The ANALYST

The Journal of the Society for Analytical Chemistry: a monthly publication dealing with all branches of analytical chemistry

Published for the Society by
W. HEFFER & SONS LTD., CAMBRIDGE

Volume 88

No. 1050, Pages 659-742

September 1963

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Volume 88, No. 1050 September, 1963

THE ANALYST

Report of the Analytical Methods Committee 1961/1962

THIS seventh Report of the Analytical Methods Committee of The Society for Analytical Chemistry reviews the progress of work during the two years 1961 and 1962, covering the period January 1st, 1961, to December 31st, 1962.

GENERAL REVIEW

During 1961, the Committee was sorry to lose Dr. J. Haslam, who resigned on his retirement from Imperial Chemical Industries Ltd.; he had been a valuable member of the Committee for many years.

The Committee was also sorry to lose, through resignation to take up another appointment, the services of Miss A. M. Parry in November, 1961. Miss Parry had been Assistant Secretary to the Committee since January, 1956; she was succeeded, in November, 1962, by Miss V. Lewis.

PROGRESS OF WORK—

The Committee can once again report steady progress in its work. Some projects have been completed and the Sub-Committees and Panels, having prepared their Reports, which will be published during the coming year, have been disbanded. Two new committees were appointed during 1961. One of these is a Sub-Committee of the Analytical Methods Committee for investigating methods for Particle Size Analysis. This Sub-Committee had

its first meeting in March, 1961, and, after nearly 2 years of vigorous work, it has prepared a comprehensive classification of methods that, it is hoped, will prove useful to chemists and physicists new to the subject and who require guidance on how to set about their task of finding suitable methods for their own particular types of materials and fields of application. This classification is being published in the March, 1963, issue of *The Analyst*. The other committee to be appointed is a new Panel of the Joint Committee of the Society (as represented by the Analytical Methods Committee) and The Pharmaceutical Society on Methods of Assay of Crude Drugs. This Panel, the seventh to be appointed by the Joint Committee, is investigating chemical methods for assaying the biologically active constituents of thyroid. An exploratory meeting was held on the subject in December, 1961, and the Panel had its first meeting in February, 1962. Unlike most sub-committees and panels of the A.M.C., which are commissioned to examine published or known methods of assay with a view to ensuring their applicability and reproducibility for reference purposes, this Panel has the more difficult task of devising a method practically *de novo*; for this purpose, its investigations are more in the nature of research on a collaborative basis.

The other existing Sub-Committees of the A.M.C. and Panels of the Joint Committee have, on the whole, been actively at work during the period under review. One of the Joint Committee's Panels—that dealing with the investigation of chemical methods for anthraquinone drugs (senna, cascara, etc.)—has resumed work again having been in suspension for nearly 2 years pending a parallel investigation on biological methods by an associated Panel (Panel 3A). Panel 3A has now unified a biological technique for senna fruit that can be used as a form of "yardstick" for correlating chemical methods with purgative activity in mice.

Another Panel of the Joint Committee—that on methods for lonchocarpus and derris (Panel 5)—completed its programme of work at the end of 1961 and its second Report, on the Colorimetric Determination of Rotenone, was published in *The Analyst* in November of that year. This Panel has now been disbanded.

The 1960 Report of the A.M.C. presented rather a gloomy picture with regard to the difficulties encountered by the Additives in Animal Feeding Stuffs Sub-Committee in its endeavour to apply known methods of analysis to animal and poultry feeds. It says much for all the members of the five Panels of that Sub-Committee that during the past two years these difficulties have been largely resolved, with the result that four of these Panels have completed their programmes and have been disbanded, and their Reports are to be published shortly. They include methods of assay for antibiotics (penicillin, chlortetracycline and oxytetracycline), for synthetic hormones (stilboestrol and hexoestrol) and for a number of vitamins (vitamin A and β-carotene, of the fat-soluble types, and vitamin B_{12}, nicotinic acid, pantothenic acid and riboflavin, of the water-soluble group of B-vitamins). Only the methods for two of the B-vitamins—choline and pyridoxin—have proved to be somewhat intractable, and it became evident that these would require lengthy and specialised investigation; some of the problems encountered are described later (see p. 665).

The fifth Panel of this Sub-Committee—that on prophylactics—has completed its work on one item, nitrofurazone, and has prepared its Report, which will be published with those on the other additives. In view of this Panel's lengthy programme, it has been decided that it should be re-organised as a Sub-Committee in its own right directly under the aegis of the A.M.C. This arrangement has enabled the Additives Sub-Committee to wind up its commitments, and it has been disbanded.

Associated with the Additives Sub-Committee is another Sub-Committee that dealt, independently, with methods for determining trace elements in fertilisers and feeding stuffs. This latter committee started its work in 1957—about a year and a half before that on additives—and it has now completed its lengthy programme of work; the collected methods for about fourteen elements are to be published early in 1963 by the Society as a separate booklet, since it is envisaged that they will be capable of application to products other than fertilisers and feeding stuffs.

Another Sub-Committee to complete part of its programme is that on Metallic Impurities in Organic Matter. In 1960, it published two Reports, on Small Amounts of Arsenic and on Methods for the Destruction of Organic Matter; during 1961, the work on a method for copper was completed and the Report is being published in the April, 1963, issue of *The Analyst*. Meanwhile, the Sub-Committee has continued with its programme and has been con-

sidering methods for zinc and mercury; after examining its specificity in the presence of certain other metals that react with dithizone, the Sub-Committee can recommend the method for zinc adopted by the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee. The determination of very small amounts of mercury in organic matter has presented a problem to analysts for a long time, since the removal of the organic matter can frequently lead to a significant loss of the metal. It is possible that radiochemical procedures (particularly isotope dilution), which are rapidly increasing in popularity and becoming more available to smaller laboratories, might be used as the basis of a reference method for mercury, and the Sub-Committee has been considering this possibility. However, because some laboratories may not have the necessary facilities for some time, the Sub-Committee thinks that a chemical method should be investigated in the first place, and collaborative work is proceeding on a method that appears to be promising and that is sensitive for measuring amounts down to half a microgram. During this collaborative work, use is being made of radiochemical techniques for monitoring the various stages during the wet-combustion procedure to check that there is no loss of mercury.

Two Reports were published in the September, 1961, issue of *The Analyst* by the Meat Products Sub-Committee. The first Report, on Nitrogen Factors for Pork, presents a vast amount of evidence from results collected from laboratories in the United Kingdom and in Europe to show that the hitherto-accepted factor of 3·6 for the conversion of meat nitrogen into meat content was too high for pork. This had meant that in the past manufacturers of pork products often had to put in more meat than necessary to ensure that analysis of their products would give results within the statutory limits; the factor of 3·45, as recommended in the Report, appears to be the best compromise on the evidence of the results presented, and it has been adopted for use by the Association of Public Analysts and by the meat trade. The second Report is a short one dealing with the Nitrogen Content of Rusk Filler. Since rusk filler is used in the manufacture of sausages and some other meat products, it was considered necessary to revise the value for its nitrogen content in view of changes that have occurred in milling practice since the second World War. The Sub-Committee is continuing its programme of work by collecting values for the nitrogen contents of beef and of chicken meat, in a similar manner to that carried out for pork. Its Report on the Nitrogen Factors for Beef has been completed and will be published in 1963, and its Report on chicken meat is being prepared.

The Chlorine in Organic Compounds Sub-Committee completed its programme of work during 1962, and its Report on the application of the oxygen-flask combustion method to the determination of pesticides containing organically-bound chlorine will be published in 1963.

It was reported in 1960 that two more Panels (set up jointly by The Society for Analytical Chemistry, the Association of British Manufacturers of Agricultural Chemicals, and the Scientific Sub-Committee of the Interdepartmental Advisory Committee on Poisonous Substances used in Agriculture and Food Storage) had been set up to study methods for determining malathion and organo-mercury residues. The Report on malathion was published in *The Analyst* at the end of 1960, and that on organo-mercury appeared in September, 1961. Another Panel set up in 1960 was that on demeton-methyl residues in fruits and vegetables; its Report was published in *The Analyst* in June, 1962.

The Joint Committee of The Pharmaceutical Society and The Society for Analytical Chemistry (represented by the Analytical Methods Committee) has continued its work during the past 2 years and, as mentioned earlier, the Panel on Lonchocarpus and Derris has been disbanded on completion and publication of its second Report. Also, as mentioned earlier, the new Panel on Thyroid (Panel 7) has now been at work for nearly a year. The Panel engaged on methods for the chemical assay of the Capsaicin Content of Capsicum and its Preparation has completed its programme of work and is preparing its second Report, the first being published in 1959. The first Report included recommended methods for the assay of the drug and for three official preparations (oleoresin, tincture and ointment). Since then, experience of the methods has revealed the need for some revisions and these are included in the second Report, together with a simpler method for ointment; a more practicable colorimetric method, involving use of Gibb's phenol reagent, has been devised for end-determination (the spectrophotometric difference method remaining the method of choice) and is also included.

Other Sub-Committees of the A.M.C. and Panels of the Joint Committee have continued with their programmes of work. Details of individual committees—their personnel and reports of work—will be found below.

S.A.C. SCHOLARSHIP—

Dr. J. H. Stevenson was appointed in October, 1960, to carry out investigation into bio-assay methods for determining pesticide residues, at Rothamsted Experimental Station. He was unable to continue his work for a second year, and his results are now awaiting publication.

ANALYTICAL METHODS TRUST—

The second of the three-year periods for the receipt of promised subscriptions to the Trust Fund ended in 1960 and, as in 1958, an Appeal to Industry to continue its financial aid of the work of the Analytical Methods Committee was made in June, 1961. The response to this appeal was extremely gratifying; 76 organisations promised donations, some of which were by Deed of Covenant for 7 years, others for 3 years and some others for 1961 only. This represented an increase of 51 over the number in 1960 and showed the wider response to the appeal.

The total amount received for 1961 was £5,665, and firm promises totalling £3,646 were made for each of the years 1962 and 1963.

ANNUAL ACCOUNTS—

The audited statements of accounts for the two financial years ending October 31st, 1961, and October 31st, 1962, are shown in Appendixes I and II, respectively.

REPORTS OF SUB-COMMITTEES OF THE ANALYTICAL METHODS COMMITTEE

PUBLICATIONS SUB-COMMITTEE

CONSTITUTION—

D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C. (*Chairman*)	*Chairman, Analytical Methods Committee*
J. B. Attrill, M.A., F.R.I.C.	*Editor*, The Analyst
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Member, Analytical Methods Committee*
D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.	*Member, Analytical Methods Committee*
H. N. Wilson, F.R.I.C.*	*Member, Analytical Methods Committee*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C. (*Secretary and Editor*)	*Editor, Scientific Publications, The Pharmaceutical Society of Great Britain*

* Resigned July, 1962.

PROGRESS OF WORK—

Much progress has been made in the collection and editing of all those recommended methods of analysis published since 1926 by the Analytical Methods Committee in its various Reports. These collected methods were published in March, 1963, under the title "Official, Standardised and Recommended Methods of Analysis," and included a revised and expanded version of the Bibliography of Standard and Recommended Methods originally published in 1951.

ADDITIVES IN ANIMAL FEEDING-STUFFS SUB-COMMITTEE

CONSTITUTION—

D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C. (*Chairman*)	*Boots Pure Drug Co. Ltd.*
A. J. Amos, O.B.E., B.Sc., Ph.D., F.R.I.C.	*Analytical and Consulting Chemist*
W. T. Barker*	*Ministry of Agriculture, Fisheries and Food*
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Public Analyst, Official Agricultural Analyst and Consulting Chemist*
R. F. Phipers, B.Sc., Ph.D.	*Cooper Technical Bureau*
S. A. Price, B.Sc., F.R.I.C.	*Vitamins Ltd.*
C. J. Regan, B.Sc., F.R.I.C.	Formerly *Chemist-in-Chief, London County Council*
W. L. Sheppard, F.R.I.C.	*Boots Pure Drug Co. Ltd.* (Formerly with *Unilever Ltd.*)
R. E. Stuckey, Ph.D., D.Sc., F.P.S., F.R.I.C.	*British Drug Houses Ltd.*
F. R. Williams†	*Ministry of Agriculture, Fisheries and Food*
Miss C. H. Tinker (*Secretary*)	

* Appointed May, 1962. † Resigned May, 1962.

TERMS OF REFERENCE—"To investigate and prepare methods for determining the amounts of additives (nutrients, stimulants and prophylactics) in animal and poultry feeding stuffs."

PROGRESS OF WORK—

Although the task was expected to prove difficult when originally undertaken by the Sub-Committee, the efforts of its Panels have led to a resolution of most of the problems. The Antibiotics, Hormones and the Vitamins (fat-soluble) Panels have completed their programmes; the Vitamins (water-soluble) Panel has completed its work on all but two of the vitamins listed in its programme. The various Reports by both these groups, giving recommended methods of assay, are being submitted for publication. The Prophylactics Panel has completed its Report on Nitrofurazone.

Since the remaining projects require a considerable amount of further investigation, either collaboratively or by individual research, it was decided to disband the Sub-Committee, which had acted purely in a steering and advistory capacity for all the Panels. This has now been done, and the opportunity is taken here to thank the members, not only of the Sub-Committee itself, but of all the Panels, for the vast amount of hard work that has been so willingly undertaken. Only the Prophylactics Panel survives to carry on its programme of work, and, to allow of its re-organisation and broadening of scope, it has been decided to reconstitute it as a Sub-Committee of the Analytical Methods Committee in its own right.

During the work of the Additives Sub-Committee it has become apparent that, in some instances, wide tolerances on the assay must be allowed; an unrealistic amount of extra work would be necessary to increase the accuracy. Throughout the work, the problem of sampling has been apparent and the Sub-Committee emphasises that the stated accuracy of a recommended method can be attained only if the analyst's sample is properly prepared; problems involved in sampling the bulk material are, however, not within the Sub-Committee's province.

ANTIBIOTICS PANEL

CONSTITUTION—

S. A. Price, B.Sc., F.R.I.C. (*Chairman*)	*Vitamins Ltd.*
A. J. Cavell, M.Sc., A.R.C.S., D.I.C., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service*
Mrs. J. Gammon, B.Sc.	Formerly *Cyanamid of Great Britain Ltd., Lederle Laboratories*
O. Hughes	*Pfizer Ltd.*
W. P. Jones, F.P.S., F.R.I.C.	*Cyanamid of Great Britain Ltd., Lederle Laboratories*
G. Sykes, M.Sc., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
J. H. Taylor, Ph.D., M.R.C.V.S.	*Cyanamid of Great Britain Ltd., Agricultural Division*
S. Varsanyi, A.I.S.T.	*Glaxo Laboratories Ltd.*
Miss A. M. Parry (*Secretary*)	

PROGRESS OF WORK—

The Panel has completed its programme of work and its Report will be published in 1963. Methods for the three permitted antibiotics (penicillin, chlortetracycline and oxytetracycline) can now be recommended for the assay of feeding stuffs, with the limitation that the identity of the antiobiotic is known and also that only *one* antibiotic is present.

Both supplements and supplemented feeding stuffs are covered. Because of the higher levels of antibiotics in the former (a few grams per pound), chemical methods of assay are recommended; for the much lower levels in feeds (a few grams per ton), microbiological methods are required.

HORMONES PANEL

CONSTITUTION—

R. E. Stuckey, Ph.D., D.Sc., F.P.S., F.R.I.C. (*Chairman*)	*British Drug Houses Ltd.*
J. Allen, A.R.I.C.	*British Drug Houses Ltd.*
L. Brealey, B.Sc., F.R.I.C.	Formerly *Boots Pure Drug Co. Ltd.*
J. A. Potter, A.R.I.C.	*Analytical and Consulting Chemist*
W. L. Sheppard, F.R.I.C.	Formerly *Unilever Ltd.*
Miss A. M. Parry (*Secretary*)	

PROGRESS OF WORK—

The Panel has completed its programme of work and its Report will be published in 1963. Only the two synthetic hormones, stilboestrol and hexoestrol, have been investigated and physico-chemical methods are recommended.

PROPHYLACTICS PANEL

CONSTITUTION—

R. F. Phipers, B.Sc., Ph.D. (*Chairman*)	*Cooper Technical Bureau*
C. W. Ballard, B.Sc., F.P.S., F.R.I.C.	*May & Baker Ltd.*
N. C. Brown, M.A., B.Sc., A.R.I.C.	*Cooper Technical Bureau*
H. G. Dickenson, B.Sc., Ph.D.*	*Ward, Blenkinsop & Co. Ltd.*
G. Drewery, B.Sc., F.R.I.C.†	*Merck Sharp & Dohme Ltd.*
A. W. Hartley, F.R.I.C.	*Spillers Ltd.*
A. Holbrook, F.R.I.C.	*Imperial Chemical Industries Ltd.* (*Pharmaceuticals Division*)
D. H. Mitchell‡	*Wellcome Chemical Works*
S. G. E. Stevens, B.Sc., F.R.I.C.	*Smith Kline & French Laboratories Ltd.*
J. A. Stubbles, B.Sc.	*May & Baker Ltd.*
Miss A. M. Parry, Miss C. H. Tinker (*Secretaries*)	

* Corresponding member. † Appointed January, 1962. ‡ Appointed April, 1961.

PROGRESS OF WORK—

The Panel has a long programme of work, complicated by the fact that the fashion in prophylactic chemicals is constantly changing, so that new substances are frequently appearing and others are likely to go out of fashion before there has been time to devise a suitable method for determining them in feeds.

