium	1·02	0·1 *N* KNO_3, 0·01% gelatin	−0·581
"	1·82	" "	−0·583
"	1·82	0·1 *N* KCl, 0·01% gelatin	−0·600
"	4·73	" "	−0·600

The same procedure has been applied extensively in plotting manually the polarograms of certain oxy-acids of sulphur. In this field of work the comments of Kolthoff[11] regarding the unsatisfactory nature of internal mercury-pool electrodes are particularly applicable. In much of this later work it has been advantageous to use relatively high concentrations (up to 10 millimolar) of depolariser. The three-electrode polarographic cell (Fig. 3) has enabled such solutions to be examined without incurring the risks that otherwise would attend the formation of mercurous sulphide films on a mercury-pool anode and without necessitating corrections to potential measurements owing to the internal resistance of the cell. This work will be reported elsewhere.

The author is indebted to Dr. W. Cule Davies for many helpful discussions and gratefully acknowledges his friendly advice and encouragement.

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February, 1952

The Reproducibility of Geometrical Correction Procedures in the Spectrophotometric Estimation of Vitamin A

By H. H. BAGNALL and F. G. STOCK

Recent assessments of the precision of geometrical correction procedures for the spectrophotometric estimation of vitamin A are discussed. The spectrophotometric data on the international standard preparation of vitamin A for the instrument used are given. Experience with recent modifications of the method, including a detailed table of the results of applying the three correction equation procedure of Cama, Collins and Morton, is described. The intra-laboratory reproducibility is indicated by the application of these three correction equations to each of three weights of oil, so giving nine values for the corrected $E^{1\%}_{1cm}$ at 328 mμ for which the average fiducial limits (P = 0·05) of the mean are $\pm$1·2 per cent. The results of a small-scale inter-laboratory test are given. The use of a factor to allow for the presence of neovitamin A is discussed.

The spectrophotometric determination of vitamin A has been discussed at some length elsewhere[1] and this paper will be concerned solely with the reproducibility of the results of geometrical correction procedures, in the belief that the new method of the B.P. Addendum 1951,[2] with some modifications, is worthy of better support than is suggested in the publication of Adamson, Elvidge, Gridgeman, Hopkins, Stuckey and Taylor.[3] Any statement about the precision of the method must surely rest ultimately upon the reproducibility of results within a single laboratory and, although it is impossible to eliminate inter-laboratory variation, the reproducibility within individual laboratories when every possible precaution has been taken must be indicated before a comparison between a number of laboratories can legitimately be made.

Cama, Collins and Morton[4] have recently derived new standard absorption curves for vitamin-A ester and alcohol using both synthetic and natural vitamin. In the same paper the spectroscopic properties of all-*trans* vitamin-A alcohol and acetate are dealt with fully and the data given should become accepted as standard for these substances. The following correction equations for eliminating the effect of irrelevant absorption, derived from the absorption curve for all-*trans* vitamin-A acetate in *cyclo*hexane, are given—

(*a*) E (corrected)* = 7 (E at 327·5 mμ − 0·405 E at 312·5 mμ − 0·595 E at 337·7 mμ),
(*b*) E (corrected) = 6·58 (E at 328 mμ − 0·412 E at 313 mμ − 0·588 E at 338·5 mμ)
and (*c*) E (corrected) = 3·52 (2 E at 328 mμ − E at 316 mμ − E at 340 mμ).

The validity of geometrical correction procedures rests upon the assumption that the irrelevant absorption at the fixation points is linearly related; this assumption has been challenged because it cannot be tested experimentally, but, as data on the reproducibility of the method are to be investigated, comment on the effective linearity of the irrelevant absorption is unnecessary.

Adamson *et al.*[3] discuss the precision of the three-point correction method. Seven laboratories assayed each of five vitamin-A oils, readings being made in duplicate with photoelectric instruments. The gross $E^{1\%}_{1cm}$ at 328 mμ values were geometrically corrected for irrelevant absorption and the conclusion, from a statistical analysis of the results, was that the limits of error of a determination of vitamin-A content in duplicate by any one of the seven laboratories were about $\pm$15 per cent. for P = 0·05; the corresponding figures for gross E values were $\pm$2 per cent. As the authors of that paper state that their concern is with the reproducibility of the method, the apparent lack of precision implied by this conclusion is somewhat disconcerting. If, however, we examine more closely the nature of the data used in the statistical calculations in relation to the deductions made from the latter, we may justifiably entertain some doubts of the value of this inter-laboratory test as a

* This equation is used in the method of the B.P. Addendum 1951.

criterion of the possible precision of the method. To take one example, a particular sample of halibut liver oil submitted to each of the seven laboratories was found to have an average irrelevant absorption of 10·7 per cent.; yet the figure furnished by one of the laboratories was *minus* 4 per cent.; it is difficult to avoid the conclusion that any results obtained by a laboratory capable of so wide a margin of error would tend to be somewhat unreliable. In any event, the inclusion of a nonsensical result such as this, from which in practice no conclusions would be drawn, is surely unjustifiable, and it seems that insufficient attention was paid to the variability of the results of the laboratory in question and from one other. Although it is not possible, in the absence of the original data, to be dogmatic, it seems likely that a significantly greater precision would have been obtained if only the more consistent results submitted by the other five laboratories had been used. Nevertheless, this experiment may be fair enough if considered as a test conducted with a view to determining *what might happen* if the Morton and Stubbs correction procedure were applied in a number of laboratories to the analytical results from particular oils; but the result gives no indication at all of the ultimate precision of the method, as at this stage laboratories

TABLE I

THE INTERNATIONAL STANDARD PREPARATION

Wavelength, mμ	All-*trans* vitamin-A acetate Solvent—*cyclo*hexane (Cama, Collins and Morton[4]), $E_{\lambda}/E_{\lambda(max.)}$	International standard preparation Compensator—cotton seed oil Solvent—*cyclo*hexane, $E_{\lambda}/E_{\lambda(max.)}$
295	0·448	0·447
300	0·555	0·555
305	0·670	0·667
310	0·806	0·806
311	0·830	0·829
312	0·846	0·846
312·5		0·857
313	0·867	0·867
315	0·894	0·890
317·5		0·916
320	0·935	0·937
322·5		0·965
325	0·985	0·988
326	0·993	0·995
327	1·000	0·998
328	1·000	1·000
330	0·989	0·991
335	0·915	0·914
338	0·853	0·857
338·5		0·843
340	0·811	0·814
345	0·695	0·700
350	0·556	0·562

vary considerably in experience, expertise and manipulative care. In fact, the paper of Adamson *et al.*[3] points to the necessity for rigid and meticulous standardisation of technique more than anything else. The Morton and Stubbs[5,6,7] correction procedure was used in the above-mentioned test; by using the revised data of Cama *et al.*[4] an experimental design is made possible that, if care be taken, should greatly increase the precision. It is interesting to compare the experiences of Adamson *et al.*[3] with those of Morgareidge, Blitz, Foy and Aaron[8] who, by the use of the procedure of the U.S.P. XIV,[9] show the magnitude of the error in a corrected E value determined on the unsaponifiable fraction to be about ±8 per cent. of the true mean for a single determination ($P = 0·05$), as deduced from data from a number of laboratories. The authors give their impression that the degree of inter-laboratory variation is gradually decreasing as more experience with the method is gained, and stress the constant attention needed to maintain spectrophotometers in their state of fine adjustment. Wider limits of error are to be expected if the U.S.P. method is used rather than that of the B.P. Addendum 1951, simply on account of the fact that the former involves saponification of the oil and hence more manipulation than the latter, which requires simple solution of the oil. If, therefore, we accept the conclusions of Morgareidge *et al.*,[8] the B.P. Addendum method should be

capable of giving results with limits of error of less than ±8 per cent., and experience leads one to believe that this is so. Rigorous attention to the calibration of the spectrophotometer is a prerequisite to all reliable results, and, when the results of inter-laboratory tests are discussed, special reference from this point of view should be made to the precautions that each laboratory has taken. Greater attention paid to this factor will eventually result in the reduction of errors to a minimum and the attainment of far better agreement between laboratories.

Experimental

The experimental data that follow, with the exception of those relating to the international standard preparation, are concerned solely with the examination of high-potency vitamin-A ester material, chiefly halibut liver oils with an approximate potency of 30,000 i.u. per g. For the estimation of vitamin A in such preparations, the method of the B.P. Addendum

Table II

Intra-laboratory reproducibility with halibut liver oils

Laboratory and sample number	Gross $E^{1\%}_{1cm}$ value		Corrected $E^{1\%}_{1cm}$ value				
			Replicates at three sets of fixation points				
	Replicates	Mean	(*a*) 312·5 mμ, 327·5 mμ and 337·7 mμ	(*b*) 313 mμ, 328 mμ and 338·5 mμ	(*c*) 316 mμ, 328 mμ and 340 mμ	Mean of nine values with P = 0·05 fiducial limits	Fiducial limits as a percentage of the mean
1*a*	16·03		13·45	13·59	13·53		
	16·00		13·41	13·25	13·67		
	15·97	16·00	13·51	13·47	13·46	13·48 ± 0·08	0·60
1*b*	16·06		13·50	13·55	13·80		
	15·92		13·55	13·29	13·42		
	15·84	15·94	13·45	13·53	13·55	13·51 ± 0·10	0·74
1*c*	16·10		13·30	13·49	13·57		
	16·18		13·69	13·68	13·51		
	15·93	16·07	12·57	12·83	12·99	13·29 ± 0·31	2·33
1*d*	15·92		12·99	12·82	12·90		
	15·94		13·33	13·29	13·48		
	15·86	15·91	13·36	13·49	13·56	13·25 ± 0·22	1·66
1*e*	15·77		12·89	13·28	13·10		
	15·72		12·77	13·04	12·98		
	15·56	15·68	13·32	13·02	13·30	13·08 ± 0·15	1·15
2*a*	15·07		13·15	13·00	12·98		
	15·00		13·29	13·26	13·40		
	14·93	15·00	13·13	13·03	13·11	13·15 ± 0·11	0·84
2*b*	15·11		13·33	12·98	12·92		
	15·06		13·16	12·83	12·90		
	15·00	15·06	12·90	13·03	12·94	13·00 ± 0·12	0·92
2*c*	15·08		12·93	13·09	12·85		
	14·98		12·66	12·73	12·47		
	14·97	15·01	13·20	12·98	13·04	12·88 ± 0·18	1·40
2*d*	15·26		13·21	13·32	13·43		
	14·99		12·97	12·76	12·77		
	14·95	15·07	13·05	12·80	13·15	13·05 ± 0·19	1·46
2*e*	15·17		13·26	13·09	13·19		
	15·01		12·98	12·91	13·14		
	14·94	15·04	12·57	12·82	12·89	12·98 ± 0·16	1·23
3*a*	15·61		12·74	12·69	12·55		
	15·66		12·69	12·88	12·50		
	15·55	15·61	12·44	12·67	12·35	12·61 ± 0·13	1·03
4*a*	15·12		13·28	13·02	13·09		
	15·01		12·76	12·48	12·52		
	15·02	15·05	12·84	12·87	12·86	12·86 ± 0·20	1·56

TABLE II—*continued*

Laboratory and sample number	Gross $E^{1\%}_{1cm}$ value		Corrected $E^{1\%}_{1cm}$ value				
			Replicates at three sets of fixation points				
	Replicates	Mean	(*a*) 312·5 mμ, 327·5 mμ and 337·7 mμ	(*b*) 313 mμ, 328 mμ and 338·5 mμ	(*c*) 316 mμ, 328 mμ and 340 mμ	Mean of nine values with P = 0·05 fiducial limits	Fiducial limits as a percentage of the mean
4b	15·08		12·92	12·92	13·05		
	15·07		13·40	13·12	13·02		
	14·97	15·04	13·07	13·04	13·02	13·06 ± 0·11	0·84
4c	15·17		13·24	13·12	13·20		
	15·03		13·22	13·08	13·10		
	15·05	15·08	13·46	13·28	13·19	13·21 ± 0·09	0·68
4d	15·31		13·67	13·64	13·67		
	15·00		12·93	12·90	12·91		
	15·04	15·12	13·07	12·93	12·88	13·18 ± 0·28	2·12
5a	15·31		12·37	11·95	12·30		
	15·20		12·45	12·52	12·44		
	15·31	15·27	12·87	12·81	13·04	12·53 ± 0·26	2·08
5b	15·87		13·29	13·14	13·17		
	15·79		13·36	13·37	13·13		
	15·73	15·80	13·35	13·14	13·15	13·23 ± 0·08	0·60
5c	15·59		13·15	13·39	13·32		
	15·49		12·90	13·05	12·99		
	15·47	15·52	12·93	12·94	13·06	13·08 ± 0·13	0·99
6a	15·62		12·74	12·77	12·63		
	15·54		12·61	12·53	12·35		
	15·44	15·53	12·32	12·37	12·45	12·53 ± 0·13	1·04
7a	13·80		11·03	11·00	11·06		
	13·70		11·00	10·92	11·02		
	13·77	13·76	11·25	11·20	11·13	11·07 ± 0·08	0·72
8a	15·97		13·41	13·36	13·13		
	15·92		13·17	12·96	13·04		
	15·84	15·91	13·24	13·13	13·34	13·20 ± 0·12	0·91
8b	15·68		13·22	13·31	13·43		
	15·64		13·44	13·26	13·47		
	15·68	15·67	13·81	13·72	13·71	13·50 ± 0·16	1·19
8c	15·68		13·10	12·79	13·13		
	15·61		12·86	12·72	12·85		
	15·59	15·63	12·61	12·79	12·80	12·85 ± 0·13	1·01
8d	15·76		13·86	13·63	13·84		
	15·62		13·42	13·36	13·66		
	15·54	15·64	13·72	13·36	13·45	13·59 ± 0·15	1·10
8e	15·45		12·96	12·84	12·93		
	15·31		12·38	12·23	12·11		
	15·28	15·35	12·51	12·46	12·59	12·56 ± 0·20	1·67
8f	15·81		13·07	13·36	13·31		
	15·74		13·40	13·29	13·20		
	15·66	15·74	13·32	12·93	13·38	13·25 ± 0·12	0·91
9a	15·71		14·46	13·92	14·20		
	15·63		14·09	13·94	13·61		
	15·65	15·66	14·03	13·98	13·99	14·03 ± 0·18	1·28
9b	15·42		14·03	13·79	13·76		
	15·59		13·90	13·68	13·93		
	15·46	15·49	13·95	13·72	13·85	13·85 ± 0·09	0·65
						Mean ($\bar{x}$) =	1·17

1951 was followed with the following modifications, *viz.*, all three correction equations recommended by Cama *et al.*[4] were used with each of three separate weights of oil dissolved in *cyclo*hexane so giving nine "corrected" values for $E^{1\%}_{1cm}$ at 327·5 to 328 mμ. The fiducial limits (P = 0·05) of the mean of the nine values were then calculated. Experience shows that the wavelength scale of the spectrophotometer can easily get out of adjustment and it is important to check frequently the position of the 4861A hydrogen line. Gridgeman[10] has drawn attention to the large errors that can be introduced by comparatively small displacements of this scale. The instrument used in these determinations was the Unicam photo-electric spectrophotometer.

