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Volume 90, No. 1075 October, 1965

Summaries of Papers in This Issue

Nitrogen Content of Rusk Filler

Report prepared by the Meat Products Sub-Committee.

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1965, **90**, 579–580.

Nitrogen Factors for Turkey

Report prepared by the Meat Products Sub-Committee.

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1965, **90**, 581.

The Chemical Assay of Senna Fruit and Senna Leaf

Recommended Methods for the Evaluation of Drugs prepared by the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Methods for the Evaluation of Drugs.

Joint Committee of the PHARMACEUTICAL SOCIETY and the SOCIETY FOR ANALYTICAL CHEMISTRY.
14 Belgrave Square, London, S.W.1.

Analyst, 1965, **90**, 582–588.

Continuous-flow Enthalpimetry

The technique of continuous-flow enthalpimetry consists in passing reacting liquids in constant, non-equivalent proportions through a mixing vessel and measuring continuously the temperature changes due to the reaction. The calorific change that occurs in such a system, and which can be detected by thermistor thermometers, is proportional to the amount of reaction product formed and thus to the molar concentration of the more dilute reactant. Continuous-flow enthalpimetry may be applied to routine quantitative chemical analysis, on-line control of processes and the measurement of heats of reaction. Advantages over the present practice of automatic titrimetric methods include simplicity of apparatus, avoidance of use of pipettes and ease of operation.

P. T. PRIESTLEY, W. S. SEBBORN and R. F. W. SELMAN
Research Laboratories, Kodak Ltd., Wealdstone, Harrow, Middlesex.

Analyst, 1965, **90**, 589–593.

Acetone as Solvent in the Enthalpimetric Titration of Acidic Substances

The enthalpimetric titration of acidity with non-aqueous alkali often results in a curve that shows only a slight change in slope at the end-point, but, if acetone is used as a solvent for the acid, it has been found to act as an "indicator." At the neutralisation point a rapid heat rise occurs owing to the formation of diacetone alcohol from the reaction of acetone with the excess of non-aqueous alkali. The conditions under which this reaction occurs have been studied and a method incorporating the best conditions is described. The method is applicable to a wide range of acidic substances, including 2,6-disubstituted phenols, keto-enols and imides, and can be used to determine traces of acidity.

G. A. VAUGHAN and J. J. SWITHENBANK
Coal Tar Research Association, Gomersal, Leeds.

Analyst, 1965, **90**, 594–599.

Hydroxylysine as a Growth Stimulant in Microbiological Assays for Lysine

Results for the lysine content of animal products, obtained by microbiological assays with *Leuconostoc mesenteroides* P60 may be too high unless the basal medium contains hydroxylysine. Evidence is presented to show that although cultures of this organism vary in the extent of their response to lysine in the presence of hydroxylysine, the effect of the latter synergist is apparent with several cultures and under many different assay conditions. A standard addition of hydroxylysine to the assay medium is therefore recommended in all microbiological assays for lysine in which the above organism is used.

A. W. HARTLEY and L. D. WARD
Spillers Limited, Central Laboratory, Cambridge.
and K. J. CARPENTER
School of Agriculture, University of Cambridge, Cambridge.

Analyst, 1965, **90**, 600–605.

Modifications and Extensions to an Existing Sequential Scheme for Determining Fall-out Nuclides in Water

Extensions and modifications to an existing sequential scheme for the radiochemical analysis of fall-out nuclides in drinking and rain water are described. This new scheme makes possible the determination, in the same sample, of manganese-54, yttrium-91, promethium-147 and zirconium-95 activities along with the more commonly determined barium, caesium, cerium and strontium fall-out nuclides.

After evaporation of the sample to dryness, conventional precipitation and solvent-extraction procedures are used to separate the various radioactive species carried on known amounts of added carriers. Mean chemical recoveries of the added carriers range from 40 per cent. for zirconium to 70 per cent. for strontium. Taking a 100-litre sample of drinking water and using low-background anti-coincidence-counting assemblies for detection of the β-particle emissions gives levels of detection ranging from 0·01 pC per litre for promethium-147 to 0·002 pC per litre for strontium-90. The manganese-54 is determined by γ-ray counting with a limit of detection of 0·02 pC per litre.

R. WOOD and L. A. RICHARDS
Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1965, **90**, 606–622.

A Simple and Rapid Titrimetric Method for the Determination of Carbon in Iron and Steel

A simple and rapid method is described for the determination of carbon in iron and steel, based on non-aqueous titration with tetra-n-butylammonium hydroxide of the carbon dioxide evolved by combustion of the sample in a stream of oxygen. The carbon dioxide is absorbed in a solution of formdimethylamide. Sources of blank value and analytical conditions required for optimum results are discussed. Results of a reproducibility test carried out on a series of certified British Chemical Standards are reported. The procedure can be made semi-automatic by use of an automatic titrimeter and this modification is described.

R. F. JONES, P. GALE, P. HOPKINS and L. N. POWELL
The Steel Company of Wales Limited, Abbey Works, Port Talbot, Glamorgan.

Analyst, 1965, **90**, 623–629.

Spectrophotometric Determination of Alkylating Agents with 4-Picoline and o-Dinitrobenzene

An accurate, sensitive spectrophotometric method has been developed for determining alkylating agents. The method involves the alkylation of 4-picoline at 100° C in 2-methoxyethanol, with subsequent reaction of the 4-picolinium cation with *o*-dinitrobenzene in the presence of alkali to produce the chromogen. With 2-phenylethanol as the solvent, the method is autocatalytic at 100° C. The variables in the procedures were studied, and the methods were compared to one another and to methods in the literature. Some preliminary studies of other possible methods for alkylating agents are discussed.

DANIEL F. BENDER, EUGENE SAWICKI and RONALD M. WILSON, jun.

Laboratory of Engineering & Physical Sciences, Division of Air Pollution, Robert A. Taft Sanitary Engineering Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio 45226.

Analyst, **1965**, **90**, 630–634.

Polarography of Thallium: Determination of Thallium in Sodium Iodide

Short Paper

V. T. ATHAVALE, S. V. BURANGEY and R. G. DHANESHWAR

Analytical Division, Atomic Energy Establishment Trombay, Bombay, India.

Analyst, **1965**, **90**, 635–637.

A Rapid Method for Separating and Determining DDT in Fat

Short Paper

J. H. P. DINGLE

Department of Agriculture, Board of Tick Control, Box C285, Lismore, N.S.W., Australia.

Analyst, **1965**, **90**, 638.

Determination of Zirconium (and Hafnium) in Niobium and other Metals with Catechol Violet: A Note

Short Paper

D. F. WOOD and J. T. JONES

Imperial Metal Industries (Kynoch) Limited, Kynoch Works, Witton, Birmingham 6.

Analyst, **1965**, **90**, 638.

Notice to Authors

THE Editor welcomes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Papers are submitted to the Editorial Committee, who decide on their suitability for publication.

Intending authors should consult the current Notice to Authors, last published in full in *The Analyst*, **1965**, **90**, 249, reprints of which can be obtained on application to The Editor, *The Analyst*, 14, Belgrave Square, London, S.W.1. All papers submitted will be expected to conform to the recommendations there laid down, and any that do not may be returned for amendment.

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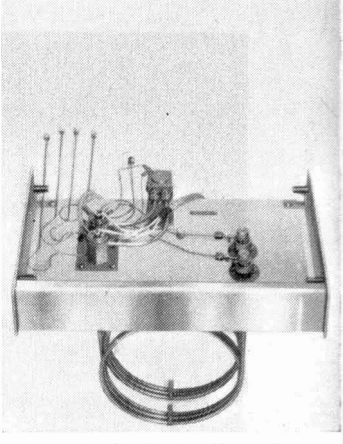

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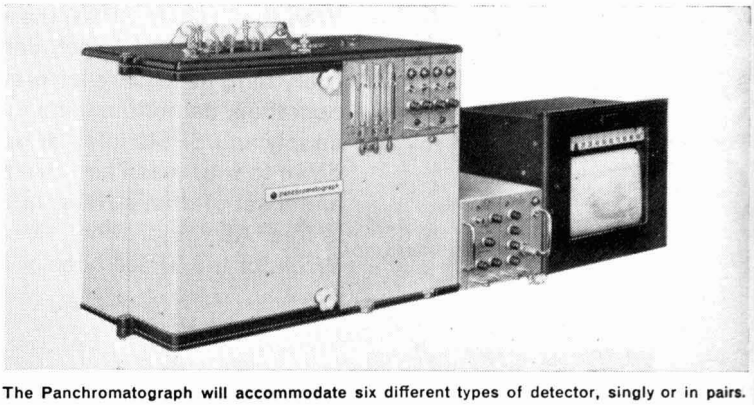

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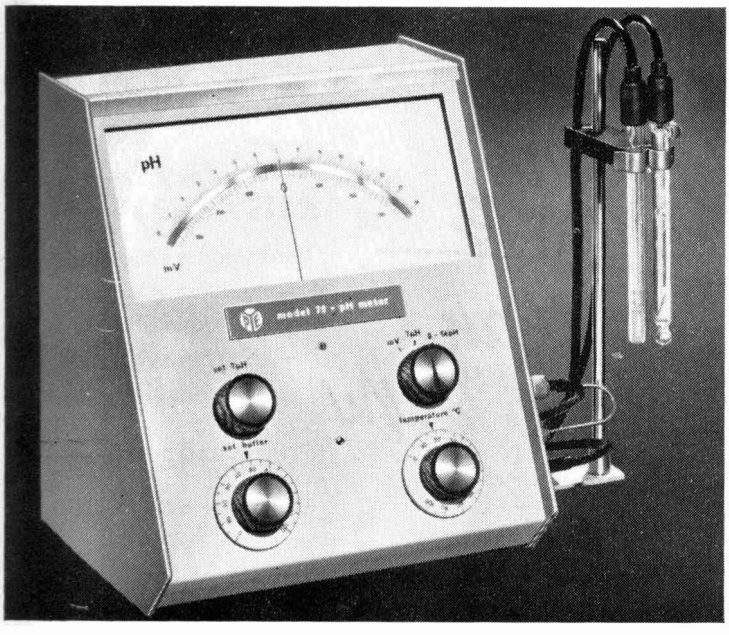

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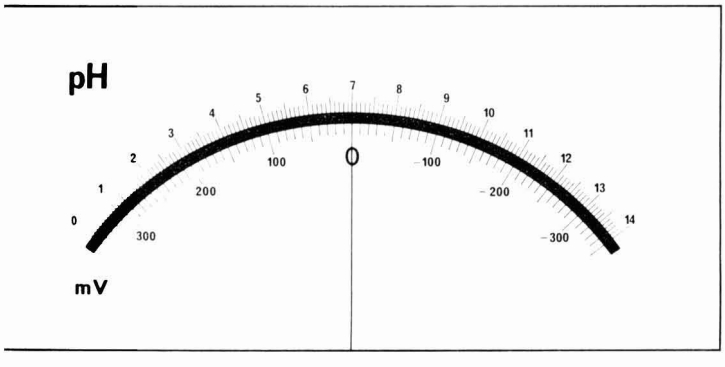

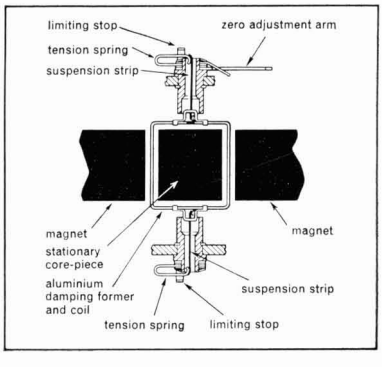

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THE ANALYST

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Content of Rusk Filler

THE Analytical Methods Committee has received the following report from its Meat Products Sub-Committee. The report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. O. Dennis, Mr. J. R. Fraser, Mr. H. C. Hornsey, Dr. A. J. Kidney, Dr. R. A. Lawrie, Mr. T. McLachlan, Dr. A. McM. Taylor and Mr. E. F. Williams, with Miss V. Lewis (resigned) and then Mr. P. W. Shallis as Secretary.

In its report[1] published in 1961 the Sub-Committee recommended on the basis of the data then available that a figure of 2·3 per cent. should be adopted as the correction for nitrogen in rusk used as an ingredient in manufactured meat products. The Sub-Committee also recommended that this figure should be reviewed periodically.

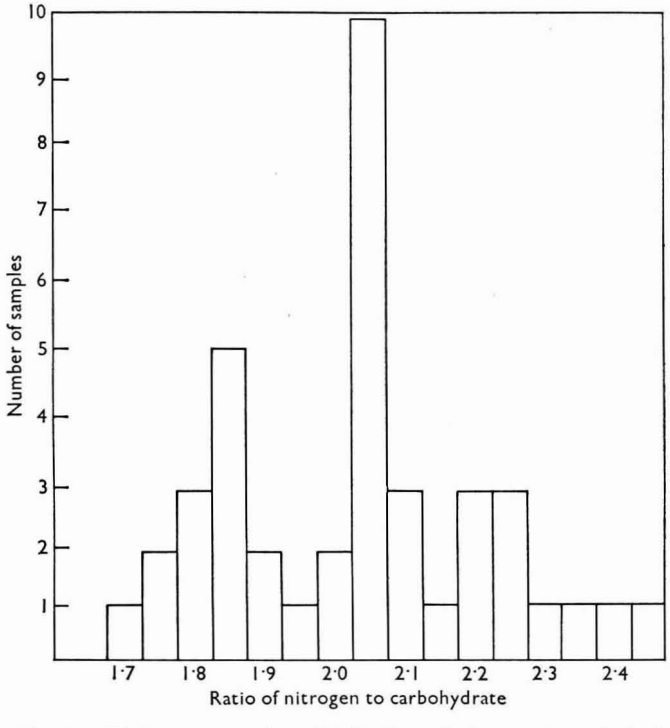


Fig. 1. Histogram showing distribution of nitrogen-to-carbohydrate ratios for 40 samples of rusk filler

Recently, Savageri and Nichols[2] published results of analyses of samples of cereal fillers used in sausages and suggested that a figure of 2·0 per cent. should be used for correction. In the meantime the Sub-Committee had been collecting further information by asking manufacturers of rusk for analytical data and by analysing samples of rusk obtained from various sources. The results of the examination of 40 samples are shown on the histogram (Fig. 1).

It will be seen that there is a considerable variability in the kinds of flour used in the manufacture of rusk. The largest number of samples had a nitrogen to carbohydrate ratio of 2·05 per cent., but a significant proportion of samples were made from a softer flour.

A certain amount of variability in the characteristics of flour used for rusk is to be expected, depending on season, trading conditions, etc. It is, however, probably safe to assume that generally a cheaper soft flour will be selected for such manufacture.

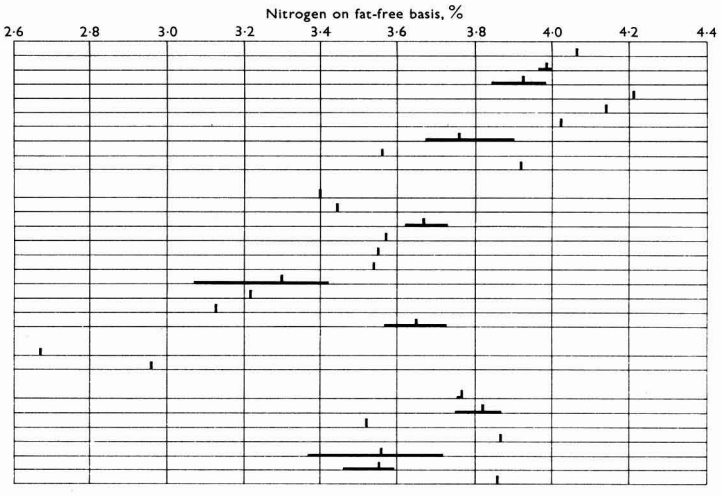
Recommendation

After reviewing the analytical results and bearing in mind the above consideration, the Sub-Committee recommends that a figure of 2·0 per cent. should be used in future as the correction for nitrogen in a rusk filler. Futher, the Sub-Committee is of the opinion that this correction should be kept under constant surveillance.

References

1. Analytical Methods Committee, *Analyst*, 1961, **86**, 560.
2. Savageri, J. V., and Nichols, B. S., *J. Assoc. Public Analysts*, 1964, **2**, 60.

		Laboratory	No. of samples		Range	Mean	
BREAST MEAT	Home killed: freed from skin, subcutaneous fat and sinews	A	1		—	4·06	
	Breast muscle	‡	2	(1m: 1f)	3·97–4·00	3·99	
	English hens: analysed after removal of skin and surface fat	B	3		3·84–3·98	3·92	
	Origin and sex unspecified	B	1		—	4·21	
	English: sex unspecified	C	1		—	4·14	3·90
	Irish: sex unspecified	C	1		—	4·02	
	*Frozen: polythene wrapped: 12 lb 9 oz to 13 lb 4 oz	D	6		3·67–3·90	3·76	
	Large white: age 12 weeks, Grade A	E	1		—	3·56	
	Large white: age 12 weeks, Grade B	E	1		—	3·92	
LEG MEAT	Home killed: freed from skin, subcutaneous fat and sinews	A	1		—	3·40	
	Leg muscle	‡	2	(1m: 1f)	3·44–3·44	3·44	
	English hens: analysed after removal of skin and surface fat	B	3		3·62–3·73	3·67	
	Origin and sex unspecified	B	1		—	3·57	
	English: sex unspecified	C	1		—	3·55	3·44
	Irish: sex unspecified	C	1		—	3·54	
	*Frozen: polythene wrapped: 12 lb 9 oz to 13 lb 4 oz	D	6		3·07–3·42	3·30	
	Large white: age 12 weeks, Grade A	E	1		—	3·22	
	Large white: age 12 weeks, Grade B	E	1		—	3·13	
	Thigh muscle: Broad Breasted Bronze	§	2	(1m: 1f)	3·57–3·73	3·65	
SKIN	Large white: age 12 weeks, Grade A	E	1		—	2·67	2·82
	Large white: age 12 weeks, Grade B	E	1		—	2·96	
WHOLE TURKEYS	Unspecified	‡	2	(1m: 1f)	3·76–3·77	3·77	
	†English hens: analysed after removal of skin and surface fat	B	3		3·75–3·87	3·82	
	English cock: analysed after removal of skin and surface fat	B	1		—	3·52	
	Uruguayan hens: analysed after removal of skin and surface fat	B	2		3·87–3·87	3·87	3·66
	*Frozen: polythene wrapped: 12 lb 9 oz to 13 lb 4 oz	D	6		3·37–3·72	3·56	
	Combination of dark and white meat from 4 birds in natural proportions	F	3		3·46–3·59	3·55	
	Representative of flesh of 50 birds: average weight 15 lb	G	–		—	3·86	



* Samples taken by proposed procedure
† Calculated
‡ U.S. Poultry and Egg Magazine, June, 1942
§ South Dakota State College, Bulletin No. 463, April, 1957

Fig. 1. Nitrogen contents of various portions of turkey. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factors for Turkey

THE Analytical Methods Committee has received the following report from its Meat Products Sub-Committee. The report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. O. Dennis, Mr. J. R. Fraser, Mr. H. C. Hornsey, Dr. A. J. Kidney, Dr. R. A. Lawrie, Mr. T. McLachlan, Dr. A. McM. Taylor and Mr. E. F. Williams, with Miss V. Lewis (resigned) and then Mr. P. W. Shallis as Secretary.

In continuation of its investigations on nitrogen factors for different kinds of meat the Sub-Committee examined all available results for the nitrogen content of turkey meat. Further analyses were carried out in the laboratories of members of the Sub-Committee and in some other laboratories, whose collaboration is gratefully acknowledged.

The sampling procedures issued to analysts were similar to those given on an earlier occasion in connection with work on the nitrogen factors for chicken.[1]

The results collected by the Sub-Committee are shown in Fig. 1. Those marked with an asterisk were obtained on samples taken by the recommended procedure, and the values thus marked in the "Whole Turkeys" section of the chart have been calculated from the results for the component parts. In addition, the values marked with a dagger in this section were obtained by a similar calculation, although the separation of the carcase into breast and other meat was not necessarily carried out in the standard manner. In other tests, for various reasons, sampling was not carried out by the prescribed method, and the resulting figures may to some extent be affected by the variation.

RECOMMENDATION

The Sub-Committee recommends the following average figures for use in the analysis of turkey products: breast meat, 3·9 per cent.; dark meat, 3·5 per cent.; whole carcase, 3·65 per cent.

ACKNOWLEDGMENT

The Sub-Committee thanks those listed below for their help and communications—

Dr. H. A. Williams.
Brand & Co. Ltd.
C. Shippam Ltd.
Crosse & Blackwell Ltd.
Professor W. J. Stadelman, Purdue University, U.S.A.
Professor L. E. Dawson, Michigan State University, U.S.A.
S. J. Palmer & Co. Ltd.

REFERENCE

1. Analytical Methods Committee, *Analyst*, 1963, **88**, 583.

Recommended Methods for the Evaluation of Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS FOR THE EVALUATION OF DRUGS

The Chemical Assay of Senna Fruit and Senna Leaf

THE Panel of the Joint Committee responsible for the preparation of this Report was set up with the following terms of reference: "To investigate methods for estimating the purgative activity of drugs and preparations of drugs containing anthraquinone derivatives with a view to recommending standard methods of assay." The constitution of the Panel, which held its first meeting in October, 1956, and subsequently suspended its work for over 2 years to allow Panel 3A to investigate the biological activities of samples under test, was: Professor J. M. Rowson (Chairman, 1956–57; resigned from the Panel 1957; re-appointed Chairman 1962), Dr. W. Mitchell (Chairman, 1957–62; resigned 1962), Professor J. W. Fairbairn, Mr. C. A. Johnson, Mr. S. C. Jolly (resigned 1962, succeeded by Miss B. Gartside), Miss H. M. Perry, Mr. H. A. Ryan, Mr. W. Smith (resigned 1962) and Mr. R. V. Swann, with, successively, Miss A. M. Parry, Miss V. Lewis and Mr. P. W. Shallis as Secretary.

After considering the published work on the evaluation of anthraquinone drugs, the Panel decided to investigate the assay of senna fruit, *Cassia senna* L. (commercial varieties: Alexandrian senna from *Cassia acutifolia* Delile and Tinnevelly senna from *Cassia angustifolia* Vahl). The investigation was then extended to the two commercial varieties of senna leaf. The anthraquinone glycosides present in both varieties of these two drugs are principally derivatives of rhein together with small amounts of a third, active non-rhein glycoside. The Panel has done much work on the estimation of the rhein glycosides, but considers that an assay of total glycosides is a satisfactory measure of the purgative activity of both drugs.

DETERMINATION OF TOTAL GLYCOSIDES IN SENNA FRUIT AND SENNA LEAF

The analytical procedure that the Panel recommends is set out in Appendix I. It is based on the published method of Kussmäul and Becker,[1] as modified by Fairbairn and Michaels.[2] During the course of repeated collaborative trials by this method, various modifications have been incorporated in order to establish an unambiguous procedure that reduced inter-laboratory variations in results. The method as described has yielded concordant results in six different laboratories, when applied to different samples of either Alexandrian or Tinnevelly senna fruit (see Table I). Similar concordant results were obtained in five of these laboratories when the method was applied to different samples of Alexandrian or Tinnevelly senna leaf (see Table II).

The validity of the chemical method of estimation as a measure of the purgative activities of these materials was tested by Panel 3A, set up to examine the purgative activities of certain of these samples by biological methods. The report of that Panel is given in Appendix III and Table VI. All potencies have been calculated relative to senna leaf sample TL1 as reference standard. The mean values for the chemical estimations of the samples (Tables I and II) have also been expressed relative to TL1 and these figures for both chemical and biological estimations are given in Table III. It will be seen that both methods of estimation place the samples in the same order of potency, but it should be noted that the estimates for the two more potent samples are about 30 per cent. higher by the chemical method and that this difference is greater than can be accounted for by the limits of error of the estimates.

OBSERVATIONS ON THE METHOD—

(*i*) Extraction of the powdered fruit or leaf with acid - alcohol is followed by removal of alcohol from an aliquot that has been rendered only slightly acid. (Sennosides are hydrolysed in strongly acid solution, but are stable in faintly acid solution.) If alcohol remains in the extract it causes loss of glycosides in stage (*ii*).

(*ii*) Free anthraquinones have little or no purgative activity and are removed by extraction with chloroform. Under the conditions of the extraction there is no significant loss of

anthraquinone glycosides; losses do occur if ether is used instead of chloroform or if alcohol is not removed in stage (*i*).

(*iii*) Anthraquinone glycosides are hydrolysed.

(*iv*) Liberated anthraquinones are extracted with ether.

(*v*) The anthraquinones are extracted with sodium hydroxide solution and a cherry-red colour is developed by heating with a standardised solution of hydrogen peroxide under controlled conditions (modified Bornträger reaction). The intensity of the resultant colour is measured spectrophotometrically at 515 mμ. The intensity of the colour developed varies if the conditions under which the colour develops are altered, especially if the concentration of hydrogen peroxide is changed. Thus when a 4 per cent. solution of hydrogen peroxide

TABLE I

RESULTS OF COLLABORATIVE ASSAYS OF SENNA FRUIT B.P.

Assays carried out by the procedure recommended in Appendix I

	Alexandrian samples				Tinnevelly samples			
	A1	A2	A3	A4	T1	T2	T3	T4
Equivalent Sennoside B, %—								
Laboratory A	7·1	4·5	5·9	7·5	3·1	2·5	2·4	3·4
B	7·0	4·5	6·0	7·5	3·2	2·5	2·4	3·3
C	7·1	5·0	6·2	7·8	3·2	2·6	2·4	3·2
D	7·2	4·7	6·0	7·6	3·3	2·5	2·5	3·2
E	6·6	4·4	5·6	7·4	2·9	2·5	2·3	3·0
F	6·9	4·7	6·1	7·8	3·0	2·6	2·5	3·3
Mean	7·0	4·6	6·0	7·6	3·1	2·5	2·4	3·2
s.d.	0·21	0·22	0·21	0·17	0·15	0·06	0·08	0·14
Ratio value (E 515/E 440)—								
Laboratory A	1·39	1·36	1·37	1·32	1·30	1·21	1·12	1·25
B	1·44	1·40	1·38	1·34	1·29	1·22	1·15	1·28
C	1·36	1·38	1·34	1·39	1·27	1·24	1·19	1·28
D	1·38	1·43	1·40	1·38	1·26	1·28	1·20	1·34
E	1·30	1·32	1·37	1·31	1·34	1·29	1·20	1·31
F	1·45	1·41	1·39	1·44	1·34	1·29	1·17	1·33
Mean	1·39	1·38	1·38	1·36	1·30	1·26	1·17	1·30
s.d.	0·06	0·04	0·02	0·05	0·03	0·04	0·03	0·03

TABLE II

RESULTS OF COLLABORATIVE ASSAYS OF SENNA LEAF B.P.

Assays carried out by the procedure recommended in Appendix I

	Alexandrian samples				Tinnevelly samples			
	AL1	AL2	AL3	AL4	TL1	TL2	TL3	TL4
Equivalent Sennoside B, %—								
Laboratory A	3·8	3·7	3·6	3·5	4·2	4·2	3·9	2·7
C	3·7	3·4	3·4	3·6	4·3	4·2	3·9	2·6
D	3·7	3·7	3·7	3·5	4·3	4·3	4·1	2·6
E	3·5	3·5	3·5	3·2	3·8	3·8	3·6	2·5
F	3·4	3·7	3·6	3·7	4·0	4·2	3·9	2·6
Mean	3·6	3·6	3·6	3·5	4·1	4·1	3·9	2·6
s.d.	0·17	0·14	0·12	0·19	0·22	0·20	0·18	0·02
Ratio value (E 515/E 440)—								
Laboratory A	1·43	1·41	1·35	1·27	1·35	1·37	1·31	1·30
C	1·43	1·23	1·35	1·35	1·40	1·33	1·32	1·29
D	1·44	1·52	1·44	1·40	1·39	1·43	1·33	1·30
E	1·25	1·39	1·36	1·36	1·31	1·30	1·40	1·39
F	1·48	1·41	1·35	1·38	1·38	1·36	1·32	1·28
Mean	1·41	1·39	1·37	1·35	1·37	1·36	1·34	1·31
s.d.	0·09	0·10	0·04	0·05	0·04	0·05	0·04	0·04

was used the $E^{1\%}_{1cm}$ value (at 515 mμ) was decreased by 1·7 per cent., and when a 2 per cent. solution was used the $E^{1\%}_{1cm}$ value was increased by 3·3 per cent. in comparison with values obtained with a 3 per cent. solution of hydrogen peroxide.