A method for nitrofurazone can now be recommended, and the Panel's Report on this will be published in 1963. The work on methods for acinitrazole, sulphaquinoxaline and Amprolium is virtually completed, and Reports are to be prepared for publication.

As mentioned above, the future work of the Panel is to be continued by the "Prophylactics in Animal Feeds Sub-Committee," which is being formed from the Panel members with additional representation from other organisations interested in the subject; Dr. Phipers will continue as Chairman of the new Sub-Committee.

VITAMINS (FAT-SOLUBLE) PANEL

CONSTITUTION—

W. L. Sheppard, F.R.I.C. (*Chairman*)	*Boots Pure Drug Co. Ltd.* (Formerly with *Unilever Ltd.*)
C. R. Louden, B.Sc., F.R.I.C.	*R. Silcock & Sons Ltd.*
H. Pritchard, M.Sc., F.R.I.C.	*Analytical and Consulting Chemist*
S. A. Reed, B.Sc., A.R.I.C.	*British Cod Liver Oils* (*Hull and Grimsby*) *Ltd.*
G. Whalley, B.Sc., F.R.I.C.	*Unilever Ltd.*
J. Williams, B.Sc., Ph.D., F.R.I.C.	*Spillers Ltd.*
Miss C. H. Tinker (*Secretary*)	

PROGRESS OF WORK—

As reported 2 years ago, the Panel has completed its work on the spectrophotometric method for both vitamin A and for β-carotene, and the Report has been approved for publication. In addition, a colorimetric method has since been tested collaboratively and is to be included in the Report as a quick routine method; however, it is not sufficiently reliable to recommend it as a reference method. The Panel has now been disbanded.

VITAMINS (WATER-SOLUBLE) PANEL

CONSTITUTION—

A. J. Amos, O.B.E., B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Analytical and Consulting Chemist*
J. E. Ford, B.Sc., Ph.D.	*National Institute for Research in Dairying*
B. M. Gibbs, B.Sc., A.R.I.C.	*Unilever Ltd.*
F. W. Norris, Ph.D., D.Sc., A.R.C.S., D.I.C., F.R.I.C.	*University of Birmingham (Department of Biochemistry)*
S. A. Price, B.Sc., F.R.I.C.	*Vitamins Ltd.*
H. Pritchard, M.Sc., F.R.I.C.	*Analytical and Consulting Chemist*
F. Clermont Scott, B.Sc., F.R.I.C.	*Vitamins Ltd.*
S. Varsanyi, A.I.S.T.	*Glaxo Laboratories Ltd.*
J. Williams, B.Sc., Ph.D., F.R.I.C.	*Spillers Ltd.*
Miss C. H. Tinker (*Secretary*)	

PROGRESS OF WORK—

The Panel has studied and tested collaboratively on animal feeding stuffs selected methods of assaying riboflavin, nicotinic acid, vitamin B_{12}, pantothenic acid, pyridoxin and choline. Because of their specificity and sensitivity, microbiological procedures have been the methods of first choice.

It has been possible to determine microbiologically nicotinic acid, vitamin B_{12}, pantothenic acid and riboflavin in unsupplemented and supplemented animal feeding stuffs with an acceptable "between-laboratories" variance, and the Panel's two reports on recommended methods for these four vitamins has been approved for publication. During the Panel's earlier investigation of the determination of riboflavin by the microbiological method recommended by the Analytical Methods Committee for foods (*Analyst*, **1946**, **71**, **397**), it was suggested that the unacceptable divergence between the results obtained in different laboratories was attributable to the extraction procedure. As a result of a study of the effect of variations in the method of extraction, it has been possible to introduce modifications that allow the riboflavin activity of animal feeding stuffs to be determined with an accuracy commensurate with those of the assay methods recommended for the other three vitamins. Collaborative studies of a microbiological method involving *S. zymogenes* have revealed that, despite its greater sensitivity, the method has no advantage over the recommended method, which relies on *L. helveticus* as the test organism.

Continued studies of the remaining two vitamins on the Panel's original list—namely, pyridoxin and choline—have failed to produce methods of assay that can be recommended for use with animal feeding stuffs. Several microbiological methods of assaying pyridoxin have been subjected to collaborative trials, but none has given results with an acceptable "between-laboratories" variance. Collaborative studies of microbiological and chemical methods of assaying choline have been no more successful.

ANALYTICAL STANDARDS SUB-COMMITTEE

CONSTITUTION—

E. Bishop, B.Sc., A.R.C.S.T., F.R.I.C. (*Chairman*)	*University of Exeter (Department of Chemistry)*
S. Andrus, A.R.I.C.	*Laporte Chemicals Ltd.*
P. R. W. Baker, M.Sc., A.R.I.C.	*Wellcome Research Laboratories*
A. G. Hill, F.R.I.C.	*British Drug Houses Ltd.*
R. M. Pearson, F.R.I.C.	*Imperial Chemical Industries Ltd. (Billingham Division)*
J. M. Skinner, B.Sc., Ph.D., F.R.I.C.	*Imperial Chemical Industries Ltd. (Billingham Division)*
W. I. Stephen, B.Sc., Ph.D., A.R.I.C.	*University of Birmingham (Department of Chemistry)*
N. E. Topp, B.Sc., Ph.D., A.R.I.C.*	*National Chemical Laboratory*
J. T. Yardley, B.Sc., F.R.I.C.	*Hopkin & Williams Ltd.*
Miss A. M. Parry, Miss C. H. Tinker }	(*Secretaries*)

* Appointed April, 1961.

TERMS OF REFERENCE—"To examine existing analytical standards and to select suitable substances."

PROGRESS OF WORK—

Continuing the critical assessment of standards for acid - base titrimetry with a view to recommending a suitable standard to IUPAC, the Sub-Committee has set up a carefully defined scale of standards. After a detailed appraisal of the material submitted for consideration, sodium carbonate was selected for initial experimental examination in view of new work on the preparation of this compound. A statistical design was set up for a collaborative assay involving four laboratories, four samples of sodium carbonate prepared by two different methods and two reference standards—atomic-weight silver and zone-refined benzoic acid. Experimental procedures were devised to give the required precision of $\pm 0{\cdot}1$ per cent., and have been tested. The first collaborative assay broke down over the difficulty of storing and transporting molar hydrochloric acid without change of concentration; this effect was later discovered to be due mainly to leakage through the stoppers of polythene bottles. The second attempt failed because of the difficulty of removing carbon dioxide from solution without simultaneous loss of traces of benzoic acid. The third assay, in which the sodium carbonate samples were related to a uniform sample of atomic-weight silver through individual hydrochloric acid samples, met with considerable success, but revealed an interesting laboratory bias of $\pm 0{\cdot}02$ per cent. Although the result of this assay fell just within the prescribed limits, a fourth and final assay on fresh samples was made, and confirmed that the samples were assaying at about 99·96 per cent. The varieties of sodium carbonate comprising the test samples did not therefore meet the requirements for a primary standard. Nevertheless, confirmation was received that the methods of assay developed for this work were giving adequate replication and that certain modifications and alternatives also reached this standard. Other sources of sodium carbonate were examined, and a fifth collaborative assay showed that samples assaying at 99·996 per cent. were available. Meanwhile, attempts were made to trace the source of the 0·04 per cent. deficiency in the earlier samples, but were met with only partial success. The latest assays, showing a scatter of 18 parts per million over 35 analyses, were regarded as satisfactory evidence that the proposed methods were suitable and that sodium carbonate was an appropriate primary standard for the calibration of solutions of strong acids. Finally, a readily accessible source of sodium carbonate, on which preliminary assays are promising, is being investigated. A final report, embodying the recommendation of sodium carbonate, together with the method of assay, is being prepared and will be made available to the IUPAC Conference in July, 1963.

A detailed examination of losses of benzoic acid has also been made, with the conclusion that such losses come within the experimental error and can readily be determined. The scatter of the assay results, however, of 200 parts per million indicate the unsuitability of benzoic acid for applications to titrimetry, although the Sub-Committee entertains no doubts as to its purity and suitability in other applications.

The possibilities of using the coulomb as a universal reference standard are being explored, and a proposal has been made to the Analytical Methods Committee that specialised research work on this project would be well worthwhile.

CHLORINE IN ORGANIC COMPOUNDS SUB-COMMITTEE

CONSTITUTION—

R. Belcher, Ph.D., D.Sc., F.Inst.F., F.R.I.C. (*Chairman*)	*University of Birmingham* (*Professor of Analytical Chemistry*)
J. H. Dunn, B.Sc., A.R.I.C.	*Plant Protection Ltd.*
K. Gardner, B.Sc., F.R.I.C.	*Fisons Pest Control Ltd.*
R. Goulden, F.R.I.C.	*"Shell" Research Ltd.*
C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
Miss A. M. G. Macdonald, M.Sc., Ph.D., A.R.I.C.	*University of Birmingham* (*Department of Chemistry*)
Miss C. H. Tinker (*Secretary*)	

TERMS OF REFERENCE—"To prepare methods for the determination of organically-bound chlorine, having special reference to commercial preparations such as pesticides."

PROGRESS OF WORK—

The work of the Sub-Committee has now been completed, and its Report will be published in 1963. The method recommended is based on the oxygen-flask combustion technique, and it has been tested collaboratively on (*a*) chlorobenzoic acid, (*b*) dieldrin 50 per cent. water-dispersible powder and (*c*) a miscible oil containing pentachlorophenol, by both the members of the Sub-Committee and other analysts who had not had experience of the technique. Results were excellent and the method can be accepted with confidence as a standard method for all but low-concentrate pesticide dusts, in which the high proportion of inorganic filler prevents complete combustion; for this latter type of product the Stepanow method should be used.

ESSENTIAL OILS SUB-COMMITTEE

CONSTITUTION—

G. W. Ferguson, B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Analytical and Consulting Chemist*
A. J. M. Bailey, B.Sc., F.P.S., F.R.I.C.	*W. J. Bush & Co. Ltd.*
H. E. Brookes, B.Sc., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
K. Field, M.Sc., Ph.D.*	*D.S.I.R., Laboratory of the Government Chemist*
D. Holness, B.A.	*Proprietary Perfumes Ltd.*
H. T. Islip, B.Sc., F.R.I.C.	Formerly *Tropical Products Institute*
P. McGregor, B.Sc., A.H.-W.C., F.R.I.C.†	*D.S.I.R., Laboratory of the Government Chemist*
T. L. Parkinson, B.Sc., Ph.D., F.R.I.C.	*Beecham Foods Ltd.*
Miss H. M. Perry, M.Sc., F.R.I.C.	*Stafford Allen & Sons Ltd.*
G. B. Pickering, M.A., B.Sc., Ph.D., A.R.I.C.	*D.S.I.R., Tropical Products Institute*
J. H. Seager, M.Sc., F.R.I.C.	*Yardley & Co. Ltd.*
S. G. E. Stevens, B.Sc., F.R.I.C.	*Smith Kline & French Laboratories Ltd.*
B. D. Sully, B.Sc., Ph.D., A.R.C.S., F.R.I.C.	*A. Boake, Roberts & Co. Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Appointed July, 1962. † Resigned July, 1962.

PROGRESS OF WORK—

Studies of methods for the evaluation of specific groups of substances in essential oils have been continued. Completion of Mr. Holness's study of the reactions of citronellol and geraniol, on formylation, and the publication of his Report (*Analyst*, 1961, **86**, 231) provided a major contribution to the proceedings of the I.S.O. meeting on Essential Oils at The Hague in May, 1961. The van Os and Elema method for determining geraniol and citronellol when these occur together has also been studied, as have been alternative methods of acylation of alcohols. However, the Sub-Committee is now much in favour of Dr. Sully's stearoylation method (*Analyst*, 1962, **87**, 940) for the determination of hydroxyl groups, and a series of collaborative tests is at present being carried out.

Investigations into the determination of carbonyl groups have continued, and a preliminary study has been made of spectrophotometric methods that have been published for the determination of citral as α,β-unsaturated aldehyde. Work has continued on the determination of phenols and some collaborative tests have been made on Demetrius and Sinsheimer's method; it is hoped that a short Report on these investigations will be published during the coming year.

MEAT PRODUCTS SUB-COMMITTEE

CONSTITUTION—

S. M. Herschdoerfer, Ph.D., F.R.I.C. (*Chairman*)	*T. Wall & Sons (Ice Cream) Ltd.*
S. Back, B.Sc., F.R.I.C.	*Crosse & Blackwell Ltd.*
P. O. Dennis, B.Sc., F.R.I.C.	*Oxo Ltd.*
J. R. Fraser, B.Sc., A.C.G.F.C., F.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist*
H. C. Hornsey, F.R.I.C.	*J. Sainsbury Ltd.*
A. J. Kidney, B.Sc., Ph.D., A.R.C.S., A.R.I.C.*	*T. Wall & Sons (Meat and Handy Foods) Ltd.*
R. A. Lawrie, B.Sc., Ph.D., F.R.I.C.	*A.R.C., Low Temperature Research Station*
T. McLachlan, D.C.M., A.C.G.F.C., M.I.Biol., F.R.I.C.†	*Public Analyst*
A. McM. Taylor, B.Sc., Ph.D., F.R.I.C.	*British Food Manufacturing Industries Research Association*
E. F. Williams, M.A., F.R.I.C.	*J. Sainsbury Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Appointed August, 1961. † Appointed June, 1962.

TERMS OF REFERENCE—"(*a*) the determination of the meat content of products containing meat; (*b*) the determination of the constituents of meat and meat products.

NOTE—The term 'meat products' to include hydrolysed protein and, if found necessary, fish pastes."

PROGRESS OF WORK—

The Sub-Committee's Reports on Nitrogen Factors for Pork and on Nitrogen Content of Rusk Filler were published in *The Analyst* in September, 1961. Work has continued on the collection, on similar lines, of values for the nitrogen contents of beef and chicken meat. The Sub-Committee's Reports on beef and chicken factors will be published in 1963.

METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

CONSTITUTION—

W. C. Johnson, M.B.E., F.R.I.C. (*Chairman*)	*Hopkin & Williams Ltd.*
J. C. Gage, B.Sc., Ph.D., F.R.I.C.	*Imperial Chemical Industries Ltd.* (*Industrial Hygiene Laboratories*)
T. T. Gorsuch, B.Sc., Ph.D., A.R.I.C.	*U.K. Atomic Energy Authority, The Radiochemical Centre*
Miss E. M. Johnson, M.Sc., A.R.I.C.	*British Food Manufacturing Industries Research Association*
R. F. Milton, B.Sc., Ph.D., M.I.Biol., F.R.I.C.	*Analytical and Consulting Biochemist*
E. J. Newman, B.Sc., F.R.I.C.	*Hopkin & Williams Ltd.*
W. G. Sharples, A.R.I.C.	*Imperial Chemical Industries Ltd.* (*Dyestuffs Division*)
G. B. Thackray, B.Sc., F.R.I.C.*	*Staffordshire County Council*
E. I. Johnson, M.Sc., F.R.I.C.†	*D.S.I.R., Laboratory of the Government Chemist*
J. F. C. Tyler, B.Sc., Ph.D., A.R.I.C.‡	*D.S.I.R., Laboratory of the Government Chemist*
Miss A. M. Parry, Miss C. H. Tinker (*Secretaries*)	

* Appointed May, 1961. † Appointed May, 1962. ‡ Resigned May, 1962.

TERMS OF REFERENCE—"To investigate the determination of small quantities of metals in organic matter."

PROGRESS OF WORK—

The Sub-Committee completed its work on the determination of trace amounts of copper in organic matter, and its Report will be published in 1963. The method for zinc adopted by the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee has been accepted as being applicable to organic matter generally, and the specificity of the method for zinc in the presence of certain other metals that react with dithizone has been confirmed.

The rather special problems attending the determination of mercury in organic matter have been considered at some length, and a programme of individual and collaborative work is in hand; use is being made of radiochemical techniques to check any losses of mercury during the various stages of the procedure.

DIRECT MICRO-DETERMINATION OF OXYGEN IN ORGANIC MATTER SUB-COMMITTEE

CONSTITUTION—

D. W. Wilson, M.Sc., F.R.I.C. (*Chairman*)	*Sir John Cass College* (*Department of Chemistry*)
P. R. W. Baker, M.Sc., A.R.I.C.	*Wellcome Research Laboratories*
Miss B. B. Bauminger, Ph.D., A.I.R.I., F.R.I.C.	*Dunlop Research Centre*
W. T. Chambers, B.Sc., Ph.D., A.R.I.C.	*British Rubber Producers' Research Association*
A. F. Colson, B.Sc., Ph.D., F.R.I.C.	*Imperial Chemical Industries Ltd.* (*Alkali Division*)
Miss M. Corner, B.Sc., F.R.I.C.*	*D.S.I.R., National Chemical Laboratory*
Miss J. Cuckney	*Imperial College of Science and Technology* (*Department of Chemistry*)
F. Ellington, B.Sc., A.R.C.S., F.R.I.C.	*National Coal Board, Coal Research Establishment*
F. J. McMurray	*Wellcome Chemical Works*
M. P. Mendoza, B.Sc., A.R.C.S.	*British Coal Utilization Research Association*
F. H. Oliver	*Parke, Davis & Co.*
H. J. Warlow	*D.S.I.R., Tropical Products Institute*
C. Whalley, B.Sc., F.R.I.C.	*Laporte Chemicals Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Deceased November, 1962.

TERMS OF REFERENCE—"To investigate the Unterzaucher method, and its modifications, for the micro-determination of oxygen."

PROGRESS OF WORK—

No progress can be reported since this Sub-Committee has been in enforced abeyance since 1960.