Results

The international standard preparation, the diluent oil being used as compensator and *cyclo*hexane as solvent, would be expected to have an $E^{1\%}_{1cm}$ at 328 mμ of 5·21 (× 1920 = 10,000 i.u. per g). Three determinations were made, separate weighings from different capsules being used.

Table III

$E^{1\%}_{1cm}$ of halibut liver oil samples

Oil number	Wave-length, mμ	Laboratory A				Laboratory B			
		$E^{1\%}_{1cm}$ replicates			Mean	$E^{1\%}_{1cm}$ replicates			Mean
1	312·5	16·51	16·46	16·44	16·47	16·42	16·50	16·24	16·39
	313·0	16·69	16·66	16·63	16·66	16·57	16·62	16·47	16·55
	316·0	17·46	17·37	17·38	17·40	17·38	17·33	17·26	17·33
	327·5	19·13	19·10	19·02	19·08	19·04	19·00	19·00	19·02
	328·0	19·16	19·14	19·02	19·11	19·09	19·00	18·95	19·02
	337·7	17·06	16·96	16·94	16·99	16·81	16·91	16·84	16·85
	338·5	16·73	16·63	16·60	16·65	16·62	16·57	16·44	16·54
	340·0	16·26	16·14	16·13	16·18	15·93	16·03	15·97	15·98
2	312·5	16·07	16·13	16·14	16·11	16·00	16·08	16·17	16·08
	313·0	16·23	16·25	16·31	16·26	16·14	16·24	16·19	16·19
	316·0	16·99	17·02	17·04	17·02	17·00	16·92	17·13	17·02
	327·5	18·60	18·61	18·66	18·62	18·55	18·59	18·80	18·64
	328·0	18·60	18·61	18·69	18·63	18·57	18·63	18·80	18·67
	337·7	16·43	16·50	16·45	16·46	16·52	16·45	16·63	16·34
	338·5	16·11	16·21	16·17	16·16	16·17	16·12	16·39	16·22
	340·0	15·67	15·60	15·68	15·65	15·60	15·59	15·76	15·65
3	312·5	19·84	19·86	20·04	19·91	19·55	19·90	19·53	19·66
	313·0	20·13	20·08	20·19	20·13	19·68	20·05	19·70	19·81
	316·0	21·08	21·04	21·11	21·08	20·60	20·95	20·85	20·80
	327·5	23·30	23·20	23·35	23·28	22·74	23·29	23·15	23·06
	328·0	23·30	23·20	23·35	23·28	22·79	23·34	23·18	23·10
	337·7	21·08	20·88	20·95	20·97	20·52	21·05	20·80	20·79
	338·5	20·60	20·60	20·68	20·63	20·20	20·66	20·53	20·46
	340·0	20·13	19·97	20·13	20·08	19·58	20·05	19·88	19·84

Rather than assume that each capsule contained exactly 0·250 g of material, a known amount was weighed from each capsule and compensated with the same concentration of diluent oil. The results were 5·104, 5·099 and 5·112, having a mean of 5·105, which agrees closely with the value of 5·09 on a weighed amount, obtained by Cama *et al.*[4] The international standard preparation according to these figures is apparently 2 per cent. deficient in activity. There is certainly something abnormal about it, because continuation of the absorption curve over the short-wave side of 300 mμ shows a very marked departure from the standard vitamin-A acetate curve. Nevertheless, the standard over the range 300 to 340 mμ is very useful as a means of checking the ratio $E_\lambda/E_{\lambda(max.)}$ at a particular wavelength, and the values agree very closely with the figures of Cama *et al.*[4] for pure all-*trans* vitamin-A acetate (Table I). For extreme accuracy it may be necessary to derive particular equations for each instrument with pure all-*trans* vitamin-A acetate.

Table II shows the results from twenty-eight samples of a brand of halibut liver oil capsules taken under the Food and Drugs Act from the same number of retail chemists' shops in Birmingham. They include various numbers of samples originating from nine different halibut liver oils. The P = 0·05 fiducial limits of the mean of nine values for the corrected

$E^{1\%}_{1\,cm}$ at 328 mμ, expressed as a percentage of the mean, vary from ± 0·60 to ± 2·33, with average value of ± 1·17. This is a representative set of data from a total of 127 samples, chiefly of halibut liver oils, examined during an analytical survey of these preparations in 1951.[11]

The intra-laboratory reproducibility indicated by the data shown in Table II suggests that by adopting the outlined experimental design an inter-laboratory test should show the precision of the method to be better than has been indicated by previous tests. In order to verify this conjecture, three halibut liver oils were analysed, and samples of the same oils were submitted simultaneously to another laboratory for analysis. The results are recorded in Tables III, IV and V. The figures under Laboratory A were obtained by us with a Unicam instrument and those under Laboratory B by the other laboratory with a Uvispek instrument.

Table IV

Corrected $E^{1\%}_{1\,cm}$ at 327·5 to 328 mμ of halibut liver oils

Oil number	Correction procedure*	Laboratory A: Corrected $E^{1\%}_{1\,cm}$ at 327·5 to 328 mμ replicates			Mean	Laboratory B: Corrected $E^{1\%}_{1\,cm}$ at 327·5 to 328 mμ replicates			Mean
1	(*a*)	16·03	16·36	15·98	16·12	16·72	15·81	16·84	16·46
	(*b*)	16·13	16·36	15·90	16·13	16·40	15·86	17·09	16·45
	(*c*)	16·26	16·78	15·96	16·33	17·14	16·26	16·44	16·61
2	(*a*)	16·23	15·85	16·38	16·15	15·64	16·02	16·46	16·04
	(*b*)	16·07	15·68	16·23	15·93	16·08	16·20	16·40	16·23
	(*c*)	15·98	16·20	16·43	16·20	15·98	16·72	16·58	16·43
3	(*a*)	19·06	19·15	19·47	19·23	18·30	18·95	20·08	19·11
	(*b*)	19·04	18·54	18·87	18·82	18·44	19·28	19·69	19·14
	(*c*)	19·00	19·03	19·26	19·10	19·01	19·86	19·82	19·56

* Fixation points: (*a*) 312·5, 327·5 and 337·7 mμ; (*b*) 313, 328 and 338·5 mμ; (*c*) 316, 328 and 340 mμ.

Table V

Mean corrected $E^{1\%}_{1\,cm}$ at 327·5 to 328 mμ and its P = 0·05 fiducial limits

Oil number	Laboratory A	Laboratory B
1	16·20 ± 0·21 (±1·30%)	16·51 ± 0·37 (±2·24%)
2	16·12 ± 0·19 (±1·18%)	16·23 ± 0·25 (±1·54%)
3	19·05 ± 0·20 (±1·05%)	19·27 ± 0·49 (±2·54%)

Table VI

Data on the international standard preparation from laboratory B

Solvent: *cyclo*hexane. Compensator: cotton seed oil ($E^{1\%}_{1\,cm}$ at 327·5 mμ = 5·13)

Wavelength, mμ	$E_\lambda/E_{\lambda(max.)}$	Wavelength, mμ	$E_\lambda/E_{\lambda(max.)}$
290·0	0·327	327·0	0·998
300·0	0·551	327·5	1·000
305·0	0·660	328·0	0·998
310·0	0·792	330·0	0·994
312·5	0·849	335·0	0·919
315·0	0·881	337·0	0·865
317·5	0·911	340·0	0·811
320·0	0·935	345·0	0·697
322·5	0·962	350·0	0·557
325·0	0·990	355·0	0·424
326·0	0·994	360·0	0·313

Discussion of results

The reproducibility of a gross E value appears to be better in laboratory A than in laboratory B, the magnitude of the fiducial limits of the mean corrected E values for laboratory B being nearly twice those for laboratory A. The agreement between the two laboratories

is good, the means of the corrected E values differing by 1 to 2 per cent. The absorption curve from laboratory B on the international standard preparation shows some disagreement with the standard curve for pure all-*trans* vitamin-A acetate of Cama *et al.*,[4] in particular over the critical 310 to 320 mμ region, and this may account in some part for the tendency of laboratory B to produce results consistently higher than laboratory A. The results, although inadequate to give a reliable mathematical estimate of the method's precision, strongly suggest that a larger-scale inter-laboratory test would show it to be satisfactory.

The results show that good intra-laboratory reproducibility can be attained by the method described, which is based on the published data of Cama *et al.*[4] The twenty-eight values for gross and corrected $E^{1\%}_{1cm}$ at 328 mμ given in Table II yield the following information—

Average gross $E^{1\%}_{1cm}$ at 328 mμ = 15·42 (× 1600 = 24,670 i.u. of vitamin A per g).

Average corrected $E^{1\%}_{1cm}$ at 328 mμ = 13·07 (× 1920 = 25,090 i.u. of vitamin A per g).

The twenty-eight samples had all been manufactured during the twelve months previous to sampling, with the exception of sample number 7*a*, whose absorption curve showed signs of oxidation of the vitamin, and it is to be observed that the vitamin-A content from the average gross $E^{1\%}_{1cm}$ at 328 mμ × 1600 approximates to that given by the average corrected $E^{1\%}_{1cm}$ at 328 mμ × 1920. This is in agreement with Morton's[12] observation that no better factor (than 1600) for fish liver oils in general could, even to-day, be chosen for converting gross $E^{1\%}_{1cm}$ values to international units, although oils showing little irrelevant absorption would be somewhat undervalued and oils exhibiting more irrelevant absorption a little overvalued. This statement, however, although valid for fresh and unoxidised oils, is not always applicable to samples submitted to a Public Analyst under the Food and Drugs Act. Many of these have deteriorated owing to oxidation of the vitamin, so necessitating the use of a correction procedure. This point has been discussed in some detail elsewhere.[1] The problem of neovitamin A causes some concern, and a factor of 1·04, based upon an assumed average content of 30 to 40 per cent. of neovitamin A, has been used[1,13] for calculating the vitamin content in international units from the spectrophotometric data. Yet if, as Harris, Ames and Brinkman[14] have recently asserted, the biological potency of vitamin A is significantly higher by 20 to 28 per cent. than that of neovitamin A, and since by a fortunate coincidence the correction procedure in the spectrophotometric estimation discounts neovitamin A to about the same extent as does the rat bio-assay, the use of such a factor will become unnecessary. Experience, as illustrated above, shows that gross $E^{1\%}_{1cm}$ at 328 mμ × 1600 approximates to corrected $E^{1\%}_{1cm}$ at 328 mμ × 1920, on average, for a number of unoxidised oils. As the 1600 factor was of biological origin, it would automatically allow for a difference in the biological activity of the *cis* and *trans* isomers of vitamin A, and it would seem, therefore, that this approximate relationship between the 1600 and 1920 factors substantiates to some extent the statement of Harris *et al.*[14] This point is rather critical, because even though the new international standard is pure crystalline all-*trans* vitamin-A acetate, the international unit is still the activity of a given weight thereof.[15] Hence, if there is a significant difference between the biological activity for all-*trans* vitamin A and neovitamin A, this must be accounted for in any expression of vitamin-A content in international units. The use of more than one set of fixation points goes some way to meet the criticism that the irrelevant absorption at these points may not be strictly linearly related; for departure from linearity will be reflected in an increase in the fiducial limits of the mean and so the effect will be lessened to some extent. Abnormally wide fiducial limits should give warning of possible peculiarities in the sample and care should then be taken before expressing an opinion about its vitamin-A content.

Conclusions

It is strongly suggested that any future inter-laboratory tests of the precision of geometrical correction procedures in estimating vitamin A would give an estimate significantly different from that given by Adamson *et al.*[3] if all the relevant factors were taken into consideration, including the examination of the absorption curves from the international standard preparation obtained by the participating laboratories and the adoption of an experimental design similar to that outlined in this paper.

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CITY ANALYST'S LABORATORY
BIRMINGHAM

January, 1952

The Choice of Doses in Bio-Assays

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The precision of a bio-asssy is enhanced by applying simple formulae to available information about the probable potency of a material, even when such information is vague. Linear response - log dose relationships only are considered.

IN most biological assays, some estimate of the potency of the test preparation (T.P.), based on previous experience or knowledge, is at hand to assist in planning the assay. The ways in which this estimate can best be used to minimise the variance component, which, in the statistical analysis of the assay data, is attributed to "difference between preparations," are formally examined in this paper, and thereby an increase in the precision of the assay according to well known principles is sought. Only assays in which the response metameter can be regarded as linearly related to the logarithm of the dose are considered, *e.g.*, assays of vitamin D by the rat curative method.

In general, assays fall into two main classes: (*a*) those in which a close estimate of the result is available beforehand, such as those on a compounded foodstuff to which the active principle is added as a concentrate under standardised conditions; and (*b*) assays in which no such close estimate is to hand, it being possible only to say that the true potency may lie somewhere between two stated limits, as in the assay of a crude fish liver oil.

In what follows it is assumed that every dose-level applies to the same number of animals, and that the linear response - log dose relationship exists over a useful, if limited, range.

CLOSE ESTIMATE OF POTENCY WITH ONE TEST PREPARATION ONLY

Let N_t levels of T.P. be compared with N_s levels of S.P. (standard preparation), in an assay with n animals per level, the sum of the responses at the qth level of T.P. being $r_{t(q-1)}$ and that at the qth level of S.P. $r_{s(q-1)}$.