When the alkaline solution is heated in the absence of hydrogen peroxide, a brownish colour is produced that changes to purplish red when the solution is cooled; the maximum $E^{1\%}_{1cm}$ value of this colour at 525 to 535 mμ is about 23 per cent. higher than that obtained under the conditions of test as described, in which a 3 per cent. solution of hydrogen peroxide is used. The Panel considered that this reaction in the absence of hydrogen peroxide was different from that when hydrogen peroxide was used, that it required further investigation and that the inter-laboratory results were more variable. They accepted the method as described in Appendix I, in which hydrogen peroxide is used, because the method, although empirical, gave consistently reproducible results.

As an alternative to the colorimetric determination of anthraquinones, an ultraviolet absorptiometric method was examined, but it was rejected as unreliable.

TABLE III

COMPARISON OF CHEMICAL AND BIOLOGICAL RESULTS

Relative evaluation of certain samples of senna fruit and senna leaf by the chemical method of Appendix I and by the biological method of Appendix III
(All results are relative to sample TL1 = 1·00)

	Relative evaluation* by—	
Sample	chemical method (from Tables I and II)	biological method (from Table VI)
A4	1·85 (1·96 to 1·74)	1·4 (1·48 to 1·33)
A1	1·71 (1·82 to 1·60)	1·39 (1·62 to 1·19)
TL2	1·00 (1·07 to 0·93)	0·95 (1·03 to 0·88)
AL1	0·88 (0·94 to 0·82)	0·86 (1·15 to 0·65)
T1	0·76 (0·82 to 0·70)	0·84 (0·97 to 0·73)
TL4	0·63 (0·67 to 0·59)	0·61 (0·64 to 0·59)
T3	0·50 (0·53 to 0·47)	0·53 (0·59 to 0·48)

* P = 0·95 limits of error.

(*vi*) The results are expressed as equivalent sennoside B, the factor of 130 being the mean value found by the Panel for sennoside B dried to constant weight at about 80° C *in vacuo* (128 - **130** - 132, s.d. = 1·9).

$E^{1\%}_{1cm}$ values at 440 mμ for the cherry-red reaction mixture are raised when impurities are present. The Panel has found that for a range of samples of senna fruit or senna leaf of good commercial quality, the values for E (515 mμ)/E (440 mμ) were: Alexandrian pod 1·30 to 1·47; Tinnevelly pod 1·25 to 1·34; Alexandrian leaf 1·23 to 1·52; Tinnevelly leaf 1·28 to 1·40. The Panel recommends that values for the percentage of glycoside present, calculated as in (*vi*), be accepted with confidence only if the ratio value is 1·25 or greater; for Tinnevelly pods of inferior quality (sample T3, Table I) ratio values of 1·20 and less were recorded. Ratio values for sennoside B were 1·35 to 1·46.

The Panel has not investigated the application of this method to the evaluation of liquid galenical preparations of senna. There is some evidence to show that in such determinations the ratio value is low and that reliance cannot be placed on the figures obtained for sennoside B content as a measure of purgative activity of these galenicals.

DETERMINATION OF RHEIN GLYCOSIDES

Much of the early work of the Panel was carried out on an extended method that determined only the carboxylic (rhein) glycosides. The refinements of this method are not considered necessary for the measurement of purgative activity of senna fruit or of senna

leaf. The details of the process are, however, outlined in Appendix II. The rhein aglycones obtained in stage (*iv*) of Appendix I are separated from non-rhein glycosides by extraction with sodium hydrogen carbonate solution. These aglycones are then determined by processes (*v*) and (*vi*). Tables IV and V record the results obtained by this method for the same pod and leaf samples as used in Tables I and II.

TABLE IV

RESULTS OF COLLABORATIVE ASSAYS OF SENNA FRUIT B.P.

Assays carried out by the procedure given in Appendix II

	Alexandrian samples				Tinnevelly samples			
	A1	A2	A3	A4	T1	T2	T3	T4
Carboxylic (rhein) glycosides as equivalent sennoside B, %—								
Laboratory A	5·9	4·0	4·9	6·6	2·7	2·2	2·2	2·9
B	5·9	4·1	5·0	6·7	2·6	2·2	2·2	2·8
C	6·0	4·2	5·4	6·8	2·8	2·2	2·0	2·8
D	6·0	3·7	4·8	6·5	2·6	2·0	1·9	2·6
E	5·7	3·9	5·2	6·3	2·6	2·3	2·1	2·6
F	6·2	4·1	5·3	6·8	2·7	2·2	2·1	2·9
Mean	6·0	4·0	5·1	6·6	2·7	2·2	2·1	2·8
s.d.	0·17	0·18	0·24	0·19	0·03	0·10	0·12	0·14
Ratio value (E 515/E 440)—								
Laboratory A	1·43	1·45	1·46	1·42	1·31	1·28	1·21	1·29
B	1·40	1·45	1·45	1·42	1·27	1·25	1·25	1·31
C	1·39	1·44	1·41	1·44	1·34	1·30	1·25	1·36
D	1·44	1·47	1·45	1·47	1·32	1·31	1·26	1·37
E	1·38	1·38	1·34	1·42	1·32	1·29	1·24	1·33
F	1·43	1·43	1·42	1·46	1·34	1·35	1·27	1·37
Mean	1·41	1·44	1·42	1·44	1·32	1·30	1·25	1·34
s.d.	0·03	0·03	0·04	0·02	0·03	0·03	0·02	0·03

TABLE V

RESULTS OF COLLABORATIVE ASSAYS OF SENNA LEAF B.P.

Assays carried out by the procedure given in Appendix II

	Alexandrian samples				Tinnevelly samples			
	AL1	AL2	AL3	AL4	TL1	TL2	TL3	TL4
Carboxylic (rhein) glycosides as equivalent sennoside B, %—								
Laboratory A	2·9	2·8	2·9	2·9	3·3	3·3	3·2	2·1
C	2·7	2·5	2·7	2·8	3·3	3·2	3·1	2·0
D	2·6	2·6	2·7	2·4	3·1	3·0	3·0	1·9
E	2·9	2·8	2·8	2·6	3·2	3·2	2·8	2·0
F	2·7	2·8	2·8	2·7	2·7	3·2	3·1	2·0
Mean	2·8	2·7	2·8	2·7	3·1	3·2	3·0	2·0
s.d.	0·14	0·14	0·09	0·19	0·25	0·11	0·15	0·02
Ratio value (E 515/E 440)—								
Laboratory A	1·50	1·44	1·40	1·31	1·41	1·40	1·34	1·32
C	1·47	1·24	1·43	1·43	1·42	1·43	1·29	1·33
D	1·54	1·55	1·43	1·45	1·40	1·43	1·37	1·37
E	1·28	1·32	1·31	1·39	1·37	1·44	1·34	1·25
F	1·50	1·49	1·45	1·41	1·39	1·41	1·35	1·36
Mean	1·46	1·41	1·40	1·40	1·40	1·42	1·34	1·33
s.d.	0·10	0·13	0·06	0·05	0·02	0·02	0·03	0·05

Appendix I

Recommended method for the chemical assay of total anthraquinone glycosides of senna fruit or senna leaf

Reagents—

Acid - alcohol—A 2 per cent. v/v solution of hydrochloric acid B.P. in 70 per cent. v/v aqueous ethanol.

Hydrochloric acid, 0·1 N *and* N.

Sulphuric acid, 10 N.

Sodium hydroxide, N.

Chloroform B.P.

Anaesthetic ether B.P.

Sodium hydrogen carbonate solution, 5 per cent. w/v, aqueous—This solution must be freshly prepared.

Hydrogen peroxide solution, 3 per cent. w/v, aqueous—Recently standardised.

Distilled water.

Procedure—

The assay procedure should be conducted throughout in subdued daylight.

(*i*) Weigh accurately about 1 g of senna pod or senna leaf in moderately fine powder, and place it in a stoppered 100-ml calibrated flask with 80 ml of acid - alcohol. Shake the mixture frequently during 1½ hours, set aside overnight, and shake again in the morning; thereafter make the total volume up to 100 ml with acid - alcohol. Allow the mixture to settle, and filter through a 12·5-cm Whatman No. 4 filter-paper in a funnel covered by a clock-glass. Dilute a 10-ml aliquot of the bright filtrate with 10 ml of water. Add about 2 ml of N sodium hydroxide, and ensure that the solution is just slightly acid to litmus. Remove the alcohol by warming on a water bath with constant stirring until the volume is about 10 ml; add 2 ml of N hydrochloric acid and dilute to about 20 ml with distilled water.

(*ii*) Place the solution in a separating funnel, and extract by vigorous shaking with three successive portions (each of 40 ml) of chloroform. Wash each of the chloroformic extracts successively with the same two portions (each of 5 ml) of 0·1 N hydrochloric acid. Discard the chloroform extracts so washed.

(*iii*) Place the solvent-extracted aqueous liquid and the two acid washings in a 100-ml conical flask, and add 13·5 ml of 10 N sulphuric acid. Attach a short air condenser to the flask by means of a glass cone and socket joint. Place the flask in a bath of vigorously boiling water (so that the water level is just above that of the liquid level in the flask), and leave it there for exactly 15 minutes. Immediately thereafter cool the flask and its contents rapidly in a stream of cold water.

(*iv*) Transfer the cooled liquid to a separating funnel, and rinse out the conical flask with two portions (each of 5 ml) of N sodium hydroxide, and finally with 5 ml of water; add these rinsings to the contents of the separating funnel.

Add 25 ml of anaesthetic ether, shake the funnel vigorously, and allow the layers to separate. Run off the lower aqueous layer, and then decant the ethereal layer, taking care to leave any insoluble brown material in the separating funnel. Add 5 ml of N sodium hydroxide to the contents of the separating funnel, and ensure that the residual brown material is dissolved in it. Thereafter return the acidic aqueous solution to the separating funnel. Repeat the entire extraction procedure (from "Add 25 ml of anaesthetic ether . . ." beginning of this paragraph) three times. Discard the acidic liquid so extracted.

Unite the four ethereal extracts in a separating funnel.

(*v*) Extract the ethereal solution with four successive portions (20, 10, 5 and 5 ml) of N sodium hydroxide. Discard the ethereal solution so extracted. Unite the alkaline extracts in a stoppered 50-ml calibrated flask, and make up to 50 ml with N sodium hydroxide. Transfer 10 ml of this solution to a glass tube (150 × 25 mm), add 5 ml of N sodium hydroxide and 0·3 ml of hydrogen peroxide, 3 per cent. w/v aqueous solution (recently standardised). At once suspend the tube in a bath of vigorously boiling water (so that the water level is just above that of the solution in the tube), and leave it there for exactly 6 minutes. Immediately thereafter cool the tube and its contents rapidly in a stream of cold water. Transfer

the cooled liquid to a stoppered 25-ml calibrated flask, and dilute to 25 ml with N sodium hydroxide. Measure the extinction (E) of the solution in a 1-cm cell at 440 and at 515 mμ against that of N sodium hydroxide in a similar cell. The readings should be taken within 30 minutes of making the solution up to volume, and particular care should be taken to avoid exposure of it to direct sunlight.

(*vi*) Calculate, as equivalent of sennoside B, the percentage of total anthracene glycosides present, on the assumption that the $E^{1\%}_{1\,cm}$ value at 515 mμ of the red solution that would be obtained from sennoside B is 130. Also calculate the ratio value E (515 mμ)/E (440 mμ). If this ratio value is less than 1·25 (1·20 for certain samples of Tinnevelly pod), reject the result.

Appendix II

Method for the chemical assay of rhein glycosides of senna fruit or senna leaf

Proceed as in Appendix I, (*i*) to (*iv*); continue—

(*iv* (*a*)) Extract the ethereal solution by vigorous shaking with successive portions (one of 10 ml, and then three each of 5 ml) of 5 per cent. w/v aqueous sodium hydrogen carbonate solution. Discard the ethereal solution so extracted.

Unite the four sodium hydrogen carbonate extracts in a separating funnel, add 20 ml of anaesthetic ether, and then add cautiously 10 ml of 10 N sulphuric acid. After the effervescence has subsided, shake the funnel vigorously, and then allow the layers to separate. Run off the lower aqueous layer, and then decant the ethereal layer, taking care to leave any insoluble brown material in the separating funnel; add 5 ml of N sodium hydroxide, and ensure that the residual brown material is completely dissolved in it. Thereafter return the acidic aqueous solution to the separating funnel. Add 20 ml of anaesthetic ether, and repeat the entire extraction procedure (from "shake the funnel vigorously, and allow the layers to separate . . ." line 3 of this paragraph) three times.

Unite the four ethereal extracts in a separating funnel.

Continue as in Appendix I, (*v*) and (*vi*), and calculate, as equivalent of sennoside B, the percentage of carboxylic (rhein) glycosides present. If the ratio value is less than 1·25, reject the result.

Appendix III

Biological estimations of senna samples by panel 3A

Biological estimations were carried out by members of the Panel,* comparisons being made against a sample of senna leaf, TL1.

The method described is that adopted by one laboratory and was followed by the others in all its essential details.

Method

A group of male albino mice of the same age and weighing not less than 18 g were assembled for repeated use, one week's rest being allowed between tests. They were submitted to an initial training period during which time they were treated with suitable doses of senna exactly as described below.

Cages—

Rectangular Perspex cages (18 × 9 × 5 inches) divided into 10 compartments and open at the top and bottom were used. The cages stood on a ¼-inch mesh wire grid raised ½ inch above sheets of blotting paper, and the tops of the cages were covered with identical grids.

Pre-treatment—

Subject to the restriction that the sums of the weights of the mice in each group were roughly equal, the mice were randomly distributed into four groups of ten, which were transferred to two cages with two mice in each compartment. No food or water was given for the

* Constitution of the Panel: Mr. K. L. Smith (Chairman), Dr. P. F. D'Arcy (resigned 1962), Dr. R. T. Brittain, Professor J. W. Fairbairn and Dr. G. A. Stewart.

first two or three hours, during which time the mice were observed and those producing wet faeces were replaced.

DOSING—

The dose was prepared by triturating the required amount of powder with boiling water, cooling the suspension and making up to volume. A volume of 0·5 ml or 1 ml was given orally to each mouse by means of a 1-ml syringe to which was attached a blunt-ended needle. The standard preparation and the sample under test were administered at two levels by giving equal volumes of the prepared extract and of a 50 per cent. dilution. A small receptacle of about 100-ml capacity and containing a soft paste prepared by mixing 10 parts of rat cubes (Diet 41B) with 7 to 10 parts of tap water was hung in each compartment so that the mice could feed freely during the test. Purgation was recognised by the production of wet faeces, which were irregular in shape, very soft and damp and which stained the blotting paper brown; when dry they adhered firmly to the paper. In contrast, normal faeces are usually fusiform in shape, firm, leave no stain, and when dry do not stick to the paper.

COUNTING THE WET FAECES—

Counts of wet faeces were carried out approximately 3, 5, 7, 9 and 24 hours after the mice had been dosed. To make the count, the food containers were removed and the cage inverted over a clean sheet of blotting paper. The appropriate "floor" grid was returned to the first sheet of blotting paper in exactly its original position. The wet faeces produced by each pair of mice were recorded, and a search was made for any faeces attached to the sides of the cage or grid, care being taken to note whether those on the grid were merely part of a wet faeces already recorded on the paper.

CALCULATIONS—

The results were interpreted by standard statistical methods relating number of wet faeces to log dose.

RESULTS

The results obtained by members of the Panel on some of the samples that had been used for the chemical assays are shown in Table VI.

TABLE VI

RATIO OF ACTIVITY OF SENNA SAMPLES TO THAT OF TL1 BY BIOLOGICAL EXAMINATION

Laboratory		A4	A1	TL2	AL1	T1	TL4	T3
H	R	1·12	n.d.	1·01	1·20	n.d.	n.d.	0·73
	w	188	n.d.	600	1206	n.d.	n.d.	441
I	R	1·39	n.d.	1·00	0·62	n.d.	0·60	0·52
	w	4829	n.d.	182	908	n.d.	7865	2402
J	R	1·57	1·39	0·81	0·73	0·84	0·73	0·60
	w	1599	882	467	2·77	1005	892	470
K	R	1·30	n.d.	0·95	1·03	n.d.	0·66	0·62
	w	804	n.d.	1032	248	n.d.	597	666
L	R	1·31	n.d.	0·99	0·71	n.d.	0·60	0·58
	w	407	n.d.	1030	524	n.d.	1764	446
Mean	R	1·40	—	0·95	0·86	—	0·61	0·53
	χ^2*	6·9	—	3·0	50·2	—	7·0	10·09
	w	7827	—	3311	252	—	11,118	1752

* Provided the value for χ^2 for 5 estimates does not exceed 9·49 satisfactory agreement at the P = 0·95 level is indicated.

w = "weight" of estimate = Reciprocal of variance of log R suitably adjusted for discordant values of χ^2.

n.d. = not determined.

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Continuous-flow Enthalpimetry*

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The technique of continuous-flow enthalpimetry consists in passing reacting liquids in constant, non-equivalent proportions through a mixing vessel and measuring continuously the temperature changes due to the reaction. The calorific change that occurs in such a system, and which can be detected by thermistor thermometers, is proportional to the amount of reaction product formed and thus to the molar concentration of the more dilute reactant. Continuous-flow enthalpimetry may be applied to routine quantitative chemical analysis, on-line control of processes and the measurement of heats of reaction. Advantages over the present practice of automatic titrimetric methods include simplicity of apparatus, avoidance of use of pipettes and ease of operation.

The use of thermometric techniques in quantitative analysis has developed in recent years along various lines, most of which have been based on the detection of titrimetric end-points. The magnitude of heat change is related to the amount of reaction product formed. The conventional method of determining heats of reaction requires knowledge of the concencentrations and thermal capacities of the reactants, and measurement of the temperature change. This method, under the name "direct-injection enthalpimetry," has only recently found application in routine quantitative analysis and is based on comparisons with standard solutions.[1]

Thermo-titrimetric methods require addition at constant rate of a titrant solution of known concentration to a titrand solution of unknown concentration, the cessation of temperature change being used to indicate the equivalence point of the titration.[2] The rate of change of temperature in such a titration is a function only of the rate of addition and concentration of the titrant, and the volume of titrant used in titration indicates the amount of reactant present in the titrand. In these circumstances the titration must be completed before the result can be evaluated. However, if the titrant solution is made the unknown sample under test, then the rate of change of temperature is related to the concentration and rate of addition of the sample and, although in practice it might be inconvenient to have the unknown in the burette, this procedure can, in fact, lead to methods of deriving the result before the titration is completed. Thus, the unknown concentration can be related to—

(*a*) the rate of change of temperature of the titrand during the titration;

(*b*) the time taken for a given temperature change; or

(*c*) the temperature change in a given time.

All of the above possibilities have been investigated and found to have useful application, but it is evident that the temperature changes are linear with respect to concentration only when volume changes and variations in titrant flow-rate are negligible. These and other disadvantages that are inherent in titrimetric methods have been mainly eliminated by using a new technique that has been termed "continuous-flow enthalpimetry."

Theory

The technique consists in passing two reacting solutions through a mixing vessel in such a way as to ensure complete and thorough mixing. One of these solutions is termed the "reagent solution," and, although its precise concentration need not be known, it must be stoicheiometrically in excess of the sample solution whose concentration is required. The proportions by volume of the two solutions are kept constant so that the temperature change due to reaction remains constant and allows measurement of the concentration of the weaker sample solution.

In order to simplify the following theory it is assumed that the thermal capacities of the reacting and product solutions are constant and each equal to K. This is a reasonable assumption for aqueous solutions with a concentration of less than M. Further, the temperatures of the reacting solutions are assumed not to fluctuate with a time period less than the throughput time in the reaction vessel.

* Presented at the First International Conference on Thermal Analysis, Aberdeen, Scotland, September 6th to 9th, 1965.

If the molar concentrations, M, and instantaneous temperatures, T, of sample, reagent and product are symbolised by the subscripts s, r and p, and if the flow-rates of the sample and reagent are x and Rx, where R is the ratio of flow-rates, then the flow-rates of sample and reagent in millimoles per second will be xM_s and xRM_r, respectively. If the heat of reaction is H kilocal per mole and $xM_s < xRM_r$ then the heat entering the system due to reaction enthalpy will be xM_sH cal per second. The heat entering the system due to unreacted solutions is $(KxT_s + KRxT_r)$ cal per second and the heat leaving the system by way of the product is $K(R + 1)xT_p$ cal per second. When equilibrium is reached the net heat change is zero, so that—

$$xM_sH + KxT_s + KRxT_r = K(R + 1)xT_p$$

This can be re-arranged to give—

$$\frac{M_sH}{K} = T_p(R + 1) - T_s - RT_r \quad .. \quad .. \quad .. \quad (1)$$

Consequently, for a given reaction (H and K constant), the molarity of the sample is a linear function of the three temperatures T_p, T_s and T_r; and also of the parameter, R, the ratio of the flow-rates. The flow-rate term, x, is not present in equation (1) and actual flow-rates are unimportant so long as the ratio R remains constant; under practical conditions, however, rapid fluctuations in flow-rates must be avoided if equilibrium is to be obtained. The heat capacity of the vessel determines the rate at which equilibrium is attained.

Practical considerations

In applying the above theoretical considerations to the construction of suitable apparatus for routine measurement of concentrations of small volumes of solutions, several practical difficulties arise. The mixing vessel must be efficient in operation, and arrangements are necessary for measuring the required temperatures: these points are dealt with in the apparatus section. From equation (1) several ways can be deduced for carrying out continuous-flow enthalpimetry.

(*i*) For sample concentrations in the range 0·01 to 1·0 M it is convenient to use equal flow-rates ($R = 1$) and to measure the three temperatures T_p, T_s and T_r. Thus, the required molarity is given by—

$$M_s = \left(T_p - \frac{T_s + T_r}{2}\right)\frac{2K}{H} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

This is the preferred method, and has been used to provide the results in the experimental section. Thermistors have been used to sense the three temperatures, and compensating circuitry has been designed[3] to compute the difference between T_p and the mean of T_s and T_r.

Calibration of the apparatus with standard solutions allows for any difference there may be between K of the reactant and K of the sample. Equalisation of the temperatures T_s and T_r is no doubt the simplest condition, but it is inconvenient for rapid working, owing to heat-transfer problems. However, only two thermometers would be required in this instance in order to measure the temperature difference $T_p - T_s$ (or T_r).

(*ii*) When the sample concentration is less than 0·01 M it is obviously undesirable to dilute this solution and it is then more convenient to make the flow-rate ratio R very small (say less than 0·01) so that dilution is negligible. In this instance the maximum possible temperature change is observed, and measurement of T_s and T_p gives the desired molarity according to the relationship—

$$M_s = (T_p - T_s)\frac{K}{H} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

It is assumed that T_r has little effect because the rate of flow of the reagent is less than 1 per cent. of that of the sample.

(*iii*) Concentrated samples may be handled by a dilution technique where $R > 100$; the effect of T_s is then negligible and the working equation now approximates to—

$$M_s = (T_p - T_r)\frac{KR}{H} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

(*iv*) Another possibility, for which a single thermistor is used, involves the addition of concentrated reagent ($R < 0{\cdot}01$) in discrete amounts at suitable intervals so that the

difference in temperature of the solutions before and after reaction may be measured; the difference leads directly to M_s on application of equation (3).

Other possibilities that have not yet been explored include—

(*v*) the use of a non-reactive solvent as carrier, the reactants being added intermittently as required (this method would be useful for maintaining constant pH); and

(*vi*) the formation of one or both of the reactants *in situ* in the stream, for example, by electrolysis.

Apparatus

The complete apparatus for continuous-flow enthalpimetry comprises a dual-channel liquid pump, a reaction vessel and a thermistor bridge with galvanometer null indicator. A peristaltic pump fitted with two identical neoprene tubes placed side by side beneath the rotating rollers has proved suitable for achieving identical flow-rates for reagent and sample: the particular pump used (Watson Marlow Ltd.) has a set of three rollers rotating at 200 r.p.m. giving delivery rates of about 15 ml per minute with 1/32-inch bore tubing. The advantage of using such a system is that it is self-priming and is easily washed out; in addition, the flow-rate ratio R remains constant, even though the actual rates may fluctuate a little.

It is necessary (see equation (2)) to measure the difference between the temperature of the product solution, and the mean of the temperatures of the reacting solutions. Although each temperature could be measured separately and the difference calculated, it is more convenient to use a multiple-thermistor bridge circuit such as the one described by Priestley,[3] which gives a direct reading of the required concentration in millimole units on a "Digidial": this circuit was used in the analyses listed under "Experimental." The sensitivity of the bridge is adjustable so as to compensate for different values of H and K, and both exothermic and endothermic reactions can be monitored.

It has been mentioned previously that the efficiency of mixing of reacting solutions must be as high as possible; it is also preferable to have a system of low thermal capacity and conductivity. Efficient mixing has been obtained by introducing the solutions through small orifices into a small mixing tube, and the desired thermal characteristics have been attained by use of Perspex. The vessel used for the work described here is shown in Fig. 1,

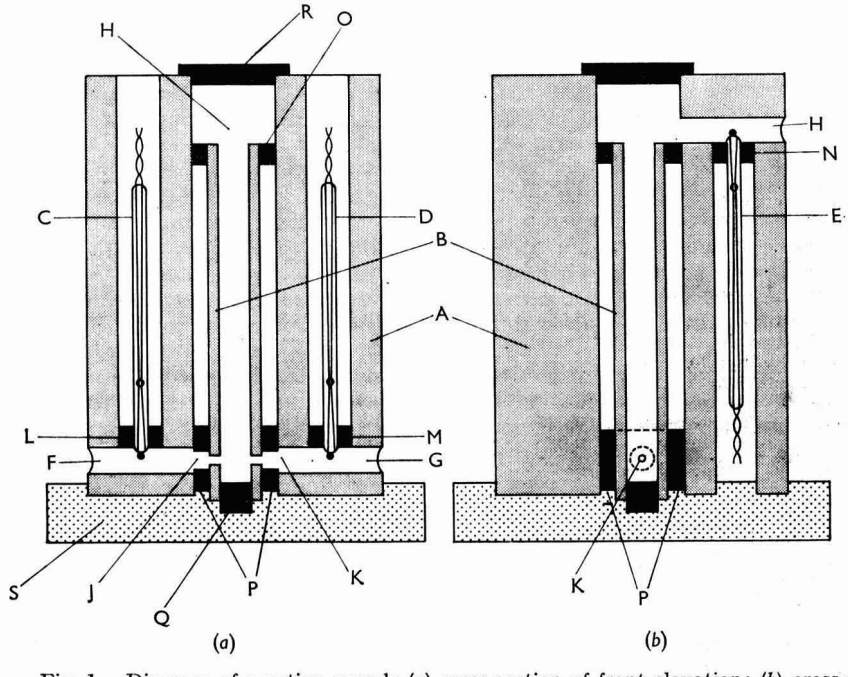

Fig. 1. Diagram of reaction vessel: (*a*) cross-section of front elevation; (*b*) cross-section of side elevation. For details of components lettered, see p. 592

and consists, in the main, of a Perspex cylinder, A, 1·5 inches in diameter by 4·5 inches long. A 9/32-inch central hole accommodates the mixing tube, B, and three $\frac{1}{4}$-inch holes enclose the thermistors, C, D and E. The inlet ports, F and G, and the outlet port, H, are drilled to accept the neoprene tubing feeding solutions from the peristaltic pump. The mixing tube is made from Perspex tube with a bore of $\frac{1}{8}$ inch, a wall thickness of 1/32 inch and $3\frac{3}{4}$ inches long. A 16/1000-inch hole (No. 78 drill) is pierced directly through this tube to give the two opposed mixing jets, J and K, at a point 0·5 inch from its lower end. Short lengths of rubber tubing, L, M, N, O and P, are used to seal the thermistors and mixing tube into their respective bores; the rubber seal, P, has two holes pierced in it, which are in line with the two inlet ports and the two mixing jets. The annular air space formed between the mixing tube and the main body acts as an insulator. Rubber plugs, Q and R, and the polythene base, S, complete the apparatus.

A second apparatus, which is being evaluated at the present time, is based on an earlier apparatus described by Ruby,[4] designed for rapid mixing. This device incorporates four tangential mixing jets, two for each solution, and shows promise of being very efficient in mixing and less vulnerable to blocking since the jets may be larger without impairing the mixing efficiency. It is also constructed in sections that may be readily dismantled for cleaning, and which also make it possible to vary the length of the mixing path between the jets and the sensor, should it be found desirable to increase the time allowed for reaction to occur.