PARTICLE SIZE ANALYSIS SUB-COMMITTEE

CONSTITUTION—

E. Q. Laws, B.Sc., F.R.I.C. (*Chairman*)	*D.S.I.R., Laboratory of the Government Chemist*
R. de B. Ashworth, M.Sc., Ph.D., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory*
D. G. Beech, Ph.D.	*British Ceramic Research Association*
C. G. L. Furmidge, B.Sc., Ph.D., A.R.I.C.	*"Shell" Research Ltd.*
H. Heywood, Ph.D.	*Director, Woolwich Polytechnic*
H. W. Hibbott	*D. R. Collins Ltd.*
J. F. Hinsley, F.I.M.*	*Edgar Allen & Co. Ltd.*
R. Howes, B.Sc.	*Chesterford Park Research Station*
R. Jackson, Ph.D.	*British Coal Utilisation Research Association*
B. H. Kaye, B.Sc., Ph.D.	*Research Council of the British Whiting Federation* (Formerly with *Nottingham & District Technical College*)
Miss C. H. Tinker (*Secretary*)	

* Appointed March, 1962.

TERMS OF REFERENCE—"To study methods of particle size analysis, to survey available instruments and to evaluate them with regard to their principles of operation and fields of application."

PROGRESS OF WORK—

The Sub-Committee held its first meeting in March, 1961, and its initial task has been to list and classify existing methods for determining particle size in the sub-sieve range (*i.e.*, below 76 μ). After 2 years' hard work this classification, comprising 74 methods, has been completed and is being published as a Review article in *The Analyst* in March, 1963. The principle of each method is briefly described and accompanied by adequate literature references.

The second, and more difficult, stage of the Sub-Committee's work will consist of some form of appraisement or evaluation of methods or apparatus, and its is envisaged that, since this will entail collaborative tests of a wide variety of apparatus, the constitution of the Sub-Committee will be supplemented by co-opted members, as and when necessary for special tests.

TRACE ELEMENTS IN FERTILISERS AND FEEDING STUFFS SUB-COMMITTEE

CONSTITUTION—

C. J. Regan, B.Sc., A.R.I.C. (*Chairman*)	Formerly *Chemist-in-Chief, London County Council*
S. M. Boden, B.Sc., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service*
S. G. Burgess, B.Sc., Ph.D., F.Inst.Pet., M.Inst.S.P., F.R.I.C.	*Scientific Adviser, London County Council*
J. H. Hamence, M.Sc., Ph.D., F.R.I.C.	*Public Analyst, Official Agricultural Analyst and Consulting Chemist*
E. I. Johnson, M.Sc., F.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist*
R. F. Milton, B.Sc., Ph.D., M.I.Biol., F.R.I.C.	*Analytical and Consulting Biochemist*
R. L. Mitchell, B.Sc., Ph.D., F.R.I.C., F.R.S.E.	*Macaulay Institute for Soil Research*
J. B. E. Patterson, M.Sc., F.R.I.C.	*Ministry of Agriculture, Fisheries and Food, National Agricultural Advisory Service*
W. L. Sheppard, F.R.I.C.	*Boots Pure Drug Co. Ltd.* (Formerly with *Unilever Ltd.*)
J. Williams, B.Sc., Ph.D., F.R.I.C.	*Spillers Ltd.*
Miss C. H. Tinker (*Secretary*)	

TERMS OF REFERENCE—"To devise appropriate methods of analysis (to be recommended for inclusion in the Regulations under the Fertilisers and Feeding Stuffs Act, 1926) for the determination of boron, cobalt, copper, fluorine, iodine, iron, magnesium, molybdenum, selenium and zinc, which can be expected to be present in fertilisers and feeding stuffs."

PROGRESS OF WORK—

By the end of 1961, the first draft of the final Report had been prepared; this comprised an introductory section, a section on general considerations and then detailed procedures for the preparation of the sample and for the determination of the eleven elements listed above. In addition, methods for chromium and nickel were added to cater for fertilisers containing sewage sludge; methods for calcium and chloride ion were also included as a result of a request from the Additives in Animal Feeding Stuffs Sub-Committee, of which this Sub-Committee acted as the Minerals Panel. For several of the elements, alternative methods are given for application in various circumstances. Publication of these collected methods is to be in the form of a separate booklet, under the title "Determination of Trace Elements, with Special Reference to Fertilisers and Feeding Stuffs," since it is envisaged that the methods will find a wider application than that for which they were originally intended; the book is being published early in 1963.

REPORT OF THE P.S. - S.A.C. JOINT COMMITTEE ON METHODS OF ASSAY OF CRUDE DRUGS

MAIN COMMITTEE

CONSTITUTION—

K. R. Capper, Ph.D., B.Pharm., F.P.S., D.I.C. (*Chairman*)	*Pharmaceutical Society of Great Britain*
J. Allen, A.R.I.C.*	*British Drug Houses Ltd.*
T. C. Denston, B.Pharm.†	*British Pharmacopoeia Commission*
J. W. Fairbairn, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C.	*University of London (Professor of Pharmacognosy)*
A. J. Feuell, B.Sc., Ph.D., A.R.I.C.	*D.S.I.R., Tropical Products Institute*
D. C. Garratt, Ph.D., D.Sc., Hon.M.P.S., F.R.I.C.	*Chairman, Analytical Methods Committee of the S.A.C.*
R. Higson, F.P.S.	*Ministry of Health (Supplies Branch)*
C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
H. C. Macfarlane, A.R.T.C.S., F.R.I.C.‡	*Analytical and Consulting Chemist*
W. Mitchell, B.Sc., Ph.D., F.R.I.C.	*Stafford Allen & Sons Ltd.*
R. F. Phipers, B.Sc., Ph.D.	*Cooper Technical Bureau*
J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S.	*Bradford Institute of Technology (Department of Pharmacy)*
D. Watt, F.P.S.	*T. & H. Smith Ltd.*
Miss C. H. Tinker (*Secretary*)	
Miss A. M. Parry (*Assistant Secretary*)§	

* Appointed February, 1962. † Appointed February, 1962.
‡ Resigned October, 1962. § Resigned November, 1961.

TERMS OF REFERENCE—"To prepare standard methods of assay of crude drugs and kindred materials."

PROGRESS OF WORK—

The Main Committee, in its steering capacity, is able to report continued progress in most of its Panels. Since its establishment in 1956, eight Panels have been set up to examine methods of assay of different drugs that are widely used but have no official or recognised assay procedure. During the past 2 years, one panel has been disbanded on completion of its programme of work and one new Panel has been set up; two other Panels have been in abeyance pending the outcome of associated work elsewhere.

Panel 5 (Lonchocarpus and Derris) was disbanded during 1961 on completion of its second Report—The Colorimetric Determination of Rotenone—which was published in *The Analyst* in November, 1961. Mention has been made earlier of the work of the new Panel on chemical methods for Thyroid (Panel 7), and further details of its progress and problems are given below.

Panel 1 (Digitalis Purpurea) was suspended in 1959 because no further progress could be made on a collaborative basis until considerable individual research had been undertaken. Since the problems encountered by the Panel have not yet been resolved elsewhere, it has has been decided to disband it; it is hoped, however, that it will be reconstituted at some later date. The other Panel that has been in suspension since 1960 is that dealing with chemical methods for anthraquinone drugs (Panel 3); as mentioned earlier this Panel began work again in December, 1962.

The three remaining Panels have continued with their programmes of work, and one of them—Panel 2 (Capsicum—Capsaicin Content)—is preparing its second, and final, Report.

PANEL 2: CAPSICUM—CAPSAICIN CONTENT

CONSTITUTION—

H. B. Heath, M.B.E., B.Pharm. (*Chairman*)	*Stafford Allen & Sons Ltd.*
C. F. G. Fost, M.P.S.	*W. J. Bush & Co. Ltd.*
C. A. Macdonald, B.Sc., F.R.I.C.	*Evans Medical Research Laboratories*
E. A. Elsbury, F.R.I.C.†	*Parke, Davis & Co.*
A. J. Middleton, B.Pharm., M.P.S., A.R.I.C.*	*Parke, Davis & Co.*
G. R. A. Short, F.P.S., F.L.S.	*W. J. Bush & Co. Ltd.*
G. I. Smales, B.Sc., F.R.I.C.	*Parke, Davis & Co.*
Miss G. M. Wells, B.Sc., A.P.I.	*Beecham Research Laboratories Ltd.*
A. J. Woodgate, B.Sc.	*Stafford Allen & Sons Ltd.*
Miss A. M. Parry, Miss C. H. Tinker (*Secretaries*)	

* Appointed May, 1962. † Resigned March, 1962.

TERMS OF REFERENCE—"To investigate methods of assay of capsicum and capsicum products with particular reference to the determination of the capsaicin content."

PROGRESS OF WORK—

Owing to the potential explosion hazards associated with the preparation of dry diazo-compounds, the Panel's work has been concentrated on perfecting an alternative colorimetric method of assay for the determination of capsaicin. This is based on the reaction of phenols with dichloroquinone-chloroimide (Gibb's reagent) to give a blue colour that can be evaluated spectrophotometrically. The Panel has also developed an assay procedure for determining capsaicin in Capsicum Wool B.P.C.

A report embodying these, together with certain amendments that have been found desirable to the originally published methods (*Analyst*, 1959, **84**, 603), is in preparation. With the completion of this report, the Panel's programme of work will come to an end.

PANEL 3: ANTHRAQUINONE DRUGS

CONSTITUTION—

W. Mitchell, B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Stafford Allen & Sons Ltd.*
J. W. Fairbairn, B.Sc., Ph.D., F.P.S., F.L.S., F.R.I.C.	*University of London (Professor of Pharmacognosy)*
C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C.	*Boots Pure Drug Co. Ltd.*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C.	*Pharmaceutical Society of Great Britain*
Miss H. M. Perry, M.Sc., F.R.I.C.	*Stafford Allen & Sons Ltd.*
J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S.	*Bradford Institute of Technology (Department of Pharmacy)*
H. A. Ryan, B.Sc., F.R.I.C.	*Westminster Laboratories Ltd.*
W. Smith, B.Sc., F.R.I.C.*	*Allen & Hanburys Ltd.*
R. V. Swann, B.Sc., F.R.I.C.	*Allen & Hanburys Ltd.*
Miss A. M. Parry, Miss C. H. Tinker (*Secretaries*)	

* Resigned September, 1962.

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."

PROGRESS OF WORK—

Since this Panel had been in suspension since May, 1960, no progress of work can be reported. As mentioned earlier in this Report, it resumed work in December, 1962, after its associated Panel (3A) on bioassay methods had submitted its report on a unified biological procedure for senna fruit that can be used as a form of "yardstick" for correlating chemical methods with purgative activity in mice.

Dr. Rowson, the Panel's original Chairman, resigned from that office in July, 1957, on his appointment to a post abroad, and Dr. Mitchell succeeded him. Now, on Dr. Rowson's return to this country, Dr. Mitchell has asked to be relieved of this duty, which he assumed on a temporary basis, and Dr. Rowson is once more the Chairman of the Panel. The Joint Committee thanks Dr. Mitchell for so ably stepping into the breach.

PANEL 3A: ANTHRAQUINONE DRUGS (BIOLOGICAL ASSAY)

CONSTITUTION—

K. L. Smith, M.P.S. (*Chairman*)	*Boots Pure Drug Co. Ltd.*
P. F. D'Arcy, B.Pharm., Ph.D., M.P.S.*	*Allen & Hanburys Ltd.*
R. T. Brittain	*Allen & Hanburys Ltd.*
J. W. Fairbairn, B.Sc., Ph.D., F.P.S., F.L.S.	*University of London (Professor of Pharmacognosy)*
G. A. Stewart, B.Sc., Ph.D., A.R.I.C.	*Wellcome Biological Control Laboratories*
Miss A. M. Parry, Miss C. H. Tinker }	(*Secretaries*)

* Resigned March, 1962.

TERMS OF REFERENCE—"To study biological methods for the assay of anthraquinone drugs."

PROGRESS OF WORK—

The reproducibility of results between laboratories for the biological assay method when mice and the same solutions and the same conditions were used has been examined. With this information, re-examination of two samples of senna pod (previously examined by Panel 3 by a chemical method) against a common sennoside standard has been made. The disagreement between laboratories beyond the limits of experimental error has been removed. A full report of these investigations was submitted to Panel 3, who made the original request for the work to be done.

PANEL 5: LONCHOCARPUS AND DERRIS

CONSTITUTION—

R. F. Phipers, B.Sc., Ph.D. (*Chairman*)	*Cooper Technical Bureau*
R. Buckley, B.Sc., A.R.I.C.	*Plant Protection Ltd.*
J. A. Dawson, B.Sc., A.R.I.C.	*D.S.I.R., Tropical Products Institute*
W. E. Drinkwater, F.R.I.C.	*Boots Pure Drug Co. Ltd.*
R. V. Foster, M.Sc., A.R.I.C.	*Cooper Technical Bureau*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C.	*Pharmaceutical Society of Great Britain*
J. T. Martin, D.Sc., F.R.I.C.	*University of Bristol (Long Ashton Research Station)*
R. A. Rabnott	*Analytical and Consulting Chemist*
F. H. Tresadern	*Stafford Allen & Sons Ltd.*
Miss A. M. Parry (*Secretary*)	

TERMS OF REFERENCE—"To investigate methods of assay of derris, lonchocarpus and their preparations, with particular reference to the determination of the rotenone content."

PROGRESS OF WORK—

The Panel continued its work on a colorimetric method for determining low concentrations of rotenone, as a routine control procedure. Optimum conditions have been established and details of the method are published in the Panel's second Report (*Analyst*, **1961**, **86**, 748), but it is not intended to be applied as a standard method.

The Panel has now completed its programme of work and has been disbanded.

Panel 6: Pyrethrum

CONSTITUTION—

W. Mitchell, B.Sc., Ph.D., F.R.I.C. (*Chairman*)	*Stafford Allen & Sons Ltd.*
E. A. Baum, D.Sc., I.C.N.*	*Pyrethrum Bureau*
H. E. Coomber, B.Sc.	*Mitchell Cotts & Co. Ltd.*
L. Donegan	*D.S.I.R., Tropical Products Institute*
M. Elliott, Ph.D.	*Rothamsted Experimental Station*
J. R. Furlong, O.B.E., Ph.D.†	*Pyrethrum Bureau*
A. D. Harford	*British Petroleum Research Centre*
S. C. Jolly, B.Sc., B.Pharm., M.P.S., A.R.I.C.	*Pharmaceutical Society of Great Britain*
W. S. Manson, B.Sc., A.R.I.C.	*Cooper Technical Bureau*
R. A. Rabnott	*Analytical and Consulting Chemist*
F. H. Tresadern	*Stafford Allen & Sons Ltd.*
T. F. West, Ph.D., D.Sc., A.M.I.Chem.E., F.R.I.C.	*Society of Chemical Industry*
Miss A. M. Parry, Miss C. H. Tinker (*Secretaries*)	

* Appointed May, 1962. † Resigned April, 1962.

TERMS OF REFERENCE—"To investigate methods of assay of pyrethrum flowers and pyrethrum extract with a view to recommending a standard chemical or physical method of assay."

PROGRESS OF WORK—

The Panel has continued its study of the mercury-reduction method. The variant of the method that has generally been used in this country was revised, and has given excellent inter-laboratory agreement of results in the hands of members of the Panel, this applying both to pyrethrum extracts and to pyrethrum flowers. Unfortunately, use of the revised version of the method by three commercial laboratories (each represented on the Panel) has not secured improved agreement between their routine results.

Independent work carried out in the laboratories of two companies represented on the Panel has shown that the Panel's variant of the mercury-reduction method records as "pyrethrin I" a significant amount of extraneous material. Several alternative procedures that appear partly to eliminate this interference have been proposed. Pending a fuller study of these, and other, possibilities, it has been decided to study the A.O.A.C. version of the mercury-reduction method, which appears to include less extraneous material.

A modified procedure (*J. Sci. Food Agric.*, 1955, **6**, 465) for the determination of "pyrethrin II" has been studied. Concurrently, it has been approved by the A.O.A.C. for inclusion in their published method.

Panel 7: Thyroid

CONSTITUTION—

C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C. (*Chairman*)	*Boots Pure Drug Co. Ltd.*
R. E. A. Drey, B.Sc., F.R.I.C.	*Wellcome Chemical Works*
Miss S. J. Patterson, B.Sc., A.R.I.C.	*D.S.I.R., Laboratory of the Government Chemist*
R. L. Clements, B.Sc.*	*D.S.I.R., Laboratory of the Government Chemist*
N. A. Terry, B.Pharm., F.P.S.	*British Drug Houses Ltd.*
C. Vickers, B.Sc., A.R.I.C.	*Boots Pure Drug Co. Ltd.*
Miss C. H. Tinker (*Secretary*)	

* Appointed December, 1962.

TERMS OF REFERENCE—"To investigate the possibility of devising a chemical method for determining the pharmacologically active constituents of thyroid."

PROGRESS OF WORK—

During the first year of its work, the Panel has concentrated on a general investigation of the problems involved in order that it might assess the possibility of success. Work has been carried out on each of the three main sub-divisions of a possible assay, namely (*i*) hydrolysis of the thyroid, (*ii*) separation of the active iodinated amino acids by paper or thin-layer chromatography and (*iii*) determination of the separated fractions.