The sum of squares to be minimised, that due to differences between preparations, is given by—

$$S.S_{\text{preps.}} = \frac{T^2}{nN_sN_t(N_s + N_t)},$$

from which it follows that an attempt must be made to make T zero. The value of T is given by the expression—

$$T = N_t \Sigma_{q=0}^{N_s-1}(r_{sq}) - N_s \Sigma_{q=0}^{N_t-1}(r_{tq}) \quad \ldots \quad (1)$$
$$= 0.$$

For the assays dealt with here, it can be assumed that—

$$r_q = \mathrm{K} \log \mathrm{P}va^q \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where K is a constant, P is the potency per unit volume (or weight), v is the lowest level dose-volume (or dose-weight), and a is the dose-ratio of the assay. Substituting equation (2) in equation (1) and expanding—

$$\mathrm{N}_s\mathrm{N}_t \log \mathrm{P}_s v_s - \mathrm{N}_s\mathrm{N}_t \log \mathrm{P}_t v_t = (0 + 1 + 2 + \ldots + \mathrm{N}_t - 1)\mathrm{N}_s \log a - (0 + 1 + 2 + \ldots + \mathrm{N}_s - 1)\mathrm{N}_t \log a,$$

which reduces to the final form—

$$\frac{\mathrm{P}_t v_t}{\mathrm{P}_s v_s} = a^{\frac{1}{2}(\mathrm{N}_s - \mathrm{N}_t)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

If $v_t = v_s$, as can often be arranged, then—

$$\mathrm{P}_t/\mathrm{P}_s = a^{\frac{1}{2}(\mathrm{N}_s - \mathrm{N}_t)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The simple relationship in equation (3) is more convenient for the practical planning of an assay than the well known statement, of which equation (1) is the mathematical expression, that the mean responses to S.P. and T.P. should be equal.[1] As is obvious without such calculations as the above, the commonly used (2 + 2) assay design requires equipotency of the two solutions, or at least of the unit doses where v_s and v_t are different. In any design, such as the (3 + 2) assay, in which $\mathrm{N}_s - \mathrm{N}_t$ is unity, the potency of the unit T.P. does should clearly be as the square root of the dose-ratio to that of the S.P.

Equation (3) is specially useful when it is desired to examine the response curve extensively, for instance, to check the range of linearity and at the same time to assay a test preparation. For example, if a (5 + 2) design is decided upon, with a dose-ratio of 2, then it would be required that—

$$\mathrm{P}_t v_t/\mathrm{P}_s v_s = 2^{\frac{1}{2}(5-2)} = 2{\cdot}83 \text{ approximately.}$$

Close estimate of potency with several test preparations

Where there are several test preparations to be assayed simultaneously the information sought usually involves orthogonal comparisons between them, such that two of them are compared, their mean is compared with a third, and so on. Also, it may be necessary to make non-orthogonal comparisons of each test preparation with the standard preparation, or with one particular test preparation. We consider here the case of only two test preparations, since it is easy to extend the results to any number.

Let the two test preparations be A and B at N_A and N_B levels, respectively, the standard preparation being at N_s levels. The orthogonal comparison of A with B involves the direct use of equation (3), which becomes—

$$\frac{\mathrm{P}_\mathrm{B} v_\mathrm{B}}{\mathrm{P}_\mathrm{A} v_\mathrm{A}} = a^{\frac{1}{2}(\mathrm{N}_\mathrm{A} - \mathrm{N}_\mathrm{B})} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

Again, following the methods of the previous section for comparing the mean of the two test preparations with the standard preparation, we have—

$$\mathrm{T}' = 0 = (\mathrm{N}_\mathrm{A} + \mathrm{N}_\mathrm{B})\Sigma_{q=0}^{\mathrm{N}_s - 1}(r_{sq}) - \mathrm{N}_s\Sigma_{q=0}^{\mathrm{N}_\mathrm{A} - 1}(r_{\mathrm{A}q}) - \mathrm{N}_s\Sigma_{q=0}^{\mathrm{N}_\mathrm{B} - 1}(r_{\mathrm{B}q}) \quad .. \quad .. \quad (6)$$

Simplification between equations (2), (5) and (6) leads to the expression—

$$\frac{\mathrm{P}_\mathrm{X} v_\mathrm{X}}{\mathrm{P}_s v_s} = a^{\frac{1}{2}(\mathrm{N}_s - \mathrm{N}_\mathrm{X})} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

where X may be A or B. Comparing equations (3) and (7), it is clear that an assay ideally designed for orthogonal comparisons between several test preparations and a standard preparation is also ideally designed for the non-orthogonal comparison of any selected test preparation with the standard preparation or, by equation (5), with any other given test preparation. Where there are more than two test preparations, the ideal relationship between every two of them should accord with equation (5) and then, provided any one test preparation obeys equation (7), the assay will have the required balance.

One test preparation of unknown potency

Assays on a test preparation of unknown potency, but to the potency of which reasonable upper and lower limits $P_{max.}$ and $P_{min.}$ can be set, fall into one or other of two ill-defined groups, according as the limits are close or wide.

Suppose an assay of vitamin D in a test preparation whose potency is expected to be anything from 2 to 4 i.u. per unit dose is to be made. In any worthwhile assay, the minimum value of N_t is 2, and adopting this figure, and even so high a dose-ratio as 4, the range of responses possible from the test preparation will correspond to 2 to 16 i.u., which will be within the linear range for most colonies. Such an assay, then, will be referred to as an example of close limits.

Alternatively, suppose the possible potency were from 2 to 20 i.u. Then, even for as low a dose-ratio as 1·5, the possible range of responses would correspond to 2 to 30 i.u. for two levels, so exceeding the linear range. This will be referred to as an example of wide limits.

Example of close limits

Assume that whatever the true potency of the test preparation, the mean responses in each T.P. dose-group shall, apart from scatter due to chance, fall within the range of the mean S.P. dose-group responses, the range of the latter being made to lie within the linear region of the log dose - response curve.

These requirements lead directly to the equations—

$$K \log P_{min.} v_t a^0 = K \log P_s v_s a^0 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

and

$$K \log P_{max.} v_t a^{N_t - 1} = K \log P_s v_s a^{N_s - 1} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

The solution of these two equations leads to two relevant conclusions, namely—

$$P_s v_s = P_{min.} v_t \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10)$$

and

$$\frac{P_{max.}}{P_{min.}} = a^{N_s - N_t} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (11)$$

Now $P_{min.}$ and $P_{max.}$ are fixed, and N_s and N_t necessarily integral, so there is only one way of making the assay fit to the desired form, and that is by adjusting the dose-ratio, a. It must be noted, incidentally, that it is desirable for N_s to exceed N_t for close-limit assays, and an example is given below to illustrate this.

One concludes that for close-limit assays, the dose of standard preparation, as measured in units of the active principle, should be made equal to that of the test preparation, assuming its potency is at the minimum limit. The values of N_s and N_t must then be chosen such that N_s exceeds N_t, and also so that N_s levels of standard preparation with the dose-ratio a calculated by equation (11) do not exceed the known linear response range.

Example—A vitamin-D assay is to be performed on a test preparation whose potency is thought to lie between the limits 2 and 6 i.u. per unit volume. The standard preparation is therefore made up to contain 2 i.u. per unit volume. The dose-ratio, given by—

$$a^{N_s - N_t} = 3$$

with a (3 + 2) design, works out to $a = 3$, and the standard preparation levels will then be 2, 6 and 18 i.u., with test preparation levels of 2 to 6 i.u. and 6 to 18 i.u.

A (4 + 2) design can be used with advantage. In this case, $a = \sqrt{3}$, and the four S.P. levels range from 2 i.u. to only 10·38 i.u. Thus, the (4 + 2) design will not, as does the (3 + 2) design, involve the risk of exceeding the upper limit of linearity.

If it were decided to use a (2 + 3) assay, *i.e.*, $N_s < N_t$, then the T.P. levels necessary might well result in one dose-group falling outside the range of linearity, and so be needlessly wasted.

Another advantage of the (4 + 2) over the (4 + 3) or (3 + 2) designs is that the smaller dose-ratio makes for a smaller spread of the fiducial limits obtained on evaluating the assay results. The (5 + 2) assay possesses this desirable attribute in even greater measure, but the (4 + 3) design, where it can be used, has the advantage of allowing a check of linearity with both standard and test preparations.

When choosing N_s and N_t, attention must be paid, as hinted above, to the practical usefulness of the resulting value of a. In this example, a (4 + 3) design cannot be used

because the required S.P. levels exceed the linear range, but a (5 + 2) design is satisfactory.

Finally, it is possible that all reasonable combinations of N_s and N_t with $N_s > N_t$ might provide impractical values of a, the dose-ratio. When this happens, these designs are not suitable for the test sample, and the designs of the next section must be used.

Example of wide limits

Assume that, whatever the true potency of the test preparation, not less than two of the mean responses in the T.P. dose-groups shall, apart from scatter due to chance, fall within the range of the mean S.P. responses, this range being made to lie on the linear portion of the response curve. In the same way as in the previous section, we may derive from these conditions the relations—

$$P_s v_s = P_{max.} v_t$$

and

$$\frac{P_{max.}}{P_{min.}} = a^{N_t - N_s}.$$

In this design it is inevitable that one or more levels of T.P. will be outside the linear range of response, and so have to be rejected from the "computation assay." The selected potency of the S.P. dose must be tied to that of the T.P. dose taken at its maximum, the values of N_s and N_t must give a useful value of a, and N_t must exceed N_s.

Example—A test preparation whose potency is thought to lie somewhere between 1 and 10 i.u. per unit volume is to be assayed for vitamin D. The S.P. is made up to contain 2 i.u. per unit dose, and the T.P. diluted to contain 0·2 to 2·0 i.u. per unit dose. A (2 + 4) design is chosen, so that $a = \sqrt{10}$, and the dose levels are—

S.P.—2 i.u., 6·32 i.u.
T.P.—0·2 to 2·0 i.u., 0·63 to 6·32 i.u., 2·0 to 20 i.u., 6·32 to 63·2 i.u.

Clearly, provided the stated limits contain the true potency, a statistically satisfactory assay can be anticipated on either a 4-point or 5-point computational basis. It will be noted that for potency limits of this order, *i.e.*, 10 to 1, only an assay for which $N_t - N_s > 1$ could possibly be used for a vitamin-D assay.

The use in the above example of a (3 + 5) design would require standard preparation levels that would invite trouble at the limits of linearity, and a better choice would be the (2 + 5) design, for which $a = \sqrt[3]{10}$. This would give enhanced precision in the result of the assay, not only because of the lower dose-ratio, but also because a smaller proportion of test preparation levels might have to be rejected through non-linearity when forming the "computation assay."

Conclusions

Some simple relationships have been derived from well-known principles, in respect of biological assays, for which a linear response - log dose relationship can be assumed. The equations can be used to enhance the precision of assays of the type to which they are applicable; particular attention has been paid to the problem of ensuring a statistically satisfactory assay when the only information available about the potency of the test substance consists of a pair of limits, however wide.

The author expresses his gratitude to Dr. E. C. Wood for checking this paper, for valuable suggestions as to its presentation, and for his encouragement and assurance that it is a good thing to place on record the theory, properly worked out, of what people have been doing in practice for a long time.

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February, 1952

The Determination of Organic Bromine Compounds in Beverages by Koenig's Reaction

By J. A. C. VAN PINXTEREN

A routine colorimetric method for determining brominated preservatives in soft drinks is described. The bromine compound is extracted with ether and decomposed and the liberated bromide is determined as cyanogen bromide by Koenig's reaction with pyridine, the extinction of the red compound at 500 mμ being measured. An apparatus has been designed specifically for isolating the cyanogen bromide. The limit of the determination corresponds to 1 mg of bromine per kilogram of soft drink.

Attention is drawn to possible interference by chlorine compounds, as cyanogen chloride also gives Koenig's reaction. Interference by eosin, which cannot always be excluded from soft drink samples, is discussed. As little as 0·2 mg of eosin per kilogram of soft drink can be detected by the fluorescence at 525 mμ of an alkaline solution prepared from the ethereal extract. Eosin can be removed by shaking the acid ethereal extract with activated Norite powder. One milligram of bromine (as brominated preservative) can be determined in presence of 100 mg of eosin per kilogram of soft drink.

Several methods of detecting small quantities of organic bromine compounds have been described in the literature (Eeckhout,[1] von Fellenberg,[2] Reith[3]). The method of von Fellenberg is also often used for determining brominated preservatives, the bromine-compound being destroyed and the bromine ion being oxidised with chloramine. The liberated bromine is combined with fluorescein to form eosin, which is determined colorimetrically. A well defined pH value is necessary throughout the determination; that Beer's law is not obeyed and reproducibility is poor are adequately shown in Table I.

TABLE I

DETERMINATION OF BROMINE BY THE FLUORESCEIN METHOD

Amount of bromide, μg	Extinction at 500 mμ
20	0·040, 0·075, 0·057
40	0·079, 0·133, 0·138
60	0·116, 0·200, 0·185
80	0·125, 0·202, 0·202
100	0·098, 0·188, 0·180

Volumetric methods include (*i*) oxidation to bromate (Reith[3]), with subsequent iodimetric titration, and (*ii*) conversion to cyanogen bromide (Lang[4]). A detailed study in this field has been made by Schuleck,[5,6] who determined small quantities of halogens by a modification of the method of Lang, with a special titration apparatus. R. F. Milton[7] drew attention to Koenig's reaction for detecting and determining small quantities of cyanogen bromide. Previously, W. N. Aldridge[8] had elaborated a colorimetric method of determining cyanide based on the same principle, namely, the formation of cyanogen bromide from cyanides and bromine. Cyanogen bromide forms a quaternary salt with pyridine that reacts with aromatic amines to form coloured dianil derivatives suitable for colorimetric measurement. Keonig's reaction is specific for pyridine as well as for the halogen cyanides, hence, an investigation was made to discover if this reaction would serve to determine bromine compounds in food products.

Experimental

For practical purposes, it was necessary to investigate—

(*i*) whether the reaction could be standardised, so that Beer's law was followed,
(*ii*) the conditions for no interference by cyanogen chloride,
(*iii*) the mineralisation of the organic compound and
(*iv*) the method of extracting the organic compound from the test sample.

STANDARDISATION OF THE REACTION—

An excess of oxidant is required to liberate the bromine from the bromide, and, although this does not influence the amount of cyanogen bromide formed in presence of potassium cyanide solution, the excess must be destroyed before proceeding. In this way it is possible to produce a measurable colour; however, the colour changes perceptibly from orange to red so that it is only possible to measure it when standards are prepared at the same time.

To overcome these difficulties and in the hope that greater precision would result with purer cyanogen bromide, the cyanogen bromide was expelled by a current of nitrogen and conducted into a mixture of pyridine and benzidine sulphate cooled to below 10° C. Ten minutes were sufficient for full development of the red colour.