Experimental

The reaction vessel was used in conjunction with the multiple-thermistor indicator[3] in order to evaluate several reaction systems. A standard solution of the sample was made up for each system and this was diluted to give a series of different concentrations. The thermistor indicator was set up in the normal way with the reagent and water and then the reagent and the highest concentration of sample. These solutions were then tested and the experimental results compared with their nominal concentrations. The results derived by this method are shown in Table I. For each analysis the experimental result is shown below its corresponding theoretical value. It should be noted that, with the equipment used, the accuracy of reading results is limited to ± 1 digit: the accuracy could obviously be improved by making use of some form of scale expander.

Table I

Comparison of observed and nominal values

Sample	Results							
Reagent, 0·6 M *sodium hydroxide*—								
Nominal values	0·050	0·100	0·200	0·300	0·400	0·500		
Hydrochloric acid	0·051	0·103	0·200	0·303	0·402	0·500		
Nitric acid	0·050	0·100	0·200	0·300	0·396	0·500		
Boric acid	0·049	0·099	0·196	0·302	0·400	0·500		
Acetic acid	0·050	0·098	0·200	0·305	0·399	0·500		
Reagent, 0·6 M *hydrochloric acid*—								
Nominal values	0·025	0·050	0·100	0·150	0·200	0·300	0·400	0·500
Sodium hydroxide	—	0·050	0·101	—	0·200	0·299	0·400	0·500
Potassium hydroxide	—	0·049	0·103	—	0·200	0·303	0·399	0·500
Ammonia solution	—	0·051	0·100	—	0·199	0·300	0·396	0·500
Pyridine	—	0·055	0·099	—	0·201	0·299	0·401	0·500
Tri-sodium orthophosphate	0·025	0·050	0·102	0·150	0·200	—	—	—
Sodium sulphite	0·025	0·050	0·106	0·153	0·200	—	—	—
Reagent, M *EDTA (tetrasodium salt)*—								
Nominal values	0·100	0·200	0·300	0·400	0·500	0·600	0·700	0·800
Barium chloride	0·099	0·199	0·300	0·395	0·500	—	—	—
Copper sulphate	—	0·100	—	0·403	—	0·600	—	—
Lead acetate	—	0·198	—	0·397	—	0·597	0·700	—
Nickel chloride	—	0·202	—	0·395	—	0·598	—	0·800
Manganese sulphate	0·100	0·201	0·298	0·400	0·500	—	—	—

By setting up the apparatus for a reaction of known enthalpy and using it at this setting with known concentrations of other reagents (samples) it is possible to determine enthalpy values for these other reagents, although, if the reactant is different, care must be taken

to correct for any difference in heat of dilution caused by the change. Alternatively, the apparatus could, of course, be set up with an electrical resistance as an absolute standard.

Table II shows values for heats of chelation of various metal ions with EDTA obtained by calculation from the results of titrations with EDTA (tetra-sodium salt), on the assumption of a value of −1·4 kcal per mole for the reaction—

$$Na^+ + Y^{4-} \rightleftharpoons Na\,Y^{3-}$$

where Y = EDTA, with the apparatus standardised on hydrochloric acid - sodium hydroxide (ΔH of −13·5 kcal per mole).

TABLE II

HEATS OF CHELATION OF METAL IONS WITH EDTA

$$(M^{x+} + Y^{4-} \rightleftharpoons MY^{(4-x)-} + yH_2O)$$

Cation	ΔH for continuous-flow enthalpimetry at 20° to 25° C	ΔH values from literature— (a)	(b)	(c)	(d)
Li^+	+2·2	—	—	+0·1	—
Be^{2+}	+2·3	—	—	—	—
$(NH^4)^+$	+4·8	—	—	—	—
Na^+	—	—	—	−1·4	—
Mg^{2+}	+4·8	+5·5	+3·1	+3·1	+2·9
Al^{3+}	+10·9	—	—	—	—
K^+	+2·2	—	—	—	—
Ca^{2+}	−5·6	−5·7	−6·5	−5·8	−2·5
Cr^{3+}	+7·3	—	—	—	—
Mn^{2+}	−6·4	—	—	−5·2	—
Co^{2+}	−3·5	−4·2	—	−4·1	—
Ni^{2+}	−7·2	−7·4	−8·4	−7·6	—
Cu^{2+}	−8·3	−8·2	−8·7	−8·2	—
Zn^{2+}	−4·5	−4·6	−5·6	−4·5	—
Sr^{2+}	−4·9	—	−4·1	−4·2	−4·1
Cd^{2+}	−9·7	−9·2	−10·1	−9·1	—
Ba^{2+}	−4·6	—	−4·8	−5·1	−4·1
La^{3+}	−3·9	—	—	—	—
Pb^{2+}	−12·7	−12·9	−14·1	−13·1	—

(*a*) Thermometric titration 20° to 25° C.[5]
(*b*) Calorimetry, 20° C.[6]
(*c*) Calorimetry, 25° C.[7]
(*d*) Potentiometric titration, 0° to 30° C.[8]

CONCLUSIONS

As far as the experimental results are concerned it is evident that the apparatus is capable of good precision. However, with the apparatus in its present form, difficulties have been experienced with precipitation reactions because of clogging or improper mixing, except at low concentrations. A result may be achieved with as little as 10 ml of sample, and only one determination is required. There is no advantage to be gained by repeating the analysis, since, in effect, continuous-flow enthalpimetry permits continuous readings to be made, which is equivalent to performing many separate titrations. Since reagent and sample are pumped directly from their reservoirs, pipettes and burettes are not required and associated errors are eliminated. Heats of reaction, ionisation and dilution may be measured without modification to the apparatus.

The apparatus may be made completely automatic in order to record continuously the molarity of a component in a plant stream; and with suitable instrumentation it should be possible to utilise the output from such a device to control chemical processes.

NOTE—The subject matter of this paper is covered by British provisional patent application 2171/64, 17th January, 1964; also a U.S. patent application, No. 425,161.

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Acetone as Solvent in the Enthalpimetric Titration of Acidic Substances*

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The enthalpimetric titration of acidity with non-aqueous alkali often results in a curve that shows only a slight change in slope at the end-point, but, if acetone is used as a solvent for the acid, it has been found to act as an "indicator." At the neutralisation point a rapid heat rise occurs owing to the formation of diacetone alcohol from the reaction of acetone with the excess of non-aqueous alkali. The conditions under which this reaction occurs have been studied and a method incorporating the best conditions is described. The method is applicable to a wide range of acidic substances, including 2,6-disubstituted phenols, keto-enols and imides, and can be used to determine traces of acidity.

A recent review by Bark[1] shows that the enthalpimetric titration of acidic substances with alkali in aqueous solution has been used for a long time but that little work has been reported on the application of this technique in non-aqueous solvents. Paris and Vial[2] have titrated bromo-derivatives of phenols and cresols in alcoholic solution with alcoholic potassium hydroxide as titrant. The same titrant has been used by Parsons[3] to titrate phenols in pyridine solution. In both these methods the graph of the temperature of the solution against the amount of titrant added, shows only a slight change in slope at the end-point. This necessitates an apparatus well-insulated from any ambient temperature changes and the precise measurement of the temperature of the solution.

In an investigation into the suitability of a number of non-aqueous solvents for use in the enthalpimetric titration of phenols, it has been found that acetone, when titrated with an alcoholic alkali, evolves a large amount of heat. Further, this evolution only takes place after acidic substances dissolved in the acetone have been titrated. The acetone thus behaves as an enthalpimetric "indicator." No other ketones have been found to behave in the same way.

The technique is analogous to that used in the aqueous titrimetry of a cation giving a small heat change at the end-point, where a second constituent is added whose heat of reaction is large and of opposite sign and which reacts after the cation being determined.[4]

Experimental

Nature of the reaction—

If a non-aqueous solution of an alkali is added continuously, for example, from a motor-driven syringe, to acetone containing an acidic substance and the temperature of the acetone is recorded and plotted against the volume of titrant added, a curve similar to that shown in Fig. 1 (*a*) is obtained. The level part of the curve represents the added acidity, and it can be seen that a marked rise in temperature occurs at the end-point; with pure acetone, this rise takes place almost immediately on addition of the alkali.

To discover the cause of the reaction acetone was purified by means of both the sodium iodide and the sodium bisulphite complexes. With both purified samples of acetone the addition of a small amount of alcoholic alkali gave rise to an exothermic reaction which indicated that the reaction was not due to the presence of an impurity. This is borne out by the small quantity of alkali required to produce the large rise in temperature: the heat produced was greater than 800 kcal per mole of potassium hydroxide.

After reaction, the purified samples of acetone were examined by gas - liquid chromatography; both contained large amounts of diacetone alcohol and other acetone condensation products. The chromatograms, apart from the acetone peak, were similar to that from a commercial sample of diacetone alcohol.

That the production of diacetone alcohol was the cause of the temperature rise was confirmed by measuring the temperature change on addition of small amounts of alcoholic alkali to a number of mixtures of acetone and diacetone alcohol. The results are shown in Fig. 2.

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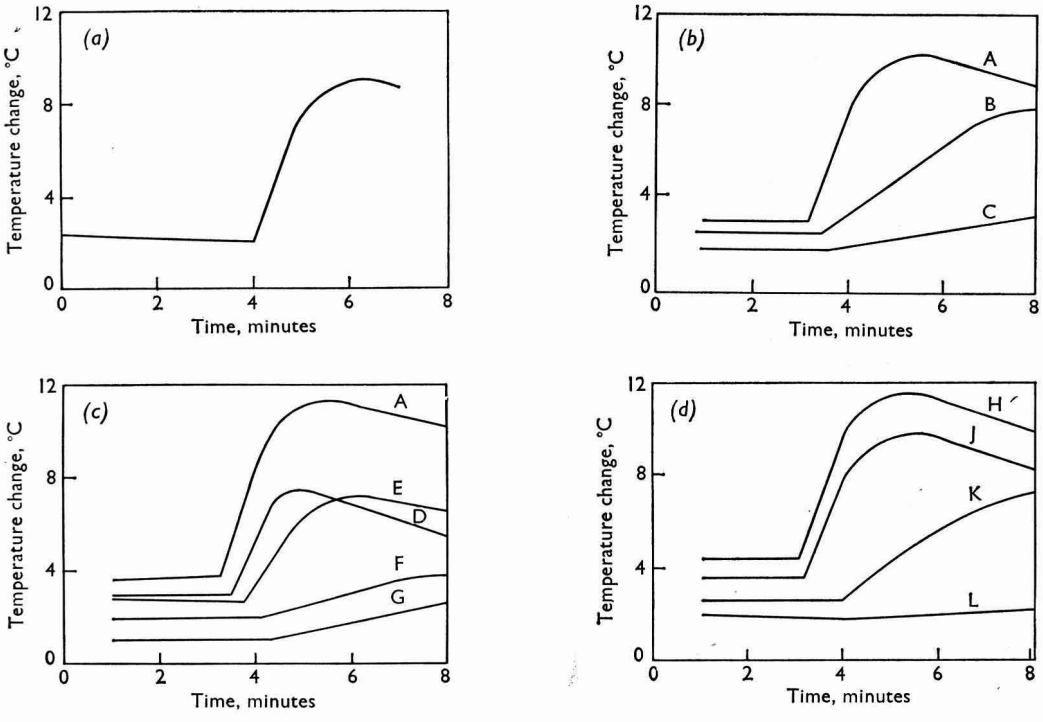

Fig. 1. Graphs of enthalpimetric titrations with acetone as solvent. (*a*) Typical titration of an acidic substance. (*b*) The effect of added water: curve A, dry acetone; curve B, acetone containing 0·2 per cent. water and curve C, acetone containing 2·0 per cent. water. (*c*) Effect of adding other solvents: curve D, 59 per cent. pyridine; curve E, 25 per cent. benzene; curve F, 50 per cent. benzene and curve G, 50 per cent. nitrobenzene. (*d*) Effect of diluting the titrant and of changing the solvent of the titrant: curve H, N potassium hydroxide in isopropanol; curve J, N potassium hydroxide in methanol; curve K, 0·1N potassium hydroxide in isopropanol and curve L, 0·01N potassium hydroxide in isopropanol

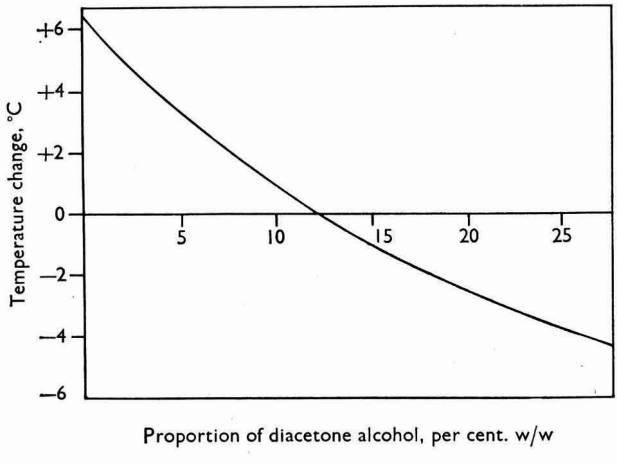

Fig. 2. Relation between temperature change after the addition of a small quantity of alcoholic alkali to a mixture of diacetone alcohol and acetone, and percentage of diacetone alcohol in that mixture

It can be seen that the diacetone alcohol gives an endothermic reaction and that the point of no heat change, *i.e.*, when the equilibrium mixture exists, is at 12·2 per cent. w/w diacetone alcohol at 21° C. This value is close to the published equilibrium value of 11·7 per cent. at 25° C (5·5 per cent. at 45° C).[5]

Effect of solvents and titrants—

The effect on the reaction of adding water or other solvents to the acetone was studied, as were the effects of using different concentrations of and different solvents for, the alkali. Figures 1 (*b*), (*c*) and (*d*) show the results obtained in the titration of 5 ml of acetone, or of acetone and solvent mixture, continuously at the rate of 0·01 ml per minute. In all experiments the heat rise started almost immediately on addition of the titrant. Fig. 1 (*b*) shows that the presence of water suppresses the rate of reaction, and about 0·2 per cent. of water is the maximum that can be tolerated. Fig. 1 (*c*) shows that the addition of an equal volume of benzene or nitrobenzene considerably reduces the reaction rate, but 25 per cent. mixtures give satisfactory end-points. Pyridine has the effect of reducing the heat rise in proportion to the amount present but the rate of reaction is the same as that of pure acetone. The effect of diluting the titrant is to reduce the rate of reaction (see Fig. 1*d*). It may be noted that isopropanol gives a slightly greater heat rise than does methanol as a solvent for the alkali.

It was also found that N butyltrimethylammonium hydroxide in methanol is as effective as N potassium hydroxide in methanol, when used as a titrant.

Materials that may be titrated by this method—

The following substances are among those that have been satisfactorily titrated. The figures in brackets show the proportion of acidic groups that were expected to be titrated in poly-acidic substances.

Carboxylic acids (*monobasic*)—acetic; stearic; benzoic

Carboxylic acids (*dibasic*)—oxalic (2:2); succinic (1:2); adipic (2:2); phthalic (2:2)

Hydroxy-acids—tartaric (2:2); citric (3:3)

Phenols (*monohydric*)—phenol; 2,6-xylenol; 2,6-di-*t*-butyl-*p*-cresol; 2-naphthol

Phenols (*polyhydric*)—quinol (2:2); resorcinol (2:2); catechol (1:2); pyrogallol (2:3)

Phenolic acids—salicylic (2:2); *p*-hydroxy benzoic (2:2); 2-hydroxy naphthoic (2:2)

Keto-enols—acetylacetone; acetoacetic ester; dimedone; diethyl malonate

Imides—succinimide; phthalimide

Passing carbon dioxide through acetone resulted in an increased acidity, which could be titrated; the increase was found to be dependent on the duration of gas flow. Evidence was also obtained that polynitro aromatic hydrocarbons could be titrated.

Method

Apparatus—

The titration vessel—Shown in Fig. 3, this consists of a tall 20-ml beaker with the 5-ml and 10-ml levels marked, and is fitted with a cap through which is fitted—(A) a glass stirrer; (B) an acetone inlet from the dispenser; (C) an inlet for air, free from carbon dioxide; (D) a titrant admission tube drawn down to a fine jet; and (E) a thermistor, Stantel type F 2311/300. The beaker is surrounded by a large beaker to act as a draught screen. The thermistor forms one arm of a bridge powered by a 1·5-V dry battery. The other arms of the bridge are two 1000-ohm resistors, R_1 and R_2, and an arm for zero adjustment consisting of a 1000-ohm variable resistance, R_3, and a 1500-ohm resistor, R_4, in series. The output of the bridge is measured by means of a Scalamp galvanometer, G.

The acetone dispenser—This consists of a 1-litre wash-bottle connected to hand bellows via a soda-lime guard tube.

The titrant-admission tube—This is connected to a 2·5-ml Hamilton gas-tight syringe No. 1002 held in a micrometer syringe holder that is driven by a synchronous motor.

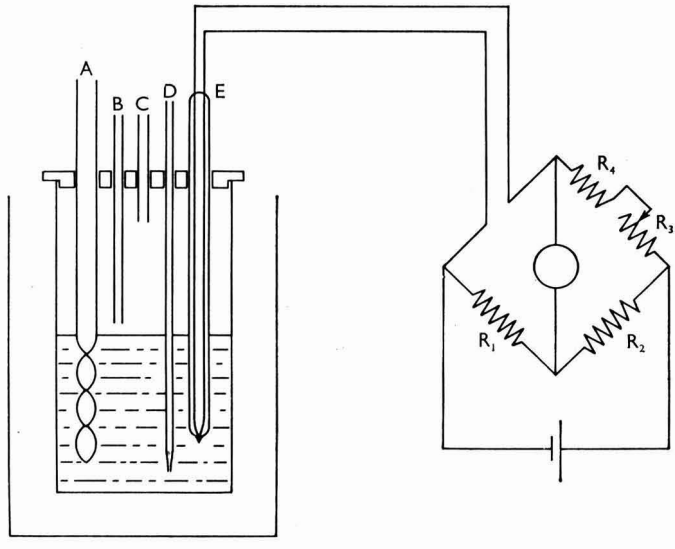

Fig. 3. Titration vessel and circuit diagram of bridge

A = Glass stirrer
B = Acetone inlet
C = Air inlet
D = Titrant tubing
E = Thermistor

R_1 = 1000 ohm resistor
R_2 = 1000 ohm resistor
R_3 = 1000 ohm variable resistor
R_4 = 1500 ohm resistor

REAGENTS—

Potassium hydroxide—A N solution in dry isopropanol.

Acetone—Dry and as free from acidity as possible.

PROCEDURE—

Weigh the sample into the dry titration vessel and, before assembling the apparatus, start the titrator and run it until a drop of titrant appears on the jet. Stop the titrator and wash the jet with acetone. Assemble the apparatus, flush it with air free from carbon dioxide and add 5 ml of acetone by means of the dispenser. Switch on the stirrer and bridge, adjusting the latter to zero if necessary. Wait for the sample to dissolve or until the galvanometer reading is steady, whichever takes the longer. Switch on the titrator, and at the same time start a stop-clock. Take galvanometer readings at 0·25-minute intervals until a marked increase in temperature occurs and then take readings for a further 3 minutes.

Plot the galvanometer readings against time and draw the best straight lines through the points before and after the point where a marked rise in temperature occurs. The intersection of these two lines denotes the end-point.

Standardise the titrant under the same conditions to determine the equivalent N potassium hydroxide delivered by the titrator in unit time with, for example, benzoic acid.

Determine a blank value to allow for the acidity in the acetone and any inertia in the titrator. Subtract this blank time from any determined values. When measuring the blank, and with low acidities, take 0·5-minute readings for about 3 minutes before starting the titrator in order that the best line may be drawn for the initial period.

RESULTS

A number of titrations of benzoic acid with approximately N potassium hydroxide in isopropanol were made to standardise the alkali.

Benzoic acid, mg	5·07	7·00	6·90	8·34	5·10	5·21	3·72
N potassium hydroxide, μl per minute	11·70	11·66	11·68	11·62	11·76	11·82	11·66

These results have a standard deviation of 0·07 μl and show that 95 per cent. of results should be accurate to within ±1·4 per cent. It is thought that most of this deviation is caused by the temperature variation of the titrant.

Duplicate determinations of the purity of a number of substances gave the following results.

	Per cent.
2,6-Xylenol	100·1, 99·6
2-Naphthol	99·8, 99·6
Salicylic acid	99·2, 101·2
Phthalimide	98·7, 98·8

The phenols contents of a number of tar oils were determined by the S.T.P.T.C. alkali-extraction method[6] and compared with results by the enthalpimetric method by assuming an equivalent weight according to the phenols expected to be present.

Material	Enthalpimetric method, per cent.	Extraction method, per cent.
Heavy creosote oil	29·6	32·4
Creosote oil	26·2	26·6
Light creosote oil	3·2	2·5
Light oil	1·5	0·5
Naphthalene oil	0·7	trace
Washed light oil	0·4	trace
Hydrogenated oil	0·02	—

The results show, that, for substances having lower phenols contents, the enthalpimetric method yields a higher result than that found with the extraction method, owing to the difficulties of measurement in the latter method.

The enthalpimetric method was compared with a non-aqueous potentiometric method in the determination of the acidity of pitch extracts. Sample weights of 20 to 30 mg were taken for the enthalpimetric method and 2 to 5 mg for the potentiometric method. In the latter method pyridine was used as a solvent with N butyltrimethylammonium hydroxide in methanol as titrant. In both instances the titrant was standardised against 2,6-xylenol.

	Percentage acidity calculated as OH				
By the enthalpimetric method ..	2·01	2·12	1·32	0·92	0·67
By the potentiometric method ..	1·80	2·04	1·00	0·94	0·68

Discussion

The ionisation of acidic substances is known to be enhanced in acetone solution,[7] and this explains why it is possible to titrate very weak acids such as 2,6-disubstituted phenols, acetylacetone and succinimide. This ionisation causes the reaction with added alkali to be rapid.

The reaction of acetone to form diacetone alcohol proceeds in three stages, hydroxyl ions having a catalytic action.

$$CH_3—CO—CH_3 \overset{OH^-}{\rightleftharpoons} CH_3—CO—CH_2^- \quad (1)$$

$$CH_3—CO—CH_2^- + CH_3—CO—CH_3 \leftrightharpoons CH_3—CO—CH_2—C(O^-)(CH_3)_2 \quad (2)$$

$$CH_3—CO—CH_2—C(O^-)(CH_3)_2 \overset{H_2O}{\rightleftharpoons} CH_3—CO—CH_2—C(OH)(CH_3)_2 + OH^- \quad (3)$$

Sykes[8] states that stage (2) is slow and consequently the neutralisation of the acids being titrated may attain completion before the formation of diacetone alcohol and the resulting evolution of heat from the reaction. Sykes also states that the reaction of acetaldehyde to form aldol follows the same pattern, but that stage (2) is fast; thus the titration of acidity in acetaldehyde should not be possible. By experiment it was shown that acetaldehyde behaved in a similar fashion to acetone in that a heat rise occurred immediately after the addition of alcoholic alkali. On addition of benzoic acid to the acetaldehyde a heat rise occurred immediately after addition of the alkali, *i.e.*, the benzoic acid was not titrated.

The action of acetone as an enthalpimetric "indicator" can therefore be explained as a diffusion of the alkali into the acetone during the titration, where it is neutralised by the acids present. This occurs with sufficient rapidity to prevent its action as a catalyst in the

slower heat-producing condensation reaction in which acetone is converted into diacetone alcohol.

This conclusion is supported by the effect of the presence of water, which would be expected to reduce the ionisation of the acetone, and by the effect of dilution with pyridine, which would be expected to enhance the ionisation.

The method is superior to the potentiometric titration of weak acids with a quaternary base hydroxide since no decomposition of the titrant can take place.[9] It should be noted that the enthalpimetric method may give a different equivalent weight for polybasic acids than that found by potentiometric titration in non-aqueous solution. For example, resorcinol and salicylic acid are dibasic by enthalpimetric titration but monobasic by potentiometric titration.

In order to utilise the "indicator" action of acetone in the enthalpimetric titration of acidity, the following points must be observed—

(*i*) the acetone must be dry;

(*ii*) the non-aqueous alkali titrant must be concentrated in order to produce a rapid rise in temperature;

(*iii*) the titrant must be added continuously with good stirring, in order to prevent a local concentration of alkali;

(*iv*) if the sample has been dissolved in another solvent, at least three times its volume of acetone must be used;

(*v*) the concentration of carbon dioxide in the acetone must be kept as constant and as low as possible.

Conclusion

The results show that the enthalpimetric method, with acetone as a solvent as well as an "indicator," can be used in the determination of a wide range of acidic substances. The method gives results comparable to those given by the non-aqueous potentiometric method and is superior to the extraction method in the determination of traces of phenols in tar oils.

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Hydroxylysine as a Growth Stimulant in Microbiological Assays for Lysine

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Results for the lysine content of animal products, obtained by microbiological assays with *Leuconostoc mesenteroides* P60 may be too high unless the basal medium contains hydroxylysine. Evidence is presented to show that although cultures of this organism vary in the extent of their response to lysine in the presence of hydroxylysine, the effect of the latter synergist is apparent with several cultures and under many different assay conditions. A standard addition of hydroxylysine to the assay medium is therefore recommended in all microbiological assays for lysine in which the above organism is used.

PETERSEN and Carroll[1] drew attention to the stimulatory influence of δ-hydroxylysine (2,6-diamino-5-hydroxyhexanoic acid) on the growth of *Leuconostoc mesenteroides* P.60 (A.T.C.C. No. 8042) under conditions of suboptimal lysine concentration, although as Bergström and Lindstedt[2] had shown earlier it does not support growth of the organism in the absence of lysine. Sauberlich,[3] in a preliminary communication without experimental details, reported that the presence of hydroxylysine in samples assayed for lysine with *L. mesenteroides* P.60 caused considerable error due to the synergistic effect, and that an enhanced response curve was obtained in the presence of a constant high level of hydroxylysine.

Other workers, however, have reported only small effects from the addition of hydroxylysine. Ågren[4] states that the amounts of hydroxylysine present, for instance, in fish have no influence on the microbiological lysine determinations; Pomeranz and Miller[5] found that DL-δ-hydroxylysine did not change the response to the presence of graded lysine doses, and the assay medium of Steele, Sauberlich, Reynolds and Baumann,[6] sold in dehydrated form for lysine assays, is not supplemented with hydroxylysine (Difco Laboratories[7]).

When discrepancies were observed, in both our own laboratories and elsewhere in the U.K., between results from microbiological assays and from Moore and Stein chromatographic determinations of the lysine content of various fish products and of meat meals, an investigation was made into the effect of hydroxylysine in the microbiological assays, under our own conditions, and of the factors that may have been responsible for the contradictory published findings. A preliminary communication has been published.[8] Our own results are, in general, in agreement with those of Sauberlich.[3]

Experimental

Hydroxylysine—

In our experiments we have used two specimens of hydroxylysine. One had been prepared by Dr. S. M. Partridge from hydrolysed gelatin, the other was supplied commercially from the same source (Koch - Light Laboratories Ltd., Colnbrook, Bucks.) as that used by Pomeranz and Miller.[5] Ion-exchange chromatography with a 133-cm column in a Technicon Amino Acid Analyzer (Technicon Ltd., Chertsey, Surrey) gave twin peaks with both these samples. These peaks occupied the same positions in the chromatogram as those of the normal and allodiastereomers of hydroxylysine as described by Hamilton and Anderson.[9] The D-allo hydroxylysine is believed to be formed by partial epimerisation at the α-carbon atom during acid hydrolysis (Greenstein and Winitz[10]). These two peaks were completely resolved by using phosphate buffers and the appropriate fractions were used to compare their relative activities in microbiological assays.

The lysine standards were prepared from L-lysine hydrochloride (*either* British Drug Houses Ltd., Poole, Dorset, *or* "chromatographically pure" grade; Mann Research Laboratories Inc., New York 6, N.Y., U.S.A.).

ASSAY CULTURES AND MEDIA—

The strains of *Leuconostoc mesenteroides* P.60 used in this work were grown and stored as agar stab cultures.[11] A sub-culture was taken from the agar stab into the same medium without agar, and incubated at 37° C for 24 hours. After centrifugation, the cells were re-suspended in sterile 0·9 per cent. saline and again spun in a centrifuge. After the supernatant liquid had been discarded and the residue re-suspended in sterile saline, each assay tube was inoculated with one drop of inoculum.