Stage (*i*), which is likely to prove the most difficult for quantitative work, has so far received only a minimum of attention. Both alkaline and enzymatic conditions of hydrolysis are being considered. Chromatographic separation of synthetic mixtures of iodinated amino acids, Stage (*ii*), has been successfully carried out by a number of different systems, and this work is now being extended to the qualitative examination of thyroid hydrolysates. For the determination of the very small amounts of iodine deriving from such separations, Stage (*iii*), several methods have been investigated. The ceric sulphate - arsenious oxide catalytic method is considered unsuitable for routine application to occasional samples, such as would be necessary for the commercial examination of thyroid. The use of ultraviolet absorption measurements (at 288 mμ) and the formation of a starch - iodine complex both show considerable promise, and these are being further investigated.

APPENDIX I

THE SOCIETY FOR ANALYTICAL CHEMISTRY ANALYTICAL METHODS TRUST ACCOUNTS FOR THE YEAR ENDED OCTOBER 31ST, 1961

Income and Expenditure Account for the Year Ended October 31st, 1961

1960					*1960*			
£	£		£	£	£	£		£
	246	Rent, Light, Heat and Telephone	272			*4461*	Subscriptions from Industry	5910
	3497	Salaries	2969				Income Tax recovered on Covenanted Subscriptions for the years 1954/55 to	
	46	Office Equipment ..	63			*955*	1958/59	—
	138	Printing and Stationery	358		*5416*			
	23	Travelling Expenses ..	33				Interest from Investments	
	136	Expenses of Meetings ..	29			*10*	(gross)	8
	42	Audit Fee and Accountancy	21			*521*	Bank Deposit Interest ..	650
	101	Postage and Petty Expenses	123				Profit on Sales of "Recommended Methods for the Analysis of Trade Effluents" received from the Society for Analytical	
4229				3868		*336*	Chemistry	105
200		Scholarship Grant and Award for Research ..		1132				
148		Contribution to Fittings and Decorations of Council Room		—				
1706		Excess of Income over Expenditure for the year ended 31st October, 1961 transferred to Accumulated Fund		1673				
£6283				*£6673*	*£6283*			*£6673*

Accumulated Fund

1960			*1960*		
£		£	£		£
15,831	Balance carried to Balance Sheet	17,504	*14,086*	Balance at October 31st, 1960 ..	15,831
			1,706	Excess of Income over Expenditure for the year ended 31st October, 1961	1,673
			39	Increase in Value on Redemption of £100 Government of Ceylon 3¼% Stock 1959	—
£15,831		*£17,504*	*£15,831*		*£17,504*

Balance Sheet at 31st October, 1961

1960			*1960*			
£		£	£	£		£
15,831	*Accumulated Fund*	.. 17,504			*Investments (at Cost):*	
2728	*Sundry Creditors*	.. 1064		*83*	£100 3½% Conversion Stock ..	83
				100	£100 3½% War Stock	100
			183			183
					(Market Value at 31.10.61, £105)	
			36		*Sundry Debtors* ..	37
					Cash:	
					At Banks on—	
				11,000	Deposit Account	11,000
				7340	Current Account	7348
			18,340			18,348
£18,559		£18,568	*£18,559*			£18,568

Signed on behalf of the Analytical Methods Trust Fund G. H. LLOYD-JACOB, *Chairman*,
J. HUBERT HAMENCE, *Honorary Treasurer.*

Report of the Auditors to the Trustees of The Society for Analytical Chemistry Analytical Methods Trust Fund

We have examined the above Balance Sheet which in our opinion gives a true and fair view of the state of affairs of the Trust Fund at 31st October, 1961.

10, New Court,
Lincoln's Inn,
LONDON, W.C.2.
16th January, 1962.

(Signed) RIDLEY, HESLOP & SAINER
Chartered Accountants,
Auditors.

APPENDIX II

THE SOCIETY FOR ANALYTICAL CHEMISTRY ANALYTICAL METHODS TRUST ACCOUNTS FOR THE YEAR ENDED OCTOBER 31ST, 1962

Income and Expenditure Account for the Year Ended October 31st, 1962

1961					*1961*			
£	£		£	£	£		£	£
		Rent, Light, Heat and			*5910*	Subscriptions from Industry ..		3787
	272	Telephone	334			Interest from Investments		
	2969	Salaries	2371		*8*	Gross	7	
	63	Office Equipment ..	13		—	Taxed (net)	52	
	358	Printing and Stationery	83					59
	33	Travelling Expenses ..	30		*650*	Bank Deposit Interest		603
	29	Expenses of Meetings ..	41			Profit on Sales of "Recommended Methods for the Analysis of Trade Effluents" received from the Society for		
	21	Audit Fee and Accountancy	21		*105*	Analytical Chemistry ..		130
	123	Postage and Petty Expenses	97					
3868				2990				
1132		Scholarship Grant and Award for Research		—				
		Excess of Income over Expenditure for the year ended 31st October, 1962 transferred						
1673		to Accumulated Fund		1589				
£6673				£4579	*£6673*			£4579

Accumulated Fund

1961			*1961*		
£		£	£		£
17,504	Balance at 31st October, 1962 carried to Balance Sheet ..	19,093	*15,831*	Balance at October 31st 1961, ..	17,504
				Excess of Income over Expenditure for the year ended 31st	
			1673	October, 1962	1589
£17,504		£19,093	*£17,504*		£19,093

Balance Sheet at 31st October, 1962

1961					*1961*				
£	£		£	£	£	£		£	£
17,504		Accumulated Fund		19,093			*Investments (at Cost):*		
		Sundry Creditors					£100 3½% Conversion Stock ..		
	21	Accountancy ..	21			*83*		83	
		The Society for Analytical Chemistry	2924			*100*	£100 3½% War Stock	100	
	1043						350 Debenhams Ltd. 10s. Ordy Shares	747	
1064	——		——	2945			£250 Imperial Chemcal Industries Ltd. Ordy Stock ..	736	
							500 Philip Hill Investment Trust Ltd. 5s. Ordy Shares	708	
							£300 Renold Chains Ltd. Ordy Stock..	685	
							300 Royal Insurance Co. Ltd. 5s. Ordy Shares	735	
							400 Wharf Holdings Ltd. Ordy Shares	706	
						183		——	4500
							(Market Value at 31.10.62, £4225)..		
						37	*Sundry Debtors* ..		26
							Cash at Banks:		
							United Dominions Trust Ltd. on		
						11,000	Deposit Account	11,000	
							Barclays Bank Limited on Current		
						7348	Account ..	6512	
						18,348		——	17,512
£18,568				£22,038	*£18,568*				£22,038

Signed on behalf of the Analytical Methods Trust Fund G. H. LLOYD-JACOB, *Chairman*,
J. HUBERT HAMENCE, *Honorary Treasurer*.

Report of the Auditors to the Trustees of The Society for Analytical Chemistry Analytical Methods Trust Fund

We have examined the above Balance Sheet which in our opinion gives a true and fair view of the state of affairs of the Trust Fund at 31st October, 1962 and is in accordance with the Books kept by the Trustees. We have verified the Investments and found them to be in order.

10 New Court,
Lincoln's Inn,
LONDON, W.C.2.
28th June, 1963.

(Signed) RIDLEY, HESLOP & SAINER
Chartered Accountants,
Auditors.

APPENDIX III

SUBSCRIBERS TO THE TRUST FUND DURING 1961 AND 1962

Albright & Wilson Ltd.
Alginate Industries Ltd.
Ashburton Chemical Works Ltd.
The Associated Octel Co. Ltd.
Associated Chemical Companies Ltd.
Associated Portland Cement Manufacturers Ltd.
Boots Pure Drug Co. Ltd.
The British Aluminium Co. Ltd.
The British Arkady Co. Ltd.
British Cod Liver Oils (Hull and Grimsby) Ltd.
The British Drug Houses Ltd.
British Oxygen Research & Development Ltd.
Cadbury Brothers Ltd.
Central Electricity Generating Board.
Cerebos Ltd.
Cooper, McDougall & Robertson Ltd.
Courtaulds Ltd.
Crosse & Blackwell (Holdings) Ltd.
Cyanamid of Great Britain Ltd.
The Distillers Co. Ltd.
Dunlop Rubber Co. Ltd.
Norman Evans & Rais Ltd.
Fisons Ltd.
A. Gallenkamp & Co. Ltd.
Glaxo Laboratories Ltd.
Goulding Fertilisers Ltd.
Arthur Guinness, Son & Co. (Park Royal) Ltd.
T. Hedley & Co. Ltd.
H. K. Heinz Co. Ltd.
E. M. Hobbs, Esq.
Hopkin & Williams Ltd.
Horlicks Ltd.
Hovis McDougall.
Huntley & Palmers Ltd.
Imperial Chemical Industries Ltd.
International Flavors & Fragrances (Great Britain) Ltd.
Johnson Matthey & Co. Ltd.
Kellogg Co. of Great Britain Ltd.
Kodak Ltd.
Laporte Chemicals Ltd.
Laporte Industries Ltd.
London County Council.
J. Lyons & Co. Ltd.
May & Baker Ltd.
Merck Sharp & Dohme Ltd.
The Metal Box Co. Ltd.
The Millers' Mutual Association
Monsanto Chemicals Ltd.
National Coal Board
Nestlé Co. Ltd.
Novadel Ltd.
Oxo Ltd.
Parke Davis & Co.
Pfizer Ltd.
The Pharmaceutical Society of Great Britain.
Procea Products Ltd.
The Pyrethrum Bureau
Quaker Oats Ltd.
Roche Products Ltd.
Rowntree & Co. Ltd.
J. Sainsbury Ltd.
Schweppes (Home) Ltd.
"Shell" Research Ltd.
Smith Kline & French Laboratories Ltd.
Stafford Allen & Sons Ltd.
John & E. Sturge Ltd.
Tate & Lyle Ltd.
Unilever Ltd.
Vitamins Ltd.
Wallace & Tiernan Ltd.
Washington Chemical Co. Ltd.
The Wellcome Foundation Ltd.
Weston Research Laboratories Ltd.
West Norfolk Farmers Manure & Chemical Co-operative Co. Ltd.
William R. Warner & Co. Ltd.
John Wyeth & Brother Ltd.

Beer's Law and its Use in Analysis

A Review

By G. F. LOTHIAN

(*University of Exeter, Department of Physics, The Washington Singer Laboratories, Prince of Wales Road, Exeter*)

If a parallel beam of homogeneous (*i.e.*, one wavelength) radiation of intensity I falls on a sufficiently thin layer (thickness *dl*) of absorbing material, a small fraction will be absorbed. If the thickness of the layer is doubled, the fraction absorbed will be doubled; this is obvious if successive layers behave independently of one another (no multiple scattering) and if each thin layer is so sparsely populated with absorbing molecules that they do not hide behind one another in the path of the beam. Thus for thin layers the fraction absorbed is proportional to the layer thickness, *i.e.*—

$$dI/I = -\mu dl \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where μ is a constant known as the absorption coefficient. (The minus sign occurs because an increase in path means a decrease in intensity). Integration of this expression gives

$$\log_e I_0/I = \mu l \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where I_0 is the incident intensity at $l = 0$. This equation may be rewritten in various ways—

$$\begin{aligned} \log_{10} I_0/I &= 0{\cdot}4343 \log_e I_0/I \\ &= 0{\cdot}4343\ \mu l \\ &= Kl \\ &= d \end{aligned} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where K is a new constant, or—

$$t = I/I_0 = e^{-\mu l} \text{ or } 10^{-Kl} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where t is the fraction transmitted.

Any of these forms expresses the law first formulated about 1730 by Lambert and by Bouguer.

If the concentration, *c*, of absorbing molecules is doubled and the path-length halved so that the total number remains the same, the absorption (d or t) will remain the same, provided that the molecules do not come so close together that they come within each other's influence, modifying the energy levels or even causing chemical changes. That is, the absorption will be a function of the total number of molecules, *i.e.*, of the product *cl*, and equation (3) may be rewritten—

$$d = \log_{10} I_0/I = Ecl \text{ or } \epsilon cl \quad .. \quad .. \quad .. \quad .. \quad (5)$$

E, ϵ are new constants, the latter symbol being used when concentration *c* is in gram-molecules per litre.

This last equation is Beer's law, first stated in 1852[1]; it includes the earlier Lambert's law. In words it may be summarised by saying that the absorption of radiation by molecules depends only on their total number. Its original formulation has been recently discussed by Pfeiffer and Liebhafsky.[2]

For a mixture of several different absorbing substances the above argument may be repeated—provided again that the mixture is sufficiently dilute—when it may be shown that the optical densities of the several components are added, *i.e.*—

$$d = (E_1c_1 + E_2c_2 + - - -)l \quad .. \quad .. \quad .. \quad .. \quad (6)$$

This is the generalised form of Beer's law used in analysis of mixtures.

Terms and symbols

d, K, E and ϵ (equations 3 and 5) are fundamental in the use of Beer's law. Their usefulness is obvious, since the value of d is proportional to concentration and path length, and the value of K is proportional to concentration. The names of these quantities are summarised, together with the definitions, in Table I. In 1942 a report in *The Analyst*[3] recommended the use of names shown in column 1 and of the symbols shown in column 4. At later dates some alternative names shown in columns 2 and 3 were recommended[4,5,6] in the U.S.A. and are now commonly used there. The absorption coefficient, μ, is in common use for absorption of x radiation, but not for optical radiations.

Table I

Terms, symbols and definitions

Term				
Society of Public Analysts[3]*	Brode[4] and Hughes[5]	National Bureau of Standards[6]	Symbol	Definition
Optical density (or extinction)	Absorbance	Absorbance	d†	$\log_{10}(I_0/I)$—logarithm of reciprocal transmission
Extinction coefficient	—	Absorbance index	K	d/l—optical density (etc.) per unit path length
Specific extinction coefficient	Absorptivity	Absorbancy index	E	d/cl—optical density for unit path length and concentration
Molecular extinction coefficient	Molar absorptivity	Molar absorbancy index	ϵ	d/cl—specific extinction coefficient (etc.) for concentration of 1 gram-mole per litre

* Now The Society for Analytical Chemistry.
† D, E or **E** sometimes used.

The validity of Beer's law

If a measurement of optical density, d, is made, with known path length l, then the concentration, c, can be calculated from equation 5 if the specific extinction coefficient (E or ϵ) is known. But before this equation can be used it is necessary to test that the equation holds under the particular conditions of a measurement. If it is found not to hold, it is possible to establish an empirical relation (calibration curve) between optical density and concentration. But it is dangerous to do this blindly; unless one knows why Beer's law is not holding, one may not know within what limits the empirical relation may be expected to apply. It is therefore most important to consider in detail reasons for departure from Beer's law.

It will be appreciated from the introductory paragraphs that Beer's law may be expected to apply for—

(1) perfectly monochromatic radiation
(2) travelling in an optically homogeneous medium (no scattering of radiation)
(3) as a strictly parallel beam.
(4) It is further necessary that the absorbing molecules are never close enough to one another or to other (impurity) molecules that the molecule structure and hence the energy levels are affected.

These four conditions are ideals to which it is possible only to approximate. The degrees of approximation necessary and the effects that appear when the limits are exceeded will now be discussed for each of these conditions in turn.

THE USE OF MONOCHROMATIC RADIATION—

The constant E (or ϵ) varies with wavelength so that, if the incident radiation contains a number of wavelengths, the wavelength distribution of the radiation reaching the deeper layers of an absorbing specimen will be modified and the conditions assumed in equation (1), that all elementary layers absorb the same fraction of radiation, is no longer true. In practice, radiation can never be perfectly monochromatic and will depart from this condition in two respects—

(*i*) Finite wavebands. It will include a continuous band of radiation extending over a finite waveband centred about the required wavelength.

(*ii*) Scattered radiation. Radiation of widely different wavelengths from the desired wavelength arising from scatter, unwanted reflections, etc., in a monochromator will also form part of the measuring beam. These two factors will be considered in turn.

Finite waveband—Fig. 1 shows spectrophotometric curves for two concentrations (ratio 4 to 1) of the same substance. If, in endeavouring to measure the optical density at the narrow long-wave peak, the "monochromatic" radiation used covers the waveband cd, it is obvious that the observed optical density will be less than the true value for the peak.

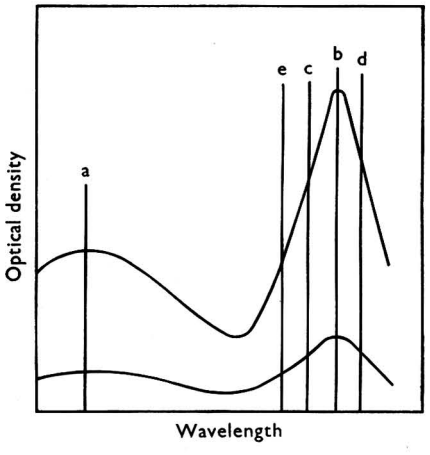

Fig. 1. Typical absorption curve with broad and narrow peaks

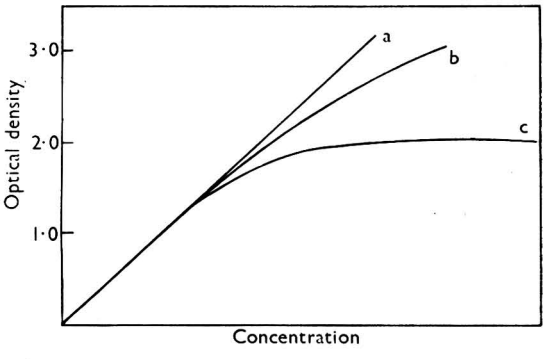

Fig. 2. Relation between optical density and concentration:

(*a*) Beer's law;

(*b*) Failure of Beer's law owing to finite waveband;

(*c*) Failure of Beer's law owing to stray radiation. Drawn to scale for 1 per cent. of stray radiation that is unabsorbed by the specimen

The difference between the observed and true maximum value will be proportionately greater for the higher concentration, from which it follows that the relation between optical density and concentration will no longer be linear, but curved as in b of Fig. 2. Calculations by Brodersen[7] have shown that even if measured optical densities are found to be proportional to concentration (curve a of Fig. 2) the observed values may be less than the "true" values for perfectly monochromatic radiation situated at the wavelength of the absorption maximum. (The difference amounted to 8 per cent. in a case calculated by him.)