With high concentrations of the cyanogen bromide the dye may be precipitated, but this can be prevented by adding 1 ml of 96 per cent. v/v alcohol to the pyridine - benzidine mixture. Less than 1 μg of bromine can easily be detected and determined with sufficient accuracy.

TABLE II

RESULTS OF KOENIG'S REACTION APPLIED TO CYANOGEN BROMIDE FROM PURE BROMIDE SOLUTIONS

Bromide ion concentration, μg	Extinction at 500 mμ with 1-cm cells
5	0·135, 0·128
7·5	0·180, 0·184
10·0	0·248, 0·248
12·5	0·317, 0·326
15·0	0·400, 0·410
17·5	0·490, 0·495
20·0	0·576, 0·578

EFFECT OF INTERFERENCE—

In macrochemical methods of separating chloride from bromide and to avoid liberating chlorine, ammonium persulphate is mostly used as the oxidant. In microchemical work, Seaber[9] found that quantities of chlorides about 10 times greater than those of bromide might interfere. Persulphate oxidation gave good results when the samples contained bromide ion only, with a linear relationship between extinction and concentration for 5 to 20 μg of bromide ion.

Interference by chloride and iodide was investigated by preparing standard solutions containing either ion, and by adding small quantities of the solutions to the standard bromide solution; the bromide determination was then carried out in the usual manner.

In the standard bromide solution there were 10 μg of bromide ion per ml; in the iodide solution, 380 μg of iodide ion per ml; and in the chloride solution, 240 μg of chloride ion per ml.

TABLE III

INTERFERENCE OF CHLORIDE AND IODIDE WITH AMMONIUM PERSULPHATE OXIDATION

Amount of interfering ion in 1 ml of bromide solution, ml	Extinction at 500 mμ with 1-cm cells	Bromine ion, calculated, μg
1·0 (iodide)	0·245	10
1·0 (iodide)	0·245	10
0·5 (chloride)	0·370	14
0·25 (chloride)	0·400	15

Although iodide ion has no influence on the results, chloride interferes seriously, so oxidation by ammonium persulphate is unsuitable.

In a communication on seawater damage, Seaber[9] recommends a saturated chromic acid solution for liberating bromine from bromide. For this purpose it seemed to be advantageous to carry out the oxidation at 100° C on a water-bath. With this procedure, up to 500 μg of chloride ion did not interfere with 10 μg of the bromide. An extinction equal to 10 μg of bromide was given by 100 mg of chloride. This error may be due to contamination of the chloride by bromide.

MINERALISATION OF THE ORGANIC BROMINE—

The bromine contents of a number of bromine compounds were found by hydrolysis with alcoholic potassium hydroxide solution and also by the micro-method of Irimescu and Chirnoga,[10] in which the compound is heated under a reflux condenser with absolute alcohol with about 100 mg of metallic sodium, diluted with water, after 3 minutes again boiled for 3 minutes and the liberated bromide titrated with silver nitrate. As is shown by Table IV, good results are given by this method.

TABLE IV

DETERMINATION OF BROMINE IN ORGANIC COMPOUNDS BY THE SODIUM - ALCOHOL METHOD

Compound	Weight, mg	Amount of 0·01 *N* silver nitrate, ml	Bromine found, %	Theoretical bromine content, %
Bromoacetanilide	11·4	5·30	37·2	37·4
	8·3	3·87	37·3	
	5·2	2·33	35·6	
o-Bromobenzoic acid	10·4	4·90	37·6	39·8
	12·6	6·32	39·9	
	6·8	3·35	39·4	
p-Bromobenzoic acid	14·9	7·43	39·8	39·8
	11·7	5·88	40·1	
Di-ethylbromoacetylurea	10·0	4·31	33·6	33·7
	9·9	4·11	33·2	
	12·9	5·44	33·7	

As the brominated preservatives are aliphatic compounds—esters of bromoacetic acid—the sodium - alcohol method can be omitted, as boiling with alcoholic potassium hydroxide can be relied upon to mineralise the bromine. However, by applying the sodium - alcohol method, destruction of organic materials is more complete. This is favourable, as traces of organic material seriously interfere with the colorimetric bromide determination, and heating to a high temperature for a long time must be avoided.

The results of colorimetric determinations carried out after the sodium - alcohol destruction and ignition for 5 to 10 minutes at 500° agreed well with volumetric measurements.

TABLE V

COMPARISON OF COLORIMETRIC AND VOLUMETRIC DETERMINATIONS OF BROMINE

Compound	Bromine	
	By colorimetric method, mg per 100 g	By volumetric method, mg per 100 g
Preservative solution I	332	332
" II	335	330
" III	559	575
	%	%
Bromo*iso*valerianylurea	36·4, 35·1	35·8

Some of the bromine in the preservative solution was present as inorganic bromine. It is easy to understand that, depending on the rate of hydrolysis, decomposition of the bromine compound can take place accompanied by a decrease in antimicrobial potency. The amount of inorganic bromine in certain preservatives gives an idea of this deterioration, as shown in Table VI.

EXTRACTION OF THE BROMINE COMPOUND—

In general the brominated preservatives can be extracted with ether in sulphuric acid medium. However, evaporation of the ethereal extract at the boiling-point of ether can

cause serious loss of the generally volatile preservative. Therefore, the evaporation must be carried out under reduced pressure and after adding alcoholic potassium hydroxide to neutralise the solution. The evaporation and destruction can be carried out in the same vessel if a Claissen flask is used.

TABLE VI

ORGANIC AND INORGANIC BROMINE CONTENT OF SOME PRESERVATIVES

Sample	Organic bromine, mg per 100 g	Inorganic bromine, mg per 100 g
Preservative solution I	280	52
" II	259	76
" III	513	19

METHOD

REAGENTS—

All reagents should be of recognised analytical purity.

Potassium cyanide—A 6·5 per cent. w/v solution in water.

Benzidine hydrochloride—A saturated solution. To prepare a colourless solution, dissolve 2 g of benzidine hydrochloride in 100 ml of boiling water, add 50 mg of Norite and filter hot. Filter again after cooling the solution in a refrigerator.

Pyridine.

Alcohol—A 96 per cent. v/v solution and a 50 per cent. v/v solution.

Chromic acid—Dissolve 150 g in 80 ml of water.

Standard potassium bromide solution—Dissolve 148·8 mg of potassium bromide in 100 ml of water and dilute 1 ml of this solution with 99 ml of water immediately before use. 1 ml of this solution $\equiv$ 10 μg of bromine.

Alcoholic potassium hydroxide—A 0·5 *N* solution.

PROCEDURE—

Add 5 ml of 4 *N* sulphuric acid to 20 ml of the soft drink freed from carbon dioxide by filtering, and shake with three 25-ml portions of ether; filter the ether over anhydrous sodium sulphate. Evaporate the ether in a 150-ml Claissen flask after adding 1 ml of alcoholic

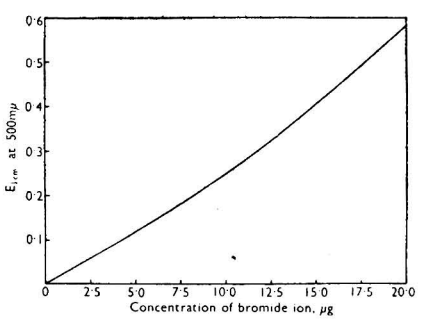

Fig. 1. Absorption of cyanogen bromide

potassium hydroxide under reduced pressure. Add to the residue 0·1 g of metallic sodium and 1 ml of absolute alcohol, and heat under a reflux condenser for 3 minutes till the sodium has dissolved. Add 3 ml of water and heat again similarly for 3 minutes. Transfer the liquid to a platinum evaporating dish about 3·5 inches in diameter and evaporate on a water-bath. Ignite the residue at a temperature of less than 500° C. Dissolve the remaining material in *N* sulphuric acid, pour into a 25-ml volumetric flash, make up to the mark and filter. Use 2 to 5-ml portions of this clear solution for the bromine determination. Add to tube A (Fig. 2) a measured quantity (2 to 5 ml) of this solution, 3 ml of potassium cyanide and 2 to 5 ml of chromic acid.

Add to tube B 4 ml of benzidine hydrochloride previously mixed with 1 ml of pyridine and 1 ml of 96 per cent. alcohol; wet the glass beads with this solution also.

Place tube A in a water-bath at 100° C and tube B in ice-water. After the tubes have been connected, pass a current of nitrogen for 8 minutes. Pour the contents of tube B into a 25-ml volumetric flask, wash B with 50 per cent. v/v alcohol and fill the flask to the mark

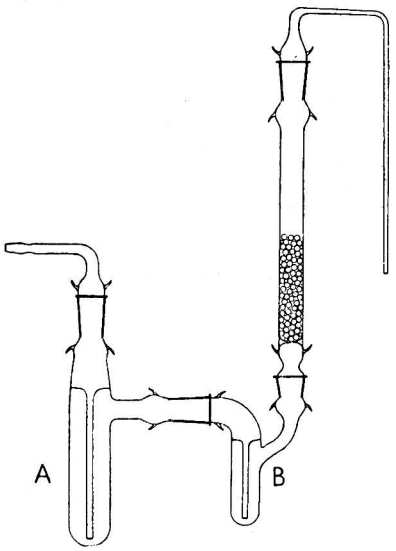

Fig. 2. The extractor

with 50 per cent. alcohol. Measure the extinction in a spectrophotometer at 500 mμ with a 1-cm cell. Compare the readings with a standard graph and calculate the bromine value (Fig. 1). Make a blank run to determine the bromine content of the reagents.

Results

Samples of pure soft drinks prepared in the laboratory were mixed with known amounts of preservatives, and the bromine was determined.

Bromine added as ester of bromoacetic acid, μg per 20 ml	74·0	50·0	62·5
Bromine recovered, μg per 20 ml	73·9	49·7	63·0

Bromine was determined in three commercial samples that were suspected to contain a preservative, and gave 3·15, 2·71 and 2·42 mg per litre.

Interference by eosin—

It is possible that the beverages contain a small quantity of eosin as a dye, which is easily detected as the compound can be extracted in acid medium with ether. After extracting the ether with sodium hydroxide, the alkaline solution shows a strong fluorescence in light of 500 to 525 mμ. In this manner as little as 0·2 mg of eosin per litre can be detected.

In the presence of eosin it is necessary to shake the ethereal extract with about 50 mg of Norite, which adsorbs it; the procedure can then be continued in the usual way.

A negligible amount of bromine was recovered after shaking pure drink samples containing 10 mg of eosin per 20 ml with Norite. To find if the Norite extracted any of the preservative, samples containing a known amount were shaken with ether and the ether was shaken with Norite; the procedure was then continued as described.

Bromine added, μg per 20 ml	100	100
Bromine recovered, μg per 20 ml	86	86

Samples containing 10 mg of eosin per 20 ml as well as preservative were examined in a similar manner with satisfactory results.

Bromine added, μg per 20 ml	50	50
Bromine found, μg per 20 ml	48	50

When the ratio of eosin to preservative is not too great, the total amount of the preservative can be recovered easily.

A single determination can be completed in about 90 minutes, and for a series, the average time for each determination is about 50 minutes.

The author wishes to express his thanks to Mr. S. Wit and to Miss Carla v.d. Plas for their valuable assistance.

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January, 1952

The Determination of the *iso*Cyanate Group in Rubber Bonding Agents

BY A. G. WILLIAMSON

A potentiometric method of determining *iso*cyanates has been devised that is based on the reaction between *iso*cyanates and di*iso*butylamine in an inert solvent. The excess of amine is titrated potentiometrically with hydrochloric acid in an aqueous organic medium.

A comparison of results with those of other methods is given.

COMPOUNDS with more than one *iso*cyanate group in the molecule are important for the production of good adhesive bonds between rubber and metals or textiles. Three volumetric methods of determining *iso*cyanates are given in the literature[1,2,3]; all involve the reaction of the *iso*cyanate with an excess of a strong amine in an inert solvent and subsequent colorimetric titration of the excess amine with an acid. *iso*Cyanate solutions are generally highly coloured and the end-point in the first two of these methods is diffuse and consequently difficult to determine reproducibly; the results are not accurate enough for this determination. Hence a procedure has been devised, based on the glass electrode.

In Germany, where *iso*cyanates were first used on a large scale, determination[1] involved the quantitative reaction of the *iso*cyanate with di*iso*butylamine, as follows—

$$R.NCO + HN.(C_4H_9)_2 \rightarrow R.NH.CO.N(C_4H_9)_2,$$

the product being a neutral substituted urea. An inert solvent was used, and the excess of di*iso*butylamine was titrated with hydrochloric acid, with methanol as diluent and bromophenol blue as indicator. The procedure developed by the author makes use of the same reaction in an inert solvent. The titration with hydrochloric acid, however, is done potentiometrically in an organic medium containing about 10 per cent. of water. By this method, the difficulties of colorimetric measurement in coloured and non-ideal solutions are avoided and sharp end-points are attained.

METHOD

APPARATUS—

Potentiometric titration necessitated minimisation of the current passed through the cell, in order to prevent polarisation at the electrodes. A glass electrode was the most

satisfactory of the indicator electrodes examined, and one was accordingly used; potential measurements were made in a valve voltmeter circuit giving an unusually low grid input current, of the order of 10^{-10} amperes. In this circuit a potentiometric cell and a potentiometer were connected in series in the grid circuit of a suitable triode valve. During titration, the anode current through the valve was kept constant by maintaining a constant grid potential. This was effected by balancing any change in cell potential with an equal but opposite potential change in the potentiometer. Hence changes in potentiometer readings measured changes in cell potential.

The apparatus used for the titrations was built in these laboratories. The circuit, however, is fundamentally similar to that of most commercial pH meters and there is no reason why such instruments should not be used with their own glass-electrode systems.

EXPERIMENTAL—

With this apparatus, the titration of di*iso*butylamine with hydrochloric acid was examined and a suitable titration medium found. In German work a mixture of monochlorobenzene (30 volumes) and methanol (100 volumes) was used. Concentrated aqueous hydrochloric acid dissolves in this medium to give normal solutions, or solutions of higher concentration if required. Titrations of di*iso*butylamine with such solutions, in the same medium, gave slow and unsatisfactory pH changes at the equivalence points, and so were useless for quantitative purposes. However, the monochlorobenzene - methanol solution proved to be miscible with 10 per cent. of water at room temperatures and titrations carried out in this medium gave very sharp end-point inflections. With this medium the titration of di*iso*butylamine and hydrochloric acid could be accurately done in *N* solutions, but less satisfactorily with 0·1 *N* solutions. Such a trend would be expected by application of the law of mass action to di*iso*butylamine hydrochloride regarded as a slightly hydrolysed salt.