Unless otherwise stated, the assay medium was prepared by re-hydration of Bacto lysine assay medium (Difco Laboratories Ltd., Detroit 1, Michigan, U.S.A.) prepared according to the instructions of Steele *et al.*[6] and the assay continued under the directions given by these authors, *i.e.*, 5 ml of medium were added per tube, lysine standard or hydrolysates added and the total volume brought to 10 ml with water. The tubes were then autoclaved for 10 minutes at 10 p.s.i. pressure, cooled and inoculated. Acid production was measured by titration after incubation for 72 hours at 37° C.

In a smaller number of assays, media were prepared freshly either to the same formula[6] or that of Pomeranz and Miller.[5] In others, steaming of the tubes replaced autoclaving, and in some runs growth was measured turbidimetrically after 24 hours incubation at 34° C.[12] The hydrolysates of the test materials for subsequent microbiological assay were prepared by the procedure of Barton-Wright.[13]

RESULTS

Over 30 assays have been carried out in this series, all showing a response to hydroxylysine. Only a few typical results can be illustrated here.

Fig. 1 shows the results of an assay in which each tube contained the Difco medium *plus* 150 μg of L-lysine together with varying levels of hydroxylysine. Even with a ratio of hydroxylysine to lysine of less than 1 to 30 there was significantly enhanced acid production. Ratios of this magnitude are found in commercial products such as fish flours and meat meals containing collagen-type proteins.

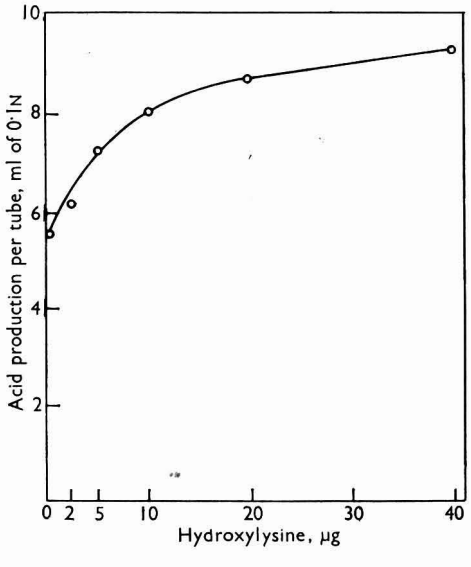

Fig. 1. Effect of increasing amounts of hydroxylysine on the acid production with 150 μg of lysine

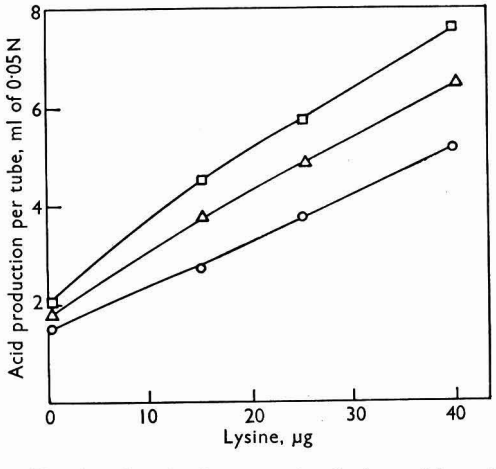

Fig. 2. Standard curves for lysine with and without additions of 5 and 25 μg of hydroxylysine per tube in Dr. Pomeranz's synthetic medium. Curve ○, lysine only; curve △, lysine *plus* 5 μg of hydroxylysine per tube; curve □, lysine *plus* 25 μg of hydroxylysine per tube

Fig. 2 shows standard curves for the lysine assay covering the low range of 0 to 40 μg of lysine per tube used by Pomeranz and Miller,[5] together with similar curves in the presence of 5 and 25 μg of hydroxylysine per tube. These results were obtained in one laboratory, A, by using Pomeranz and Miller's synthetic medium[5]; similar, but not so linear, response curves were obtained in another laboratory, B, that used re-hydrated Difco medium with and without 25 μg of hydroxylysine per tube.

Fig. 3 shows a standard curve for lysine continued to a much higher level of lysine per tube, and the corresponding curve with 40-μg additions of hydroxylysine in each tube. This shows the tendency for the hydroxylysine-supplemented curve to become flatter at the higher levels and thus become closer to the unsupplemented curve. In other experiments, in which lysine levels were increased to 300 and 600 μg of lysine per tube, the hydroxylysine effect then became negligible, but under these conditions the high acid production, amounting to as much as 12 ml of 0·1 N acid per tube, may be inhibiting any further increase by the bacteria. In practice, lysine concentrations of this order would not be used in lysine assays and would not yield valid results.

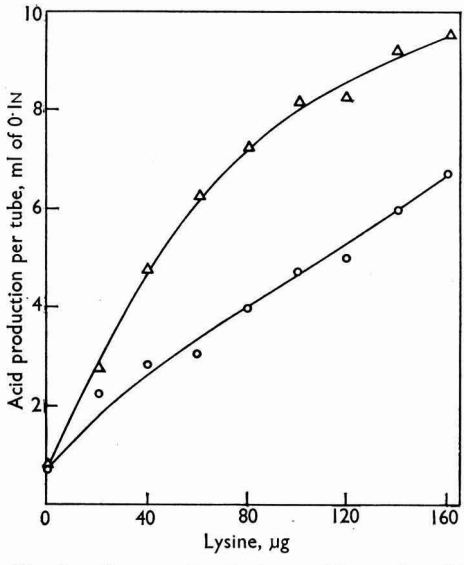

Fig. 3. Curves for lysine with and without standard additions of 40 μg of hydroxylysine per tube in Difco medium. Curve ○, lysine only; curve △, lysine *plus* 40 μg of hydroxylysine per tube

Turbidimetric readings after either 24 or 72 hours showed at least as great a proportional response to hydroxylysine as had acid production. In some instances the lysine curve (without added hydroxylysine) gave actually less turbidity after 72 hours than after only 24 hours, whereas the reverse was the case in the presence of hydroxylysine. This may be related to the report that hydroxylysine can inhibit the lysis of lysine-deficient cells.[14]

Steaming of the tubes prior to inoculation, as contrasted with autoclaving, resulted in slightly improved, *i.e.*, straightened, standard curves for lysine in the absence of hydroxylysine, but the response to added hydroxylysine was unchanged.

EFFECT OF VARIATION IN CULTURE—

The cultures of *Leuconostoc mesenteroides* P.60 as used in laboratories A and B showed minor differences in their behaviour with lysine and with lysine in the presence of hydroxylysine, particularly in the linearity of the response. Towards the end of the study, the culture used in laboratory A gave successively poorer standard curves, with early flattening off; hydroxylysine still gave some response, but the curves remained unsatisfactory even with this addition.

A private communication from Dr. L. P. Ederzeel* led to an exchange of cultures. Fig. 4 shows the response curves of the Dutch organism to lysine with and without hydroxylysine additions, obtained in laboratory A. This illustrates the very high acid production with Dr. Ederzeel's culture and also the excellent linearity up to relatively high lysine levels without added hydroxylysine, found in both laboratories. However, even here, added hydroxylysine has given a small but consistent further response. A difference in slope and acid production was found in the two Cambridge laboratories, but this was within the

* Institute for Fishery Products, Ijmuiden, The Netherlands.

expected inter-laboratory variation in standard curves, due perhaps to media differences, temperature variations and other details of technique.

Dr. Pomeranz also kindly supplied a specimen of the culture from his laboratory, where previously no response to hydroxylysine had been found. The results from one trial in laboratory A are also illustrated in Fig. 4, and the results in laboratory B were essentially similar. Clearly the culture on these occasions had shown a large response to hydroxylysine and Dr. Pomeranz (private communication) has also now observed a similar effect.

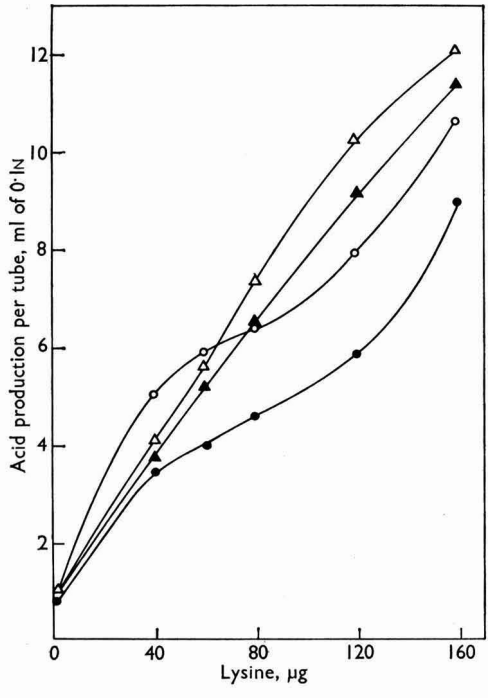

Fig. 4. Standard curves for lysine with and without hydroxylysine. Assay 76 with Dr. Pomeranz's culture: curve ●, lysine only; curve ○, lysine *plus* 20 μg of hydroxylysine. Assay 80 with Dr. Ederzeel's culture: curve ▲, lysine only; curve, △ lysine *plus* 25 μg of hydroxylysine

COMPARISON OF NORMAL AND ALLO-HYDROXYLYSINE—

A 133-cm Technicon column packed with Chromo-bead resin was equilibrated with 0·2 N acetate buffer at pH 3·4 before it was loaded with 1 mg of hydroxylysine (Koch - Light Laboratories Ltd.). Elution was carried out with 0·1 M sodium phosphate buffer (pH 7·5) at 21° C, the eluate being taken to a fraction collector. Aliquots from each tube were analysed with ninhydrin, and twin peaks were found completely resolved at a point between 8¼ and 9½ hours from the start of elution. The chromatogram is shown in Fig. 5 and calculation showed that the proportions present were 49·5 per cent. normal and 50·5 per cent. allo isomer. The tubes having the highest concentration were bulked to yield solutions of the two isomers each having a concentration of 50 μg per ml for testing their microbiological activity. Table I shows that the two hydroxylysine isomers after incubation at 37° C for 72 hours in Difco medium were equally active in stimulating the response to 50 μg of lysine.

These results indicate that the extent of the epimerisation occurring during acid hydrolysis will not influence the synergistic effect of hydroxylysine derived from a protein. An equimolecular mixture of the two epimers is reported to result from prolonged refluxing with mineral acid.[9] This has been confirmed in our own experiments.

TABLE I

RESPONSE TO HYDROXYLYSINE EPIMERS

Acid production was measured after 72 hours' incubation

Acid production, ml of 0·1 N from—		
50 μg of lysine	50 μg of lysine *plus* 20 μg of normal hydroxylysine	50 μg of lysine *plus* 20 μg of allo hydroxylysine
2·7	3·7	3·5
2·9	3·8	3·9
2·7	3·9	3·8
2·7	3·7	3·8
2·8	3·9	3·9
Mean 2·76	*3·80*	*3·78*

EFFECT OF STANDARD ADDITIONS OF HYDROXYLYSINE TO THE LYSINE-ASSAY MEDIUM—

After the various results reported above on the effect of hydroxylysine on the lysine standard curve had been considered, a standard addition of 50 μg of hydroxylysine per tube was adopted as the proposed supplementation of the medium. This is equivalent to an addition of 10 mg of hydroxylysine per litre.

TABLE II

SUMMARY OF RESULTS OBTAINED WITH ANIMAL-PROTEIN MATERIALS

Test material	Hydroxy-lysine, g per 16 g of N (column)	Ratio, hydroxy-lysine to lysine	Lysine, g per 16 g of N		
			Leuconostoc (Difco medium)	*Leuconostoc* (Difco medium) *plus* 50 μg of hydroxylysine per tube)	Column separation and colorimetry
Cod fillets No. 34 ..	0·022	1 to 420	9·7	9·5	9·2
Fish flour No. X385 ..	0·21	1 to 37	10·7	7·9*	7·7
Fish offal	0·55	1 to 13	8·05†	7·1†	7·2
Meat meal No. MM18 ..	0·33	1 to 20	10·0	7·0	6·7
Meat greaves	0·55	1 to 13	7·9†	6·65†	7·05
Gelatin	1·40	1 to 3	5·9	4·35	4·05

* Variable results have been obtained for this sample: 8·8 g per 16 g of N was obtained in an early assay reported in our preliminary communication.[8]

† Results from assays in which the culture supplied by Dr. Ederzeel was used, *i.e.*, the culture showing least response to hydroxylysine.

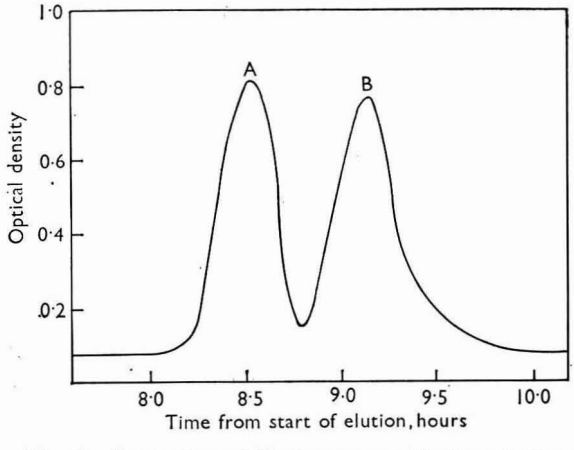

Fig. 5. Separation of diastereomers of hydroxylysine on a 133-cm Technicon ion-exchange resin column with 0·1 M phosphate buffer. Peak A, normal isomer; peak B, allo isomer

Results for a range of animal products assayed with and without this supplement are given in Table II. It is seen that the addition of hydroxylysine reduces the values obtained by microbiological assay and brings them much closer to those obtained by column chromatography.

Discussion

We believe that the synergistic effect of naturally occurring hydroxylysine in hydrolysates used for the microbiological assay of lysine with *Leuconostoc mesenteroides* may have been the cause of too high values having been reported in the past. In some laboratories in which the phenomenon has been encountered and recognised, hydroxylysine additions to the medium have been standard practice (*e.g.*, S. A. Price, Vitamins Ltd., private communication).

No attempt has been made in our laboratories to explain the biochemical significance of this unusual effect of hydroxylysine, but that aspect has been studied by Smith and Henderson[14] with both *Streptococcus faecalis* and *Leuconostoc mesenteroides*. Lindstedt[15] showed that supplements of synthetic hydroxylysine were ineffective in promoting growth of lysine-deficient rats, and Sinex and Van Slyke[16] have also discussed the source and state of the hydroxylysine of collagen. Carpenter and Milner (unpublished) have found no synergistic effect of hydroxylysine on the growth response of chicks to lysine.

It seems clear that cultures of the organism may vary in the extent to which they respond to hydroxylysine: and that successive cultures may change their character in the course of time—influenced perhaps by the frequency with which they have been subcultured and the medium used.

In all our experiments with different cultures and with variations in media, the synergistic effect of hydroxylysine has always been evident, and with the knowledge now available it seems only prudent to include hydroxylysine in the medium for the assay of lysine with *Leuconostoc mesenteroides* as a routine precautionary measure.

One of us (K.J.C.) is indebted to the U.S. Department of Agriculture for a grant towards research, of which this formed a part.

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Modifications and Extensions to an Existing Sequential Scheme for Determining Fall-out Nuclides in Water

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Extensions and modifications to an existing sequential scheme for the radiochemical analysis of fall-out nuclides in drinking and rain water are described. This new scheme makes possible the determination, in the same sample, of manganese-54, yttrium-91, promethium-147 and zirconium-95 activities along with the more commonly determined barium, caesium, cerium and strontium fall-out nuclides.

After evaporation of the sample to dryness, conventional precipitation and solvent-extraction procedures are used to separate the various radioactive species carried on known amounts of added carriers. Mean chemical recoveries of the added carriers range from 40 per cent. for zirconium to 70 per cent. for strontium. Taking a 100-litre sample of drinking water and using low-background anti-coincidence-counting assemblies for detection of the β-particle emissions gives levels of detection ranging from 0·01 pC per litre for promethium-147 to 0·002 pC per litre for strontium-90. The manganese-54 is determined by γ-ray counting with a limit of detection of 0·02 pC per litre.

Since the latter half of 1957 a monitoring scheme has been in operation in the United Kingdom, whereby the fall-out derived radiochemical content of drinking water from selected Undertakings has been measured.[1] Until July 1st, 1961, this work was carried out by the Atomic Energy Research Establishment, Harwell. From then on the responsibility for the collection and radiochemical analysis of samples was taken over by the Laboratory of the Government Chemist, acting as agent for the Ministry of Housing and Local Government and its counterparts in Scotland and Northern Ireland.[2] Caesium-137 (half-life, 30 years) and strontium-90 (half-life, 28 years), the most hazardous radionuclides from the point of view of health, are regularly determined in this survey. The shorter-lived strontium-89 (half-life, 51 days) has been determined when present, *i.e.*, during nuclear-weapon tests and until such time as it has decayed away. With the resumption of nuclear testing during 1961–1962, the Ministry of Housing and Local Government requested the determination of additional fall-out nuclides in special samples of drinking water. This paper describes the methods by which several of these additional radionuclides were determined by extending an existing sequential scheme of analysis for fall-out nuclides in water.

Several problems are posed when fall-out radionuclides in drinking water are determined. Firstly, the method used must be capable of coping with the problems of isolating the milligram amounts of carriers *plus* their respective activities from waters of widely differing characteristics. For a hard water, this may mean separating the carriers from as much as 30 g of total solids in the sample. Also, the levels of the individual fall-out activities in certain waters, *e.g.*, well waters, are extremely low, so that large volumes have to be used.

Several sequential schemes of analysis have been proposed for the separation and determination of individual radionuclides from a complex mixture of fission products.[3,4] However, that devised by Osmond and co-workers[5] and its subsequent revised version[6] was essentially designed for water samples. It has been successfully used for the determination of radiocaesium and radiostrontium in drinking waters, first by the Atomic Energy Research Establishment (A.E.R.E.) and later in the Laboratory of the Government Chemist. Essentially this scheme involves evaporating a 100-litre sample to dryness and isolating the milligram amounts of carriers *plus* their respective activities by classical chemical procedures. In addition, this sequential scheme includes the determination of cerium and barium radionuclides. Since it was obviously desirable to determine as many radionuclides as possible in the same sample, the existing scheme was extended in this Laboratory. This has permitted yttrium-91, zirconium-95, promethium-147 and other rare-earth nuclides all to be determined in the same sample as the original nuclides. Manganese-54, although not a fission product, has also been included in this scheme on account of its presence in fall-out.[7]

A separate 5-litre sample of drinking water is taken for the determination of any naturally occurring elements that are present in amounts sufficient to affect the chemical yields of the added carriers, *viz.*, strontium and, sometimes, manganese.

Experimental

As a result of experience gained here in the routine determination of radiocaesium and radiostrontium in drinking waters, and to accommodate the extensions described here, some modifications have been made to the original A.E.R.E. method.[5,6] Fig. 1 shows the complete sequential scheme incorporating the general separation steps of the original method and the proposed extensions. The specific decontamination steps for the individual radionuclides are not shown. The numbers in parentheses in the text refer to those of the steps or fractions in Fig. 1. For the sake of continuity and to avoid constant cross-reference to the original method of Osmond and co-workers,[5,6] the complete experimental details of the whole scheme in Fig. 1 are given in the Method. The sections for barium, caesium and strontium purification and isolation are substantially unchanged except for a few minor modifications.

Preliminary treatment of a water sample—

Difficulty has been found in the initial treatment of the dried residue from a 100-litre sample of hard water. Using the method as it stood resulted in a large insoluble portion requiring sodium carbonate fusion. This difficulty has been resolved by baking the dried residue at 120° C for several hours to fix the silica, and then water-extracting the soluble salts from the residue. The residue is then treated as usual with hydrofluoric and perchloric acids to remove silica, with subsequent dissolution in acid. The water and acid extracts then go forward to the chemical separation. The very small amount of residue is rendered soluble by a carbonate fusion.

The presence of zirconium slightly complicates the normal successive aqueous and acid leaches of the carbonate-fusion mixture. Zirconium converted to sodium zirconate in the fusion is immediately hydrolysed by the aqueous leach to sandy insoluble zirconium hydroxide. Dissolution of this insoluble residue can be accomplished by treatment with hydrofluoric acid. The soluble portion is added to the zirconium fraction, **(15)**, and any small amount of insoluble material still remaining can be discarded. This solid is mainly platinum black arising from attack on the platinum dishes used in the preliminary treatment of a water sample. The activity retained on the solid is negligible compared with that in the complete sample.

Development of extensions and modifications to the sequential scheme—

The development of the proposed extensions and modifications proceeded in several ways—

(*i*) By using simulated water samples, each new or modified fraction of the sequential scheme was run through to check on the recovery of added radionuclides. Table I lists the results obtained.

(*ii*) By using a further series of simulated water samples, decontamination experiments from possible radioactive contaminants were carried out on each individual fraction of the sequential scheme. Table II lists the results obtained.

Table II

Decontamination factors of the individual fractions of the sequential scheme from possible contaminants

	Decontamination factor from—					
Fraction	^{90}Sr - ^{90}Y	^{144}Ce - ^{144}Pr	^{95}Zr	Ra D,E,F	Thorium *plus* daughters	^{147}Pm
Cerium ..	2×10^4	—	$>2{\cdot}3 \times 10^4$	3×10^3	2×10^4	3×10^3
Rare earth ..	6×10^4	$1{\cdot}4 \times 10^4$	$>2{\cdot}3 \times 10^4$	10^3	4×10^3	—
Yttrium ..	—	$1{\cdot}6 \times 10^3$	$>1{\cdot}5 \times 10^4$	10^3	4×10^3	$2{\cdot}8 \times 10^3$
Zirconium ..	$1{\cdot}3 \times 10^5$	8×10^4	—	$>2 \times 10^4$	$5{\cdot}7 \times 10^4$	2×10^5

(*iii*) Rain water samples containing high concentrations of fall-out activity were examined by using the sequential scheme.

All separated activities were thoroughly checked for radiochemical purity by means of decay or measurements of transmission through an aluminium absorber, or both. Table III lists the results obtained.

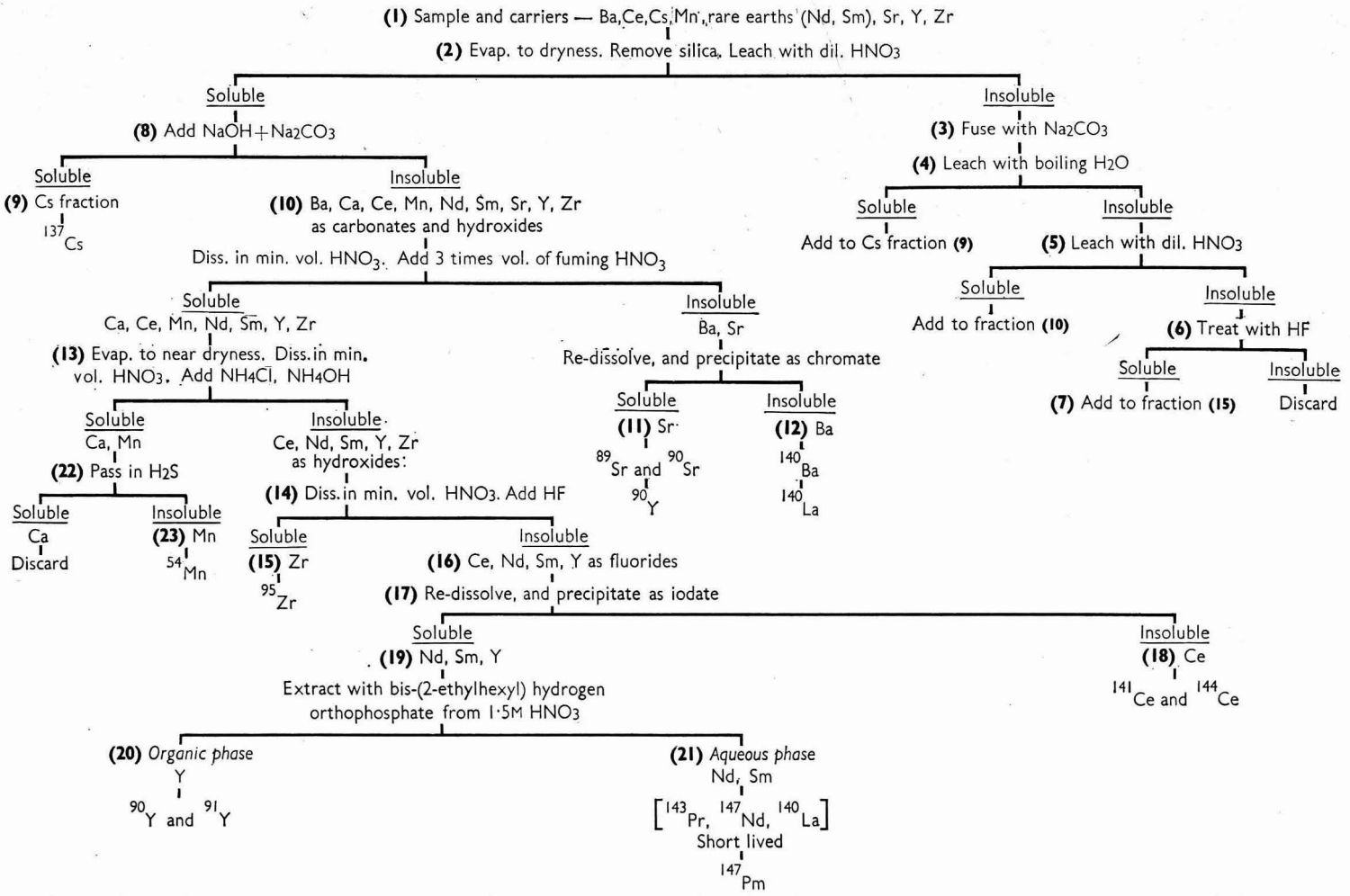

Fig. 1. Sequential separation scheme for the isolation of individual radionuclides from a water sample. The numbers in parenthesis are for ease of relating the stages and fractions described in the text

TABLE I

RECOVERY OF ADDED RADIONUCLIDES FROM SIMULATED WATER SAMPLES

Nuclide	Disintegrations per minute added to each sample	Disintegrations per minute recovered from sample*					Percentage overall error in sample				
		1	2	3	4	5	1	2	3	4	5
^{54}Mn	4440	4397	4258	4351	4541	4595	−1·0	−4·1	−2·0	+2·3	+3·5
^{91}Y	1983	2065	1926	2116	2038	1866	+4·1	−2·9	+6·7	+2·8	−5·9
^{95}Zr	1014	1087	1022	1072	1072	1025	+7·2	+0·8	+5·7	+5·7	+1·1
^{144}Ce - ^{144}Pr	1848	1845	1838	1881	1819	1844	−0·2	−0·5	+1·8	−1·6	−0·2
^{147}Pm	10,240	10,580	9962	10,543	9600	10,400	+3·3	−2·7	+3·0	−6·2	+1·6

* Sample 1 was distilled water, samples 2, 3, 4 and 5 were drinking waters containing 2·5, 15·0, 97·0 and 90·0 mg of calcium hardness per litre, respectively.

TABLE III

EXPERIMENTALLY OBSERVED HALF-LIVES AND β-RAY TRANSMISSION OF RADIONUCLIDES ISOLATED FROM RAIN-WATER SAMPLES (A, B, C, D)

Nuclide	Half-life	Observed half-life				Percentage transmission* of standard				Percentage transmission* of sample			
		A	B	C	D	A	B	C	D	A	B	C	D
^{89}Sr	51d	50d	51d	50·5d	50d	—	—	—	—	—	—	—	—
^{90}Y (^{90}Sr)	64·2h	64·0h	64·2h	63·0h	63·2h	65·8	65·8	65·8	65·8	64·8	65·7	66·2	65·4
^{91}Y	57d	58d	57d	58d	†	43·5	43·5	43·5	—	42·5	43·3	43·6	†
^{95}Zr	65d	65d	64·5d	64d	65d	16·5	16·5	16·5	16·4	16·4	16·4	16·5	16·1
^{137}Cs	30y	—	—	—	—	27·7	26·1	27·1	26·9	27·6	26·4	27·7	27·1
^{140}La (^{140}Ba)	40·2h	‡	40h	38·8h	39·1h	—	—	—	—	—	—	—	—
^{144}Ce - ^{144}Pr	285d	285d	285d	285d	285d	47·3	45·9	42·3	42·8	47·7	45·3	42·4	42·8
^{147}Pm	2·6y	—	—	—	—	31·7	31·8	31·7	—	32·0	31·3	32·9	†

* Using the aluminium absorbers specified on p. 620.
† Not determined.
‡ Not present in sample.

The range of and the mean chemical recoveries of added carriers from the various types of water sample examined by using the procedure described here are listed in Table IV.