It follows from all this that a spectrophotometric curve obtained with a monochromator passing a finite waveband will have its maxima depressed and its minima raised. The "true" curve can be estimated approximately from an observed curve by a graphical method first given by Paschen in 1897 and more recently explained by Slater[8] and also given in various text-books on spectrophotometry. Of course no treatment of observations can reveal detailed structure within the waveband used for the measurements.

The maximum waveband permissible, if Beer's law is to hold, thus depends on the measurements being made. Conditions are less stringent at a broad peak (a of Fig. 1) than at the

narrow peak b of this figure; the former is obviously more satisfactory to use for analytical purposes. In atomic-absorption spectrophotometry, the width of an absorption line may be about 0·02 Å, and the source of radiation has to be a hollow-cathode discharge giving emission linewidths appreciably less than this. For measurements made at the peak of the α band ($\lambda = 5770$ Å) of oxyhaemoglobin, a bandwidth of 2 to 3 Å would be desirable—this is not easy to attain with a monochromator and a source giving a continuous spectrum, and it is preferable in determining haemoglobin to avoid this narrow absorption band. But with measurements on potassium chromate at the broad maximum at $\lambda = 3720$ Å, a bandwidth of 50 Å will leave Beer's law valid within about 1 per cent. It is obvious from Fig. 1 that the higher the concentration the sharper a maximum becomes, and efforts to increase precision by working at high optical densities (differential spectrophotometry) must mean a narrower waveband if Beer's law is to be maintained.

Stray radiation—In general a monochromator passes, in addition to an intensity I_0 of radiation of the required wavelength, an intensity S of stray radiation of other wavelengths. If a specimen whose optical density is being measured transmits this stray radiation without any absorption, the apparent or measured value will be

$$d_{appt} = \log_{10} \frac{I_0 + S}{I + S}$$

in place of the value given by equation (5). As the concentration approaches infinity, when according to Beer's law d should approach infinity, the apparent optical density value will now approach a maximum value of

$$d_{appt} = \log_{10} \frac{I_0 + S}{S}$$

and a curve of optical density against concentration will now be as c in Fig. 2. If the stray radiation is 1 per cent. of I_0, the maximum value of apparent optical density will be 2·0.

Departure from Beer's law from this cause can be a frequent source of error. It is dangerous to use empirical calibration curves such as c of Fig. 2. This is partly because the curve may change from day to day with changing instrumental conditions (changing values of S due to dust, tarnished surfaces, etc.). But, in addition, the measurement will be affected by the presence of impurities; to take an extreme instance, if a specimen being measured contains, unknown to the operator, impurities completely transparent at the working wavelength, but which completely absorb the stray radiation of other wavelengths, the calibration curve c of Fig. 2 will be replaced by the straight line a—and the operator will not know this!

Tests for the effective presence of stray radiation may be made in one of several ways—

(*a*) By making measurements to construct a calibration curve as in Fig. 2 under conditions where one knows that Beer's law ought to be applicable. Some of the first workers to describe the use of this method were Hogness, Zscheile and Sidwell[9] who found, using their own instrument at $\lambda = 3700$ Å with a hydrogen-discharge lamp, a value for S/I_0 equal to 0·004. A modified form of this method is to insert in the beam a specimen having a narrow absorption band at such a thickness that one would expect zero transmission, and then to measure the resulting intensity of radiation or the optical density of the specimen.

(*b*) An alternative method, complementary to the first, is to endeavour to reduce the stray radiation by inserting a filter having high transmission at the required wavelength and low transmission at the wavelengths at which stray radiation might be expected. With a double-beam instrument the filter should, of course, be inserted into both beams. If the use of such a filter then brings about a change in the measured value of optical density, the presence of stray radiation is to be inferred, and it would be desirable to retain the added filter for measurements under these conditions.

(*c*) Pritchard[10] has described how to determine the stray radiation of various wavelengths for various wavelength settings, by passing pure radiation from a double monochromator into the spectrophotometer.

The effective amount of stray radiation is likely to be great (S/I_0 large) when the effective value of I_0 is small. This will be most likely to occur when working at wavelengths near the

limits of usefulness of the radiation source, the optical system or the detector. For example, a tungsten-filament lamp *can* be used at $\lambda = 3500$ Å, but the energy here is so small compared with the emission at longer wavelengths that there is grave risk of measurements of optical density being grossly in error. Thus a specimen whose absorption is small throughout the visible spectrum and continuously increasing in going to shorter wavelengths in the ultraviolet, may well show a completely spurious absorption maximum at, say, $\lambda = 3700$ Å; a smaller apparent optical density at $\lambda = 3500$ Å being measured because the energy here may be mostly visible radiation. Similar difficulties occur in the far infrared, where the emission of one of the usual incandescent sources is very small compared with the emission in the region $\lambda = 1$ to $3\ \mu$. Likewise, measurements with a quartz optical system near its useful limit of $\lambda = 2000$ Å, or a photomultiplier near its long wave limit in the red, need careful consideration before being passed as valid. This is not the place to discuss how to reduce the amount of stray radiation; but the examples mentioned are intended to emphasise the need to test for Beer's law under the exact conditions used in subsequent measurements—in particular: nature of specimen, source of radiation, slit width, dispersing system, radiation detector and alignment of components. Goldring, Hawes, Hare, Beckman and Stickney[11] have published some curves showing the effects of stray radiation on measurements of potassium chromate; in their paper they discuss this and other sources of error in some detail, and include a useful bibliography of 19 references.

PARALLELISM OF BEAM—

In the introduction we have presupposed a parallel beam—an ideal that can never be obtained. A perfectly parallel beam must come from a point source and can carry only an infinitesimal amount of energy. In practice a beam of finite angular size (see Fig. 3) must be used. For a beam passing through a specimen at an angle θ to the axis the path length and hence the optical density will be increased by a factor $1/\cos\theta$. If the extreme value of θ through the specimen is 9°, the optical density will be increased by 1 per cent. ($1/\cos 9° = 1{\cdot}01$). If the refractive index of the specimen is 4/3, the corresponding angle outside the specimen will be $\theta = 12°$; this is greater than the semi-angle of beam in most commercial spectrophotometers, so that the finite angle will not in general be a source of error. But if efforts are made to increase precision by working at high optical densities (say $d = 3$) to a precision of 0·005, as is sometimes done in differential spectrophotometry, the finite angular size of beam may well cause departure from Beer's law. The effect is analogous to that arising from a finite waveband, but is complementary to the latter—the central axial beam giving a minimum optical density.

As will be seen in the next section, the angular size of beam is more important when scattering specimens are being measured.

NON-HOMOGENEOUS SPECIMENS—SCATTERING IN THE SPECIMEN—

A specimen may be fortuitously turbid; *i.e.*, it may include some suspended or colloidal material as an impurity in what would ideally be a homogeneous solution. Provided the turbidity is small, the optical densities due to absorption and scattering are additive, and it is often possible to determine the latter separately by extrapolation of measurements at adjacent wavelengths where the absorption is zero.

Much more must be said about specimens that inherently scatter radiation—colloidal solutions and suspensions, where it is the light scattering material in which one is interested. Such measurements may be made for various purposes—

(1) for chemical analysis—as in the determination of sulphate as barium sulphate,
(2) as a means of determining particle size,
(3) to determine the actual absorption of the material forming (part of) the particles —*e.g.*, the absorption of powdered materials incorporated in a pressed disc of potassium bromide or the absorption of pigment in plant or animal cells.

The argument used in the opening paragraphs to deduce Beer's law may be repeated for a suspension or colloid simply by speaking of "particles" instead of molecules. One assumes that some or all of the radiation incident on a particle is lost from the transmitted beam—by absorption or by scattering or refraction into another direction. The fraction so lost depends in a complicated way on a number of factors; the matter can only be summarised here by saying that for a perfectly parallel beam the obscuring power of a single particle

is not equal to its projected area A, as might be expected from geometrical optics, but to KA, where K (quite distinct from the K in Table I) is often known as the scattering area coefficient. The value of K (somewhere between 0 and 5) is a function of—

(*i*) particle size relative to wavelength;

(*ii*) particle shape and orientation in the beam;

(*iii*) refractive index of the particle relative to the surrounding medium;

(*iv*) the true absorption of the particle.

In practice the angles θ_s and θ_d of the incident and measured beams must be finite (see Fig. 3) so that some of the scattered radiation will be picked up by the detector, reducing the effective opacity; *i.e.*, reducing the coefficient K. Thus K is also a function of—

(*v*) the beam angles θ_s and θ_d.

If all these five conditions remain constant so that K remains constant, Beer's law may be expected to hold provided—

(1) The beam angles θ_s and θ_d of Fig. 3 are so small that the path lengths are not significantly different for the various beams through the specimen—as already discussed for homogeneous specimens.

(2) Secondary scatter may be neglected. Radiation scattered out of the observed beam may suffer secondary scatter back into the observed beam. This secondary scatter will be more pronounced at high optical densities, reducing their value and giving an optical-density - concentration curve as in b of Fig. 2. The larger the angles θ_s and θ_d the lower the optical density at which such failure of Beer's law will set in.

The effects of these considerations on measurements made for the three different purposes listed on p. 682 are discussed below in turn.

Chemical analysis by turbidimetric measurements—In the determination of barium sulphate, Treon and Crutchfield,[11] for example, found Beer's law to hold for the largest optical densities they used (up to 1·0) at $\lambda = 5700$ Å. But this conclusion would be useful only under the conditions of their work—type of spectrophotometer and its adjustment and the exact technique they used in preparing their suspensions. It is not really possible to be sure of repeating the technique exactly from day to day, and it is essential that all measurements be comparative—an unknown concentration being measured by comparison with known concentrations not very different from the unknown, the two suspensions being prepared side by side at the same time. Working in this way, one does not rely on the applicability of Beer's law except for interpolation over a small range.

Determination of particle size—For this sort of work it is desirable to have the angles θ_s and θ_d of the incident and emergent beams as small as possible in order to approximate to the conditions ($\theta_s = \theta_d = 0$) under which the scattering properties have been calculated.[13]

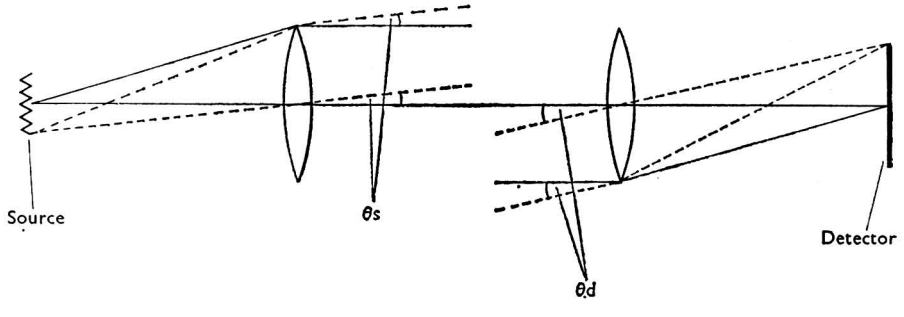

Fig. 3. Illustration of the finite angular sizes of the beam incident on a specimen—semi-angle θ_s—and of the beam received by the detector—semi-angle θ_d

Goulden[14] has described modifications of commercial instruments with this end in view and has made measurements to determine the size of the fat globules in milk. As explained above, such small angles help to maintain Beer's law to higher values of optical density. It is of course important to check by measurements at several concentrations or cell lengths that Beer's law is holding under the conditions of measurement. If secondary scatter is affecting the measurements they cannot, without correction, be used to calculate particle size.

Determination of absorption curves of granulated materials—More or less successful attempts have been made to deduce true absorption curves from transmission measurements on granulated materials; for example, haemoglobin in whole blood cells[15,16] and chlorophyll in cells of the alga, Chlorella.[17] But the theory developed so far involves some approximations, and at present it must be said that in general it is not safe to assume that absorption curves obtained from such measurements give the true curve that would be obtained from a homogeneous solution of the absorbing compound.

If the measurements are to be a function of the absorption only and not of the scattering properties, it is necessary to include all the scattered radiation in the measurement. Ideally this may be done by surrounding the specimen with an integrating sphere. But the theory and practice of this arrangement are complicated, and the method is not in general use. A good approximation is to use a translucent diffusing surface at the exit face of the specimen cell to direct a representative sample of the forward scattered radiation into the detector.

With these conditions of observation Beer's law may be expected to hold, but only in the limited sense that optical density will be proportional to the number density (number per unit area) of a given type of particle provided this is small enough for secondary scatter not to come into play. The optical densities will not be proportional to the values that would be obtained for the same amount of material uniformly dispersed in a homogeneous solution. This is because of the "sieve effect." Some of the beam of radiation passes outside the absorbing particles to reach the detector unabsorbed. This means that for a given number of particles the optical density will not exceed a limiting value, however great the absorption in each particle; hence absorption peaks will be depressed. It may be shown[18] that this effect disappears as the absorption of a single particle approaches zero. A consequence of this is that powdered materials (as in the potassium bromide pressed-disc method) should be ground as fine as possible. In addition, peaks may be slightly shifted because of refractive index changes near an intense absorption band. And if not enough of the scattered radiation is picked up, an absorption peak may well disappear altogether when a pigment is localised in cells.[16,19]

MOLECULAR INTERACTIONS—

When the concentration is sufficiently increased, an absorbing molecule can no longer be regarded as being isolated from other similar molecules by empty space (gas samples) or by an infinite ocean of solvent molecules. The energy levels, and hence the absorption spectrum, will become modified when molecules are in sufficient concentration to influence one another and consequently to reduce the influence of surrounding solvent molecules. In the classical language of chemistry this modification is explained as a chemical change that may be described as a change of polymerisation, isomerisation, ionisation, hydrolysis, etc. These effects form too vast a subject to be dealt with in this review and are appropriate to reviews on these individual chemical topics. It must suffice to say that, if a lack of proportionality of optical density and concentration cannot be ascribed to any of the causes already discussed in some detail, a chemical change must be considered.

A short mention should, however, be made of the case of absorption in a gas under changing pressure. As the pressure of a gas is increased, an absorption band is in general broadened with the result that the specific extinction coefficient at the centre of the band is depressed, *i.e.*, Beer's law is no longer valid. But it is found that even when this is so, the total area under an absorption band ($A = \int K d\nu$ or $\int \mu d\nu$ when the integration is carried out over the whole range of frequencies, ν, covered by the band) is often approximately proportional to the total concentration of absorbing molecules. This may be regarded as a modified version of Beer's law. Some spectrophotometers provide mechanical integrating attachments for determining this integral. The use of the area under an absorption curve for analytical work has been discussed in several original papers; see, for example, Oswald,[20] Ramsay[21] and Mills and Thompson.[22] These last workers show how, even when the spectro-

meter will not completely resolve a band, a correct value for A can be obtained by extrapolation to zero partial pressure by measurements on mixtures with an inert non-absorbing gas.

Determination of absorbing materials

The use of Beer's law for determining a single absorbing component (equation 5) and of mixtures of several such components (equation 6) is common practice and is well covered in the various text-books on spectrophotometry, and so needs little consideration here.

As already explained, it is desirable to make measurements at the peaks of broad bands for several reasons: (*a*) At a *peak* so that the sensitivity is a maximum and interfering effects of absorption due to impurities is a minimum, and further so that a small error in wavelength setting will cause minimum change in optical density—measurement on the steep slope of a band (e in Fig. 1) is bad practice. (*b*) As already explained the use of a *broad* peak reduces troubles due to finite waveband.

For measurements on mixtures of n components, n measurements at n different wavelengths are needed to solve n equations of the type of equation (6). The wavelengths need to be chosen for maximum sensitivity—at each of the n wavelengths one component having a large absorption and the others having small absorption. Simplified methods of solution are given in various text-books.

The range of optical density in which a measurement is made can be adjusted by suitable choice of cell lengths. Much has been written about the optimum value of optical density to choose. For photographic and visual spectrophotometry a high value is, in general, the best. Various tests show that, with simple assumptions about the limiting sensitivity of a photoelectric or thermo-electric detector, the optimum precision is attainable at an optical density of 0·43. But it has also been shown[23] that this conclusion is modified by taking account of the fact that a measurement of optical density involves several operations—including setting a galvanometer or similar scale with no radiation falling on the detector, then with a blank cell (solvent) in the beam and finally with the absorbing specimen in the beam. These considerations may need to be further modified by reason of instability in the radiation source (voltage fluctuations). It is my opinion that the optimum optical density depends on so many factors—voltage stability and level of photo-electric (or thermo-electric) current—that an optimum value of d cannot be usefully calculated, but must be determined in any particular investigation by trial and error determination of repeatability of observations.