On the basis of these results, several *iso*cyanate solutions were reacted with approximately twice the theoretically equivalent quantities of 2 *N* di*iso*butylamine solution, and the excess of amine was titrated with a *N* hydrochloric acid solution, prepared by dissolving concentrated aqueous hydrochloric acid in monochlorobenzene - methanol solution (30 volumes + 100 volumes). Pure phenyl *iso*cyanate, toluene 2:4-di*iso*cyanate (Desmodur T), and Vulcabond TX (a proprietary poly*iso*cyanate of undisclosed composition marketed by Imperial Chemical Industries Limited) were used. Satisfactory accuracy was attained, provided that a Lunge-Rey or similar weight-pipette was used for sampling the more reactive of the compounds.

Owing to the comparatively high cost of di*iso*butylamine, some experiments were made with distilled piperidine, as used by Stagg in his colorimetric method,[2] as the reactant base, but it proved to be less satisfactory.

Table I shows the results obtained from the analysis of a sample of phenyl *iso*cyanate by (*i*) the colorimetric method developed by Stagg,[2] (*ii*) the potentiometric method given here, with piperidine, and (*iii*) the potentiometric method given here, with di*iso*butylamine.

TABLE I

ANALYSIS OF A SAMPLE OF PHENYL *iso*CYANATE

*iso*Cyanate found		
By colorimetric method, %	By potentiometric method with piperidine, %	By potentiometric method with di*iso*butylamine, %
96·5	100·0	98·0*
97·1	99·1	97·7*
94·6	101·8	98·4
97·3	102·0	98·6
96·1	102·3	98·9
	98·2	98·7
Mean = 96·3%	Mean = 100·6%	Mean = 98·4%

* In these two estimations an old bottle of di*iso*butylamine, which had been sampled frequently over a period of about nine months, was used in order to check the stability of the reagent. The effect on results was significant, lowering the results by just under 1 per cent., so that careful storage and use are required to give consistent results from the same reagent over long periods, even though blank determinations are necessarily made.

The three methods give results with the same sample of phenyl *iso*cyanate that are significantly different, the potentiometric method with di*iso*butylamine giving results intermediate between results from the other two methods.

The variability of the method with di*iso*butylamine and potentiometric titration is less, according to these figures, than the variability of the other methods, the estimated standard deviation of a single determination being about 0·5 per cent. compared with 1·0 per cent. for the other methods.

PROCEDURE—

Dissolve sufficient concentrated hydrochloric acid, sp. gr. 1·18, to make a normal solution in a medium consisting of monochlorobenzene (30 volumes) and methanol (100 volumes), and standardise this solution by direct two-phase colorimetric titration with aqueous standard alkali solution, using methyl orange as indicator.

Take a 1·5 to 2 *N* solution of di*iso*butylamine in monochlorobenzene and transfer exactly 10 ml to a small flask. Add sufficient monochlorobenzene to bring the final volume of inert solvent to about 20 ml and weigh, *e.g.*, from a Lunge-Rey pipette, sufficient of the *iso*cyanate solution to react with 5 to 7 ml of the di*iso*butylamine solution. Agitate the flask gently, then cork it and set it aside for about 10 minutes to complete the reaction. Pour the contents of the flask into a titration beaker provided with an automatic sitrrer, wash the flask with a known quantity of fractionated methanol, equivalent to 100 volumes for 30 volumes of inert solvent present in the reaction flask, and add the washings to the beaker. Add 13 volumes of water, slowly, with stirring.

Insert the glass electrode and one limb of an agar bridge connected to a calomel electrode in the solution. Couple the cell so formed into the valve voltmeter circuit. With continuous stirring, titrate the excess of di*iso*butylamine with a standard normal solution of hydrochloric acid made up from concentrated aqueous acid and a medium containing 30 volumes of monochlorobenzene to 100 volumes of methanol. In the region of the equivalence point, take readings after the addition of each drop of titrant and plot the cell potential against the amount, in millilitres, of titrant added. The equivalence point is accurately found from the resulting curve. Carry out a blank determination, omitting the *iso*cyanate.

In some *iso*cyanate samples, carbamyl chlorides formed as intermediates in the *iso*cyanate preparation may be found as impurities. These interfere as they do in other methods based on the reaction of the *iso*cyanate with an amine. The true *iso*cyanate content of such a mixture can be found by combining the results of potentiometric titration with those of gravimetric determination as given by Stagg.[2]

The author wishes to thank the Firestone Tyre and Rubber Company Limited (through the courtesy of Mr. M. M. Heywood) for a gift of phenyl *iso*cyanate, and the Chief Scientist of the Ministry of Supply and the Council of the Research Association of British Rubber Manufacturers for permission to publish this note.

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November, 1951

An Ester-Fractionation Method for the Component Fatty Acid Analysis of Mixtures Containing Hydroxy Fatty Acids

By K. T. ACHAYA and S. A. SALETORE

An ester-fractionation method, not involving acetyl values, for the analysis of natural or synthetic mixtures containing hydroxy fatty acids is illustrated by reference to a synthetic mixture containing 70 per cent. by weight of the mixed fatty acids of groundnut oil and 30 per cent. by weight of ricinoleic acid. Saturated acids are removed by the usual lead salt - alcohol separation; oleic - linoleic acid mixtures are not separated from ricinoleic acid, presumably because of the insolubility of the lead salts of ricinoleic acid in light petroleum owing to mixed salt formation, but a concentrate of oleic (containing linoleic) acid can be separated from one of ricinoleic (containing linoleic) acid by two precipitations of the urea-insoluble adducts of the oleic - linoleic mixture in methyl alcohol. The composition of the original mixture is calculated from the values of the saponification equivalents and iodine values of the fractionated methyl esters of the saturated and oleic - linoleic acid extracts and of the fractionated acetylated methyl esters of the ricinoleic acid extract. Results agree well with theory.

Although Riley,[1] and Gupta, Hilditch and Riley[2] have recently published a method for the component fatty acid analysis of castor oil, the method was exceptional in that it dealt with hydroxy fatty acids at concentrations of over 90 per cent.; moreover, linoleic acid was determined by the spectrophotometric technique, which is not readily accessible to many laboratories. The literature reveals that when a hydroxy fatty acid is encountered in a fat, the fat analysis is never satisfactory.[3,4,5] Consequently a method for the routine analyses of these natural fats or of any mixture containing hydroxy fatty acids was required. In the procedure described here no acetyl values, which require large quantities of material for accurate determination in replicate, are necessary after the initial determination; subsequent ester fractionation followed by the usual determination of saponification equivalents and iodine values of each fraction suffice, and so bring these mixtures into line with other fats, from an analytical point of view.

In the earlier stages of this work, a synthetic mixture of 70 parts by weight of the mixed fatty acids of *Annona squamosa* seed fat (iodine value 85·9) recently analysed in these laboratories,[6] with 30 parts by weight of fairly pure ricinoleic acid was used as test material; subsequently, the analysis was carried out on another synthetic mixture in which the mixed fatty acids of groundnut oil (I.V. 92·0) replaced those from *A. squamosa* in the same proportion by weight. Saturated acids were easily separated by the usual lead salt - alcohol procedure. It is essential, if subsequent ester fractionation is to be amenable to mathematical treatment without determining acetyl values, that the hydroxy acid be separated from oleic - linoleic acids, and it was hoped when the work was started to effect separation by making use of the insolubility of the lead salts of ricinoleic acid in light petroleum. For, Lewkowitsch[7] states ". . . lead ricinoleate is insoluble in low-boiling light petroleum. It is therefore possible to separate ricinoleic acid from other unsaturated acids." Williams and Bolton[8] have shown that lead oleate also crystallises slowly from light petroleum, but small quantities of oleate could have been allowed for. Experience showed, however, that the formation of mixed salts of the divalent lead (as against mutual solubility effects, which were ruled out by separate experiments) result in only very small lead salt precipitates of little use. Various differential solubility experiments with light petroleum also proved fruitless. With the new reagent urea,[9,10] which gave insoluble adducts with oleic but not with ricinoleic acid (as shown separately with the pure acid), separation of two groups was effected by two precipitations in methyl alcohol solution. Methylation and fractionation of the acids derived from the precipitated urea adducts through an electrically-heated and packed column indicated that these consisted of about 90 per cent. of oleic, 6 per cent. of linoleic and 3 per cent. of residual

saturated acids. The soluble urea adduct group consisted of ricinoleic and linoleic acids, the methyl esters of which boil at similar temperatures at reduced pressures; acetylation of these methyl esters, whilst leaving the linoleate unaffected, raised the boiling-point of the ricinoleate by about 35° C at a pressure of 10 mm of mercury,[11] lowering, in consequence, the iodine value of the ricinoleate to 71·7 and the apparent saponification equivalent to 177·0. Fractionation of these acetylated methyl esters gave fractions progressively richer in ricinoleate, with a sharply falling sequence of iodine values, and saponification equivalents that were easily calculated on an iodine value basis; the final figures indicate a composition of about 70 per cent. of ricinoleic and 30 per cent. of linoleic acid in the fraction. No decomposition of the acetylated esters was noticeable either here or in a pilot experiment, in which the acetylated methyl esters of the mixed fatty acids of a sample of castor oil were fractionally distilled.

Experimental

Pilot experiment—

The feasibility of fractionating acetylated esters was tested; the mixed fatty acids (Sap. Equiv. 296·3; I.V. 87·2) from a sample of commercial castor oil (Sap. Equiv. 307·9; I.V. 85·8; n_D^{25} 1·4770; acetyl value 151·5) were first methylated and then acetylated by the usual procedures. The acetylated methyl esters (Sap. Equiv. 184·2; I.V. 74·3; n_D^{25} 1·4542; acidity equivalent to 0·5 per cent. of acetic acid) were fractionally distilled, at a reduced pressure of about 0·6 to 0·8 mm of mercury, through an electrically-heated and packed column of the Longenecker type,[12] and determinations of the saponification equivalent and iodine value (Wijs method, 30 minutes) of each of the ten fractions were made, with the results shown in Table I. The peak values, shown by fractions 8 and 9, correspond closely to the theoretical values for acetyl methyl ricinoleate (Sap. Equiv. 177·0; I.V. 71·7), whilst the general trend of iodine values is compatible with the presence of about 4·5 per cent. of linoleic acid in castor oil.[2]

Table I

Fractional distillation of the acetylated methyl esters of castor oil mixed fatty acids through an "E.H.P." column at a pressure of 0·6 to 0·8 mm

Fraction	Weight of ester, g	Boiling-point, °C	Sap. equiv.	I.V.
1	0·776	151	203·2	72·7
2	0·881	154	201·0	78·4
3	1·609	170	197·4	78·2
4	2·353	181	188·7	76·7
5	2·074	184	186·2	75·5
6	2·344	184	181·6	74·7
7	2·638	185	180·5	73·9
8	2·124	186	179·3	72·9
9	2·319	186	181·4	72·7
10	4·374	Residue	190·5	76·3
Total ..	21·492			

Preparation of a synthetic mixture—

Mixed fatty acids from Annona squamosa—A 15 per cent. solution of a sample of previously-analysed oil[6] in low-boiling light petroleum was kept for 3 days at 10° C to precipitate toxic matter (Harper, Potter and Gillham[13]). The solution was decanted from the resinous precipitate, the solvent removed and the mixed acids recovered; Sap. Equiv. 279·8 and I.V. 85·9.

Ricinoleic acid—Cold-drawn castor oil from a local mill was substantially freed from diricinoleo-glycerides by a method based on that of Panjutin and Rapoport,[14] with light petroleum. The acids from the resulting triricinolein were set aside at 10° C for several days, in which time about 1 per cent. of dihydroxystearic acid separated (Achaya and Saletore[15]) and was filtered off. The analysis of the resulting ricinoleic acid was as follows—

	Sap. equiv.	I.V.	n_D^{25}	Acetyl value
Found	295·8	85·0	1·4704	165·8
Theoretical	298·0	85·2	1·4703	165·0

Synthetic mixture—The mixed fatty acids from *Annona squamosa* were mixed with ricinoleic acid in the proportion of 70 to 30 by weight. The mixture had a saponification equivalent of 284·3, an iodine value of 85·4 and a calculated acetyl value of 49·7.

SEPARATION PROCEDURE—

Lead salt separation from alcohol—The usual lead salt - alcohol technique[16] with 89·49 g of the mixture gave 20·65 g (23·1 per cent.) of mainly saturated acids of I.V. 21·5, and 68·84 g (76·9 per cent.) of mainly unsaturated acids of I.V. 104·8.

Lead salt separation from light petroleum—The mainly unsaturated fatty acids were converted into lead salts through the potassium soaps (Lewkowitsch[7]), dried, heated with a reflux of 5 parts of low-boiling light petroleum (40° to 60° C) for an hour and kept overnight at 15° C. Only about 3·16 g (4·6 per cent.) of the material separated; remixing and repetition gave only 7·4 per cent. precipitation, although theoretically about 38 per cent. of ricinoleic acid was present. This was not the effect of a "mutual solubility" of lead oleate, or linoleate, on lead ricinoleate as the solubility (0·4 per cent.) of lead ricinoleate in low-boiling light petroleum only went up to 2·0 per cent. when an equal weight of the lead salts prepared from the mainly unsaturated acids from *A. squamosa* seed oil (from a previous lead salt run, I.V. 105·0) was present. The low precipitation, in spite of the insolubility, to some degree, of lead oleate in light petroleum,[8] can, therefore, only be due to the formation largely of mixed salts of the divalent lead with ricinoleic and oleic or linoleic acids.

Attempts at separation with light petroleum—Pure ricinoleic acid is soluble at 0° C in about its own volume of low-boiling light petroleum, any excess of solvent separating out above. Separation of the hydroxy acid from other acids by this means was not possible, as even the addition of a small quantity of another unsaturated acid caused complete solubility, and only after much dilution with solvent was a small quantity of ricinoleic acid precipitated. It was also found that old, possibly polymerised, ricinoleic acid sometimes gave misleading extracts from light petroleum. Experiments on similar lines with pure acetyl ricinoleic acid or acetyl methyl ricinoleate were also unsuccessful.