TABLE IV

CHEMICAL RECOVERY OF ADDED CARRIERS FROM WATER SAMPLES

Type of sample		Added carriers: Ba	Ce	Cs	Mn	Rare earth	Sr	Y	Zr
Rain	Range, per cent.	30 to 65	50 to 60	50 to 70	40 to 80	40 to 55	35 to 70	40 to 60	20 to 50
	Mean, per cent.	45	54	59	50	44	50	43	40
Soft drinking water	Range, per cent.	50 to 80	30 to 70	60 to 80	70 to 90	40 to 50	70 to 80	30 to 40	20 to 60
	Mean, per cent.	65	50	73	80	45	78	32	45
Hard drinking water	Range, per cent.	—	40 to 50	60 to 80	40 to 90	40 to 90	70 to 80	40 to 50	30 to 60
	Mean, per cent.	—	45	70	60	50	77	45	40

Cerium fraction—In the original procedure,[5] the cerium was decontaminated from zirconium activity first by a fluoride precipitation and then by two zirconium iodate scavenges, with the cerium in the three-valency state. Trouble was experienced in obtaining quantitative zirconium iodate scavenge precipitates, probably due to the complexing effect on zirconium of traces of fluoride carried through from the initial fluoride precipitation.

It has been found that any zirconium not precipitated as the iodate, while the cerium was in the tervalent state, subsequently comes down with the ceric iodate and follows the cerium through the oxalate precipitation, giving an erroneously high chemical recovery for cerium and incomplete decontamination from traces of zirconium activity. The zirconium iodate precipitations are also an important step in the removal of the natural species of bismuth, lead and thorium present in water samples. Failure to include the zirconium iodate scavenge precipitates in the radiochemical assay of a rain sample resulted in contamination of the cerium and to a lesser extent the yttrium and rare-earth sources with lead and bismuth-210.

It appeared that in the original procedure[5] insufficient complexing of the traces of fluoride occurred before these zirconium iodate precipitations were performed. In the subsequent procedure[6] by the same workers, this was circumvented by carrying out the zirconium iodate scavenges before the fluoride precipitations, an alteration that, however, could not be made use of in this present work. Instead it has been found here that addition of boric acid at all stages of the cerium-purification procedure after the initial separation of the cerium from zirconium by fluoride precipitation, **(14)**, together with the use of sodium hydroxide instead of ammonia for the hydroxide precipitations at those stages, effectively removed traces of fluoride. This allows the subsequent zirconium iodate precipitations to proceed quantitatively (see Tables II and III for results).

Manganese fraction—Although manganese-54 ($t_{\frac{1}{2}}$, 290 days) is not a fission product, being formed probably by radioactivation of the nuclear-weapon casing, it appears in fall-out at the present time.[7] It has been included in this sequential scheme for this reason. Unlike other nuclides determined here, manganese-54 is not a β-particle emitter, but decays by electron capture with subsequent emission of a γ-ray having a 0·84-MeV photopeak. The primary concern, therefore, in the decontamination of the manganese fraction has been to remove traces of other interfering γ-ray-emitting nuclides that may be found in rain or drinking water.

After the initial separations in the proposed sequential scheme involving the removal of the caesium, strontium and barium fractions, the manganese is found along with the lanthanides, zirconium and a variable amount of calcium. Separation of the manganese fraction is effected by a hydroxide precipitation in the presence of an excess of ammonium chloride, in which conditions it stays in solution along with the calcium. The subsequent radiochemical and chemical decontamination of the manganese fraction from other interfering species has been achieved in the main by adapting a sulphide separation scheme.

Fig. 2 shows the γ-ray photopeak of manganese-54 separated from a rain sample by the proposed procedure. Although this rain contained a considerable activity due to zirconium-95 *plus* niobium-95, ruthenium-106 and cerium-144, the only peak appearing above background is that due to manganese-54.

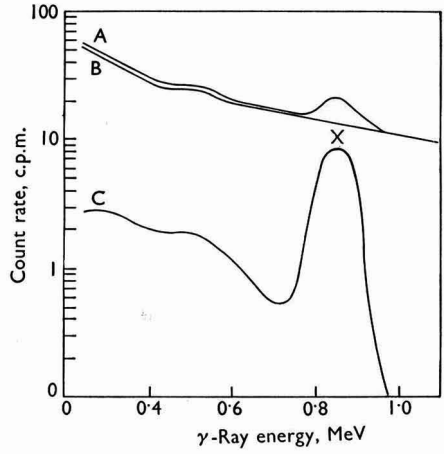

Fig. 2 γ-Ray spectrum of manganese-54 separated from other fall-out nuclides in a rain-water sample. Curve A, sample *plus* background; curve B, background; curve C, curve A *minus* curve B; peak X, manganese-54 photopeak at 0·84 MeV

Zirconium fraction—Zirconium is separated from the lanthanides by making use of the fact that its fluoride is soluble. Further decontamination of the zirconium-95 fraction from interfering activities, including that of its daughter niobium-95, is obtained by a series of steps involving successive barium fluorozirconate precipitations.[8] Certain water samples examined by the proposed procedure contained iron and aluminium. These elements follow zirconium through the sequential scheme and are precipitated as barium fluoro complexes along with the barium fluorozirconate. Extraction of the iron with di-isopropyl ether before the separation of the zirconium from the lanthanides obviates part of this difficulty, and the aluminium can be removed by virtue of its solubility in excess of sodium hydroxide solution. The zirconium is finally precipitated as the mandelate, an additional safeguard against any aluminium being carried, and ignited to the oxide in which form it is counted. On account of the low levels of zirconium-95 found in certain waters, low-level anticoincidence β-particle counting techniques are employed in its measurement rather than the usual γ-ray counting.

Niobium-95 has not been included in this sequential scheme, mainly on account of the insoluble nature of most niobium salts and the consequent difficulty of maintaining the element in solution through the separation involved in Fig. 1. However, it has been noted[4] that carrier-free niobium follows zirconium carrier, and that its recovery is more reproducible when niobium carrier is not added until the final stages of the procedure. In view of this, addition of niobium carrier before the first barium fluorozirconate precipitation and subsequent work-up of the supernatant liquor would allow niobium-95 to be isolated and subsequently measured by γ-ray counting.[9]

Yttrium and rare-earth fractions—The yttrium and rare-earth fraction is initially separated as a soluble iodate from the insoluble ceric iodate. A separation of the yttrium and rare-earth fractions has been worked out by making use of published figures[10,11] for their distribution coefficients in the system di-(2-ethylhexyl)orthophosphoric acid (in heptane) - nitric acid. The yttrium fraction is extracted into the organic phase from 1·5 M nitric acid, the rare-earth elements remaining in the aqueous phase. No attempt has been made in this work to isolate chemically the individual rare-earth activities.

In fall-out less than 16 months old, the yttrium fraction would contain yttrium-91 ($t_{\frac{1}{2}}$, 57 days). If the time between the separation of the yttrium from strontium and counting of the former is only a few days, yttrium-90 ($t_{\frac{1}{2}}$, 64 hours), the daughter of strontium-90, would also be present. A decay curve of the yttrium source would then have to be resolved into the yttrium-90 and yttrium-91 components, or, alternatively, the short-lived isotope can be allowed to decay completely (16 days) before the yttrium-91 is counted.

The rare-earth fraction could contain a mixture of activities, principally, the lanthanum-140 ($t_{\frac{1}{2}}$, 40 hours), daughter of barium-140 ($t_{\frac{1}{2}}$, 12·8 days), praseodymium-143 ($t_{\frac{1}{2}}$, 13·8 days) and the neodymium-147 ($t_{\frac{1}{2}}$, 11·6 days) - promethium-147 ($t_{\frac{1}{2}}$, 2·6 years) pair. Promethium-147 can be measured after the decay of the shorter-lived activities, which anyway would only be present in fall-out of recent origin. Since promethium does not exist in a stable form, a mixture of the elements of adjacent atomic number, neodymium and samarium, is used to carry the radioactive promethium-147 and the other rare-earth activities.

TIME SCHEDULE FOR THE PROPOSED PROCEDURE—

The time required for a complete radiochemical analysis of a water sample depends on the type of sample. By using a suitably thermally shielded 10-litre flask on a 1200-watt heating ring, an evaporation rate of 1 litre per hour can be maintained. The final evaporation and preliminary treatment of the residue of a rain or soft-drinking-water sample in a platinum dish can be completed in 1 to 1½ days, but a hard-water sample requires at least an extra day. A skilled operator can carry out the sequential scheme itself in 3 to 4 days; again an extra day should be added for hard-water samples, for which extra, time-consuming precipitations are required at some stages. The time needed to obtain a series of counts for the resolution and positive identification of all the radionuclides capable of being determined by this present scheme is in our experience up to 3 months.

METHOD

APPARATUS—

Demountable Perspex filter-stick, 16 mm in internal diameter—This filter-stick takes a 21-mm diameter filter-paper. Use two filter-papers together in the filter-stick. After filtration, separate the two papers and allow them to come to equilibrium with the atmosphere for 2 hours before weighing. The assumption is made that both papers gain or lose the same weight during the filtration and drying process.

γ-Ray-counting equipment.

Low-background (approximately 1 count per minute) anticoincidence β-particle counting assemblies.[5]

Infrared lamps—Use these for all evaporations unless otherwise stated.

Centrifuge bottles and tubes—Use 250-ml capacity bottles and 40-ml capacity graduated tubes according to the volumes met with at each step in the procedure.

REAGENTS—

Use analytical-reagent grade reagents whenever possible, and check them for the presence of radiochemical contamination. Do this indirectly by carrying out blank runs, with carriers present, of the various stages in the procedure and count the various sources obtained.

Barium, caesium, cerium, manganese, neodymium, samarium, strontium, yttrium and zirconium carrier solutions—Use nitrates or oxides to prepare standardised solutions in M nitric acid, to contain 10 mg of element per ml of solution.

Antimony, barium, cadmium, cerium, cobalt, iron, lead, neodymium, samarium, strontium, tellurium, yttrium, zinc and zirconium hold-back carrier solutions—Use potassium antimonyl tartrate, telluric acid and the nitrates or oxides of the other elements to prepare solutions containing 2·5 mg of element per ml of solution.

Niobium hold-back carrier solution—Prepare, to contain 2·5 mg of niobium per ml of solution, by dissolving niobium oxide in hot 40 per cent. hydrofluoric acid. Store the solution in a polythene bottle.

Bis-(2-ethylhexyl) hydrogen orthophosphate in heptane, 0·75 M—Equilibrate this solution by washing it three times with an equal volume of 3 per cent. hydrogen peroxide prepared by diluting 30 per cent. hydrogen peroxide with 1·5 M nitric acid. The peroxide-scrubbed heptane solution of the acid is stable and can be stored.[11]

Di-isopropyl ether—Equilibrate this by washing it with half its volume of hydrochloric acid, sp.gr. 1·18.

Bismuth tri-iodide reagent—Dissolve 20 g of bismuth tri-iodide and 20 g of sodium iodide in 50 ml of water and 2 ml of glacial acetic acid. Filter off any insoluble solid.

Bismuth tri-iodide wash solution—Add 1 ml of bismuth tri-iodide reagent to 9 ml of ice-cold 10 per cent. acetic acid solution. Prepare freshly before use.

Chloroplatinic acid solution, 10 per cent. w/v, aqueous.

Iodate wash solution—Dissolve 8 g of potassium iodate in 1 litre of 4 M nitric acid.

Diethyl ether - hydrochloric acid, sp.gr. 1·18 (1 + 5, v/v) mixture.

Mandelic acid solution, 16 per cent. w/v, aqueous.

Mandelic acid wash solution—Dissolve 5 g of mandelic acid in 100 ml of 0·5 N hydrochloric acid.

Sulphide wash solution—Add a few millilitres of ammonium sulphide solution to a 1 per cent. w/v solution of ammonium chloride. Prepare freshly before use.

Poly(vinyl acetate) (Gelva) solution, 5 per cent. w/v, methanolic.

Standard radiochemical solutions of the radionuclides to be determined.

Di-(o-hydroxyphenylimino)-ethane, saturated methanol solution—This solution is stable up to 1 month.

PROCEDURE—

(1) *Preliminary treatment of a water sample*—Add 40 mg each of barium, caesium, cerium, strontium and zirconium, 20 mg each of manganese and yttrium, 10 mg each of neodymium and samarium carriers and 20 ml of nitric acid, sp.gr. 1·42, to the collection vessel before sampling. A 100-litre sample of a drinking water is generally taken. The volume of a rain sample is conditioned by rainfall and the size of collectors, but, when **(2)** possible, a 10-litre sample should be taken. Evaporate the samples to 0·5 to 1 litre on a boiling-ring. Complete the evaporation in a platinum dish, taking care to transfer to the dish any solids adhering to the evaporation flask.

If the calcium hardness does not exceed 30 mg per litre, proceed as follows—

Break up the dried residue, treat it with 20 ml of 40 per cent. hydrofluoric acid and take to dryness. Add a further 20 ml of 40 per cent. hydrofluoric acid and 10 ml of 72 per cent. w/v perchloric acid and take to dryness. Break up the residue, add 10 ml of 72 per cent. w/v perchloric acid and evaporate until no further fumes are evolved, completing this last operation on a hot plate. Extract the residue with 20 to 30 ml of hot 30 per cent. nitric acid, transferring the slurry to a centrifuge tube. Spin the tube and retain the supernatant liquor for step **(8)** and the residue for step **(3)**.

For a sample containing more than 30 mg of calcium hardness per litre, add 50 ml of nitric acid, sp.gr. 1·42, to the dried residue, and again take to dryness. Bake the dried residue in an oven for 3 to 4 hours at 120° C. Break up the residue and extract it with 50-ml portions of hot water, spinning off in a centrifuge the insoluble material each time. Continue until an almost colourless extract is obtained, (3 or 4 extracts are normally required). Combine the extracts and retain them for step **(8)**. Return the insoluble residue to the platinum dish and treat with hydrofluoric and perchloric acids as described above for the residue from a soft water.

(3) Transfer the residue, mixed with 5 to 6 times its volume of anhydrous sodium **to** carbonate, to a platinum crucible and fuse until the melt is clear. Cool and leach **(7)** successively with boiling water, **(4)**, and hot 30 per cent. nitric acid, **(5)**. Combine the aqueous leach with the main caesium fraction, **(9)**, and the acid leach with the main insoluble carbonate and hydroxide fraction, **(10)**. Should the insoluble residue after the first sodium carbonate fusion exceed 10 to 20 mg, then carry out a second fusion as above.

Return any remaining insoluble material to the platinum crucible with the minimum of water, take to dryness, add 5 ml of 40 per cent. hydrofluoric acid and heat gently on a hot plate for a few minutes, **(6)**. Transfer the mixture to a Lusteroid tube, spin it in a centrifuge and retain the supernatant liquor, **(7)**, for addition to the zirconium fraction, **(15)**. Discard any remaining solid.

It has been found in practice[12] that the preliminary treatment of a rain sample containing a large amount of foreign insoluble material is best carried out following that described above for a drinking water containing more than 30 mg of calcium hardness per litre.

(8) *Sequential separation scheme*—Combine the water and acid extracts from step **(2)**, make them alkaline with sodium hydroxide solution and then add solid sodium carbonate with stirring. Spin the mixture in a centrifuge and wash the precipitate, **(10)**, thoroughly with water. Combine the supernatant liquor, washings and aqueous leach, **(4)**, and retain them for caesium separation, **(9)**.

(10) Combine the acid leach, **(5)**, from the sodium carbonate fusion and the precipitate of carbonates and hydroxides, **(10)**, and add the minimum volume of 30 per cent. nitric acid to ensure complete dissolution. To this solution add three times its volume of fuming nitric acid. Stir and cool in ice for 30 minutes. Spin the mixture in a centrifuge and retain the supernatant liquor, **(13)**, for the separation of cerium, yttrium, zirconium, manganese and the rare-earth elements. Retain the nitrate precipitate for the separation of barium, **(12)**, and strontium, **(11)**.

(13) Evaporate the fuming nitric acid solution to near dryness in a porcelain basin. Dissolve the residue in the minimum volume of 30 per cent. nitric acid, and transfer it to a centrifuge tube or bottle. (The addition of 1 ml of hydrogen peroxide may be necessary to ensure complete dissolution of this residue. Boil the mixture to destroy the excess of peroxide.) Add 2 g of ammonium chloride for each 10 ml of solution and precipitate the hydroxides with the minimum volume of carbonate-free 18 M ammonia solution. Spin the mixture in a centrifuge immediately, and reserve the supernatant liquor for the separation of manganese, **(22)**. Re-dissolve the hydroxides in the minimum volume of 30 per cent. nitric acid, and transfer the solution to a tube if a bottle has been in use. Add an equal volume of water, the requisite amount of ammonium chloride and re-precipitate the hydroxides with the minimum volume of carbonate-free 18 M ammonia solution. Spin the mixture in a centrifuge and add the supernatant liquor to that previously obtained, **(22)**.

Again dissolve the hydroxides in the minimum volume of 30 per cent. nitric acid. Precipitate the hydroxides with carbonate-free 18 M ammonia solution. Spin the mixture in a centrifuge and discard the supernatant liquor. Repeat this until the supernatant liquor contains no calcium. (Test with ammonium carbonate.)

(14) Dissolve the hydroxides in the minimum volume of 30 per cent. nitric acid, transfer the solution to a Lusteroid tube, dilute to 15 ml and add 2 ml of 40 per cent. hydrofluoric acid with stirring. Heat the solution in a water-bath to coagulate the fluoride precipitate, **(16)**, and spin the mixture in a centrifuge. Transfer the supernatant liquor to a Lusteroid tube, and retain it for zirconium separation, **(15)**. Wash the fluoride precipitate with 5 ml of water, adding the washing to the main zirconium fraction.

(17) Dissolve the fluoride precipitate in 5 ml of 5 per cent. boric acid solution *plus* a few drops of nitric acid, sp.gr. 1·42, with warming. Transfer the solution to a glass centrifuge tube with water washings, and dilute to 15 ml. Precipitate the hydroxides with a slight excess of sodium hydroxide solution. Spin the tube in a centrifuge, and reject the supernatant liquor. Wash the precipitate with water, spin it in a centrifuge and discard the washings. Dissolve the precipitate in the minimum volume of 30 per cent. nitric acid, add 2 ml of 5 per cent. boric acid, dilute to 15 ml and repeat the hydroxide precipitation and washing.

Re-dissolve the precipitate in the minimum volume of 30 per cent. nitric acid, add 2 ml of 5 per cent. boric acid, 2 ml of zirconium hold-back carrier solution and a few drops of hydrogen peroxide solution. Boil to destroy the excess of peroxide, cool and add an ice-cooled mixture of 7 ml of nitric acid, sp.gr. 1·42, and 25 ml of 7 per cent. potassium iodate solution. Cool the mixture in ice for 30 minutes with occasional stirring, spin it in a centrifuge, filter the liquor through a fast paper and reject the zirconium iodate precipitate. Heat the solution in a boiling-water bath, cautiously add a little solid sodium bromate and continue heating to coagulate the yellow ceric iodate. Cool the mixture slightly and spin it in a centrifuge. Transfer the supernatant liquor to another tube and retain it for yttrium and rare-earth separation, **(19)**. Wash the iodate precipitate with 10 ml of iodate wash solution, spin it in a centrifuge and reject the washings. Retain the cerium iodate for subsequent purification, **(18)**.

(9) *Caesium purification and isolation*—Acidify the caesium fraction with glacial acetic acid, boil on a hot plate to drive off carbon dioxide and reduce in volume to at least 300 ml. Filter off and reject any aluminium hydroxide or silica that separates at this stage. Dissolve 5 g of sodium nitrite in the solution and cool in ice. Add, with stirring, 25 ml of a freshly prepared saturated solution of sodium cobaltinitrite, and continue cooling for 30 minutes. Spin the mixture in a centrifuge and reject the supernatant liquor. Using 10 per cent. acetic acid solution, wash and transfer the cobaltinitrite precipitate to a centrifuge tube, spin it in a centrifuge and reject the washings.

Dissolve the precipitate by warming it with 2 to 3 ml of 30 per cent. nitric acid. Dilute the solution to 10 ml and make alkaline with 50 per cent. sodium hydroxide solution. Spin the mixture in a centrifuge, and transfer the supernatant liquor to a fresh tube. Wash the cobalt hydroxide with 5 ml of water, spin it in a centrifuge and combine the washings with the previous supernatant liquor. Discard the precipitate. Acidify the solution with glacial acetic acid, boil off any carbon dioxide and evaporate to 8 to 10 ml in a boiling-water bath. Add a further 0·5 ml of glacial acetic acid, cool the mixture thoroughly in an ice-bath and add 2 ml of bismuth tri-iodide reagent. Stir and continue cooling for 5 minutes. Spin the mixture in a centrifuge and reject the supernatant liquor. Dissolve the precipitate in 1 to 2 ml of 30 per cent. nitric acid and boil the solution in a water-bath to remove iodine. Dilute to 10 ml, and add 50 per cent. of sodium hydroxide solution. Spin the mixture in a centrifuge and transfer the supernatant liquor to a fresh tube. Wash the bismuth hydroxide with 5 ml of water, spin it in a centrifuge and combine the washings with the previous supernatant liquor. Discard the precipitate.

Acidify the solution with glacial acetic acid, reduce in volume to 8 to 10 ml and repeat the precipitation with bismuth tri-iodide reagent. Spin the mixture in a centrifuge, and reject the supernatant liquor. Wash the caesium bismuth iodide complex successively with ice-cold 10-ml portions of bismuth tri-iodide wash solution and ethanol. Spin the mixture in a centrifuge, and reject both washings. Dissolve the precipitate in 1 to 2 ml of 30 per cent. nitric acid, and boil the solution in a water-bath to remove the iodine. Make up to 10 ml with 30 per cent. nitric acid, and add 10 ml of water. Cool the solution in ice, and add 1 to 2 ml of 10 per cent. chloroplatinic acid solution. Stir the mixture and continue cooling for 10 minutes. Spin it in a centrifuge, and reject the supernatant liquor. Wash the precipitate with 10 ml of ice-cold 15 per cent. nitric acid, spin the mixture in a centrifuge, and reject the washings. Slurry the precipitate with ice-cold ethanol, filter the liquid through a tared Whatman No. 42 filter-paper, by using a filter-stick, and wash the caesium chloroplatinate with two 5-ml portions of ethanol. Allow the residue to air dry, weigh it as the chloroplatinate and calculate the chemical recovery. Mount the solid on a planchet with Gelva, and count both normally and through a 50-mg per sq. cm aluminium absorber.

(11) to (12) *Barium and strontium purification and isolation*—Dissolve the nitrate precipitate in the minimum volume of water and make up to 10 ml or the nearest multiple of 10 ml. Add 25 ml of fuming nitric acid for each 10 ml of aqueous solution. Stir the mixture and cool it in ice for 30 minutes. Spin it in a centrifuge, and reject the supernatant liquor. Repeat the barium and strontium nitrate precipitation with decreasing volumes of water and acid, still in the ratio of 10 + 25, v/v, water - acid mixture, until the volume of nitrate precipitate is no more than 1 ml in the graduated centrifuge tube.

Dissolve the strontium and barium nitrates in 10 ml of water, and neutralise the excess of acid with 6 M ammonia solution with methyl red indicator. Add 1 ml of 30 per cent. acetic acid and 2 ml of 25 per cent. ammonium acetate solution, dilute to 25 ml, heat in a boiling-water bath and add 1 ml of 30 per cent. sodium chromate solution. Continue heating with occasional stirring to coagulate the precipitate of barium chromate. Cool, spin the mixture in a centrifuge and transfer the supernatant liquor to a centrifuge tube and retain it for the separation of strontium, **(11)**.

Barium purification and isolation—To the barium chromate add 1 ml of hydrochloric acid, sp.gr. 1·18, dilute to 5 ml and add a few drops of hydrogen peroxide. Boil until the solution colours green and then to destroy the excess of hydrogen peroxide. Dilute to 15 ml, add 1 ml of iron hold-back carrier solution, place the mixture in a boiling-water bath for a few minutes and make alkaline with carbonate-free 18 M ammonia solution and boil until the supernatant liquor is colourless. Spin the mixture in a centrifuge and filter the supernatant liquor through a fast paper into a fresh tube. Discard the precipitate. To the supernatant liquid add 1 ml of strontium hold-back carrier solution, and then add a few millilitres of 18 M ammonia solution and an excess of solid ammonium carbonate. Spin the mixture in a centrifuge and reject the supernatant liquor.

Dissolve the carbonates in the minimum volume of 30 per cent. hydrochloric acid, and add 15 ml of the ether - hydrochloric acid mixture. Stir and cool in ice for 10 minutes. Spin the mixture in a centrifuge, and reject the supernatant liquor. Dissolve the barium

chloride in the minimum volume of water and re-precipitate the barium chloride as before. Dissolve the barium chloride in 10 ml of water, add 1 ml of yttrium carrier solution, and store for at least 13 days to allow the lanthanum-140 to grow into radiochemical equilibrium with barium-140. (Yttrium is used to carry the lanthanum-140 activity in preference to natural lanthanum, which exhibits a small natural activity due to lanthanum-138.)

Heat the solution in a boiling-water bath, and add sufficient carbonate-free 18 M ammonia solution to precipitate the yttrium. Note the time of precipitation. Spin the mixture in a centrifuge, and transfer the supernatant liquor to another tube. Dissolve the precipitate in the minimum volume of 30 per cent. nitric acid, dilute the solution to 10 ml, heat it in a boiling-water bath for a few minutes before re-precipitating the yttrium hydroxide with carbonate-free 18 M ammonia solution. Spin the mixture in a centrifuge, add the supernatant liquor to that from the previous precipitation, add a few millilitres of 18 M ammonia solution and solid ammonium carbonate, warm to dissolve the excess of reagent and to coagulate the barium carbonate precipitate. Reserve until the yttrium fraction has been treated.

Re-dissolve the yttrium hydroxide precipitate in the minimum volume of 30 per cent. nitric acid and add 20 ml of 8 per cent. oxalic acid solution. Heat the mixture in a water-bath to coagulate the precipitate. Cool, and filter the liquid through a Whatman No. 42 filter-paper in a filter-stick. Wash the precipitate successively with two 10-ml portions each of water and methanol. Ignite the precipitate to the oxide in a platinum crucible. Slurry the oxide with methanol, and filter the liquor through a tared Whatman No. 42 filter-paper in a filter-stick. Allow the residue to air dry, weigh it as yttrium oxide and calculate the chemical recovery. Mount it on a planchet with Gelva, and count it at regular intervals to check on the decay of the lanthanum-140.

Cool and filter the reserved barium carbonate precipitate on a tared Whatman No. 42 filter-paper in a filter-stick. Wash the precipitate successively with two 10-ml portions each of water and methanol. Allow the residue to air dry, weight it as barium carbonate and calculate the chemical recovery. Mount it on a planchet with Gelva. Count the barium source 13 days later, and then at intervals to follow any decay due to barium-140. In the absence of decay, any count is probably due to radium daughter products.

Strontium purification and isolation—To the supernatant liquor containing the strontium fraction, add an excess of 18 M ammonia solution and solid ammonium carbonate. Spin the mixture in a centrifuge, and reject the supernatant liquor. Dissolve the strontium carbonate in the minimum volume of 30 per cent. nitric acid, dilute the solution to 10 ml, boil off the carbon dioxide, add 1 ml of barium hold-back carrier solution and repeat the barium chromate precipitation as above. Spin the mixture in a centrifuge, and filter the supernatant liquor through a fast paper into a fresh centrifuge tube. Reject the precipitate. Re-precipitate the strontium fraction by adding an excess of 18 M ammonia solution and solid ammonium carbonate to the supernatant liquor.

Dissolve the precipitate in the minimum volume of 30 per cent. nitric acid, make up to 10 ml with water, and add 25 ml of fuming nitric acid. Stir the mixture and cool it in ice for 30 minutes. Spin it in a centrifuge, and reject the supernatant liquor. Dissolve the precipitate in 10 ml of water, and test for the continued presence of calcium (see below). Repeat the strontium nitrate precipitation until calcium is absent. Add 1 ml of iron hold-back carrier solution, heat the solution in a boiling-water bath for a few minutes, and make the solution alkaline with carbonate-free 18 M ammonia solution. Spin the mixture in a centrifuge, and filter the supernatant liquor into another tube. Reject the precipitate. Just acidify the supernatant liquor with 30 per cent. nitric acid, add 1 ml of yttrium carrier solution and store the solution for at least 16 days to allow the yttrium-90 to attain radiochemical equilibrium with strontium-90.