References

1. Beer, A., *Ann. Phys. Chem.*, 1852, **163**, 78.
2. Pfeiffer, H. G., and Liebhafsky, H. A., *J. Chem. Educ.*, 1951, **28**, 123.
3. Cooper, B. S., Gillam, A. E., Lothian, G. F., and Morton, R. A., *Analyst*, 1942, **67**, 164.
4. Brode, W. R., *J. Opt. Soc. Amer.*, 1949, **39**, 1022.
5. Hughes, H. K., *Anal. Chem.*, 1952, **24**, 1349.
6. U.S. National Bureau of Standards, *Circular No.* 484, Washington, 1949.
7. Brodersen, S., *J. Opt. Soc. Amer.*, 1954, **44**, 22.
8. Slater, J. C., *Phys. Rev.*, 1925, **25**, 783.
9. Hogness, T. R., Zscheile, F. P., and Sidwell, A. E., *J. Phys. Chem.*, 1937, **41**, 379.
10. Pritchard, B. S., *J. Opt. Soc. Amer.*, 1955, **45**, 365.
11. Goldring, L. S., Hawes, R. C., Hare, G. H., Beckman, A. O., and Stickney, M. E., *Anal. Chem.*, 1953, **25**, 869.
12. Treon, J. F., and Crutchfield, W. E., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 119.
13. A number of tables showing values of scattering area coefficient, K, for particular values of particle size (relative to wavelength), refractive index and extinction coefficient have been published (and are continuing to appear) in various journals. See for example—
    Bouwkamp, C. J., *Rep. Prog. Physics*, 1954, **17**, 35. (This paper contains an extensive bibliography).
    Lowan, A. N., *Nat. Bur. Stand., Appl. Math. Series*, 1949, No. 4.
    Penndorf, R. B., *J. Opt. Soc. Amer.*, 1962, **52**, 896.
14. Goulden, J. D. S., *Brit. J. Appl. Phys.*, 1961, **12**, 456.
15. MacRae, R. A., McClure, J. A., and Latimer, P., *J. Opt. Soc. Amer.*, 1961, **51**, 1366.
16. Lothian, G. F., and Lewis, P. C., *Nature*, 1956, **178**, 1342.
17. Latimer, P., *Science*, 1958, **127**, 29.
18. Lothian, G. F., "Absorption Spectrophotometry," Hilger & Watts Ltd., London, 1958, p. 38.
19. Adams, G. A., Bradley, R. C., and Macallum, A. B., *Biochem. J.*, 1934, **28**, 482.
20. Oswald, F., *Z. Elektrochem.*, 1954, **58**, 345.
21. Ramsay, D. A., *J. Amer. Chem. Soc.*, 1952, **74**, 72.
22. Mills, I. M., and Thompson, H. W., *Proc. Roy. Soc., A*, 1955, **228**, 287.
23. Gridgeman, N. T., *Anal. Chem.*, 1952, **24**, 445.

Received *April 24th*, 1963

The Application of Flying-spot Scanning to Particle Size Analysis in the Formulation of Pesticides*

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Pesticides are usually applied as dusts or sprays in which particle size is one factor that controls the impaction on and coverage of the target surface and the amount of drift of toxic material away from the target. Most pesticide sprays are formulated as emulsions, or suspensions of solid particles in water; the particulate size of these dispersions is important in determining both their relative stability and the biological toxicity of the deposits they produce on the target surface.

Particle size analysis in the pesticide field necessitates the study of a wide variety of particulate matter under many different conditions, and it has been found possible to use the "flying spot" automatic scanning technique to cover many of the problems involved. The advantages and disadvantages of this automatic method of particle sizing are discussed with particular reference to the sampling requirements to obtain the greatest accuracy in the results.

One of the essential functions of formulation is to make possible the distribution of extremely small amounts of pesticide over large surface areas. The most obvious way of achieving this is to reduce the particle size of the pesticide so that its surface area approaches that of the target surface. It is impossible to do this to the toxicant alone, but, if its bulk can be extended by dilution with biologically inert solids or liquids and this larger amount is applied in the form of extremely fine particles, a satisfactory distribution of pesticide becomes possible. Thus, the usual ways in which pesticides are applied are as sprays or as dusts. Aqueous sprays are usually preferred when reasonable amounts of water are available, but few pesticides are soluble in water, and so they have to be formulated as emulsions or as suspensions of solid particles. The formulation and application of pesticides is concerned, therefore, with the dispersion of particulate material in various ways, the most important of which are solid particles in air (dusts), oil droplets in water (emulsions), solid particles in water ("wettable powders") and sprays of these last two dispersed in air. A control over the particle size in all these types of dispersions may be important in determining the biological efficiency of the pesticide application.

Sprays—

The droplet spectrum formed when a liquid is sprayed into air can vary considerably with the spray equipment both in terms of the actual size of the drops and in the range of drop size found in the spray cloud. Both these factors may influence spray performance in several ways. The effect of droplet size on coverage of the target surface is illustrated in Fig. 1. This shows the area (in acres) covered by 10 and 50 gallons of an ideal spray (when all the droplets are the same size), the coverage being calculated on the assumption that each droplet will impact and spread to cover a circle whose diameter is 4 times that of the original droplet. A spread factor of this magnitude is an average figure for aqueous sprays containing a reasonable wetting agent on foliage. For many pests, good coverage of the target surface is essential for satisfactory control, but the total leaf area of an acre of crop may be anything from 1 to 30 acres. To provide a spray coverage equivalent to the crop area, either extremely large volumes of spray must be used or the spray must be composed of extremely small droplets. The modern tendency is to apply smaller and smaller volumes of spray per unit area, and between 10 and 50 gallons to the acre is common; a drop size of less than 100 μ is therefore desirable.

However, the process of spraying is not as simple as this, because, if small droplets are used, drift of the droplets away from the target may become serious. Fig. 2 shows the

* Presented at the meeting of the Society on Wednesday, February 6th, 1963.

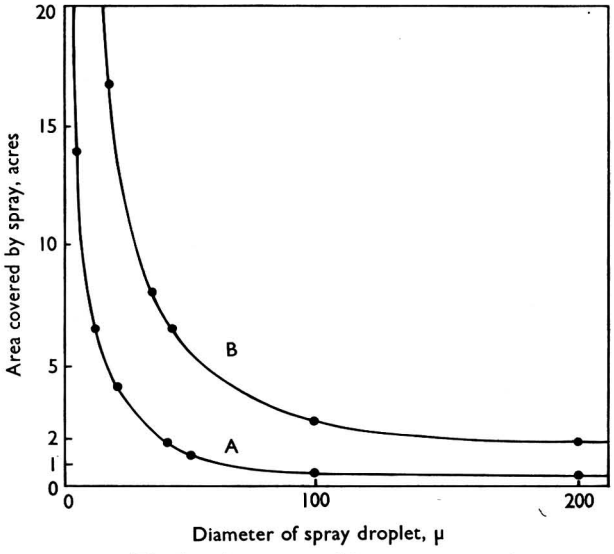

Fig. 1. Area covered by spray, assuming a spread factor of 4 × diameter of drop: curve A, 10 gallons; curve B, 50 gallons

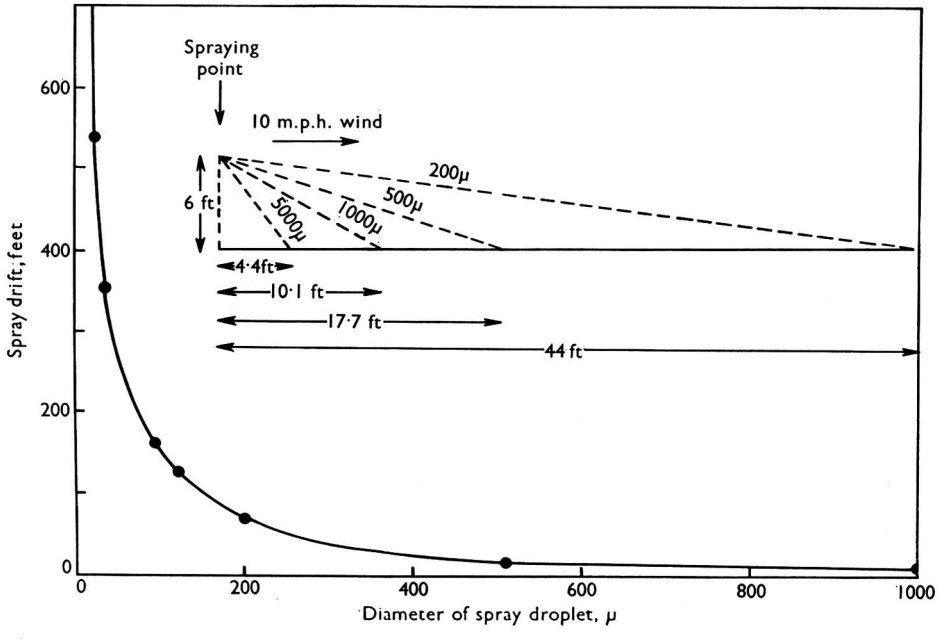

Fig. 2. Influence of drop size on spray drift

variation of spray drift with droplet size, the spray droplets being assumed to be initially stationary at 6 feet above ground level in a 10 m.p.h. wind. The spray drift is shown by the horizontal distance that the droplets travel before reaching the ground. In practice, 10 m.p.h. is about the maximum wind speed in which spraying would be carried out, but under these circumstances drops as large as 200 μ may drift a considerable distance.

The droplet size is one of the factors that influence the impaction efficiency of spray droplets and the retention of liquid on sprayed surfaces. It is impossible to generalise on the optimum drop size required for impaction on agricultural spray targets, because the latter vary so considerably in size, surface characteristics, degree of packing and in their movement relative to the spray droplets. The larger the drop size, the more readily will the drops impact on foliage, so that if it is desired that the spray should penetrate through thick foliage or dense undergrowth, then extremely small drop sizes are required.

The droplet size also governs the toxicity of a spray to flying insects. Work on the effects on flying mosquitoes of droplets of lubricating oil containing 8 per cent. of DDT shows that the maximum activity per unit weight of spray is found with a droplet size just over 10 μ; drop sizes either larger or smaller show much reduced activity.[1] A similar drop size - toxicity effect is found with many other flying pests, and this is important in the formulation of the aerosol generators that are now so widely used. Such formulations consist of a liquefied propellent gas, mixed with a solution of the toxicant in a non-volatile oil and held under pressure in a metal container. On releasing the pressure, the propellent gas vaporises and produces extremely fine droplets of the non-volatile oil; the droplet size is controlled by the proportion of propellent to oil and by the dimensions of the nozzle through which it is released.

Dusts—

These have not, so far, been studied as thoroughly as sprays, but similar considerations apply in respect to drift and coverage of target. Dusts for foliage application would normally have a particle size between 50 and 70 μ, and so drift is again a serious problem unless the dusts are applied under still or extremely light wind conditions. One important factor in the application of dusts is the relative size of toxicant particles to filler particles. If considerable differences in size exist, toxicant and filler may settle out of the dust cloud in different areas and thus produce a patchy cover of toxicant.

The size of the particles in a dust cloud has a considerable effect on their impaction on foliage, their adhesion and on their resistance to weathering. Generally, impaction is improved as the particle size is increased, but resistance to weathering (by wind or rain) is decreased.

Emulsions and suspensions—

Most pesticides are formulated as water-dispersible oils (usually called emulsifiable concentrates) or as water-dispersible powders (wettable powders). The essential requirements in a wettable powder are that it should flow freely, even after prolonged storage, and that it should suspend well in water. The particle size can affect both these properties, and it is also one of the factors that determine the ease with which the toxicant is deposited on to the sprayed surface once the spray has impacted. The biological efficiency of the toxicant deposit is also governed by its particle size; in general, the smaller the particles, the better the results.

Similar considerations apply to the size of the oil globules in oil-in-water emulsion sprays.

In studying emulsion behaviour, it is valuable to be able to measure the rate of coalescence of emulsion globules in the dilute emulsion systems used in practice. This rate of coalescence is related to the ease of formation of the emulsion, its stability on standing and its behaviour after impaction on a plant surface. In a similar way, it is interesting to measure the rate of aggregation of solid particles in suspensions of pesticides.

Particle size analysis

Research into the formulation of pesticides necessitates the study of an extremely diverse range of particulate systems. Several methods are available for determining particle size,[2] but most of these methods are limited to one type of particulate system, *e.g.*, dusts in air or solid particles suspended in water. Many of these methods are used in studying specific formulation problems, *e.g.*, the over-all stability of emulsions and suspensions is usually estimated by fractional decantation methods. These have the advantage that the results can be directly related to the extent to which the dispersed phase will settle-out in the spray tank. Several other sedimentation methods are equally satisfactory for studying this type of system, but such methods are not generally applicable to all the particulate systems involved in the formulation and application of pesticides. For example, the measurement of spray droplet size can be satisfactorily carried out only by collecting the droplets

on a suitable surface and sizing them. This method, in which the sample is placed under a microscope and the particles are compared by means of a suitable eye-piece graticule, has been used to a considerable extent, but it is really too tedious and slow to be satisfactory except in those instances when few samples have to be measured or when the particle size is reasonably uniform. Even then the method tends to be inaccurate because of the eye-strain that is involved.[3,4]

These difficulties can be overcome by using an automatic method of counting and sizing the samples, and, if such methods are sufficiently versatile, they may be used for many of the particulate studies besides spray droplet sizing that are important in the formulation of pesticides. The flying-spot system[5,6] of scanning the sample was selected as being the most versatile method, with an instrument based on the flying-spot microscope and developed by Messrs. Rank-Cintel Ltd.

FLYING-SPOT PARTICLE RESOLVER—

The operation of the flying-spot particle resolver is shown diagrammatically in Fig. 3. A 700-line scanning raster, produced on the face of the scanning tube, is passed into the optical system. For convenience, this usually consists of a standard optical microscope, but, if lower magnifications are desired, it can be replaced by a simple projection lens. The image of the scan raster is focused on the sample to be examined, the scanned area decreasing as the magnification increases. The amount of light passing through the sample, when transparent backgrounds are used, or reflected from the sample if it is opaque, varies according to the optical density and configuration of the objects on the sample; these changes in light intensity are detected by a multiplier photocell, in which they are converted into an electrical signal. This signal is fed to the video amplifier, the output of which modulates the monitor cathode-ray tube, and thus a magnified image of the sample is produced on the monitor screen.

Automatic counting of all the particles within the scanned field is carried out by using the extra units shown in Fig. 3. If only one particle is on the sample slide when this is

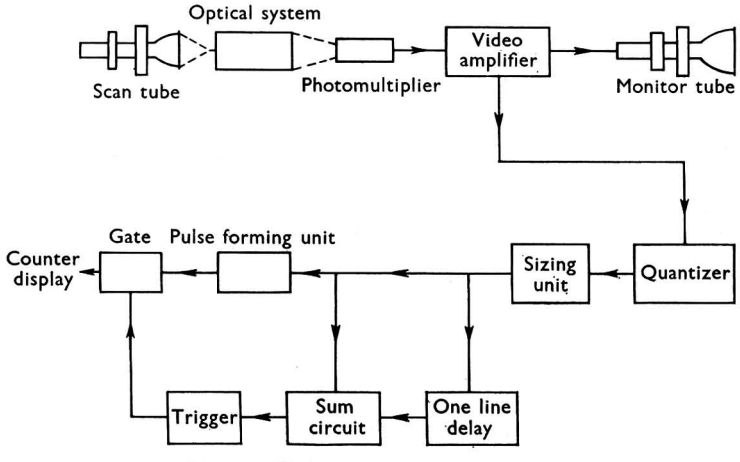

Fig. 3. Flying-spot particle resolver

scanned by the flying spot, an intercept pulse will be produced which passes to the quantizer. This unit will pass on the pulse only if it is greater than a predetermined voltage level; it thus prevents counts from spurious particles and random noise. From here the pulse passes to a magnetic memory system (the one-line delay), the sum circuit and the pulse-forming unit where it is suitably shaped. After shaping, the pulse passes through an electronic gate, which is normally open, and then operates a dekatron counter tube. Acceptance of a count pulse is shown on the monitor screen by a bright spot appearing on the image of the particle. If this particle is larger than one picture element, *i.e.*, greater than the distance between two adjacent scanning lines, the flying spot will scan the same particle for a second time on its next scan line. A similar sequence of events occurs, but this time the sum circuit receives the new pulse from the quantizer plus the pulse from the previous scanning line, which is

released from the one-line delay unit. This state of coincident inputs produces an inhibiting pulse that shuts the gate and prevents a second count being recorded for the same particle.

Thus, in any given field every particle, however large, will be counted only once. It should be noted that this is an ideal situation and that, should the profile of the particle present a re-entrant along the line of the scan, two counts will be recorded from the same particle. Also, aggregates or particles that are not separated by more than two picture elements may be counted falsely.

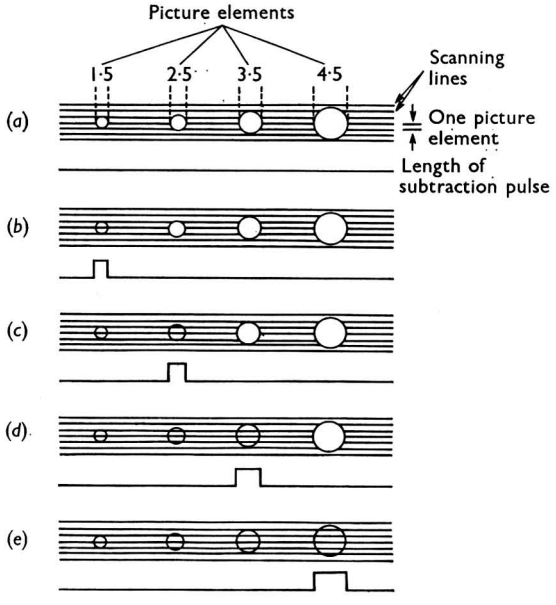

Fig. 4. Operation of sizing unit :

(*a*) Sizing unit set on O; no subtraction pulse is produced and therefore all four particles are counted.