Separation of urea adducts—Preliminary experiments showed that pure ricinoleic acid forms no insoluble urea adducts in methyl alcohol, so a small portion of the mixture was taken in this solvent and to it was added 5 parts by weight of urea in saturated methyl alcohol solution. Each portion was worked up as described below to give nearly 40 per cent. of material of I.V. 82·4, n_D^{25} 1·4603, probably containing oleic and saturated acids, while a repeat urea separation yielded a further 8·3 per cent. of material of I.V. 89·5, n_D^{25} 1·4600—clearly mainly oleic acid; the remainder, of n_D^{25} 1·4724, contained the ricinoleic and linoleic acids.

As the mixture had become badly oxidised at this stage, the analysis was run through in full on a fresh artificial mixture.

METHOD

PREPARATION OF THE MIXTURE—

Mix the fatty acids from groundnut oil (pressed in the laboratory from fresh market seed) and ricinoleic acid, obtained from triricinolein derived from castor oil (as described above), in the weight ratio of 70 to 30; the characteristics of the materials used in one experiment were as follows—

	Sap. equiv.	I.V.	Acetyl value	n_D
Groundnut oil mixed fatty acids, A ..	288·0	92·0	—	1·4551 (40° C)
Ricinoleic acid, B	299·4	85·0	165·8	1·4702 (25° C)
Mixture, A:B = 70:30	291·4	90·1	49·7 (calc.)	1·4572 (40° C)

PROCEDURE—

Lead salt separation from alcohol—Proceed as usual[16]; this yielded two portions from 92·49 g of the fatty acid mixture, in a typical determination—

	Weight, g	Percentage, w/w	I.V.	n_D^{25}
Insoluble lead salt acids	14·67	15·9	17·0	—
Soluble lead salt acids..	77·82	84·1	104·3	1·4646
Total acids	92·49	100·0	—	—

Urea adduct separations—Slowly add, with mechanical stirring, about 1200 ml of a saturated solution of urea in methyl alcohol to 75·85 g of the soluble lead salt acids in a 5-litre beaker. About 225 g or 3 times as much urea as the weight of acids is required. Filter the precipitate by suction and wash with methyl alcohol saturated with urea. Decompose it with water, preferably slightly acidulated if the recovery of urea is immaterial, and extract the fatty acids with ether; treat the filtrate, after recovery of methyl alcohol, similarly. Two typical fractions were—

	Weight, g	Percentage, w/w	I.V.	n_D^{25}
Insoluble urea adduct acids	25·74	27·8	83·9	1·4587
Soluble urea adduct acids	52·08	56·3	109·3	1·4686
Total acids	77·82	84·1	—	—

Treat the soluble urea adduct acids similarly, this time with enough saturated urea solution to contain 100 g of urea. The precipitate and filtrate were worked up with the materials of the above typical experiment and gave—

	Weight, g	Percentage, w/w	I.V.	n_D^{25}
Insoluble urea adduct acids	12·80	13·8	103·2	1·4627
Soluble urea adduct acids	39·28	42·5	111·4	1·4710
Total acids	52·08	56·3	—	—

Final fractions—Combine the two urea adduct-soluble acids to give three final fractions. The fractions after resolution of the typical synthetic mixture were as shown in Table II.

TABLE II

FINAL FRACTIONS OBTAINED BY RESOLUTION OF THE SYNTHETIC MIXTURE

Characteristics of synthetic mixture—S.E. 291·4; I.V. 90·1; Acetyl value 49·7 (calc.); n_D^{25} 1·4572

	Fraction	Weight, g	Percentage, w/w	I.V.	n_D^{25}
Lead salts insoluble in alcohol ..	*S*	14·67	15·9	17·0	—
Urea adducts insoluble in methanol ..	*U*	38·54	41·6	90·3	1·4597
Urea adducts soluble in methanol ..	*R*	39·28	42·5	111·4	1·4710
Totals		92·49	100·0		

Ester fractionation—Methylate and fractionate *S* through the electrically-heated and packed column and treat as usual. Similarly methylate and fractionally distil *U*, which consists of oleic and linoleic acid, as is clear from its iodine value and refractive index, and then calculate the composition of fraction *U* on a basis of palmitic and oleic - linoleic esters, the latter of I.V. 91·3. Methylate fraction *R*, which consists of ricinoleic and linoleic acids and then acetylate the neutral methyl esters by boiling under a reflux condenser for 2 hours with twice as much by weight of acetic anhydride. The acetylation has the effect of increasing the difference in boiling-point, iodine value and apparent saponification equivalent of the two components; the neutral acetyl esters on fractionation give a series of fractions characterised by rapidly falling iodine values, easily calculated on this basis to compositions progressively richer in ricinoleate. Table III summarises the three fractionations in the typical experiment.

RESULTS AND DISCUSSION

Component fatty acid composition—Assembly of the data from the typical determination gave the figures shown in Table IV.

No ambiguity attaches to the analytical figures and mathematical treatment of fractions *S* and *U*, which are quite typical of esters of these types. Fraction *R* has been calculated as consisting entirely of methyl ricinoleate and linoleate, but it is possible that traces of oleic acid are present; if this is so, the calculations will lead to larger proportions in each fraction of a linoleate - oleate mixture, of lower iodine value than that of linoleate, whatever their individual proportions, than the amounts at present recorded as pure linoleate. This will lead in turn to smaller quantities of ricinoleate right down the line, and so to a smaller total than that recorded here; as the percentage of ricinoleic acid calculated is in excellent accord

TABLE III

FRACTIONAL DISTILLATION OF ESTERS THROUGH AN "E.H.P." COLUMN AT A PRESSURE OF 0·6 TO 0·8 mm

Fraction S, methyl esters—

Fraction	Weight, g	Boiling-point, °C	S.E.	I.V.	Saturated acids: Myristic	Palmitic	Stearic	Arachidic	Behenic + lignoceric	Unsaturated acid, oleic
*S*1	0·788	115	258·7	11·1	0·376	0·310	—	—	—	0·102
*S*2	1·628	123	278·7	7·8	—	1·076	0·404	—	—	0·148
*S*3	1·508	125	279·1	10·1	—	0·958	0·373	—	—	0·177
*S*4	1·236	132	287·7	11·8	—	0·413	0·653	—	—	0·170
*S*5	1·433	134	295·9	12·7	—	0·080	1·041	—	—	0·212
*S*6	1·293	137	306·8	13·8	—	—	0·635	0·450	—	0·208
*S*7	1·016	138	308·4	15·7	—	—	0·414	0·416	—	0·186
*S*8	2·127	143	314·4	23·5	—	—	0·215	1·329	—	0·583
*S*9	3·164	Residue	360·5	23·7	—	—	—	—	2·290	0·874
Total *S*	14·193				0·376	2·837	3·735	2·195	2·290	2·660
	Percentage as esters				2·7	20·1	26·5	15·6	16·2	18·9
	Percentage as acids				2·6	20·0	26·5	15·6	16·4	18·9

Fraction U, methyl esters—

Fraction	Weight, g	Boiling-point, °C	S.E.	I.V.	Saturated acid, palmitic	Unsaturated acids: Oleic + linoleic		Unsaponifiable matter
*U*1	1·77	146	290·8	71·8	0·38	1·39		—
*U*2	1·32	150	291·2	80·8	0·15	1·17		—
*U*3	2·57	152	293·9	87·4	0·11	2·46		—
*U*4	3·54	152	293·0	84·6	0·26	3·28		—
*U*5	5·30	153	293·2	89·2	0·12	5·18		—
*U*6	3·64	153	295·6	90·4	0·04	3·60		—
*U*7	2·89	154	294·5	90·8	0·01	2·88		—
*U*8	2·57	154	293·4	91·3	—	2·57		—
*U*9	2·24	155	305·6	85·7	—	2·17		0·07
*U*10	6·56	Residue	309·3	86·5	—	6·28		0·28
Total *U*	32·40				1·07	29·02	1·96	0·35
	Percentage as esters				3·3	89·6	6·0	1·1
	Percentage as acids				3·3	89·6	6·0	1·1

(C_{18} unsaturated esters taken as I.V. 91·3, S.E. 295·9)

Fraction R, acetylated methyl esters—

Fraction	Weight, g	Boiling-point, °C	S.E.	I.V.	Unsaturated acid, linoleic	Hydroxy acid, ricinoleic	Unsaponifiable matter
*R*1	0·71	120	223·3	123·6	0·36	0·35	—
*R*2	1·42	128	234·1	132·7	0·86	0·56	—
*R*3	1·51	146	223·8	124·1	0·78	0·73	—
*R*4	2·39	160	225·4	126·2	1·29	1·10	—
*R*5	2·86	162	220·0	120·4	1·38	1·48	—
*R*6	4·56	164	209·0	113·7	1·89	2·67	—
*R*7	5·35	168	199·3	102·6	1·64	3·71	—
*R*8	3·54	170	188·2	87·5	0·55	2·99	—
*R*9	4·13	172	186·8	83·2	0·47	3·66	—
*R*10	10·06	Residue	200·2	79·7	—	8·90	1·16
Total *R*	36·53				9·22	26·15	1·16
	Percentage as acetylated esters				25·2	71·6	3·2
	Percentage as esters				27·6	68·9	3·5
	Percentage as acids				27·5	68·9	3·6

(experimental 29·9 per cent., theory 30·0 per cent.) with the original amount, it follows by indirect inference that oleic acid cannot be present in more than a very small amount in fraction *R*. A third urea adduct precipitation would no doubt ensure complete removal of oleic acid together with a further quantity of linoleic acid.

TABLE IV

COMPONENT FATTY ACIDS OF THE SYNTHETIC MIXTURE

Acid	Insoluble lead salt acids, *S*	Insoluble urea adduct acids, *U*	Soluble urea adduct acids, *R*	Total acids	Percentage excluding unsaponifiable matter, w/w
	15·9%	41·6%	42·5%		
Saturated—					
Myristic	0·42	—	—	0·42	0·4
Palmitic	3·18	1·37	—	4·55	4·6
Stearic	4·21	—	—	4·21	4·3
Arachidic	2·49	—	—	2·49	2·5
Behenic + lignoceric	2·60	—	—	2·60	2·7
Unsaturated—					
Oleic	3·00	37·25	—	40·35	41·1
Linoleic	—	2·52	11·68	14·20	14·5
Hydroxy—					
Ricinoleic	—	—	29·28	29·28	29·9
Unsaponifiable matter	—	0·46	1·54	2·00	—

The accordance with theory is very satisfactory. Ricinoleic acid, as just indicated, is in close agreement, whilst a sample of groundnut oil fatty acids of a composition corresponding to the figures for saturated, oleic and linoleic acids found here would have an iodine value of 91·3 compared with the actual I.V. of 92·0. The weight percentage composition (saturated acids, 20; oleic acid, 59; linoleic acid, 21) is also a likely one (Jamieson, Baughman and Brauns[17]; Griffiths, Hilditch and Jones[18]).

We wish to thank Dr. S. Husain Zaheer, Director of these laboratories, for his encouragement in this work.

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CENTRAL LABORATORIES FOR SCIENTIFIC AND INDUSTRIAL RESEARCH
HYDERABAD, INDIA

December, 1951

Notes

THE DETECTION AND DETERMINATION OF SMALL AMOUNTS OF INFLAMMABLE HYDROCARBONS IN COMBUSTIBLE MATERIALS

In cases of suspected arson, the writer has been frequently requested by the Royal Canadian Mounted Police to examine certain materials such as charred paper, fabrics, sacking, wood and so on, for the presence of volatile inflammable hydrocarbons. In all instances where there was an odour of gasoline (petrol), kerosene (paraffin oil) or other light hydrocarbon it was possible to separate the inflammable substance by the following procedure, which is based on a method already known for the determination of hydrocarbons in alcohol (Hoff[1]). It has also been possible to estimate approximately the amount present and to identify the liquid by micro or semi-micro methods. This procedure has given more satisfactory results than ordinary steam distillation.

Procedure—

Weigh a sample of the suspected material, to the nearest gram, and place it in a wide-mouthed boiling flask. Add sufficient of 95 per cent. v/v alcohol to saturate the material thoroughly and leave a slight excess, *e.g.*, 100 g of jute sacking requires 200 ml of alcohol. Set the flask aside for some time, preferably overnight, add three times as much water as alcohol to the contents, mix well and distil slowly through a vertical condenser fitted with a straight adapter. The adapter is inserted as far as possible into the top of a 50-ml burette. When 20 ml of distillate have been collected, substitute another burette and collect a further 20 ml. The whole of the hydrocarbons (up to 5 to 6 per cent.) will then have passed over if the rate of distillation has been well regulated, but, as a precaution, another fraction may be collected. For 2·5 per cent. or less of hydrocarbons the distillation is allowed to proceed at 2 to 3 ml per minute, but for higher percentages at 5 ml per minute. Add 15 ml of potassium dichromate solution (one-sixth saturated) and then 2 ml of hydrochloric acid, sp.gr. 1·18, to the contents of each burette. Stopper the burettes with rubber bungs and mix the contents by inverting repeatedly. Set the burettes aside overnight, when the hydrocarbons will separate and the aqueous layer will assume an olive green colour, and then measure the total volume of hydrocarbons. When the volume of the hydrocarbons has been determined, separate the hydrocarbons from the aqueous layer, by using the burettes as separating funnels, and combine them.

Test the inflammability of one drop of the hydrocarbons and determine their refractive index, specific gravity and boiling range, which are generally sufficient for their identification.

The reason for using much less potassium dichromate solution than is required for the complete oxidation of alcohol is obscure. In the original method published by Babington and Tingle[2] no mention was made why 15 ml of a one-sixth saturated potassium dichromate solution was chosen as the diluting agent. Certainly the partial oxidation results in a much better physical separation and there is a great advantage in having a green aqueous layer.

References

1. Hoff, R. W., *Analyst*, 1934, **59**, 687.
2. Babbington, F. W., and Tingle, A., *Ind. Eng. Chem.*, 1919, **11**, 555.

Customs Excise Laboratory
National Research Council
Ottawa, Canada

J. M. Macoun
January, 1952

DETECTION OF SEA WATER INFILTRATION

Over-pumping in recent years of wells and boreholes in the citrus groves behind the town has resulted in the water supplies of Famagusta becoming extremely bitter and saline and, consequently, unsuitable to drink.

Detailed analysis of sea water at the coast and of the water from three Municipal boreholes, approximately 90 feet deep, a half mile from the sea and sunk in calcium-rich Pleistocene sand dunes, gave the results shown in Table I.