Heat the solution in a boiling-water bath, and add sufficient carbonate-free ammonia solution to precipitate the yttrium. Note the time of precipitation. Then following exactly the procedure as described in the barium purification and isolation section, isolate the strontium fraction as carbonate and the yttrium as oxide. Calculate the strontium and yttrium chemical recoveries, making allowance for the natural strontium content of the water sample.

Count the strontium source immediately with a 110-mg per sq. cm aluminium absorber in position and then normally after at least 16 days' interval. Count the yttrium source normally at regular intervals to check on the decay of yttrium-90 and also through a 110-mg per sq. cm aluminium absorber.

Test for the presence of calcium[13]—Take 6 drops of chloroform in a semi-micro test-tube. Add 2 drops of the solution under test, 4 drops of distilled water, 4 drops of a saturated methanolic solution of di(*o*-hydroxyphenylimino)-ethane and 2 drops of 10 per cent. sodium hydroxide solution. Cover the test-tube with a polythene disc, and shake it for 10 seconds. Add 2 drops of 10 per cent. sodium carbonate and shake the tube for a further 10 seconds. A red colour in the chloroform layer indicates the presence of calcium. (It is advisable to compare the colour in the chloroform layer with that produced by 2 drops of strontium carrier solution (2·5 mg per ml) treated in the same way. The test as performed above detects down to 0·2 per cent. of calcium contamination in a strontium solution.)

Determination of natural calcium and strontium—Evaporate an aliquot of carrier-free water, sufficient to yield 30 to 40 mg of calcium carbonate, to 100 ml. Add 20 ml of 8 per cent. oxalic acid solution and adjust the pH to 4 with 18 M ammonia solution with bromocresol green as indicator, and maintain the solution at 60° C for 1 hour. Cool, decant most of the supernatant liquor through a filter-stick containing a Whatman No. 42 filter-paper, and then filter off the calcium oxalate. Wash this well with water and methanol. Transfer the precipitate and paper to a tared silica crucible and ignite at 500° ± 25° C to a constant weight of calcium carbonate.

Carefully add a few drops of 30 per cent. nitric acid to the crucible. Cover with a watch-glass until effervescence ceases. Wash down the glass and sides of the crucible with a little water, and evaporate the solution to dryness. Determine the strontium content of the residue by X-ray fluorescence[14] or flame photometry.[15]

(15) *Zirconium purification and isolation*—To the zirconium-containing solution, **(15)**, add 1 ml each of cerium, yttrium and niobium hold-back carrier solutions and 2 ml of 40 per cent. hydrofluoric acid with stirring (polythene rod). Warm the mixture to coagulate the fluoride precipitate, spin it in a centrifuge and transfer the supernatant liquor to another Lusteroid tube. Reject the precipitate. Add 1 ml of nitric acid, sp.gr. 1·42, and 5 ml of barium carrier solution (20 mg per ml) to the supernatant liquor. Stir the mixture and warm it to coagulate the barium fluorozirconate precipitate. Add further drops of barium carrier solution and hydrofluoric acid to ensure complete precipitation, cool the mixture in an ice-bath for 15 minutes, spin it in a centrifuge and reject the supernatant liquor. Wash the precipitate with 5 ml of 0·05 M nitric acid, and reject the washings. Dissolve the barium fluorozirconate precipitate in 5 ml of 5 per cent. boric acid *plus* a few drops of nitric acid, sp.gr. 1·42, with warming. Re-precipitate the barium fluorozirconate by adding 2 ml of barium carrier solution and 2 ml of hydrofluoric acid, as previously. Reject the supernatant liquor. Again wash the residue with 5 ml of 0·05 M nitric acid, and discard the washings. Dissolve the precipitate as before with boric and nitric acids.

Transfer the solution to a glass centrifuge tube with the minimum of washing, and add 1 drop of sulphuric acid, sp.gr. 1·84. Spin in a centrifuge the precipitate of barium sulphate, and test the supernatant layer with a further drop of sulphuric acid to ensure complete precipitation. Spin the mixture in a centrifuge again, if necessary, and filter the liquor through a No. 541 Whatman filter-paper into a fresh tube. Discard the precipitate. To the filtrate add an excess of 50 per cent. of sodium hydroxide solution dropwise, and stir. Spin the mixture in a centrifuge and discard the supernatant liquor. Wash the zirconium hydroxide with 5 ml of water, spin it in a centrifuge and reject the washings.

Dissolve the precipitate in 1 to 2 ml of diluted hydrochloric acid (1 + 1), and then dilute the solution with water to 10 ml. Heat it in a water-bath at approximately 90° C, and add 10 ml of 16 per cent. mandelic acid solution in 1-ml portions over 30 minutes. Cool the mixture in an ice bath, filter the liquor through a Whatman No. 42 filter-paper in a filter-stick and wash the zirconium mandelate with two 10-ml portions of the mandelic acid wash solution and then with two 10-ml portions of methanol. Ignite the precipitate to zirconium oxide in a platinum crucible by using a Méker burner. Make

the oxide into a slurry with methanol, and filter the liquor through a tared Whatman No. 42 filter-paper in a filter-stick. Allow the residue to air dry, weigh it as the oxide and calculate the chemical recovery. Mount it on a planchet with Gelva, and count it immediately, both normally and through a 30-mg per sq. cm aluminium absorber.

(18) *Cerium purification and isolation*—Dissolve the ceric iodate, **(18)**, in 1 to 2 ml of hydrochloric acid, sp.gr. 1·18, with warming, dilute to 10 ml with water and add about 1 ml of 100-volume hydrogen peroxide. Heat the solution for several minutes, cool, add an excess of 18 M ammonia solution and place the mixture in a boiling-water bath until the yellow ceric hydroxide is precipitated. Spin the mixture in a centrifuge, and reject the supernatant liquor. Dissolve the hydroxide in the minimum volume of nitric acid, sp.gr. 1·42, and a few drops of hydrogen peroxide with warming, add 2 ml of 5 per cent. boric acid and 1 ml each of yttrium and zirconium hold-back carrier solutions. Boil the mixture for a few minutes to destroy the excess of hydrogen peroxide. Cool and repeat the iodate separations as before, **(17)**. Reject the zirconium iodate precipitate and the final supernatant liquor after precipitation of the ceric iodate. Wash the ceric iodate with iodate wash solution as before, and then dissolve the iodate in the minimum volume of hydrochloric acid, sp.gr. 1·18, dilute the solution to 10 ml with water, heat it in a boiling-water bath and add 4 per cent. ammonium oxalate solution, reducing the acidity slightly with a few drops of 6 M ammonia solution, if necessary, to aid precipitation of the cerous oxalate. Spin the mixture in a centrifuge and reject the supernatant liquor. Re-dissolve the precipitate in the minimum volume of nitric acid, sp.gr. 1·42, dilute the solution to 25 ml with water and warm it to coagulate the cerous oxalate, again adding a few drops of 6 M ammonia solution if necessary.

Cool the mixture and filter the liquor through a Whatman No. 42 filter-paper in a filter-stick, washing the residue twice with methanol. Ignite the precipitate to ceric oxide in a platinum crucible. Make the residue into a slurry with methanol, and filter the solid off on to a tared Whatman No. 42 filter-paper in a filter-stick. Allow the residue to air dry. Weigh it as the oxide, and calculate the chemical yield. Mount it on a planchet with Gelva, and count with and without a 200-mg per sq. cm aluminium absorber in place above the source, in order to resolve cerium-141 and cerium-144.

(19) *Yttrium purification and isolation*—To the supernatant liquor, **(19)**, containing the yttrium and rare-earth fraction, add an excess of 50 per cent. sodium hydroxide solution with stirring. Spin the mixture in a centrifuge, and reject the supernatant liquor. Wash the precipitate with 10 ml of water, and reject the washings. Dissolve the precipitate in the minimum volume of 30 per cent. nitric acid and add 1 ml of cerium hold-back carrier solution. Add a mixture of 7 ml of nitric acid, sp.gr. 1·42, and 25 ml of 7 per cent. potassium iodate solution. Heat the solution in a boiling-water bath, and then cautiously add a little solid sodium bromate and continue heating to coagulate the ceric iodate. Cool the mixture in ice, spin it in a centrifuge and filter off and reject the precipitate. To the filtrate, add 50 per cent. sodium hydroxide solution, spin the mixture in a centrifuge, and reject the supernatant liquor. Wash the hydroxides with 10 ml of water, and reject the washings.

Dissolve the hydroxides in the minimum volume of 30 per cent. nitric acid, and, with methyl red as indicator, add 2 M ammonia solution until just alkaline. Boil off the excess of ammonia. Add standard nitric acid (10 M) sufficient to make the solution 1·5 M with respect to nitric acid. Transfer it to a small separating funnel, and wash the centrifuge tube with an equal volume of 0·75 M bis-(2-ethylhexyl) hydrogen orthophosphate in heptane solution, and then add it also to the separating funnel and extract for 2 minutes. Allow the phases to separate, run off the aqueous phase into a second separating funnel and extract it for 2 minutes with a further equal volume of 0·75 M bis-(2-ethylhexyl) hydrogen orthophosphate in heptane solution. Allow the phases to separate. Run off the aqueous phase into another separating funnel, and retain it for the separation of the rare-earth fraction, **(21)**.

(20) Combine the organic phases, and extract them with three successive equal volumes of N nitric acid. Discard the aqueous extracts. Re-extract the yttrium from the combined organic phases with 10 M nitric acid. Two 5-ml portions of acid each shaken for 2 minutes are usually sufficient. Combine the acidic extracts, and wash them with 5 ml of heptane. Reject the heptane washings.

Transfer the acidic extract to a centrifuge tube, and precipitate the yttrium hydroxide with ammonia solution. By following the procedure through oxalate to oxide as described for the yttrium fraction in the section on the barium purification and isolation, isolate the yttrium oxide and calculate the chemical recovery. Mount the oxide on a planchet with Gelva, and count it at regular intervals to permit resolution of the yttrium-90 and yttrium-91 to be made. After not less than 16 days from the initial count, recount the source through a 110-mg per sq. cm aluminium absorber to check the transmission of the yttrium-91.

(21) *Rare-earth purification and isolation*—Dilute the aqueous rare-earth-containing fraction, **(21)**, in a separating funnel with half its volume of water. Extract the aqueous phase twice with half its volume of 0·75 M bis-(2-ethylhexyl) hydrogen orthophosphate in heptane solution. Discard the organic phases. Wash the aqueous phase with 5 ml of heptane. Reject the heptane washings.

Transfer the aqueous phase to a centrifuge tube and precipitate the rare-earth elements as hydroxides with ammonia solution. By following the procedure through oxalate to oxide as described for the yttrium fraction in the section on the barium purification and isolation, isolate the rare-earth oxides. Calculate the chemical recovery on the basis that the final oxide precipitate contains a ratio of samarium to neodymium identical to the ratio of the initially added carriers. Count the rare-earth source at regular intervals over several weeks to permit successive resolution firstly of the 40-hour half-life lanthanum-140, and secondly the 13·8-day half-life praseodymium-143 - 11·6-day half-life neodymium-147 pair from the 2·6-year half-life promethium-147. When the short-lived rare-earth activities have decayed away, check the identity of the promethium-147 by recounting it through a 7-mg per sq. cm aluminium absorber.

(22) *Manganese purification and isolation*—Pass hydrogen sulphide gas for several minutes into the previously separated supernatant liquor from stage **(22)** containing the manganese fraction. Spin the mixture in a centrifuge, and reject the supernatant liquor. Wash the precipitate into a centrifuge tube with the minimum volume of the sulphide
(23) wash solution. Spin the tube in a centrifuge, and reject the washings. Add 10 ml of ice-cold diluted hydrochloric acid (1 + 10) to the precipitate, and stir it for 2 minutes. Spin the mixture in a centrifuge, and transfer the supernatant liquor to a fresh tube and reject any insoluble residue. To the supernatant liquor, add 1 ml each of cadmium, lead, antimony, cobalt, zinc and tellurium hold-back carrier solutions. Pass in hydrogen sulphide gas for a few minutes, dilute the liquor to twice its volume, boil it and pass in more hydrogen sulphide. Spin the mixture in a centrifuge, filter the liquor into a 50 ml beaker and reject the precipitate.

Boil off the hydrogen sulphide, transfer the filtrate to a centrifuge tube, add 2 g of ammonium chloride for each 10 ml of solution and a slight excess of carbonate-free ammonia solution. Spin off, immediately, in a centrifuge and discard any precipitate. Pass hydrogen sulphide gas for several minutes into the supernatant liquor. Spin the mixture in a centrifuge, and reject the supernatant liquor. Wash the precipitate with 10 ml of the sulphide wash solution. Spin the mixture in a centrifuge, and reject the washings. Add 10 ml of ice-cold diluted hydrochloric acid (1 + 10) to the precipitate, and stir it for 2 minutes. Spin the mixture in a centrifuge, and filter off the solid, transferring the filtrate to a 50-ml beaker. Reject the precipitate.

Boil off the hydrogen sulphide from the filtrate, transfer the liquor to a fresh centrifuge tube and add an excess of 50 per cent. sodium hydroxide solution. Spin the mixture in a centrifuge, and reject the supernatant liquor. Wash the manganese hydroxide precipitate with 10 ml of water. Spin the mixture in a centrifuge, and reject the washings. Dissolve the precipitate in the minimum volume of nitric acid, sp.gr. 1·42, and dilute the solution to 10 ml with water. Boil it and add solid sodium bromate in small portions, continuing boiling until all the manganese dioxide is precipitated. Cool, spin the mixture in a centrifuge and discard the supernatant liquor. Wash the precipitate well with two 10-ml portions of water, spin the mixture in a centrifuge and reject the washings. Re-dissolve the manganese dioxide in 0·5 ml of nitric acid, sp.gr. 1·42, and a few drops of hydrogen peroxide, and transfer the solution to a polythene-capped glass specimen-tube (5·7 cm high and 1·5 cm in internal diameter) with the minimum of water washings. Heat the solution in a water-bath to destroy the excess

of peroxide. Dilute the solution accurately to 5 ml with water. Count it in a γ-ray counter.

After counting, transfer by pipette 1 ml of the solution from the tube to a 100-ml calibrated flask, and dilute it to the mark. Determine the chemical recovery of manganese colorimetrically as potassium permanganate,[16] taking account of the total manganese content of the water sample.

Treatment of samples containing large amounts of iron or aluminium—If a sample is known or suspected to contain large amounts of iron or aluminium, or both, relative to the amounts of added carriers, then proceed as follows—

After removal of traces of calcium, dissolve the hydroxides from stage **(13)** in the minimum volume of hydrochloric acid, sp.gr. 1·18, add a further 2 to 3 ml of acid and transfer the solution to a small separating funnel. Rinse the centrifuge tube with a volume of di-isopropyl ether a few millilitres in excess of the volume of the aqueous phase, and add it to the separating funnel. Extract for 2 minutes, allow the phases to separate and discard the organic phase. Repeat the extraction a further two times, using the same volume of ether. Reject the ethereal phases. Transfer the aqueous phase to a fresh centrifuge tube, and boil out any dissolved ether. Precipitate the hydroxides with an excess of 50 per cent. sodium hydroxide solution. Spin the mixture in a centrifuge, and discard the supernatant liquor. Wash the precipitate with 10 ml of water, and discard the washings. Now proceed to the fluoride precipitation, **(14)**.

CALCULATION—

Details of the calibration of counters and the counting methods used for measuring barium-140, cerium-141 and cerium-144, caesium-137 and strontium-89 and strontium-90 have already been described.[5,6] Similar techniques can be used for measuring promethium-147, yttrium-91 and zirconium-95 activities.

Prepare a graph of weight of source against efficiency of counting, with the appropriate radiochemical standard solution, for each counter to be used in the measurement of any particular nuclide. For the zirconium-95 calibration, remove the niobium-95 daughter by means of a double barium fluorozirconate precipitation (see back to section on zirconium purification and isolation, **(15)**) before preparing a series of sources. Then count these sources as soon as possible before further niobium-95 grows in.

No attempt has been made here to calibrate counters for praseodymium-143 or neodymium-147, since these two nuclides have approximately similar half-lives and β-particle emission energies, making resolution by decay or transmission impossible. Use a barium-140 source weight - efficiency of counting curve to obtain an approximate counting efficiency for this pair.

When calibrating the counters, determine the transmission of β-particle emissions of the nuclides listed below through the indicated aluminium absorbers—

Nuclide	Absorber, mg per sq. cm
Promethium-147	7
Zirconium-95	30
Caesium-137	50
Strontium-89 and strontium-90	110
Yttrium-90 and yttrium-91	110
Cerium-144 + praseodymium-144	200

The concentration of any nuclide in a water sample at the time of sampling, A_0, is given by—

$$A_0 = C \times \frac{1}{Y} \times \frac{1}{E} \times \frac{1}{D} \times \frac{1}{V} \times \frac{1}{2{\cdot}22} \text{ pC per litre,}$$

where C is the counting rate in counts per minute corrected for lost counts and background,

Y is the fractional chemical recovery (in the determination of barium-140 and strontium-90, an extra chemical recovery factor, that of the yttrium carrier, Y_1 is included in the expression for A_0),

E is the fractional counter efficiency corresponding to the weight of the source and the particular nuclide counted,

D is the decay factor for the time interval between sampling and counting for the particular nuclide and

V is the volume of sample in litres.

Check the radiochemical purity of each source by using the appropriate aluminium absorber and also by decay measurements if practicable.

Calibrate the γ-ray-counting equipment with the standard manganese-54 solution under the same conditions of geometry to be used in the counting of the samples. Determine the photo-peak efficiency for the 0·84-MeV γ-ray of manganese-54.

The concentration of manganese-54 in a water sample is given by the expression for A_0 above, but when, in this instance, C is the total counts per minute under the photo-peak corrected for lost counts and background, and E is the photo-peak efficiency.

Results

The procedure described above has been applied to both rain- and drinking-water samples. The concentrations of various fall-out nuclides in samples of London rain water have been determined since September, 1961, the scope of the radiochemical analyses being increased as methods were worked out for further nuclides.[12] The purification and isolation procedure for the rare-earth fraction had not been finalised before the short-lived members of this group were no longer to be found in rain samples. However, the lanthanum-140, the praseodymium-143 - neodymium-147 pair and the promethium-147 components of an irradiated uranium sample were successfully resolved by following the decay of the rare-earth source at regular intervals.

Various drinking-water samples have been examined radiochemically, by making use of this and another (unpublished) procedure designed to include nuclides, *e.g.*, those of ruthenium, tellurium, antimony and tin, that could not be conveniently included in the sequential scheme described here. The object of this exercise was to provide a balance sheet for a water sample between its total β-particle activity, and the sum of the activities of the individual nuclides present, all expressed as strontium-90.[17,18]

The sensitivity of detection of the various nuclides depends on the sample size and duration of counting time. The limits of detection are listed below, and were obtained by using an anti-coincidence β-particle-counting assembly having a background of 1 count per minute—

Nuclide	^{140}Ba	^{144}Ce	^{137}Cs	^{54}Mn	^{147}Pm	^{89}Sr	^{90}Sr	^{91}Y	^{95}Zr
Limit of detection, pC per litre ..	0·01	0·006	0·003	0·02	0·01	0·006	0·002	0·004	0·005

The limits of detection were calculated from the values for A_0 for (*a*) a 100-litre sample, (*b*) a count equivalent to three standard deviations of a background count of 3000 minutes' duration, (*c*) a mean chemical recovery as recorded in Table IV, (*d*) a counting efficiency corresponding to mean chemical recovery for the particular nuclide and (*e*) a time interval of 1 month between sampling and counting (decay factor). The limits of detection for manganese-54 are expressed with respect to the γ-ray-counting equipment.

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A Simple and Rapid Titrimetric Method for the Determination of Carbon in Iron and Steel

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A simple and rapid method is described for the determination of carbon in iron and steel, based on non-aqueous titration with tetra-n-butylammonium hydroxide of the carbon dioxide evolved by combustion of the sample in a stream of oxygen. The carbon dioxide is absorbed in a solution of formdimethylamide. Sources of blank value and analytical conditions required for optimum results are discussed. Results of a reproducibility test carried out on a series of certified British Chemical Standards are reported. The procedure can be made semi-automatic by use of an automatic titrimeter and this modification is described.

The conventional method of determining carbon content,[1] whereby a sample is burnt in a current of oxygen, and the evolved carbon dioxide absorbed in soda asbestos and weighed, has remained unchallenged for many years as the most suitable procedure for the routine analysis of ferrous materials. When the carbon content is less than 0·05 per cent., more sensitive techniques must be used. These include the modified gravimetric[2] and low-pressure pneumatic[3] methods and the use of various instruments[4,5,6] that utilise the principles of conductimetric, coulometric and infrared gas analysis.

In this paper we propose an alternative method for the routine determination of carbon in ferrous material, by the use of a non-aqueous titrimetric method to determine the carbon dioxide evolved during combustion of the sample in oxygen. This method is faster than the conventional one, requiring only 3 to 4 minutes for a single determination. The apparatus is quite simple and can be constructed at relatively small cost using standard laboratory glassware. The procedure can be semi-automated by using a commercial automatic titrimeter to determine the end-point. This modification is described.

The method described is now in use on a routine basis at this laboratory and has replaced the conventional gravimetric method for determining carbon in ferrous materials. The rapid analysis times, together with the comparable accuracy at low carbon levels, gives the non-aqueous titrimetric method a distinct advantage over the low-pressure pneumatic method for use on a routine basis, since the latter method requires 12 to 15 minutes for the analysis of a single sample.

Preliminary work

Blom and co-workers[7] described a very simple method for determining carbon in steel by non-aqueous titration. Their method is based on absorption in pyridine of the carbon dioxide evolved during combustion in oxygen and subsequent titration of the absorbed carbon dioxide with sodium methoxide in pyridine - methanol solution. The method required a rather elaborate cell for absorption of the carbon dioxide and had the disadvantage that the pyridine used for absorption constituted a toxic hazard. These difficulties were overcome by Grant, Hunter and Massie,[8] who used a simple cell system wherein the evolved carbon dioxide was absorbed in formdimethylamide and titrated with potassium methoxide in benzene - methanol mixture. They also improved the end-point by using thymolphthalein as the indicator.

The Grant, Hunter and Massie modification to the non-aqueous titration was not very successful due to the difficulty encountered in obtaining complete absorption of the carbon dioxide in the formdimethylamide solution. This was overcome by adding a small amount of mono-ethanolamine as originally proposed by Blom.

Although these modifications were a distinct improvement on the original procedure recommended by Blom and others, the value of 0·44 mg of carbon dioxide per 1·0 ml of 0·02 N potassium methoxide proposed by Grant, Hunter and Massie for calculating the carbon content, is not compatible with our findings. All the work carried out by us clearly indicates that the conversion factor is 0·88 mg of carbon dioxide per 1·0 ml of 0·02 N potassium methoxide and this is in complete agreement with the calculations of Blom and his colleagues.

During the titration with potassium methoxide a fine white precipitate of potassium methyl carbonate is formed. This tends to adhere to the sides of the absorption vessel, obscuring the end-point, and clogging the jet of the burette. This necessitates frequent stripping down and cleaning of the apparatus. The problem has been overcome by using tetra-n-butylammonium hydroxide as the titrant,[9] since this reagent does not form insoluble reaction products.

Scope of the method

With a 1-g sample and 0·02 N tetra-n-butylammonium hydroxide, the method is suitable for steels having carbon contents in the range 0·01 to 0·25 per cent. By adjustment of reagent strength and taking smaller sample weights, or both, the range can be extended to 3·0 per cent. of carbon or higher, in iron samples.

Principle—

The material to be analysed is burnt in a stream of oxygen in the presence of a flux that assists combustion. The carbon dioxide evolved is absorbed in a solution of formdimethylamide containing a small amount of mono-ethanolamine and subsequently titrated with standard tetra-n-butylammonium hydroxide. Thymolphthalein is used as the indicator.

The reaction is stoicheiometric and proceeds as below—

$$C\begin{matrix}O\\O\end{matrix} + (CH_3.CH_2.CH_2.CH_2)_4NOH \longrightarrow (CH_3.CH_2.CH_2.CH_2)_4\text{–}N\text{–}O\text{–}\overset{\overset{OH}{|}}{C}=O$$

Reagents—

Formdimethylamide—General-purpose reagent grade.

Mono-ethanolamine—General-purpose reagent grade.

Manganese dioxide—Prepare as per B.S. 1121, Part 11, 1948.

Tetra-n-butylammonium hydroxide, 0·10 N in benzene/methanol.

Tetra-n-butylammonium hydroxide, 0·02 N—Dilute 200 ml of the 0·10 N reagent to a volume of 1 litre with analytical-reagent grade benzene.

Thymolphthalein indicator—Prepare a 0·10 per cent. w/v solution of thymolphthalein in anhydrous methanol.

Absorption solution—Prepare this solution by mixing formdimethylamide, mono-ethanolamine and thymolphthalein indicator in the ratio 150 + 1 + 1.

Description of apparatus—

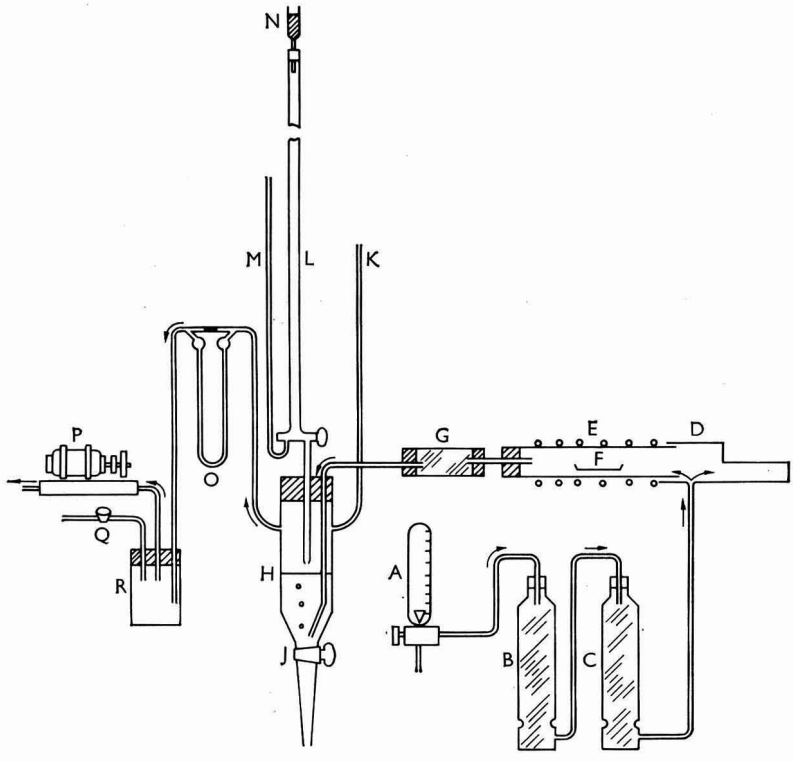
The apparatus is shown in Fig. 1. There are three main sections.

Oxygen purification train—The oxygen input is adjusted to a flow of 1 litre per minute by means of the regulator, A. The oxygen is passed through magnesium perchlorate, B, to remove water vapour, and soda asbestos, C, to remove any carbon dioxide present, before entering the combustion furnace.

Combustion furnace—The combustion furnace, E, is a resistance-type furnace capable of continuous operation at 1100° to 1250° C. The purified oxygen enters the furnace via a brass tuyère, D, open at the end to facilitate rapid insertion of the combustion boat. Details of the tuyère are shown in Fig. 2. On entering the furnace the oxygen stream splits into two parts. One stream is drawn through the apparatus at a rate of 150 ml per minute by means of a small electric pump, P, at the end of the combustion train. The excess of oxygen escapes to atmosphere through the open-ended tuyère, and forms a curtain of oxygen that effectively prevents air entering the combustion tube. The rate at which the oxygen stream is drawn through the apparatus can be regulated by means of tap, Q, to the required setting on the calibrated flowmeter, O. Any water vapour or sulphur gases evolved during combustion are removed, before entry into the absorption cell, by passing the gas through a prolong, G, containing magnesium perchlorate and manganese dioxide.

Absorption cell—The cell is constructed from the lower half of a 150-ml separating funnel. Side arms can be attached by any competent glass blower. A two-holed rubber bung accommodates the inlet tube for delivery of the products of combustion and a 10-ml microburette for delivery of titrant. The absorption solution is introduced into the cell via one side arm and gases are pumped off to the atmosphere through the other. The evolved carbon dioxide

is absorbed in the formdimethylamide solution and titration is carried out as the gases are evolved. On completion of the titrations, waste solution is drained away through stopcock J. All solutions are protected from atmospheric contamination by means of guard tubes containing soda asbestos. All connections to the cell and burette are made with flexible thin-walled polythene tubing.