(*b*) Sizing unit set on 2; the subtraction pulse is equivalent to 2 picture elements and no particle is counted whose size is less than this value. Therefore three particles are counted.

(*c*) Sizing unit set on $2\sqrt{2}$; no particle is counted whose size is less than 2·83 picture elements; therefore two particles are counted

(*d*) Sizing unit set on 4; only one particle is counted.

(*e*) Sizing unit set on $4\sqrt{2}$; no particles are counted.

The automatic sizing is performed by the sizing unit, which produces a pulse of selectable width that corresponds to a given size of particle on the sample. Any particle whose size is less than that selected will not be recorded during a field count, so that by successive counts with selected pulses of progressively greater width a complete size analysis of the sample in the field can be obtained. The standard pulse widths obtained from the sizing unit are given in terms of picture element size, and increase in a $\sqrt{2}$ progression; the picture element size, of course, varies with the magnification used in the optical system. The operation of the sizing units is illustrated diagrammatically in Fig. 4.

When counting, one scan across the field takes 8 seconds, and a further complete scan has to take place to re-set the sizing unit. The total time taken to count each size range is, therefore, 16 seconds, and a complete size analysis of the field may take up to 3 minutes (if all the size ranges have to be explored). Normally, between 10 and 25 fields must be sized to obtain a statistically sound size analysis of the whole sample, the number of fields depending on the particle density of the sample and on its degree of heterogeneity. Preparation of the instrument for a particular type of sample may take from 15 to 30 minutes; many similar samples can be measured with little further adjustment of the instrument. The total time taken to count and size a sample is usually between 10 and 60 minutes.

ACCURACY AND RELIABILITY OF THE METHOD—

The accuracy of the instrument in counting and sizing any particular sample will depend on two main possible sources of error. First, errors within the instrument itself, *i.e.*, those inherent in its design or those due to faulty operation. Measurement on the reliability and accuracy of the instrument showed it to be somewhat better than visual measurement on similar samples, and operating errors, with reasonable experience, were negligible.[4]

The second and by far the more important source of error in automatic counting and sizing lies in the nature of the sample. It has already been shown that aggregates and irregularly shaped particles can produce spurious counts, and it must be remembered that any form of artificial scanning can never be as discriminating as the human eye. An ideal sample for automtic counting with this instrument should have the properties listed below.

1. A reasonable degree of contrast between particle and background; a minimum of 10 per cent. optical contrast and preferably more.

2. The particles should have clear-cut, well defined edges; any haziness or reduction in contrast towards the edges will affect the length of the pulse that the particle produces, and this cannot always be overcome by adjustment of the instrument.

3. The optical contrast should be reasonably uniform over each individual particle, *e.g.*, emulsion globules that appear under transmitted light as black rings with light centres will be counted as several particles.

4. The particles should be regular in shape and preferably circular (or spherical). Ellipsoidal or irregular shapes can be counted and sized to produce a mean or average figure provided the particles are randomly distributed on the sample. Very irregularly shaped particles may be counted more than once; the counts are registered by a bright spot appearing on the monitor screen so that these false counts can be seen and may be corrected visually.

5. The sample must not be overcrowded, because the scan will not differentiate between the individual particles in groups and aggregates. On the other hand, the samples should not be too sparse, or the total count per field would be low and many fields would have to be counted and much time wasted. The errors due to overcrowding may not be very important when a number frequency is to be measured, but when volume or mass parameters are to be calculated (and this is frequently the parameter of greatest interest in the pesticide field) the errors can be extremely serious.

To summarise, the speed, reliability and degree of accuracy of the automatic instrument are superior to those of visual counting. The instrument is versatile in both the range of particulate matter it can measure and in the range of magnification that can be used. Particles from 1 μ upwards may be measured by transmitted light and from 10 μ upwards by reflected light. These ranges may be extended by using an intermediate photographic process.

Thus the use of such an instrument removes most of the difficulties involved in the measurement of particle size, but it tends to increase the difficulties involved in obtaining a sample of suitable quality. It is the quality of the sample that is important; normal sampling errors can be reduced because the increase in speed of counting permits more particles to be measured than is usually possible with visual counting.

SAMPLING—

The sampling of sprays and dusts can be carried out in the laboratory by allowing them to settle out in a sedimentation chamber on to suitable collecting surfaces. The density of particles on the sample can be readily controlled to avoid undue overcrowding. Dust particles

are relatively easy because they can be collected and measured directly on glass slides. Spray droplet sampling is more complicated in that the droplets will tend to spread when they impact on a collecting surface, and the droplets themselves must form a coloured stain that provides sufficient contrast to distinguish them from the background. In agricultural sprays, the latter requirement is best covered by adding a dye to the spray liquid; the degree of spread is dependent to a large degree on the nature of the sampling surface. Collection on various types of paper surfaces, commonly used in droplet sampling, is unsatisfactory for automatic counting, because the edges of the stains are always diffuse and the spread factor is often extremely large. Glazed photographic paper gives stains that are ideal for automatic counting, but there the stain size varies with the impaction velocity of the drop as well as with its size. Up to the present, siliconed surfaces, as described by Courshee,[7] have proved to be the most satisfactory in terms of quality of sample and constancy of spread factor.

Under practical spraying conditions in the open, sampling is very much more difficult and it is almost impossible to get a perfect size spectrum of a spray or a dust cloud. Besides the difficulties of sampling under isokinetic conditions, the size of a practical spray or dust cloud makes it virtually impossible to get samples representative of the whole cloud.

The automatic scanning technique is particularly valuable in determining the degree of cover obtained on sprayed or dusted surfaces. This is not necessarily concerned with the measurement of particles or microscopic stains because, with sprays particularly, the droplets may run together to give a more continuous film of deposit. Deposit areas can be measured by ignoring the counting and sizing circuits and electronically timing the total period during one scan that the scanning spot is obscured by the deposit. The time necessary for one complete scan with a completely clear field can also be measured, and the ratio of these two times gives the proportional area covered by the deposit. The samples for such measurements may be collected on artificial surfaces, but the most valuable information on coverage can only be obtained by studying the deposit on the surface that is used in practice, *i.e.*, the leaf. The measurement of samples collected on leaf surfaces presents serious difficulties, but these can be overcome by incorporating a fluorescent material into the spray or dust and then photographing the sprayed or dusted leaves in ultraviolet light.[8] The areas of deposit show up black on a light background, as shown in Fig. 5, and the photographic negative can be directly scanned to measure deposit area. Considerable practical difficulties arise in selecting the most appropriate fluorescent tracer to use in a particular pesticide formulation and the way to incorporate it into the formulation. It is essential that it will trace the pesticide deposit closely, because the area covered by pesticide deposited from sprays may be less than the total area wetted by the aqueous phase of the spray.

The measurement of oil droplets and solid particles dispersed in water poses some rather different problems. The automatic scanning of such particles is reasonably straightforward provided that the particles all lie in the same plane and, for emulsions, that the dispersed phase is suitably coloured. The first of these requirements can be met by placing a sample of the emulsion or suspension in a suitable cell, *e.g.*, a haemacytometer slide, and allowing the particles to rise to the surface or settle to the bottom of the cell before measurement. If the particles are extremely small and subject to Brownian motion, the sample can be photographed and the measurements made from the negative.

One of the greatest difficulties in this sampling process lies in extracting the sample from the bulk emulsion or suspension in a way such that it completely reproduces the particle size spectrum at the point of sampling. The samples are usually required at successive time intervals from the moment of preparation and at various depths below the surface of the emulsion or suspension, but the act of removing a sample can cause a considerable disturbance. Also aggregates or large emulsion globules in the extracted sample may be broken down by rapid passage through tubes or by passage through a narrow aperture.

This brief review of the most usual types of particulate matter encountered in pesticide formulation has dealt essentially with aqueous dispersions. However, it is also necessary to consider sprays of oil solutions, suspensions of solid particles in oil, and even multiple dispersions of suspensions of solid particles in oil that is itself emulsified in water. The measurement of particle size in any of these systems remains the same in principle, but different sampling problems may be involved. The measurement of particle size by automatic methods is fairly straightforward, but it is the obtaining of good samples that raises the greatest difficulties.

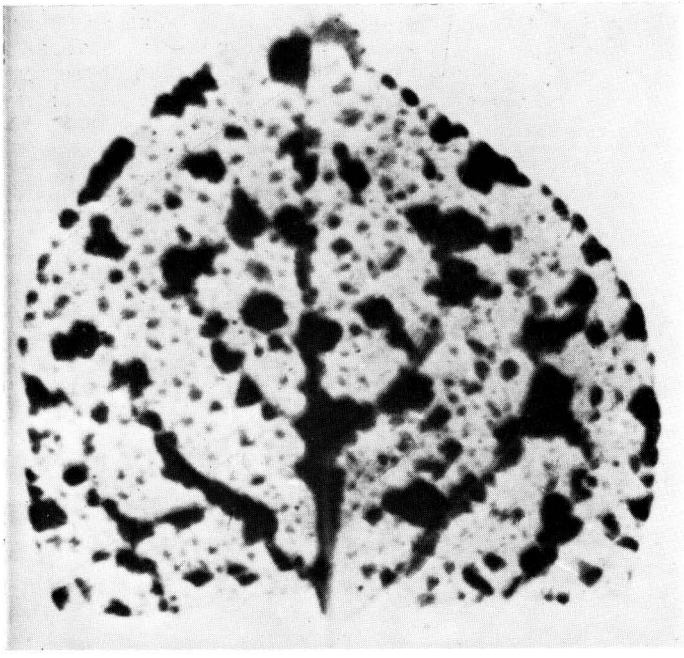

Fig. 5. Photograph of a fluorescent spray deposit on a leaf surface under ultraviolet light

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References

1. La Mer, V. K., Hochberg, S., Hodges, K., Wilson, I., Fales, J. A., and Latta, R., *J. Colloid Sci.*, **1947**, **2**, 539.
2. Analytical Methods Committee, *Analyst*, **1963**, **88**, 156.
3. Courshee, R. J., *Brit. J. Appl. Phys.*, 1954, Suppl. No. **3**, **5**, S.161.
4. Furmidge, C. G. L., *Ibid.*, **1961**, **12**, S.268.
5. Taylor, W. K., *Ibid.*, **1954**, Suppl. No. **3**, **5**, S.173.
6. Causley, D., and Young, J. Z., *Research*, **1955**, **8**, 430.
7. Courshee, R. J., and Byass, J. B., National Institute of Agricultural Engineering, Report No. **32**, **1953**.
8. Staniland, L. N., *J. Agric. Eng. Res.*, **1959**, **4**, 110.

Received *March 21st*, **1963**

The Continuous Automatic Microbiological Assay of Antibiotics

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The principle of continuous culture of the test organism is applied to the AutoAnalyzer method for the microbiological assay of antibiotics by measurement of respiratory carbon dioxide. The use of *E. coli* as a general purpose test organism is described, and the method is extended to the assay of high potency samples of benzylpenicillin, streptomycins, neomycin, novobiocin and cephalosporin C. A modification is suggested for samples of lower potency. In comparison with a conventional agar-diffusion method (8 × 8 quasi-latin square), the AutoAnalyzer results showed no bias and, with duplicate recording of each sample, were of greater precision.

AN AutoAnalyzer instrumental system for the microbiological assay of tetracyclines and polyene antibiotics has been described by Haney *et al.*[1] and Gerke, Haney and Pagano.[2] The method is based on measurement of the carbon dioxide resulting from free respiration by the test organism during a fixed incubation time and of the depression of respiration by graded concentrations of the antibiotic to be determined. In preliminary trials with the method it became clear that the instrumental system was satisfactory, but we encountered considerable difficulties in preparing a standard inoculum of a test organism (*Escherichia coli*) similar to that described for the assay of tetracyclines. It has been specified[1,2] that the organism be grown by overnight culture in a vigorously shaken medium; the cells are then collected by centrifugation, washed and re-suspended in fresh cold nutrient medium. Alternatively, the culture may be suitably diluted without centrifugation.[3] The inoculum produced in this way is maintained at 0° to 3° C during the working day in order to reduce to a minimum respiration of the cells in the inoculum reservoir.

The number of cells produced by overnight culture and their vigour depend on the degree of aeration the medium receives, and any variation in aeration necessitates a corresponding variation in the concentration of cells required for the working inoculum to maintain constant production of carbon dioxide in the AutoAnalyzer system. Moreover, the degree of sensitivity towards a given antiobiotic appeared to vary with the numbers of cells obtained, and this made it necessary to re-check for each inoculum the concentration of antibiotic required to give the working mid-point of the dose - response curve. Considerable time was often required at the beginning of the working day to establish the inoculum dilution and antibiotic concentration required for both standards and samples.

To assay certain antibiotics to which *E. coli* grown in this way is not sensitive we wished to use *Bacillus subtilis*. When grown overnight, cultures of *B. subtilis* contain a large proportion of spores, and a suitably diluted inoculum showed a rapid decline in ability to produce carbon dioxide when maintained at 0° to 3° C. Much younger cultures (4 hour), predominantly in the vegetative form, showed a similar rapid decline in vigour.

These difficulties led us to consider the possibility of continuous culture of the test organism; if successful, this would provide a vigorous and consistent inoculum for the assay and eliminate the need for maintaining a supply of ice-cold inoculum. The size of the reservoir required for continuous growth of the inoculum depends on the generation time of the organism at the incubation temperature and on the throughput of nutrient medium. With the short generation time of *E. coli* (about 20 minutes), a reservoir volume of 50 ml and the continuous addition of 0·8 ml per minute of sterile medium permits a suitable concentration of organisms to be maintained, and the whole unit can be accommodated in the AutoAnalyzer incubation bath. Vigorous aeration serves to mix this volume of culture and to remove both the respiratory carbon dioxide as it is formed and culture in excess of that required for the assay. The sensitivity of the organism grown in this way towards certain antibiotics, notably cephalosporin C and, to a lesser extent, benzylpenicillin, differs markedly from that of the same organism grown in overnight cultures.

METHOD

APPARATUS—

AutoAnalyzer—This consists of a modified sampling unit, a 15-channel proportionating pump, an incubation bath at 37° C, a colorimeter fitted with a 15-mm tubular flow-cell and 550-mμ filters and a single-pen recorder.

Fit the sampler with a reverse sampling crook and with a modified cam lever to provide a 2½-minute sample aspiration time. The constant-level device (see Fig. 2) should be clamped to the sampler, so that formaldehyde is aspirated for 24 seconds in each three-minute cycle.

Fix to the lower side of the cover of the bath four support pillars, approximately 7 inches in length, through the four central holes provided in the cover, and attach the lower end of the pillars to a 4-inch diameter disc of non-corrodible metal, suitably drilled. To one inlet of the cover attach 100 feet of polythene tubing, 0·110 inch internal diameter (Portex tubing 54B, Portland Plastics Ltd., Hythe, Kent), coiling it round the supports. Attach a glass T-joint to the other end of the tubing. To another inlet in the cover attach a short length of polythene tubing, a ½-length mixing coil and another length of polythene tubing to connect with the lower limb of the T-joint. Connect the remaining limb of the T-joint to two mixing coils in series and then to one of the outlets in the cover. For making polythene-to-glass connections, the ends of the tubing may be softened by brief immersion in boiling water, the tubing being then pushed over the glass fitting and secured with thin copper wire.

Fit the prepared inoculum reservoir (see below) through the hole in the rear of the incubation bath-top, and clamp in position.

Magnetic diaphragm air-pump—Obtainable from Chas. Austen Pumps Ltd., Byfleet, Surrey.

Inoculum reservoir and fittings—See Fig. 1.

Reagent reservoirs—Pyrex bottles, of 5-litre capacity, fitted with stoppers, each provided with one glass tube reaching to the bottom and an inlet connected to a gas wash-bottle containing sodium hydroxide solution.

REAGENTS—

Prepare all reagents with carbon dioxide free distilled water. Reagents 1, 3 and 4 are conveniently stored in 5-litre containers protected from atmospheric carbon dioxide.

1. *Sulphuric acid*, N—Add 0·1 per cent. v/v of MS Silicone Antifoam Emulsion RD (Hopkin & Williams Ltd., Chadwell Heath, Essex) to N sulphuric acid.

2. *Carbonate buffer*—Dissolve 56 g of analytical-reagent grade sodium hydrogen carbonate and 35 g of analytical-reagent grade anhydrous sodium carbonate in sufficient water to produce 1 litre.

3. *Buffered phenolphthalein reagent*—Add 7·0 ml of a 1 per cent. w/v solution of phenolphthalein in methanol to about 900 ml of water, and mix. For assay with *E. coli* 397E as test organism, add 5·5 ml of carbonate buffer, and dilute with water to 1 litre. Add 0·25 ml of capryl alcohol, and shake well. Adjust the strength of this reagent as described on p. 698.

4. *Diluent*—Add 0·1 per cent. v/v of Tween 20 to distilled water, and mix.

5. *Formaldehyde solutions*, (a) 1 *per cent. w/v*, (b) 0·1 *per cent. w/v*.

(*a*) Dilute 2·5 ml of a 40 per cent. w/v solution of formaldehyde to 100 ml with diluent.

(*b*) Dilute solution (*a*) (1 + 9) with diluent.

NUTRIENT MEDIA—

Medium 1—Prepare from the materials listed below.