Bromine was determined by the method of Haslam and Moses[1] after precipitating calcium and magnesium from a suitable volume of sample by adding a known slight excess of bromine-free sodium carbonate and sodium hydroxide, boiling, filtering, washing, neutralising the filtrate and evaporating to 50 ml in the same manner as used by Haslam, Allberry and Moses[2] for salt deposits.

The constancy of the chlorine - bromine ratio indicated that the increased salinity is due to sea water, and this is confirmed by isochlors plotted from results from other wells and boreholes in the area, but a very marked base-exchange reaction has occurred, converting about 75 per cent. of the sodium chloride in the contaminating sea water into chlorides of calcium and magnesium.

TABLE I

ANALYSIS OF WATERS FROM FOUR SOURCES

	Source			
	Sea water	Borehole 1	Borehole 2	Borehole 3
pH	8·7	7·3	7·3	7·3
$CaCO_3$, %	0·0135	0·0155	0·0170	0·0160
$CaSO_4$, %	0·146	0·0242	0·0048	0·0106
$CaCl_2$, %	nil	0·185	0·123	0·092
$MgSO_4$, %	0·238	nil	nil	nil
$MgCl_2$, %	0·424	0·252	0·165	0·144
NaCl, %	3·19	0·160	0·078	0·076
Bromine, %	0·00745	0·00127	0·00079	0·00067
Total chloride as chlorine, %	2·25	0·403	0·249	0·213
Chlorine/Bromine	302	318	315	318
Sea water calculated from bromine figures, %	100	17	11	9
Sea water calculated from chlorine figures, %	100	18	11	9

Although the source of increased salinity was obvious, the results may be of interest to other analysts as they emphasise that the chlorine - bromine ratio is a much more reliable index of sea water contamination of water supplies than is a ratio involving calcium, magnesium or sodium contents, since the cations of a water can be readily modified by base exchange with sodium zeolites, such as occurs with the Thanet sands, or with zeolitic sands containing calcium and magnesium, as occurred in the case recorded above.

Owing to the recent prohibition of any drilling in the area it has not been possible to obtain samples of the water-bearing sand for further study.

I express my thanks to the Director of Medical and Health Services, Cyprus, for his permission to publish this note.

REFERENCES

1. Haslam, J., and Moses, G., *Analyst*, 1950, **75**, 343.
2. Haslam, J., Allberry, E. C., and Moses, G., *Ibid.*, 1950, **75**, 352.

GOVERNMENT LABORATORY
NICOSIA, CYPRUS

D. BRADWELL
February, 1952

THE CHROMATOGRAPHIC SEPARATION OF A MIXTURE OF THE HYDROXIDES OF LITHIUM, SODIUM AND POTASSIUM

THE techniques of both column and paper chromatography have been successfully applied to the separation of alkali hydroxides of lithium, sodium and potassium. For column chromatography, Whatman cellulose ashless powder is suitable as the adsorbent, and ethyl alcohol containing 15 to 20 per cent. of water can be used as the developing solvent. As the chromatographed substances are colourless, fractional elution and indicator addition methods are used for ascertaining the positions of the bands. The eluate is collected in various fractions, and each fraction is examined both with an alkali indicator (phenol red or bromothymol blue) and with a special reagent. A suitable alkali indicator is added to the developing solvent so as to indicate the positions by coloured bands. A more or less uniform packing of cellulose powder was effected by pouring a thick slurry of the cellulose powder in ether into the glass column and removing the ether by suction. For a satisfactory separation, 0·1 ml of the mixture, in which the strength of each alkali is approximately 0·1 *N*, is used. The cellulose column is 6·5 cm long and 1 cm in diameter. On developing the column with about 30 ml of alcohol (containing 15 to 20 per cent. of water), the mixture separates into three distinct bands; the uppermost is constituted of lithium hydroxide, the middle one of potassium hydroxide and the lowest diffuse one of sodium hydroxide.

The separation of alkali chlorides has already been effected by paper chromatography,[1,2,3] but the R_F values of the alkali metal hydroxides on paper chromatograms with ordinary

solvents were found to be too low and close to each other to be of any practical use, *e.g.*, with alcohol containing 10 per cent. of water, R_F values of lithium, potassium and sodium hydroxides are 0, 0·03 and 0·03, respectively. An increase in water content of the solvents no doubt increased the R_F values, but those of potassium and sodium hydroxides were still too close. Hence, the method of continuous development[4] for several hours was adopted and found to be expedient, following the descending method, on Whatman No. 1 filter-paper and with alcohol containing 15 per cent. of water. For indication, the dried filter-paper was sprayed with a suitable indicator; phenolphthalein for higher concentrations of the alkalis and phenol red or bromo-thymol blue for lower concentrations. With phenolphthalein, the alkali hydroxides were visible as red spots, with phenol red they appeared as orange spots and with bromo-thymol blue as greenish-blue spots, the last two being on a yellow background. After development for 24 hours, the alkali hydroxides were found to just separate, lithium hydroxide moving little, potassium hydroxide about 3 to 5 cm and sodium hydroxide about 6 to 8 cm. In 36 to 48 hours all the three were found to be fairly well separated from each other. In the absence of solvent boundary the R_F values could not be measured; further, the distance travelled by each greatly depended on the original concentration of the solution used.

The diffuse character of the spots on the filter-paper and the dependence of their rates of movement on the concentrations of the alkalis are evidently due to the adsorptive property of the cellulose of the filter-paper. This is clearly so with column chromatography when cellulose powder has been used. The significance of the adsorptive capacity of cellulose has been emphasised by the author[5] as well as by other workers.

My sincerest thanks are due to Dr. B. Banerjee, Senior Research Fellow, Bose Institute, Calcutta, for kind interest and encouragement and to Dr. S. K. Mukherjee, Lecturer, University College of Science and Technology, Calcutta, for criticism and discussion.

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1. Burstall, F. H., Davies, G. R., Linstead, R. P., and Wells, R. A., *J. Chem. Soc.*, 1950, 516.
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4. Miettinen, J. K., and Virtanen, A. I., *Acta Chem. Scand.*, 1949, **3**, 459.
5. Burma, D. P., and Banerjee, B., *Science and Culture*, 1950, **15**, 363.

PHYSICAL CHEMISTRY LABORATORY
BOSE RESEARCH INSTITUTE
CALCUTTA

D. P. BURMA
January, 1952

Ministry of Health

CIRCULAR 14/52*

The Ice-Cream (Heat Treatment, etc.) Amendment Regulations, 1952.

This circular (price 2d.), dated May 1st, 1952, draws attention to the Ice-Cream (Heat Treatment, etc.) Amendment Regulations, 1952, by virtue of which the high temperature short time (H.T.S.T.) method of heat treatment is now permitted. The circular gives details of a nitrite test for the determination of the holding time, i.e., the time taken by the fastest particle of the mix to traverse the holding section, for H.T.S.T. plant.

Nitrite Test—The Minister of Health is advised that the holding time can accurately be measured by the use of an injection test using a sodium nitrite agent. This test, devised by the plant manufacturers, corresponds to the nickel chloride injection test for the checking of holding time of milk H.T.S.T. pasteurisation plants, but unlike the latter test, where water is used as the medium, the sodium nitrite test must be applied to the actual ice-cream mix. This is necessary because the conditions of flow in the holder are likely to be different if water is used.

Particulars of the nitrite test are also set out in the April, 1952, issue of the *Monthly Bulletin of the Ministry of Health*. It is not contemplated that the nitrite test will normally need to be applied except at the time of installation, or when any alteration to the plant is made, since normal wear of the plant should tend to increase, rather than decrease, the time taken by the mix to pass through the holding section. Ice-cream which contains any added nitrite should not be sold for human consumption. The Minister is advised that this would be a contravention of the Public Health (Preservatives, etc., in Food) Regulations, 1925–1950.

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

British Standards Institution

NEW SPECIFICATIONS*

B.S. 604:1952. Graduated Measuring Cylinders. Price 2s.
B.S. 605:1952. Distillation Receivers (Including Crow Receivers). Price 2s.
B.S. 1751:1952. General Purpose Glass Stopcocks. Price 2s.
B.S. 1792:1952. One-mark Graduated Flasks. Price 2s.

AMENDMENT SLIP*

A printed slip bearing Amendments to a British Standard has been issued by the Institution as follows—

PD 1378—Amendment No. 3 (April, 1952) to B.S. 632:1950. Raw Linseed Oil for General Purposes.

DRAFT SPECIFICATIONS

A few copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and can be obtained on application to the Secretary, Society of Public Analysts and Other Analytical Chemists, 7-8, Idol Lane, London, E.C.3.

Draft Specification prepared by Sub-Committee PVC/1/8—Chrome Pigments.
CO(PVC) 1682—Draft B.S. for Brunswick or Chrome Greens (Pure and Reduced) for Paints (Draft Revision of B.S. 303).

Draft Specification prepared by Sub-Committee RUC/10/8—Testing of Solid Rubber.
CO(RUC) 1483—Draft B.S. Methods of Testing Raw Rubber and Unvulcanized Compounded Rubber.

Draft Specification prepared by Technical Committee FCC/4—Solvents and Allied Products.
CO(FCC) 1943—Draft B.S. for Butyl Acetylricinoleate.

The Composition of Jamaica Rum†

THESE notes supply information concerning the legislation in Jamaica on rum and on the composition of Jamaican rum.

The Excise Duty Law, No. 73 of 1941, contains, *inter alia*, the following definition—

"Rum" means spirits distilled solely from sugar cane juice, sugar cane molasses, or the refuse of the sugar cane, at a strength not exceeding 150 per cent. proof spirit.

Under section 29 of the Excise Duty Law, no rum shall be coloured with any colouring matter save cane sugar caramel.

The Rum (Ether Control) Law, Cap. 181, as amended by Article 2 of a proclamation made under section 5 of the Jamaica (Constitution) Order in Council, 1944, and dated the 17th day of November, 1944, empowers the Governor in Executive Council from time to time to fix the maximum ether content of rum manufactured in the island. By definition under this law, "ether content" means the number of grams of total esters, calculated as ethyl acetate, in every hundred litres of absolute ethyl alcohol contained in rum at a temperature of 60° F. By a Proclamation issued under this Law on the 28th of February, 1935, the maximum ether content was fixed at 1600.

The object of limiting the ester content of rum was to prevent the manufacture and export of very high ester rum to countries, chiefly Germany, where it was mixed with potato spirit, or

* Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London, S.W.1.

† Abridged from Bulletin No. 1, 1951, Department of Government Chemist. "Notes on Jamaica Rum." By W. L. Barnett, Government Chemist, Jamaica.

other cheap spirits, diluted and bottled for the African market, where it was sold as Jamaica rum. Prior to this limitation, rums with an ester content between 4000 and 6000 were made specially for the German trade.

Three types of rum are made in Jamaica. These are classified as high ester rum, flavoured rum and common clean rum. High ester rum is not dealt with in these notes. Generally speaking, common clean rum is made by the fairly rapid fermentation of a wash made from a mixture of molasses with dunder and skimmings from a previous distillation. Flavoured rum is made by distilling a slowly fermented wash composed of a mixture of molasses with dunder, skimmings, acid and flavour. By "acid" is meant the sour liquor that results from the fermentation of cane juice with dunder and skimmings in the presence of cane trash. "Flavour" is somewhat the same as "acid," but contains in addition, dunder that has undergone acidic fermentation from a previous crop. There is no clear line of demarcation between the two types; a distiller can produce rum with any desired qualities by paying attention to the preparation of the wash and its fermentation.

The rum now made in Jamaica differs from the full-bodied and highly-flavoured rums made prior to 1914. Consequently, any data in the literature before that time cannot be applied to rums made now. The rum made in a small distillery from sugar cane that was always of the same variety would possess a characteristic flavour associated with that particular distillery. Instead of a large number of small distilleries and sugar cane estates, we have to-day a small number of large central distilleries. The small pot stills have been replaced by large stills. The older sugar canes, liable to mosaic disease, have been replaced by new, resistant varieties. The fermentation yeasts are also different from those formerly used.

The flavour of rum is due chiefly to its compound esters. The predominant ester in rum is ethyl acetate, but it contributes little to the distinctive flavour, which is due to compound esters of higher aliphatic acids.

In 1903 and 1904 a large number of rum samples were examined by Mr. Charles Allen, who showed that the superior flavour of the higher-priced rums was due to their higher content of compound esters as compared with common clean rums. The average results were as follows—

FLAVOURED RUMS

Parish	Parts per 100,000 of abs. alcohol: Esters as ethyl acetate	Acidity as acetic acid
Trelawny	954	21
St. James	660	57
Westmoreland	401	20

COMMON CLEAN RUMS

Parish	Parts per 100,000 of abs. alcohol: Esters as ethyl acetate	Acidity as acetic acid
St. Andrew	250	59
St. Catherine	209	41
Clarendon	286	21
St. Elizabeth	204	9

As many rums known to come from Jamaica are much lower in secondary constituents than formerly, a tentative suggestion has been put forward that a minimum of volatile acid plus esters of 130 grams per 100 litres of absolute alcohol should be adopted as a standard for Jamaica rum. In this connection it should be made clear that a large proportion of the rum exported in puncheons from Jamaica has a total of less than 130 for esters plus volatile acidity.

About thirty years ago the esters in Jamaica rum were nearly always in excess of the higher alcohols, as determined by the Allen - Marquardt method. This is not so to-day; when, as shown in Table I, the higher alcohols are about the same or more than the esters. These rums were only slightly coloured and had low obscurations. They were estate rums packed in puncheons for export and not blends. On arrival at their destination these rums would be diluted, blended, coloured and bottled in such a way as to make their origin unrecognisable.

TABLE I

ESTATE RUMS, PUNCHEONS

Years 1947 to 1949 Strength about 36° O.P.

Grams per 100 litres of abs. alcohol

Volatile acidity	Esters	Aldehydes	Furfural	Higher alcohols*
15	64	22	7	396
27	139	9	9	345
18	83	29	10	310
14	45	22	6	249
23	98	23	4	281
38	209	30	6	209
27	124	24	3	202
32	133	24	3	261
38	278	19	3	234
21	284	20	5	194
18	226	20	6	206
17	154	20	8	171
18	171	20	5	178
16	52	25	4	215
17	105	55	7	221
10	51	35	5	260
7	43	14	4	176
8	58	33	5	206
22	77	20	6	336
37	75	21	10	392
8	90	25	22	471
8	136	13	7	151
10	60	9	3	135
5	49	12	7	146
5	66	15	7	123
9	36	8	4	253

* By the Allen - Marquardt method.