A = Oxygen-flow regulator, 0 to 10 litres per minute
B = Magnesium perchlorate
C = Soda asbestos, 14 to 20 mesh
D = Brass tuyère
E = Resistance-type furnace
F = Combustion boat
G = Prolong, containing manganese dioxide and magnesium perchlorate
H = Absorption cell
J = Stopcock to waste
K = Flexible polythene delivery tube from absorption-solution reservoir
L = 10-ml microburette, fitted with PTFE stopcock
M = Flexible polythene delivery tube from titrant reservoir
N = Guard tube, containing soda asbestos
O = Flow-meter, O to 300 ml per minute
P = Electric pump
Q = Flow-rate control
R = Pump-regulator system

Fig. 1. Schematic diagram showing apparatus and oxygen-flow system

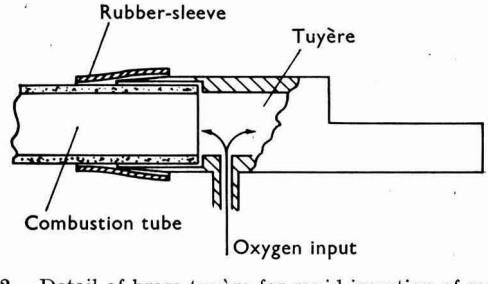


Fig. 2. Detail of brass tuyère for rapid insertion of sample

STANDARDISATION OF TETRA-N-BUTYLAMMONIUM HYDROXIDE—

For routine use the 0·10 N tetra-n-butylammonium hydroxide must be diluted and the strength adjusted to 0·02 N. This solution can be standardised in either of two ways.

(*a*) *Standardisation with benzoic acid*—Adjust the oxygen input to 1 litre per minute and regulate the pump so that oxygen at the rate of 150 ml per minute is drawn through the apparatus. Introduce 20 ml of absorption solution into the cell, and neutralise with tetra-n-butylammonium hydroxide to a blue colour. Weigh 15 to 20 mg of benzoic acid and transfer it to the absorption cell. The solution becomes decolorised. Titrate with tetra-n-butylammonium hydroxide to a blue end-point.

1 ml of 0·02 N tetra-n-butylammonium hydroxide ≡ 2·441 mg of benzoic acid.

(*b*) *Standardisation with calcium carbonate*—Weigh about 10 mg of high-purity calcium carbonate into a pre-ignited combustion boat, insert it into the hot zone of the furnace and carry out the determination as under "Procedure." Titrate the evolved carbon dioxide against tetra-n-butylammonium hydroxide. Carry out a blank determination and deduct this from the titre.

1 ml of 0·02 N tetra-n-butylammonium hydroxide = 0·88 mg of carbon dioxide.

In practice the standardisation of the titrant is carried out with benzoic acid. Calcium carbonate or standard steel samples are used to check that the apparatus is functioning in a satisfactory manner.

ANALYTICAL METHOD

PROCEDURE—

Adjust the oxygen input to 1 litre per minute and regulate the pump so that oxygen at a rate of 150 ml per minute is drawn through the apparatus. Introduce 20 ml of absorption solution into the cell and neutralise with the titrant to a blue end-point. Weigh 1 g of sample into a pre-ignited combustion boat, add flux in the form of a strip of lead foil, and introduce the boat into the hot zone of the furnace via the open-ended tuyère. As combustion products are evolved the absorption solution becomes decolorised and must be titrated with the tetra-n-butylammonium hydroxide to maintain the blue colour. Carry out a blank determination and deduct this value from the titre. Calculate the carbon content of the sample from the equation—

$$\text{Carbon, per cent.} = \frac{(A - B) \times N \times 1{\cdot}2}{W}.$$

where A = titre, in ml,
B = blank titre, in ml,
N = normality of titrant, and
W = weight of sample, in g.

NOTES ON PROCEDURE—

1. Each day, before using the apparatus, rinse out the burette and flexible connections with titrant.

2. The absorption solution may be used for more than one sample. This depends on the carbon content and the solution should be replaced when approximately 10 ml of titrant has been added.

3. All flexible connections should be made with thin-walled surgical-grade polythene tubing that is not subject to attack by the reagents. Under no circumstances should rubber or PVC tubing be used in contact with the reagents.

4. The sample weight should be adjusted, depending on the amount of carbon present. For steels of low carbon content the sample weight should be increased, and for high carbon content the sample weight should be reduced. When iron and steel samples with a carbon content greater than 1·0 per cent. are analysed it is sometimes more convenient to use 0·10 N tetra-n-butylammonium hydroxide. This avoids adding an excessive volume of titrant. Suitable titrant strengths and sample weights for the different ranges of carbon content are given below—

Carbon range, per cent.	Titrant 0·01 N	0·02 N	0·10 N
	g	g	g
Less than 0·01	5	—	—
0·01 to 0·02	—	3	—
0·02 to 0·25	—	1	—
0·25 to 1·0	—	0·25	1
1·0 to 3·0	—	0·05	0·50
3·0 *plus*	—	—	0·25 or less

5. When the sample weight is increased, the oxygen-input rate must be increased by an additional 1 litre per minute during the burn for each 1-g increase in sample weight, to provide adequate reserves of oxygen for combustion.

6. The freshly prepared 0·02 N tetra-n-butylammonium hydroxide should be allowed to stand for 24 hours before standardisation and use.

7. If possible the burette tap should be made of PTFE. If a glass tap is used this should be lubricated with a PTFE aerosol spray and not with Vaseline or silicone grease as these are subject to attack by the reagents.

Experimental

Several factors were studied to obtain the analytical conditions necessary for optimum results.

Blank determination—

In order to obtain accurate results when steels of very low carbon content are analysed it is necessary that the blank value be kept to a minimum. Three major sources of blank value were investigated; those due to oxygen, combustion boats and some of the more common fluxes. Experiments were made to determine the extent of the blank value from each source during the course of a routine determination. The results are shown in Table I.

Table I

Blank determination

The blank value is expressed in terms of per cent. of carbon on the basis of a 1-g sample weight and a 4-minute analysis time

Source of blank value	Carbon, per cent. (average)
Oxygen	0·00072
Pre-ignited combustion boat: cooled and stored in desiccator before use	0·00096 to 0·00144
Pre-ignited combustion boat: cooled in a stream of oxygen and used immediately	Nil
Flux—0·50 g of tin powder	0·00065
—strip (7 cm × ½ cm) of lead foil	0·00165
—1·0 g of red lead	0·00690

Tin powder gives the lowest blank value of the fluxes examined. It should consequently be used for analysis of steels with very low carbon contents. In practice, however, when samples that have carbon contents in the range 0·02 to 0·25 per cent. are analysed, it is more convenient to use strips of lead foil as the flux, and to take the slightly higher blank value into account when calculating the amount of carbon present in the sample.

Absorption capacity of formdimethylamide solution—

In order to determine the amount of carbon dioxide that will be absorbed by the formdimethylamide solution, before it has to be discarded, several individual weighings of the British Chemical Standard 237/1 (0·104 per cent. carbon) were analysed as given under "Procedure." The carbon dioxide evolved from the successive weighings was absorbed in a single 20-ml aliquot.

It was found that after the addition of 10 ml of tetra-n-butylammonium hydroxide the results obtained on successive samples of the 0·104 per cent. carbon standard progressively decreased. After the addition of 10 ml of titrant it is essential to discard the absorption solution and replace it with a fresh 20-ml aliquot of formdimethylamide solution.

The decrease in absorption capacity after addition of 10 ml of the titrant is due to the dilution effect of the added titrant. To check this, individual aliquots of the absorption solution were diluted by addition of 0, 10, 20 and 30 ml of benzene. The carbon dioxide evolved from individual samples of the B.C.S. 237/1 were passed into each of the aliquots and titrated with 0·02 N tetra-n-butylammonium hydroxide. The carbon content as determined by titration was found to decrease in the same order as dilution of the aliquots. Similar results were obtained when this test was repeated with B.C.S. 247/3 (3·0 per cent. carbon) and 0·10 N tetra-n-butylammonium hydroxide as titrant.

The main factor that affects the absorption capacity of the formdimethylamide solution would, therefore, appear to be the extent to which this solution is diluted by addition of the titrant. In practice a 20-ml aliquot of absorption solution is taken. It is found advisable to discard this after approximately 10 ml of titrant have been added, irrespective of the strength of the titrant.

VARIATION IN OXYGEN FLOW-RATE THROUGH ABSORPTION CELL—

The rate at which the oxygen and products of combustion flow through the absorption cell must be controlled. On one hand too rapid a flow will result in incomplete absorption of the carbon dioxide in the formdimethylamide solution, and, in certain circumstances, it is even possible to pull air into the system through the open-ended tuyère. Too slow a rate, on the other hand, will lead to a very long analysis time and make the method unsuitable for routine use. Best results were obtained by using flow-rates between 100 and 200 ml per minute. The flow-rate was therefore standardised at 150 ml per minute.

AUTOMATIC END-POINT DETERMINATION

The apparatus described in the first section of this paper is of a simple form, easily constructed, and capable of giving rapid and very accurate results in the hands of routine operators.

The titration can be followed by potentiometric means. The inclusion of an automatic titrimeter, having an electrode system suitably modified for titration in non-aqueous media, gives the procedure a great degree of flexibility; this permits the equipment to be used very efficiently by a single operator.

The E.I.L. Model 24 automatic titrimeter provides a simple and effective means to carry out routine titrations. It has two sets of input connections, and, if necessary, a second apparatus can be run from the one control unit.

It is not necessary to describe this instrument, or its operation, in great detail, as it is a standard commercial type and is fully described in the manufacturer's literature.* It embodies an electronic pH meter, the output of which is used to control a set of relays that operate a solenoid valve. The valve acts as the tap of a 10 ml microburette. During the course of a titration the bulk of the titrant is delivered at a fast rate, and this changes to a slow delivery before the end-point. At the end-point, titration terminates automatically. At this stage a green light on the unit flashes on to warn the operator.

It is important that the solenoid valve, which is in contact with the reagents, be constructed from polypropylene or other material that is not susceptible to attack by the reagent. A simple modification by the manufacturer is necessary to allow the commercial instrument to be used for non-aqueous titrations of the type described.

The electrode system consists of a spade-type platinum electrode and a saturated potassium chloride in methanol calomel electrode. The former is inserted through the rubber bung closure of the absorption cell and is immersed in the formdimethylamide solution while the rather bulkier calomel electrode is mounted outside the cell. Contact is made with the solution by way of a potassium chloride in methanol salt bridge.

The titration curve obtained potentiometrically during the combustion process is shown in Fig. 3.

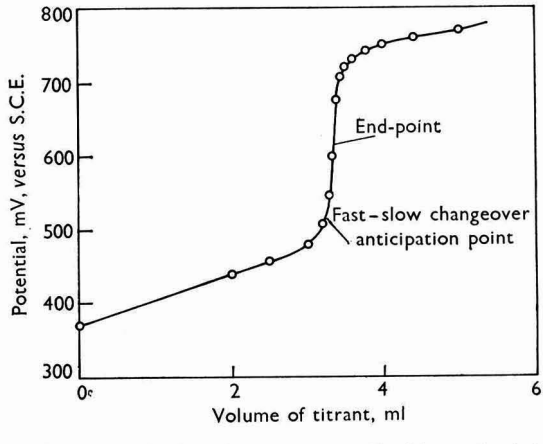


Fig. 3. Curve for potentiometric titration of carbon dioxide evolved during combustion

* Electronic Instruments Ltd., Richmond, Surrey.

REPRODUCIBILITY AND ACCURACY—

Twenty determinations were carried out on each of four British Chemical Standard samples to evaluate the reproducibility and accuracy of the method. The samples covered the range 0·001 to 3·0 per cent. carbon. The results obtained are shown in Table II.

TABLE II

REPRODUCIBILITY OF THE NON-AQUEOUS TITRIMETRIC METHOD

B.C.S. No.	Carbon, per cent. Certificate	Titrimetric	Spread	Standard deviation	Coefficient of variation
260/2	0·001	0·0013	0·0011 to 0·0015	±0·00011	8·46
237/1	0·104	0·103	0·100 to 0·105	±0·0015	1·46
238/1	0·210	0·208	0·205 to 0·211	±0·0017	0·87
247/3	3·00	3·00	2·95 to 3·11	±0·043	1·40

CONCLUSIONS

The use of non-aqueous titrimetry provides a very fast and extremely accurate method for determining the carbon content of ferrous materials. The use of an open-ended tuyère, as described, for rapid introduction of the samples into the combustion furnace permits carbon determinations to be completed in 3 to 4 minutes. At low levels, *i.e.*, 0·01 per cent. carbon, analysis times of 2 to 3 minutes are possible.

The apparatus described in the first section of the paper can be constructed at very little cost from materials readily available in most laboratories. It is extremely simple to operate and requires very little maintenance.

The introduction of a commercial automatic titrimeter to automate the end-point, whilst not essential, does permit the equipment to be used to better advantage by a single operator.

The method is extremely versatile and may be used to determine the carbon content of materials other than iron and steel.

We thank Mr. Ir. A. Wassdorp of the Royal Dutch Steelworks for supplying details of the tuyère used for rapid sample insertion, and Electronic Instruments Ltd., Richmond, Surrey, for their help in making the procedure semi-automatic. We also thank the Directors of the Steel Company of Wales Limited for permission to publish this paper.

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Spectrophotometric Determination of Alkylating Agents with 4-Picoline and *o*-Dinitrobenzene

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An accurate, sensitive spectrophotometric method has been developed for determining alkylating agents. The method involves the alkylation of 4-picoline at 100° C in 2-methoxyethanol, with subsequent reaction of the 4-picolinium cation with *o*-dinitrobenzene in the presence of alkali to produce the chromogen. With 2-phenylethanol as the solvent, the method is autocatalytic at 100° C. The variables in the procedures were studied, and the methods were compared to one another and to methods in the literature. Some preliminary studies of other possible methods for alkylating agents are discussed.

Alkylating agents have been shown to be present in urban air, in air polluted by various sources, in automobile exhaust and in cigarette tar.[1] Many pesticides, chemical carcinogens and anticarcinogens are alkylating agents, as pointed out previously.[1] More recently, the carcinogenic properties of alkylating agents such as nitrogen mustards, sulphur mustards and alkanesulphonate esters,[2] epoxides, ethyleneimines and lactones,[2,3] and nitroso compounds[4] have been reviewed. The anti-fertility effects of alkanesulphonate esters have been studied,[5] and alkylating agents synthesised as possible anti-carcinogens have been surveyed.[6,7,8,9]

The spectrophotometric determination of alkylating agents has been accomplished by using a substituted-pyridine reagent such as 4-(4-nitrobenzyl)pyridine.[10] This reagent reacts with alkylating agents to produce a substituted *N*-alkylpyridinium cation.

The 4-(4-nitrobenzyl)pyridine procedure has been used in both its original[10,11,12,13] and autocatalytic[1] forms to determine many alkylating agents. The sensitivity of the original 4-(4-nitrobenzyl)pyridine procedure[10] is relatively low (molecular extinction coefficient of 7300). Several autocatalytic methods, in which various hydrazones were used, have produced high sensitivity, with molecular extinction coefficients as high as 1,500,000 for some alkylating agents.[1] These procedures, however, yield a poorer reproducibility than the present method, do not obey Beer's law for alkyl iodides, and require a special high-temperature (180° C) bath.

In this study, an accurate, sensitive, reproducible method that obeys Beer's law and that utilises a readily available boiling-water bath has been developed. The method involves the alkylation of 4-picoline and the subsequent reaction of the 4-picolinium salt with *o*-dinitrobenzene. The method can also be used autocatalytically at 100° C. The non-catalytic and autocatalytic methods are compared with one another and with methods from the literature. Some preliminary experiments involving possible future methods for alkylating agents are discussed.

Method

Reagents and apparatus—

The chemicals were obtained from commercial sources and purified when necessary. The solutions were kept refrigerated and wrapped in aluminium foil. The *o*-dinitrobenzene reagent solutions are usually stable for at least a week; the 4-picoline solutions must be prepared fresh daily, or more often if they become coloured.

A Cary Model 14 spectrophotometer was used.

2-Methoxyethanol procedure—

One millilitre of sample dissolved in 2-methoxyethanol was placed in a small centrifuge tube or test-tube, and 0·5 ml of 60 per cent. w/v 4-picoline in 2-methoxyethanol was added. The solution was heated for 30 minutes at 100° C in a water-bath, and cooled; 0·5 ml of 10 per cent. w/v *o*-dinitrobenzene in 2-methoxyethanol and 2 ml of 29 per cent. w/w tetraethylammonium hydroxide in methanol were added. The solution was swirled and placed in a 3-ml cell with a 1-cm path length; spectrophotometric readings were made immediately.

2-PHENYLETHANOL PROCEDURE—

One millilitre of sample in 2-phenylethanol and 0·5 ml of 10 per cent. w/v 4-picoline in 2-phenylethanol were heated in a calibrated centrifuge tube for 45 minutes at 100° C in a water-bath. After the solution had been cooled, 0·5 ml of 5 per cent. w/v *o*-dinitrobenzene and 2 ml of 29 per cent. w/w tetraethylammonium hydroxide were added. The solution was swirled and transferred to a 3-ml cell (1-cm path length); readings were made immediately and again after 5 minutes, because lower concentrations apparently react more slowly.

RESULTS AND DISCUSSION

MECHANISM OF REACTION—

The alkylation of 4-picoline produces the *N*-alkylpicolinium cation. The hydrogen atoms on the methyl group of the cation are more reactive than the hydrogen atoms on the methyl group of 4-picoline and can therefore react with various active-hydrogen reagents such as *o*-dinitrobenzene[14,15] or 1,4-naphthoquinone.[16] This general reaction of an *o*- or *p*-dinitro aromatic reagent with a test substance containing active hydrogen has been used previously for the determination of aryl - alkyl and dialkyl ketones containing the $-COCH_2-$ group,[17] compounds containing the cyclopentadiene $-CH_2-$ grouping,[14] and polynitro aromatic compounds.[15] The mechanism for these reactions has been discussed in those papers, but the exact nature of the chromogen is somewhat uncertain.[14,15]

2-METHOXYETHANOL PROCEDURE—

The concentrations of reagents, heating time and temperature were thoroughly studied; the method presented here is the most sensitive, and it produces the slowest-fading chromogen and least-coloured blank solution. Since the resulting coloured solution fades approximately 10 per cent. per 5 minutes, readings must be made immediately. The complex produced obeys Beer's law over the entire optical-density range of the instrument (see Fig. 1).

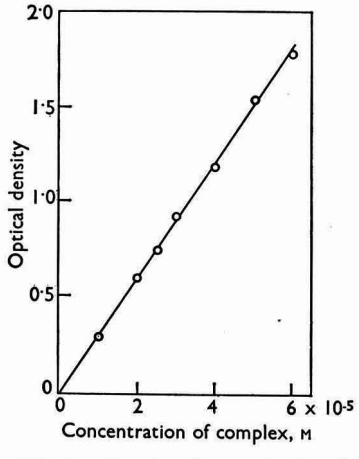

Fig. 1. Beer's Law graph for the determination of 1-iodobutane with 4-picoline and *o*-dinitrobenzene in 2-methyoxyethanol

The blank solution measured against water is slightly coloured, absorbing at 581 mμ. The wavelength maxima shifted towards shorter wavelengths as lower concentrations of tetraethylammonium hydroxide were used. For example, the maximum was at 610 mμ for 17·5 per cent. w/w tetraethylammonium hydroxide in methanol and at 400 mμ for 11·5 per cent. w/w and 6 per cent. w/w tetraethylammonium hydroxide.

The 2-methoxyethanol procedure is fairly sensitive for the alkanesulphonate esters, which are becoming increasingly important as more is learned about their carcinogenic, mutogenic, anti-mitotic and anti-fertility properties.[2,5]

2-PHENYLETHANOL PROCEDURE—

The reaction variables were investigated with 2-phenylethanol as the solvent instead of 2-methoxyethanol. Although the fading rate is approximately the same, a slight increase in optical density sometimes occurred at lower concentrations before fading. The calibration curve is not linear for 1-iodobutane, and the blank solution is slightly darker than that obtained with 2-methoxyethanol. As the percentage of tetraethylammonium hydroxide was changed, a shift to shorter wavelength occurred, as in the 2-methoxyethanol procedure, but was not as pronounced. Two wavelength maxima were found in many instances; the longer maximum (618 mμ) was of higher molecular extinction coefficient than the shorter (519 mμ).

Chloro compounds apparently do not react in 2-phenylethanol; however, a variety of chloro compounds has not yet been tested. The position of the wavelength maximum for the reaction with 1-bromo-octadecane in 2-phenylethanol may be due to the formation of some other type of chromogen.

EVIDENCE FOR AUTOCATALYTIC BEHAVIOUR—

The use of 2-phenylethanol as the solvent appears to produce an autocatalytic effect at 100° C, whereas the previously reported autocatalytic methods for alkylating agents involved higher temperatures (180° C). The autocatalytic effect is suspected in the 2-phenylethanol procedure because of the following evidence—

(*i*) The molecular extinction coefficient increases with decreasing concentration, an increase similar to that reported previously for autocatalytic methods.[1]

(*ii*) Although the wavelength maxima are the same for 1-iodobutane in both solvents, the approximate average molecular extinction coefficient in 2-phenylethanol is three to four times greater than that in 2-methoxyethanol (see Table I).

(*iii*) The molecular extinction coefficients of iodo compounds in 2-phenylethanol are much greater than those of bromo, tosyl and mesyl compounds (see Table II).

TABLE I

COMPARISON OF THE 4-PICOLINE - *o*-DINITROBENZENE METHODS AND LITERATURE METHODS IN THE DETERMINATION OF 1-IODOBUTANE

Procedure	λ_{max}	$\epsilon \times 10^{-3}$	Relative standard deviation	Limit of detection*	Sensitivity†	Time for procedure, minutes
2-Methoxyethanol	618	30	2·03‡	2·21	7·5	35
2-Phenylethanol	618	120§	10·3¶	0·59	29·1	50
4-(4-Nitrobenzyl)pyridine[10]	565	7·3	—	43	2·6	50
4-(4-Nitrobenzyl)pyridine[1] ..	558	120	4·3	0·6	30	5

* Total weight, in micrograms, of 1-iodobutane in test solution to give an optical density of 0·1 in a 1-cm cell.

† Sensitivity $= \dfrac{\epsilon \times 10^{-3}}{\text{Dilution factor}}$

‡ From Beer's law curve.

§ Average over wide range of concentrations.

¶ For $1{\cdot}26 \times 10^{-5}$ M 1-iodobutane.

TABLE II

COMPARISON OF ALKYLATING AGENTS AND METHODS

Compound	2-Methoxyethanol procedure— 10^{-5} M	λ_{max}, mμ	$10^{-3}\epsilon$	2-Phenylethanol procedure— 10^{-5} M	λ_{max}, mμ	$10^{-3}\epsilon$
Iodoform	42·5	612	0·7	1·25	625	130·0
Iodoacetic acid	1·15	625	32·4	1·25	625	120·0
1-Iodo-octadecane	2·18	617	32·2	2·5	622	72·4
1-Bromo-octadecane ..	1·6	620	20·8	2·5	591	12·0
1-Chloro-octadecane ..	13·6	618	0·75	12·5	—	—
Ethyl methanesulphonate ..	5·23	618	33·3	10·3	625	11·4
Ethyl *p*-toluenesulphonate..	1·49	622	30·5	2·5	617	15·2

COMPARISON OF PROCEDURES—

Table I shows that the 2-phenylethanol procedure is four times more sensitive than the 2-methoxyethanol procedure. The 2-methoxyethanol procedure, however, is five times more reproducible, as the relative standard deviations show. The two procedures are also compared in Table II, which shows that the general considerations of the relative reactivity in the previously reported[1] autocatalytic reactions are true for the 2-phenylethanol procedure.

Comparison of these methods with those previously developed for alkylating agents is shown in the determination of 1-iodobutane in Table I. The non-catalytic, 2-methoxyethanol procedure is three times as sensitive as the non-catalytic 4-(4-nitrobenzyl)pyridine procedure,[10] and yields a 20-fold lower determination limit and an absorption maximum further towards the blue. The 2-methoxyethanol procedure is more than twice as reproducible as the previously developed autocatalytic procedures,[1] as indicated by the relative standard deviations, and the Beer's law relationship is linear.

The autocatalytic 2-phenylethanol procedure is comparable to most of the previous autocatalytic methods.[1]

A distinct advantage lies in the possible differentiation of the particular type of alkylating agent by comparison of the results obtained by the two methods. Table II shows significant differences between the non-catalytic and autocatalytic procedures. For example, for the autocatalytic procedure there is (*a*) an increase in molecular extinction coefficient for iodo compounds, (*b*) a decrease in molecular extinction coefficient and a possible shift in the wavelength maximum for the bromo compounds, (*c*) an apparent absence of reaction for the chloro compounds and (*d*) a decrease in molecular extinction coefficient for the tosyl and mesyl compounds, with essentially no change in wavelength maxima.

INTERFERENCES—

The presence of active-hydrogen compounds, such as fluorene, *N*-alkylated alkylaza-heterocyclic hydrocarbons and other active-hydrogen compounds, would constitute interference by their action in the colour-forming step. Since the test is for any compound that is capable of alkylation, there are by definition no positive interferences in the first step of the reaction.

A more complete treatment of interferences and negatives in *N*-alkylation reactions can be found elsewhere.[1] Table II shows that chloro compounds may provide a false negative in the 2-phenylethanol procedure and bromo compounds may affect the wavelength maxima.

ADDITIONAL STUDIES—

Preliminary studies indicate that quinones, especially 1,4-naphthaquinone with cyclohexylamine as the base, and aromatic aldehydes may be substituted for *o*-dinitrobenzene. Substituting 2,6-dibromoquinonechlorimide for *o*-dinitrobenzene and using hydrochloric acid instead of base has been moderately successful.

Pyridine has been used as a reagent for determining methyl chloride in a complicated glass assembly.[18] Simplification and application to alkylating agents in general appears possible.

A specific method for alkyl iodides appears promising. Iodobutane in 2-methoxyethanol was treated successively with aqueous bromine - potassium bromide solution, heat, salicylic acid in acetic acid and phenothiazine in acetic acid. The spectrum of the phenothiazine free-radical[19,20] was obtained in the sample solution with a molecular extinction coefficient of 15,000 at 517 mμ. This reaction may be useful with electron paramagnetic resonance, since a paramagnetic chromogen is produced.

The *N*-alkylation of azaheterocyclic compounds with subsequent spectrophotofluorometric analysis is in need of further study.

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SHORT PAPERS

Polarography of Thallium: Determination of Thallium in Sodium Iodide

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Considerable work has been done[1,2] on the polarography of the thallium(I) ion in different supporting electrolytes. However, very little work on the reduction of thallium(III) has been reported,[3,4] probably because the reduction of thallium(III) coincides with the dissolution of mercury. Obviously, the reduction of thallium(I) to the amalgam state is the most suitable for any analytical purpose.

In the preparation of thallium-activated sodium iodide crystals, a knowledge of the concentration of thallous iodide in various parts of the crystals is often required. A volumetric method, based on the precipitation of thallium and re-dissolution before titration, is known, but a large amount of the sodium iodide sample (10 to 15 g) is required.[5,6]

The present paper deals with the polarographic determination of thallium in sodium iodide.

The choice of the supporting electrolyte depends on the way the sample of sodium iodide is dissolved and the subsequent chemical processing. The supporting electrolyte chosen in this instance is therefore a mixture of sodium sulphate and sulphuric acid. Polarography of thallium in concentrated sulphuric acid (17 M) has been reported.[7] Polarographic work in which sodium sulphate is used as the supporting electrolyte has also been reported,[8] although not very adequately. Since the sample is dissolved in nitric and sulphuric acids, the solution will consist of sodium sulphate and free sulphuric acid. A detailed study of the mixture of sodium sulphate and sulphuric acid as supporting electrolyte was therefore necessary.

Experimental

A Du-Bellay polarograph was used. The temperature was kept constant at 30° ± 0·1° C by means of an electronically operated thermostat.

A conventional H-cell with agar - agar bridge connecting the test solution and the saturated saturated calomel electrode was used.

The value of m and the drop time of the capillary were 1·625 mg per second and 4·8 seconds, respectively.

The sodium sulphate and sulphuric acid used were of analytical-reagent grade.