TABLE II

BOTTLED RUMS FOR EXPORT

Years 1947 to 1949 Strength 30° U.P.

Grams per 100 litres of abs. alcohol

Volatile acidity	Esters	Aldehydes	Furfural	Higher* alcohols
80	146	121	11	441
40	103	70	8	320
65	116	93	10	557
64	122	79	11	370
64	124	105	12	415
24	113	57	16	640
7	75	53	1	444
2	38	58	2	385
11	221	109	16	444
5	59	83	7	308
5	18	34	2	341
31	96	25	5	157
22	70	31	3	133
33	73	50	3	167
4	97	25	1	148
20	24	7	2	85
49	132	22	7	141
44	106	22	5	166
16	114	24	2	261
26	132	24	5	115
17	94	29	4	111
64	55	106	6	544
73	116	83	11	432

* By the Allen - Marquardt method.

TABLE III

BOTTLED RUM FOR EXPORT—15° U.P.

Grams per 100 litres of abs. alcohol

Obscuration	Volatile acidity	Esters	Aldehydes	Furfural	Higher alcohols*
3·8	59	217	65	7	746
5·1	61	188	54	6	615
5·5	73	153	52	9	555
3·5	64	167	48	8	783
6·6	63	163	56	10	985
5·5	47	110	48	7	985
3·7	47	147	47	10	925
3·2	60	192	52	7	634
4·2	61	162	49	7	763
4·2	69	197	57	7	676

* By the colorimetric method. (See "Alcohol" by C. Simmonds, MacMillan & Co., London, pp. 414–415.) These results are about 50 per cent. higher than those by the A.O.A.C. method.

Although the composition of rums bottled for export by individual distilleries in Jamaica is fairly constant, no analytical standards can be set that will serve to distinguish as Jamaican a rum that has been exported, blended, and bottled away from Jamaica.

F. L. OKELL

Book Reviews

OFFICIAL METHODS OF ANALYSIS OF THE SOCIETY OF LEATHER TRADES' CHEMISTS. Second Edition. Pp. vi + 200. Croydon: Society of Leather Trades' Chemists. 1951. Price 17s. 6d.

The first edition of this book was published in 1938 under the auspices of the International Society of Leather Trades' Chemists. The fact that only 13 years later a second edition is called for is evidence of its value. As the I.S.L.T.C. was wound up in 1947, publication of this second revised edition has been undertaken by the Society of Leather Trades' Chemists, Croydon, and is in English only, whereas the first edition was also in French. It consists of 16 sections.

In a foreword, drawn up by the Council of the Society, it is stated that most of the Official methods in the first edition have been embodied in the new edition with modifications and additions as approved by the Society. The Baldracco method of detannisation has been omitted. The Official Method of Quantitative Tannin Analysis is now identical with the former Provisional Official Method of the British Section of the old Society. The age-old subject of the method of determining sulphuric acid in vegetable-tanned leather is apparently still of interest and the Procter - Searle method is a little more precisely defined than it was when the details were agreed and drawn up by representatives of the Fighting Forces and the B.L.M.R.A. in 1927. The two more modern methods depending on pH measurements are also described in a little more detail. The determination of chromium in leather by oxidation with perchloric acid is now included as official. There is a slight mistake at the end of this paragraph on p. 106. It should read "removal (of iron) is unnecessary when the titration is carried out with 0·1 *N* ferrous ammonium sulphate."

The most important difference between the new edition and the old is the inclusion of a section of 21 pages on the physical testing of leather. It comprises such determinations as thickness, tensile strength, grain strength, apparent density, water absorption, buckle-tear, hardness and compression. These embody the decisions of the Society's special committee on physical testing, which has been sitting for some years.

There is some slight inconsistency to be found. Sometimes reference to the literature is given, *e.g.*, Burton's method for Buffer Capacity, but not for used lime-liquors or the physical tests and so on. A useful innovation is that the letterpress is confined to the left-hand pages throughout, the right-handed pages being left blank for notes or changes or additions, which are bound to arise as time goes on. It would be helpful if at the head of every page the Section number could be given.

The book is of course essential to a wide chemical public, as much to those in the industry as to those outside it, such as leather users.

R. F. INNES

TEXTBOOK OF ORGANIC CHEMISTRY. By LOUIS F. FEISER and MARY FEISER. Pp. viii + 741. Boston: D. C. Heath & Company. London: George G. Harrap & Co. Ltd. 1950. Price 35s.

It is rather startling to find in a book that is expressly stated to be designed to cover a one-year introductory course in organic chemistry references to haemin, cortisone and streptomycin. The idea of introducing these complex or topical substances is doubtless to stimulate interest by showing how such compounds fit into the general structure of our subject.

The method of approach is the not unfamiliar one of progressive building up from aliphatic hydrocarbons, thence to alcohols, halogen compounds, acids, carbonyl compounds and amines; later, a similar sort of sequence follows for the aromatic series. Interposed at suitable places are chapters on more general topics, *viz.*, stereochemistry, petroleum, ring formation, rubber, carbohydrates, fats, proteins, heterocyclic compounds, dyes, synthetic fibres and plastics, and physiologically active agents; of these it can be said that the first is particularly well set out, without in any way detracting from the merits of the others. Should the student have any spare time, his attention is directed at every stage to further reading of appropriate articles or books. Each chapter carries a number of problems (for which answers are given in another place) and a general summary, usually of 2 or 3 pages, for purposes of revision. The prefatory warning that the subject requires a lot of hard, systematic study is not out of place in view of the time factor mentioned above.

There is an immense amount of information packed clearly in every chapter (quite apart from little asides such as the origin of the name "phorone") and he would be a remarkable chemist who would not learn, rather than merely remember, something from a careful study of this "first-year" book. The publishers have played their part with the authors in presenting an eminently readable volume at a modest cost. B. A. ELLIS

Publications Received

TEXTBOOK OF ORGANIC CHEMISTRY. By GEORGE HOLMES RICHTER. Third Edition. Pp. vii + 762. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1952. Price $6.75; 54s.

TRAITÉ DE MANIPULATION ET D'ANALYSE DES GAZ. By HENRI GUÉRIN. Pp. vi + 636. Paris: Masson et Cie. 1952. Price 4500 fr. (paper); 5100 fr. (cloth boards).

ENERGY AND ANGLE DISTRIBUTION OF THE PHOTOPROTONS FROM DEUTERIUM. National Bureau of Standards Circular 515. By MARTIN WIENER. Pp. ii + 13. Washington: U.S. Government Printing Office for U.S. Department of Commerce. 1951. Price 15 cents.

APPLIED STATISTICS. Volume I, No. 1, March, 1952. Pp. 80. London and Edinburgh: Oliver & Boyd Ltd. Subscription price 25s. per annum.
A Journal of the Royal Statistical Society.

A GUIDE TO FILTER PAPER AND CELLULOSE POWDER CHROMATOGRAPHY. By J. N. BALSTON and B. E. TALBOT. Edited by TUDOR T. G. JONES. Pp. 145. London: H. Reeve Angel & Co. Ltd.; Maidstone, Kent: W. & R. Balston Ltd. 1952. Price 8s.

VERMICULITE. By E. R. VARLEY, Ph.D., B.Sc., D.I.C., A.M.I.M.M. Pp. iv + 70. London: H.M. Stationery Office. 1952. Price 7s. 6d.

AN EXPLAINING AND PRONOUNCING DICTIONARY OF SCIENTIFIC AND TECHNICAL WORDS. By W. E. FLOOD, M.A., Ph.D., and M. WEST, M.A., D.Phil. Pp. viii + 397. London: Longmans, Green & Co. 1952. Price 12s. 6d.

PORTRAITS OF PAST PRESIDENTS

THE custom of supplying Portraits of Past Presidents to Members of the Society and subscribers to *The Analyst*, which was discontinued during and since the war years, is to be restored.

Unfortunately it is no longer possible to supply these photogravure reproductions with all copies of *The Analyst*, as was done in the past, but a sufficient number will be printed to supply gratis copies to all who make application in advance to the Editor, *The Analyst*, 7 & 8, Idol Lane, London, E.C.3.

The first to be prepared is that of Mr. F. W. F. Arnaud, and orders for this portrait, on which the printing number will depend, should be sent in before August 30th, 1952.

HER MAJESTY'S COLONIAL SERVICE

A VACANCY exists for a Pollution Inspector (27106/61/52) in Uganda. The post is on contract terms with salary, according to qualifications and experience, in the scale £585 to £1,320 per annum. A gratuity of 15% of 9/10ths of basic salary is payable upon satisfactory completion of 30 36 months' contract, and in addition a temporary cost of living allowance, at present 25% of basic salary, subject to maximum of £250 per annum. Outfit allowance of £30. Air passages covering officer and family; quarters at rental not exceeding 10% of basic salary; Income Tax at low local rates; medical attendance free for officer and family (if basic salary below £1,140 per annum). Candidates should possess a degree in Chemistry and preference will be given to those who have had experience in the treatment of polluting liquids or in the control of pollution of surface waters. Duties include advising under the control of the Labour Commissioner on the dangers of industrial and sewerage pollution of the atmosphere and waters of the Protectorate and measures to be taken to avert these dangers. Officer may also be required to assist Chief Factories Inspector in chemical problems arising in factories, etc., not always connected with Pollution dangers. Intending candidates should apply in writing to the Director of Recruitment (Colonial Service), Colonial Office, Sanctuary Buildings, Great Smith Street, London, S.W.1, giving brief details of their age, qualifications and experience. They should mention this paper and quote the reference number (27106/61/52).

METALLURGIST required by Tin Mining Company in Malaya and Thailand. Degree in metallurgy or mining and experience in ore dressing essential. Details of age (not over 45 years), education and experience to be sent to Box No. 3816, THE ANALYST, 47, Gresham Street, London, E.C.2.

METALLURGICAL CHEMIST with practical works experience required for Tin Smelting Works in Malaya. University Degree or equivalent age 23–30 years. Provident Fund. Passage and quarters provided. Details of age, education and experience to Box No. 3817, THE ANALYST, 47, Gresham Street, London, E.C.2.

GRADUATE (Male) in Chemistry, Physics or Engineering, age 25–30, with industrial experience not necessarily in Packaging. Starting salary according to age and experience, but not less than £550 p.a. Participation in F.S.S.U. The position entails the speedy assessment and solution of current problems in industrial packaging and the ability to write reports for the layman is essential. Applications in writing quoting reference DPTO to Printing, Packaging and Allied Trades Research Association, Randalls Road, Leatherhead, Surrey.

MESSRS. MEREDITH & DREW LIMITED, Biscuit Manufacturers, The Highway, London, E.1, invite applications for the position of Works Chemist at the following factories: Halifax, Oldham and Newmarket. Applicants should have a B.Sc. or A.R.I.C., preferable with some industrial experience in foodstuffs. Reply in the first instance to Chief Personnel Officer of the Company at their London address.

CHEMIST, B.Sc., A.R.I.C., required by food manufacturing company in London for analysis, raw material control, and research work. Experience in food industry desirable. Write age, education, qualification and experience to Box No. Z.G.437, Deacon's Advertising, 36, Leadenhall Street, London, E.C.3.

C.A.V. LTD., Acton, London, W.3, require immediately candidates to fill the following vacancies in their Metallurgical and Chemical Laboratories:—(1) Junior Metallurgical Chemist for production and development work in the laboratories at Acton. (2) Junior Metallurgical Chemist or Metallurgist for production department at Rochester. (3) Two Junior Metallurgists for mechanical testing and general investigation work at Acton. Applicants who have completed their National Service are preferred. Candidates living outside the London area who are invited for interview, will have their travelling expenses paid. Applications to the Personnel Manager.

F.R.I.C. Branch E for responsible post with London Public Analyst and Consultant. Good pay and prospects for capable man. Box No. 3818, THE ANALYST, 47, Gresham Street, London, E.C.2.

CHEMIST with bacteriological experience required at Cambridge Factory or Bacteriologist prepared to work on Industrial Effluent disposal. Salary according to qualifications and experience. Write (quoting Ad.714), stating age, experience and salary required to Personnel Manager, Pest Control Limited, Bourn, Cambridge.

LABORATORY TECHNICIAN (M.28081.G) required by the Colonial Insecticide Research Department, Tanganyika, for one tour of 24–36 months in the first instance. Salary (including present temporary allowance of 25%) in scale £687 a year rising to £1,050. Outfit allowance £30. Free passages and liberal leave on full salary. Superannuation Scheme. National Health Service Superannuation rights may be preserved. Candidates should be members or associates of the Institute of Medical Laboratory Technology and must have had experience in an analytical chemical laboratory. Apply at once by letter, stating age, full names in block letters, and full particulars of qualifications and experience, and mentioning this paper to the Crown Agents for the Colonies, 4, Millbank, London, S.W.1, quoting on letter M.28081.G. The Crown Agents cannot undertake to acknowledge all applications and will communicate only with applicants selected for further consideration.

CHEMISTS. A large company with interests in animal products, including foodstuffs, fats, textiles and leather invites applications for posts in new control and research laboratories in North London.

(A) Experienced Analytical Chemist.

(B) Chemist to specialise on oils and fats.

Permanent positions with good prospects. Pension Scheme. Salary according to qualifications. Write giving age and particulars, experience, to Box No. 3815, THE ANALYST, 47, Gresham Street, London, E.C.2.

THE Research and Development Department of the Associated Ethyl Company has a vacancy for an Assistant Analytical Chemist who has an Hons. Degree or its equivalent and has had some experience in an anlytical laboratory. Applicants should preferably be under 30 years old. The starting salary offered will be dependent on qualifications and experience. Apply to the Personnel Manager, The Associated Ethyl Company Limited, Northwich, Cheshire.

PARTNERSHIP. A well-established firm of Analysts, London, seeks working partner. Age 35 onwards. Good qualifications, preferably including F.R.I.C. Branch E, and references essential. Drug experience an asset. Moderate available capital only necessary. Please give full particulars in strict confidence. Box No. 3819, THE ANALYST, 47, Gresham Street, London, E.C.2.

Volume 77. No. 916, Pages 333-388 July, 1952

THE ANALYST

THE JOURNAL OF
THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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Printed and Published for the Society of Public Analysts and Other Analytical Chemists by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, F. L. Okell, 7 8, Idol Lane, London, E.C.3.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47, Gresham Street, London, E.C.2.

Entered as Second Class at New York, U.S.A., Post Office.