Results and discussion

Thallium(I) waves in sulphuric acid medium—

The effect of changing the sulphuric acid concentration on the half-wave potential and diffusion current of the thallium(I) ion was investigated. The concentrations of the thallium(I) ion and thymol in the final solution were 15·48 μg per ml and 0·08 per cent., respectively; the results are given below—

Sulphuric acid concentration, per cent. v/v	8	6	4	2	1
Diffusion current, μA	0·29	0·30	0·32	0·34	0·34

The half-wave potential remained constant at −0·45 volt *versus* the saturated calomel electrode. However, the diffusion current showed an increase with decreasing concentration of sulphuric acid: an 8 per cent. decrease in the sulphuric acid concentration increases the diffusion current by 11 per cent. The polarographic curves obtained with a lower concentration of sulphuric acid were better shaped than those with higher concentrations of sulphuric acid.

Thallium(I) waves in sodium sulphate medium—

The effect of changing the sodium sulphate concentration on the half-wave potential and diffusion current of the thallium(I) ion was investigated. The concentration of the thallium(I)

ion and thymol in the final solution were 15·48 μg per ml and 0·08 per cent., respectively; the results are given below—

Sodium sulphate concentration, per cent. w/v ..	4·0	2·0	1·0	0·5	0·25
Diffusion current, μA	0·31	0·31	0·31	0·32	0·32

The changes in diffusion current are less than the changes observed when the concentration of sulphuric acid was varied. The various percentages of sodium sulphate correspond to different sample weights of sodium iodide that would be processed for the determination of thallium content. It is necessary to observe changes, if any, in diffusion current when different sample weights are taken.

THALLIUM(I) WAVES IN MIXTURES OF SULPHURIC ACID AND SODIUM SULPHATE—

The sulphuric acid concentration was varied and the sodium sulphate concentration kept constant, and vice versa. The results are given in Table I. With a higher sodium sulphate concentration (4 per cent.), any changes in the sulphuric acid concentration are without any effect, the diffusion current remaining constant at 0·24 μA. Further, with a 2 per cent. concentration of sodium sulphate, changes in sulphuric acid concentration have no effect, although the value of the diffusion current is different (0·26 μA). In other words, for a fixed concentration of sodium sulphate, any change in the sulphuric acid concentration does not have any effect on the diffusion current, but for a fixed sulphuric acid concentration the reverse is true. This observation is important from the analytical aspect, since in practice it is very difficult to control exactly the sulphuric acid concentration after the fuming. However, since the sample of sodium iodide is weighed, the amount of sodium sulphate is fixed.

TABLE I

CHANGES IN WAVE CHARACTERISTICS WITH CHANGES IN THE MIXTURE OF SODIUM SULPHATE AND SULPHURIC ACID

The concentrations of the thallium(I) ion and thymol in the final solution were 13·27 μg per ml and 0·08 per cent., respectively.

Sodium sulphate concentration, per cent. w/v	Sulphuric acid concentration, per cent. v/v	Half-wave potential ($E_{\frac{1}{2}}$), volts *versus* saturated calomel electrode	Diffusion current (i_d), μA
4	6	−0·45	0·24
4	4	−0·45	0·24
2	6	−0·45	0·26
2	4	−0·45	0·26
2	1	−0·45	0·26

INTERFERENCES

Antimony, copper, bismuth, cadmium and iron are reported to have half-wave potentials near the half-wave potential of thallium, either in sulphuric acid or sodium sulphate supporting electrolyte. The interference of these elements in the supporting electrolyte of sulphuric acid and sodium sulphate was therefore studied. Lead interference is ruled out, because of the sulphuric acid treatment. Copper, bismuth and iron were found not to interfere in the determination of thallium(I) as there were no reduction waves for these elements from −0·1 to −0·7 volt. Cadmium showed a reduction wave, but it follows that of the thallium(I) wave, the half-wave potential being −0·7 volt. If the ratio of cadmium to thallium(I) is much greater than 1, then cadmium might interfere in the determination of thallium(I). However, these considerations do not arise for sodium iodide crystals, in which thallium is an added constituent (0·05 to 0·20 per cent.) and cadmium could be present only as a trace impurity in pure sodium iodide.

TREATMENT OF SODIUM IODIDE SAMPLE—

A known weight of sample (1 to 2 g) was taken in a 400-ml beaker. Owing to its hygroscopic nature, the sample was weighed by difference from a weighing bottle. About 20 ml of water were put into the beaker and 3 ml of nitric acid, sp.gr. 1·42, were added to the solution dropwise and with constant stirring. The solution was evaporated on a sandbath until about 1 ml was left, and

the same treatment was repeated with 3 ml of nitric acid to ensure complete decomposition of the iodide.

Two millilitres of analytical-reagent grade hydrochloric acid, sp.gr. 1·18, were added, the solution evaporated to pastiness, and then fumed twice with 3-ml portions of sulphuric acid to copius fumes. Intermediate treatment with hydrochloric acid was found necessary in order to obtain a clear solution after fuming with sulphuric acid. The beaker was cooled, 20 ml of water added and then 3 ml of a 5 to 6 per cent. aqueous solution of sulphur dioxide added to reduce thallium(III) to thallium(I). Any excess of sulphur dioxide was removed by heating the solution on a sand-bath and reducing the volume to half. The solution was then diluted to 25 or 50 ml, depending on whether the weight of the sample was 1 or 2 g.

Exactly similar treatment was given to a solution containing a weighed amount of high-purity grade thallous iodide (obtainable from Semi Element Inc., U.S.A.) and the linearity of diffusion current with concentration was tested by using 4 per cent. sodium sulphate with about 0·5 ml of sulphuric acid in 25 ml of solution as the supporting electrolyte.

TABLE II

DETERMINATION OF THALLIUM IN SODIUM IODIDE

Sample	Weight, g	Thallium added, per cent.	Thallium recovered or found, per cent.
Pure sodium iodide	2·0000	0·200	0·200
Pure sodium iodide	2·0000	0·100	0·102
Pure sodium iodide	2·0000	0·050	0·049
Thallium-activated sodium iodide	1·6098	—	0·042*
Thallium-activated sodium iodide	1·0803	—	0·210†
Thallium-activated sodium iodide	Various; from 0·8210 to 3·6576	—	0·29, 0·29, 0·32 0·29, 0·28, 0·28, 0·28, 0·25‡

* Volumetrically determined value, 0·042 per cent.
† Volumetrically determined value, 0·195 per cent.
‡ Mean value, 0·285 per cent.; standard deviation (inclusive of errors due to absorption of moisture by sodium iodide in successive weighings), 0·019 per cent.

Recovery of thallium was tested by adding a weighed amount of thallium iodide solution of appropriate concentration to an extra-pure sodium iodide sample. The samples were treated as outlined above and the wave heights of thallium thus obtained were compared with the wave heights obtained by adding the same amount of thallium, as nitrate, to the equivalent amounts of sodium sulphate and sulphuric acid supporting electrolyte. The results are given in Table II. Some of the values for the samples were also tested by the volumetric method.

One of the samples was selected for statistical analysis. The standard deviation obtained was 0·019 per cent. for a sample of mean thallium(I) content of 0·285 per cent. The error due to moisture absorption during repeated weighings (eight times) is included in these results.

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A Rapid Method for Separating and Determining DDT in Fat

By J. H. P. DINGLE
(*Department of Agriculture, Board of Tick Control, Box C285, Lismore, N.S.W., Australia*)

Glass chromatographic columns, 42 × 4·5 cm, are packed as given below—

Attach the tube at the bottom of the column to a vacuum line, place the open end of the column into a container of Celite, turn on vacuum to suck the Celite up into the column. With the aid of a rubber stopper on the end of a rod, firmly pack 6 inches of Celite into the column; this method produces a firmly packed column quickly and cleanly. Then place the column on a filter flask connected to a vacuum line. Next add 70 ml of sulphuric acid, sp.gr. 1·84, to the column and suck it into place.

The fat sample is treated as follows—

If the determination is requested on a dry basis, melt the fat and weigh 10 g of melted fat into a 200-ml squat beaker. Add 20 ml of carbon tetrachloride and 10 ml of sulphuric acid to the beaker and thoroughly mix whilst warm or warmed, if necessary, to facilitate mixing. To this mixture add 5 gm of sodium sulphate and Celite until the mixture becomes grainy, this will fill about three-quarters of the beaker. Then wash this mixture into the top of the Celite in the column, which has already been packed and is now mounted on a 1-litre filter flask connected to a vacuum line, with light petroleum. Collect at least 200 ml of light-petroleum elutate in a 200-ml squat beaker. Blow-off the solvent with an electric fan in a wind tunnel. The DDT is now sufficiently free from interfering substances to permit its determination by any of the well known methods such as paper chromatography, gas chromatography, a colorimetric method or determination by infrared spectroscopy at 777 cm^{-1}.

This method of separating DDT from fats is superior to the original Davidow method in that it will handle 10 gm of fat and can be made, with small modification, to handle as much as 50 gm of fat, whereas the original Davidow column would not handle more than 5 gm. It is much quicker and cleaner than the original Davidow[1] method, as 20 complete determinations can be made in 1 day. The method gives a better recovery with less loss than the objectionable aceto-nitrile or dimethyl formamide clean-ups, which are more costly. Recoveries are better than 98 per cent., leaving DDT virtually free from interfering matter, so that its determination is easily carried out by colorimetry, gas chromatography, paper chromatography, or infrared spectroscopy, but it is not quite good enough for a direct determination by ultraviolet spectroscopy. We now use infrared spectroscopy.

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Received *March 16th*, 1965

Determination of Zirconium (and Hafnium) in Niobium and Other Metals with Catechol Violet: A Note

By D. F. Wood and J. T. Jones
(*Imperial Metal Industries (Kynoch) Limited, Kynoch Works, Witton, Birmingham* 6)

The authors have received numerous enquiries regarding the source of supply of the tri-n-octyl-phosphine oxide used in the procedure they described[1] for extracting zirconium (and hafnium) ions, and were unaware of certain difficulties when the paper was submitted for publication.

One laboratory has reported that their analysts failed to obtain any development of colour when attempting to prepare a calibration graph according to our description. We have since demonstrated that this was due to the use of an unsuitable grade of tri-n-octylphosphine oxide.

For the guidance of other analysts who may experience similar difficulties, the tri-n-octyl-phosphine oxide used by the authors was supplied by Kodak Ltd., Kirkby, Liverpool, and this particular reagent, which is manufactured by their American associates, Distillation Products Industries, has always proved satisfactory.

It is possible, however, that some analysts may have had no difficulty with other supplies of this reagent.

Reference

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Received *July 1st*, 1965

Book Reviews

ANALYSIS INSTRUMENTATION—1964. Edited by L. FOWLER, R. J. HARMON and D. K. ROE. Pp. x + 340. New York: Plenum Press. 1964. Price $14.50.

This volume represents a selected portion of the papers presented at the 10th Annual Symposium on Analysis Instrumentation organised by the Instrument Society of America in June, 1964. It is therefore in the hands of the public within nine months of the date of the meeting. In order to achieve this commendable objective it has been produced by a photo-offset process at approximately 2/3 reduction from double-column elite typescript. The production is excellent and diagrams and photographs are very well reproduced.

The desirability of publication of conference proceedings as a separate book is questionable. It may be commercially profitable, and for sharply defined, one-subject meetings it is sometimes an advantage to have the papers collected in one volume. On the other hand, it adds to the troubles of the scientist struggling against the overwhelming flood of literature. He is not usually able to afford to purchase such publications for private use. If he attends the conferences and receives a copy of the proceedings, this has increased the cost of registration to such a point as to discourage attendance. Someone has had his work severely interrupted by the burden of editing. It is an excessively expensive method of publication, and the publication delay is usually at least twice and often as much as five times as long as in the normal process of publication in a recognised journal. It has been said, with reason, that there are too many journals. There are also too many conferences, and to publish separate proceedings of these aggravates an already serious situation. Surely if the papers are worth publishing they can be submitted to the refereeing system of the acknowledged journals in the normal way, and can appear in the permanent literature.

The present volume has appeared quickly enough to be useful. It contains 28 papers grouped under eight diverse headings, but having the common ground of a more or less intimate connection with process control or industrial applications. As usual in such publications the quality and importance of the contributions is variable: some are lengthy and weighty, others are ephemeral in the extreme, and there is rather a lot of trivial matter which would have been weeded out by referee attenuation under normal publication procedure. Nevertheless, this volume will find a place in the larger industrial libraries and may generate useful ideas among process control and plant chemists. E. BISHOP

PROGRESS IN INFRARED SPECTROSCOPY. Volume 2. Edited by HERMAN A. SZYMANSKI. Pp. iv + 298. New York: Plenum Press. 1964. Price $12.50.

It is usual to publish in book form the proceedings of conferences and this volume continues the practice, presenting a collection of review articles based upon papers invited for the Sixth and Seventh Institutes in Buffalo in 1962 and 1963.

While needless proliferation of scientific literature is now deplored in general terms, such publications may attract the enthusiasm of new participants to the field, as well as acquainting established workers with topics that have not been their special preoccupation and should thus not be too hardly judged. It is in these terms that the present volume should be regarded.

The first rate review of infrared spectra of crystals would find a worthy place in a more theoretical treatise and yet bears the stamp of a real attempt to interpret this subject for those new to it. Polarised infrared spectroscopy is similarly well treated as is the much neglected field of the near infrared.

Discussion of arsenic trichloride as a solvent will be of interest, but only to specialists. The data correlations that abound in the organic field are here added to by useful contributions of their type on organophosphorus compounds and organometallic spectra. There is a good review of the spectra of biological materials with a well arranged bibliography.

The two articles on ultraviolet and visible spectra have nothing to do with the infrared field and their style is more appropriate to the bibliography in an admittedly well kept laboratory notebook.

It is to be regretted that the very high quality of printing and layout has not been matched in the planning of material to be included. This is a volume to which many will wish to refer, but few can have such a diversity of interests that they will wish to posses it. R. C. SEYMOUR

INTERPRETED INFRARED SPECTRA. Volume 1. By HERMAN A. SZYMANSKI. Pp. viii + 293. New York: Plenum Press. 1964. Price $10.75.

The object of this work, as given on the cover, is to help the reader to interpret infrared spectra with the object of identification of compounds, and for this purpose the author has assembled a considerable amount of information. The value of some of the information, *e.g.*, tables of absorption bands for methane, ethane propane, etc., for readers interested in identification seems doubtful, and one feels that greater care should have been exercised in the presentation of the data.

The volume has sections entitled Alkanes, Alkenes, Benzene ring compounds, Cyclopropane, Cyclobutadiene, Cyclopentane and Cyclohexane derivatives. It seems doubtful whether or not compounds other than hydrocarbons should be referred to in the text of the first two sections, and compounds like ethylene oxide and tetrahydrofuran can hardly be considered as cyclopropane or cyclopentane derivatives, however desirable this may appear from the spectroscopy standpoint.

Each section is sub-divided under the headings Vibrational analysis, Correlation tables and Discussion of spectra; the last heading refers to spectra that follow on subsequent pages. This discussion of spectra would be more useful if the comments were printed as captions under the appropriate spectra and thus the necessity for reference back from spectra to text would be avoided. The spectra themselves show a certain lack of uniformity in presentation and whereas the text deals with compounds other than hydrocarbons the spectra are exclusively those of hydrocarbons; these are not necessarily typical of compounds substituted with more polar groups. A wider choice of spectra in the last five sections would have served the purpose of illustrating the limitations of infrared correlations as well as their advantages.

To summarise, one feels that this book could be of some value in restricted fields where the type of compound may be suspected or known, but with entirely unknown compounds, application of the spectroscopic correlations without appreciation of limitations of these will only produce a structural formula that may prove a surprise for many preparative chemists.

A. HAMER

RADIOISOTOPES AND THEIR INDUSTRIAL APPLICATIONS. By H. PIRAUX. Pp. xiv + 614. Netherlands: Philips Technical Library. Distributed in the U.K. and Eire by Cleaver-Hume Press Ltd. (Macmillan & Co. Ltd.), London. 1964. Price 72s.

The text of this book has obviously suffered in translation from the original, since in places the language is stilted, in others the exact meaning is difficult to decipher, and occasionally it is positively misleading, although some of the discrepancies could be attributed to faulty proof reading. The early chapters might have been better if condensed and presented in appendices, as the industrial reader is likely to be more interested in the applications than the mechanisms of radioactivity, whereas the scientific reader is already familiar with the latter. A further point here is that although the chapters on biological effects, radiological protection and working conditions form a useful general guide, they are not entirely applicable in British industry, which must comply with the Ionizing Radiations Regulations.

However, once the early chapters are past, the second half of the book makes interesting reading, giving a comprehensive survey of the industrial applications of radioisotopes. Although this survey is at times haphazard and shows some bias towards those applications with which the author is presumably more familiar, it is nonetheless useful, as it gives a picture of the applications in use on the Continent.

There are two main weaknesses in the book. Firstly, the author claims an extensive bibliography, which, in fact, amounts to less than 70 references. In one field alone, activation analysis, over 1000 publications including five books are available, but not one is cited. Secondly, the chapter on instrumentation reads rather like an advertising magazine, dealing with virtually only one brand of equipment, and gives a totally distorted picture of the available instruments. Perhaps this is understandable in a book published on behalf of an instrument manufacturer. It is a pity that the author did not take the opportunity to broaden the bases of these two aspects at this stage.

The most serious criticism of the book, however, lies in the colour supplement at the end. In the author's own words—

"Some industrialists, obsessed by the idea of danger which they usually overestimate, hesitate to adopt new methods,"

but despite this, the death process of a worker involved in a *nuclear incident* at Los Alamos is vividly illustrated, although—

"such massive doses are never present in industrial applications."

The publication of these pictures, particularly in association with a book of this nature, can only increase the apprehension of industry and of the public in general with regard to radioactivity.

Priced at 72s., and with several alternative books available, this can hardly be termed a "best buy," but it is a useful book for the reference library. D. GIBBONS

GOLD: RECOVERY, PROPERTIES, AND APPLICATIONS. Edited by EDMUND M. WISE. Pp. x + 367. Princeton (New Jersey), Toronto, New York and London: D. Van Nostrand Company Inc. 1965. Price 93s.

As its title implies, this book deals with gold from a very wide variety of aspects, giving comprehensive accounts of sources and recovery, physical, chemical, and metallurgical properties, and applications. The scope is typified by citing some chapter headings, which include Inorganic Chemistry of Gold, Organogold Complexes, Gold Alloy Systems, Gold in Medicine, Gold Brazing Alloys or Solders, Gold in Glass, and Gold in Electron Tubes and Transistors.

There are included an introductory chapter on Analytical Methods and chapters on The Determination of Gold and Spectrographic Determination of Trace Impurities in Gold. The classical methods of fire-asaying are not included, though the relevant references are given, but other methods for determining gold, *e.g.*, gravimetric, titrimetric and colorimetric, are dealt with critically and in some detail with an adequate bibliography of 79 references. Techniques for the spectrographic determination of trace impurities in gold are fully described; suitable line pairs and typical working curves are given.

For anyone interested in gold, this book provides a fund of information. The analyst who consults it may very well be tempted to read very much further than the relatively small section dealing with analytical methods. F. M. LEVER

FUSED SALTS. Edited by BENSON ROSS SUNDHEIM. Pp. x + 435. New York, San Francisco, Toronto and London: McGraw-Hill Book Company. 1964. Price 148s. 6d.; $18.50.

In a sense, the study of molten salts has come of age. After a long and erratic development, it has shed many of its experimental hazards and difficulties and there is now an extensive literature covering a wide range of measurements, especially in the temperature range 150° to 950° C. Within these limits there are many stable inorganic salts that fuse to form clear, colourless liquids and that are excellent solvents. They may safely be contained in glass or silica tubes and, unless strict temperature control is required, any simple form of heating will suffice.

Such has been the rate of progress in this field that already this book takes much of the elementary material for granted and is concerned to review several established physicochemical aspects of the subject. There is a vigorous and stimulating chapter by Bloom and Bockris on Structural Aspects of Ionic Liquids and a clear and authoritative account of their Thermodynamic Properties by Førland. There is then an account of Transport Properties by Sundheim, which, as expected, involves some heavy-weight encounters with the formalism of irreversible thermodynamics. These three chapters constitute the larger part of the book.

There follows several more specialised accounts of Electrochemistry (Laitinen and Osteryoung), Spectroscopy (Gruen), Solutions of Metals in Melts (Corbett) and Reaction Kinetics (Duke). Each of these chapters is excellent in its own way and reflects the continuing researches in these fields by the various authors.

Within its own terms of reference the book is therefore to be strongly recommended. It appeared shortly after a similar book, "Molten Salt Chemistry," edited by M. Blander, and together they represent necessary and stimulating reading for anyone investigating the *physical* properties of these systems.

Neither book has much time for the simpler facts pertaining to these systems and their inorganic, preparative and analytical chemistry has yet to be exploited and recorded. The dominance of the physical chemists no doubt stems from the instrumental procedures involved in many of the early studies and from the long existing electrochemical processes underlying the electrowinning of such metals as aluminium and now uranium, titanium and tantalum. The full benefits of this technology (N.B. the new fused-salt reactor at Oak Ridge) are yet to come and this may be even more true of purely chemical aspects. The unusual oxidation states and the absence of solvolysis reactions *per se* suggest new procedures and new products that this book does not hint at. There may well be a similar future for organic reactions.

However, this book is concerned less with chemical reactions than with physical interactions. Its value lies in its clear and authoritative account of the simpler molten-salt systems that already exist. It is necessary reading for those who are contemplating research in the field; it is enjoyable and not too arduous for those who are not. G. J. HILLS

ELECTRONS AND CHEMICAL BONDING. By HARRY B. GRAY. Pp. xvi + 223. New York and Amsterdam: W. A. Benjamin Inc. 1964. Price (cloth) $8.80; (paper) $4.35.

The stated aim of "Electrons and Chemical Bonding" is to provide an introduction to modern theories of chemical bonding for undergraduate students of chemistry. The introductory chapter entitled Electrons in Atoms, starts with a brief presentation of the Bohr theory of the hydrogen atom, followed by a very sketchy account of the Schrödinger wave equation. The only explanation given of the term "Hamiltonian Operator" is that "it represents the general form of the kinetic and potential energies of the system," and it is not made clear that the wave equation has an infinite number of solutions, of which only some (the eigenfunctions) are acceptable, and which correspond to the allowed energies of the system. The chapter does, however, contain good drawings of the boundary surfaces of the *s*-, *p*-, *d*-, and, unusually, *f*-orbitals, and there is a very lucid explanation of how the terms arising from a particular electronic configuration in the Russell - Saunders coupling scheme are derived.

Succeeding chapters discuss the bonding in various classes of molecules, starting with hydrogen and proceeding through homonuclear and heteronuclear diatomic, linear triatomic, trigonal planar, tetrahedral, trigonal pyramidal and angular triatomic molecules. A unique feature of the book is that it adopts the molecular-orbital approach from the start, and uses it to describe the bonding not only in diatomic molecules, but in the more complicated molecules as well. Comparisons are made in each chapter with the valence-bond descriptions of the molecules. Surprisingly, in view of the title of the book, there is remarkably little discussion of the nature of the chemical bond in terms of the balance between nuclear-electronic attractions and internuclear and inter-electronic repulsions; atomic orbitals are linearly combined to form bonding and antibonding molecular orbitals, and no further questions are asked until a belated final message informs us that, after all "we have only provided a workable language for the laws of chemical bonding, not a theory which gives an exact accounting of the forces that hold atoms together." Students of the book are assumed to know no group theory, though symmetry principles are used in finding the correct combination of atomic orbitals for forming molecular orbitals. Despite the success of molecular-orbital theory in rationalising, for example, the paramagnetism of the oxygen molecule, one wonders whether the construction of qualitative molecular-orbital energy-level diagrams for molecules such as BF_3, CH_4 and NH_3 really adds anything to a student's understanding of them, or has any advantage over the pictorial simplicity of the valence-bond method. No indication is given of the accuracy of the placing of the molecular-orbital energy levels in the diagrams. The chapter on organic molecules contains little that is not done better and more fully elsewhere, but the final chapter on bonds involving *d*-orbitals contains a useful, brief account of the calculation of ligand-field splitting parameters (10Dq) from the observed spectra of transition-metal complexes. This chapter also contains some references to original literature, whereas the other chapters list general review references only. A useful feature of the book is the extensive listing of bond lengths, dissociation energies, spectroscopic states and dipole moments of the molecules discussed, taken from sources such as Sutton, Cottrell and Herzberg. Each chapter contains worked and supplementary problems.

For about the same price as the paper-back edition, and less than half the price of the stiff-cover edition, the undergraduate student can buy Coulson's "Valence," which will give him a better understanding of electrons and chemical bonding. Professor Gray's book is no substitute for this, but it does have value in showing how molecular-orbital theory can be used to describe the bonding in various molecules. M. A. BENNETT

Errata

AUGUST (1964) ISSUE, p. 553, 14th line from foot of page. *For* "2,2′-iminodipropionitrile" *read* "3,3′-iminodipropionitrile".

IBID., p. 555, 10th line. *For* "2,2′-iminodipropionitrile" read "3,3′-iminodipropionitrile".

MARCH (1965) ISSUE, p. 174, 1st line under heading "DETERMINATION OF MOLYBDENUM IN ALLOYS." *After* "molybdenum in" *insert* "4 ml of concentrated hydrofluoric acid and".

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ANALYST

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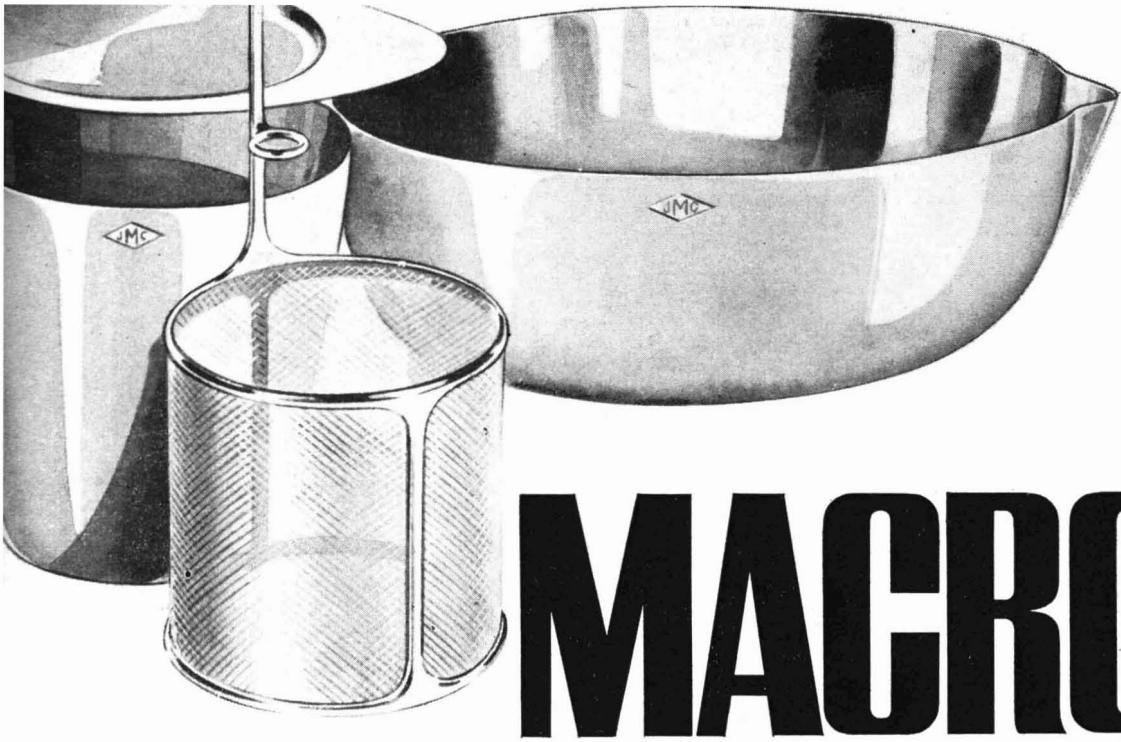

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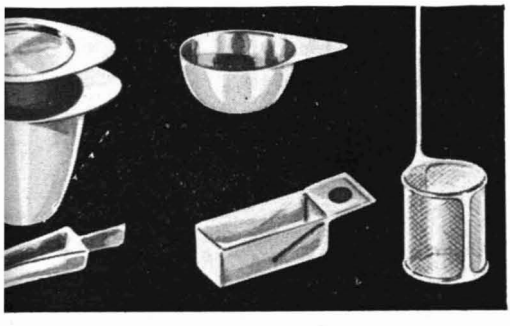

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