Dehydrated Penassay broth (Difco*)	17·5 g
Tryptone (Difco*)	10·0 g
Yeast extract (Difco*)	5·0 g
Polypropylene glycol, P2000	0·02 ml
Distilled water	to 1 litre

* Equivalent materials can be substituted, but they may not necessarily give the same yield of carbon dioxide, the same sensitivity to the antibiotic or the same dose - response slope.

Sterilise 1·5-litre amounts in 80-oz bottles in an autoclave at 121° C (15 lb. per sq. inch) for 20 minutes. The bottles should be covered with aluminium foil during the sterilisation and then sealed immediately with sterile rubber caps. Store, preferably at 4° C.

Medium 2—Prepare exactly as described for medium 1 except for the substitution of 2 ml of Tween 20 per litre in place of the polypropylene glycol. Sterlilise 2-litre amounts in 80-oz bottles. Store, preferably at 4° C.

TEST ORGANISMS—

As a general-purpose test organism, suitable for assaying many antibiotics, *E. coli* 397E has proved satisfactory, but the principle of continuous culture is of general application to other organisms including spore-formers. For such organisms the volume of the reservoir and the input of medium may be adjusted so that the inoculum used in the assay consists almost entirely of organisms in the vegetative form. Whatever organism is selected it must be capable of rapid growth and respiration and must be of sufficient sensitivity to the antibiotic being assayed. The sensitivity should be assessed under continuous culture conditions, since other methods for measuring the sensitivity to an antibiotic are not necessarily relevant.

Maintain test organisms as agar-slope cultures, fresh 24-hour slope cultures being prepared as required for the assay.

PREPARATION AND CONTINUOUS CULTURE OF INOCULUM—

Attach non-absorbent cotton filters to the air-inlet tube of the inoculum reservoir (see Fig. 1) and to the inlet side of the fittings for the reservoir of medium 1. Wrap the fittings, G, of the reservoir assembly in aluminium foil, and cover the tops of tubes C and E with foil. Sterilise the whole assembly in an autoclave at 121° C (15 lb. per sq. inch) for 20 minutes.

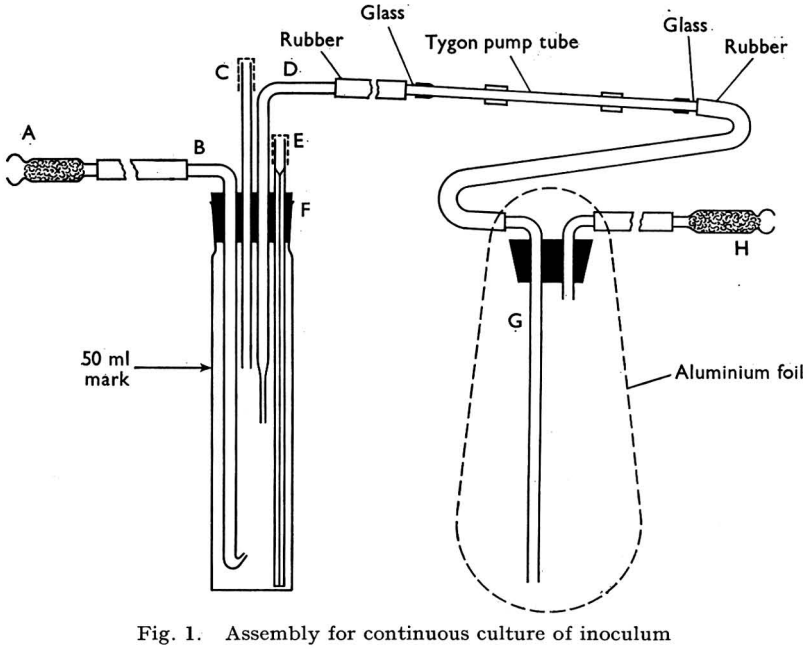

Fig. 1. Assembly for continuous culture of inoculum

When required for use, aseptically remove the foil film from assembly G, and insert into a bottle of sterile medium 1. Slightly ease out the plug on the inoculum reservoir, and by means of air-pressure applied to the filter, H, blow over 50 ml of medium 1 into the inoculum reservoir.

Aseptically remove 5 ml of medium from the inoculum reservoir, and add it to an overnight agar-slope culture of the test organism. Suspend the organisms in the medium, and

transfer the suspension to the inoculum reservoir. Re-secure the plug and fittings, ensuring that the jet of the air-inlet tube B is directed round the circumference of the reservoir and away from the bottom of the inoculum tube E. Immerse the inoculum reservoir in the incubation bath, and clamp in position, so that the level of liquid in the reservoir is below the level of the water in the bath. Insert the Tygon pump tube into one channel of the Auto-Analyzer pump, and start the pump. Connect the air-pump to filter A, and pass a steady stream of air (about 40 litres per hour) into the inoculum reservoir. Allow the system, assembled as described below, to reach equilibrium, as will be shown by the production of a constant amount of respiratory carbon dioxide during the fixed incubation time of the assay. For organisms with a short generation time, 6 hours should be sufficient, but it is convenient to allow equilibration to proceed overnight.

The inoculum can be renewed at the end of each working day or allowed to continue for several days by aseptically transferring the assembly G to a fresh bottle of medium 1 each day. Alternatively, a reservoir of medium 1 large enough to last for a week may be attached, provided contamination of the medium and inoculum reservoir can be prevented.

ASSEMBLY OF AUTOANALYZER—

The assembly shown in Fig. 2 incorporates a preliminary 1 + 9 dilution stage. This is optional but convenient for the assay of solid samples with potencies approaching those of the pure antibiotics, since the need for making many accurate dilutions by hand is thereby avoided. If, however, greater sensitivity is desired, the preliminary dilution stage can be omitted, as shown in Fig. 3, or a smaller dilution can be substituted. Whatever arrangement is adopted, it is essential that the variable amount of air admitted by the alternating operation of the antibiotic and formaldehyde sample tubes be eliminated from the system. Only liquid may be permitted to enter the re-sampling tube.

If the preliminary dilution does not exceed about 1 + 9 the whole manifold can be pumped satisfactorily with one pump, but it may be more convenient to use a separate pump for the medium 1 and the preliminary dilution stage, particularly if a larger preliminary dilution is desired.

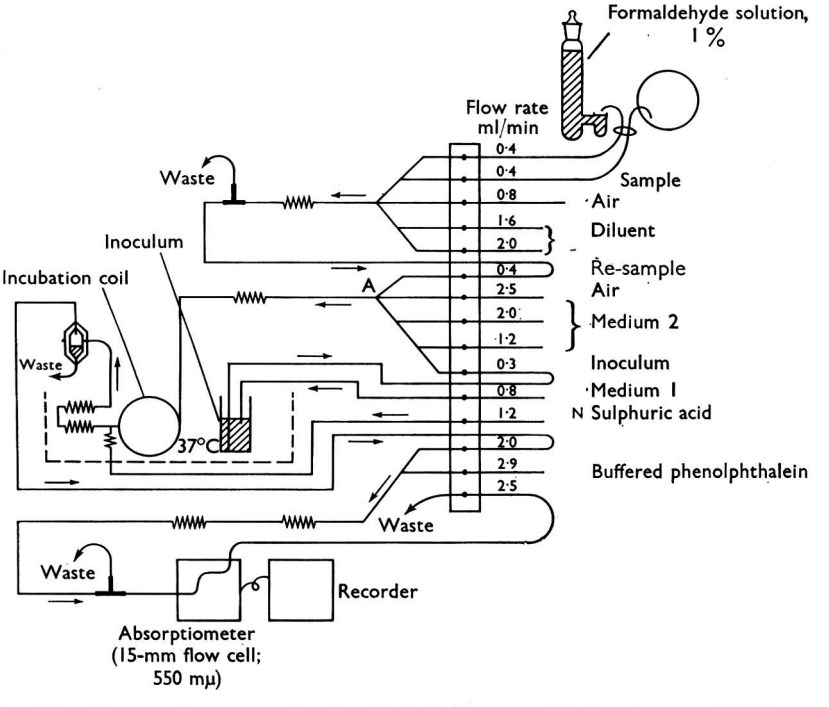

Fig. 2. Flow diagram for automatic microbiological assay of high-potency antibiotic samples

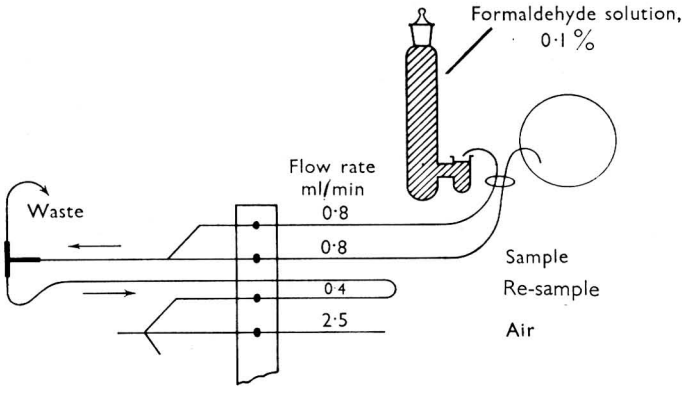

Fig. 3. Partial flow diagram for increased sensitivity

Apart from the continuous culture assembly the rest of the AutoAnalyzer arrangement is substantially that suggested by Haney, Gerke, Madigan, Pangano and Ferrari.[1] However, we prefer to carry out the gas - liquid separation at 37° C with the vacuum-jacketed separator shown in Fig. 4; this, if mounted immediately on top of the incubation bath, permits visual examination of its performance. The substitution of a 15-mm tubular flow-cell in place of the 10-mm conventional one gives improved separation of samples and a better dose - response calibration curve.

The glass cactus-fitting, A (see Fig. 1), must be of sufficiently wide bore to generate air bubbles large enough to maintain a regular bubble pattern throughout the incubation coil.

To set up the AutoAnalyzer for the assay, fill the constant-level device with 1 per cent. formaldehyde solution (or 0·1 per cent. formaldehyde solution if the preliminary dilution is omitted, as shown in Fig. 3), and connect tube E of the inoculum reservoir to the inoculum tube of the manifold with 0·045-inch bore polythene tubing. This connection should be as short as possible, to minimise the continued growth of the organisms before joining the medium 2 and diluted sample streams.

Connect the appropriate tubes to the diluent, N sulphuric acid, medium 2 and reagent reservoirs, making the connection to the buffered phenolphthalein reagent with 0·045-inch bore polythene tubing. Set the sample-plate in operation at 20 tests per hour, with water in the sample-cups. When peaks begin to be recorded, adjust the strength of the buffered phenolphthalein reagent with either carbonate buffer or distilled water containing 0·7 per cent.

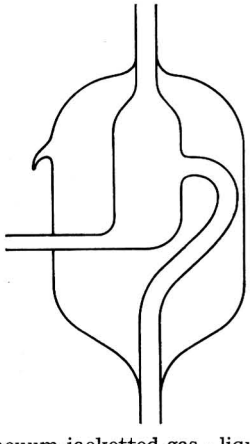

Fig. 4. Vacuum-jacketted gas - liquid separator, working capacity, approximately 0·7 ml

v/v of a 1 per cent. w/v solution of phenolphthalein in methanol, so as to obtain peak transmissions of 90 per cent.

Determine the concentration of the antibiotic under test required to give the mid-point of the dose - response curve (50 per cent. transmission) by placing a series of suitable dilutions in the sample-cups, each dilution being placed in three successive cups. Approximate mid-point concentrations for some antibiotics (with the preliminary 1 + 9 dilution) can be read from Fig. 5. Re-establish the mid-point concentration (medium standard) weekly or when any change is made in the manifold. Once this is established the concentration of the buffered phenolphthalein reagent may be adjusted to give 50 per cent. transmission with the medium standard in the sample cups.

Haney *et al.*[1] recommended that the concentration of the high and low standards, required to establish the slope of the dose -response curve, be $1\frac{1}{2}$ times and $\frac{2}{3}$ of the concentration of the medium standards, respectively. For the arrangement shown in Fig. 2 and with continuous inoculum culture, a narrower range of concentration is necessary to retain the responses on the approximately linear portion of the dose - response curves (preferably within the limits of 35 to 65 per cent. transmission).

The permissible ratio depends on the slope; for streptomycin, suitable concentrations are $\frac{6}{5}$ and $\frac{5}{6}$ of that of the medium standard, giving, for example, cup concentrations of 3·0, 2·5 and 2·08 mg per ml for the three standards.

At the end of each working day disconnect the inoculum tube from the inoculum reservoir, and plug the top of tube E. Pass 2 N sodium hydroxide through the inoculum, medium 2 and buffered phenolphthalein tubes for 5 minutes, and then pump diluent through to clear any deposit from the incubation and mixing coils.

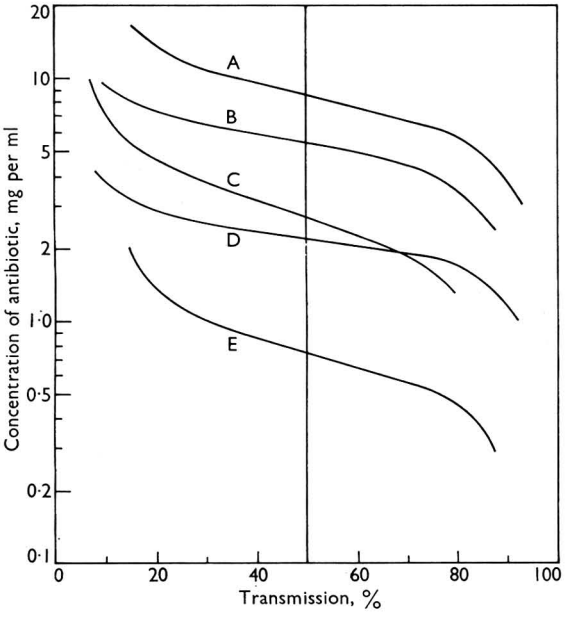

Fig. 5. Calibration graphs : curve A, novobiocin; curve B, sodium benzylpenicillin; curve C, streptomycin sulphate; curve D, sodium cephalosporin C; curve E, neomycin sulphate

ASSAY DESIGN—

In the design proposed by Haney *et al.*[1] each sample-plate is loaded with a series of 4 high, 4 low and 4 medium standards, 5 samples and 2 medium standards, with the last two (5 samples and 2 medium standards) being repeated three more times. Each sample

is assayed in duplicate. The responses of the medium standards are connected on the recording by a line (the drift line), and the responses of all samples are measured as differences from this line. The slopes of the dose - response curve above and below the medium standard are determined, and these permit calculation of the potencies of samples as differences from that of the medium standard.

Many different assay designs are possible according to the replication, and hence the precision, desired in relation to throughput of samples. In one design, which permits mathematical correction for drift, the slope of the dose - response curve over the narrower range of potencies suitable for the AutoAnalyzer arrangement described above is first established with 4 low and 4 high standards. The remaining sample-plate positions are then filled with two similar 16-position patterns, each of 6 samples in duplicate and 4 medium standards. The samples and standards are so arranged that the second eight solutions in each pattern are in the reverse order of the first eight. Thus solutions in the order A to H are immediately followed by the same solutions arranged H to A. The mean response for each sample and for the four medium standards is then independent of drift, if it is assumed that this is linear over the 48 minutes required for recording the 16 responses. The medium standards may occupy any positions in the pattern, but are conveniently placed in positions B and F.

Both the designs considered above are one-level (1 × 1) assays, in which the slopes of the dose - response lines for the standard and for every sample are known to be the same or when this may be assumed. Moreover, with *E. coli* 397E as test organism similar slopes are given by different antibiotics (Fig. 5), and it is therefore necessary that the qualitative composition of the sample be known.

Calculation of results—

Read off from the recording the percentage transmission (T%) for the low and high standards, ignoring for each the first of the four responses. Calculate the mean response for each standard, determine the difference, D, and calculate the slope constant, K, from the equation $K = \frac{R}{D}$, where R is the log of the dose ratio (high to low). The slope constant represents on a log scale the concentration difference for each 1 per cent. transmission over the working range and is positive or negative according to whether the response of a sample (in T%) is numerically less or more than that of the medium standard.

Calculate the mean responses for each sample and for the four medium standards. Determine the difference in T% from the medium standard for each sample, and multiply

Table I

Streptomycin assays

Sample number	Prepared strength of streptomycin sulphate, mg per ml	Amount of streptomycin found by using— AutoAnalyzer 1,* mg per ml	AutoAnalyzer 2,* mg per ml	AutoAnalyzer 3,† mg per ml	Agar-plate method 1,‡ mg per ml	Agar-plate method 2,‡ mg per ml
1	3·00	3·01	3·00	2·95	2·85	3·05
2	3·50	3·46	3·52	3·42	3·47	3·46
3	3·20	3·17	3·20	3·08	3·30	3·28
4	3·10	3·08	3·09	2·99	3·07	3·03
5	2·90	2·83	2·93	2·79	2·99	2·88
6	2·60	2·60	2·54	2·61	2·54	2·43
7	2·70	—	2·64	—	2·76	2·87
8	3·16	—	3·17	—	3·11	3·41
9	3·20	—	3·34	—	3·30	3·27
10	3·52	—	3·60	—	3·51	3·51
11	2·86	—	2·90	—	3·03	2·85
12	2·78	—	2·77	—	2·93	2·78

* Calculated by the proposed method.
† Calculated by the method of Haney *et al.*[1]
‡ The results of an 8 × 8 quasi-latin square design with *B. subtilis* 841 as test organism.

by K, maintaining the correct sign. Add this to (or subtract it from, as appropriate) the log of the potency of the medium standard. Convert to the antilog to give the concentration of the sample solution, and calculate the result on the original sample.

The results shown in Table I were obtained on a series of accurately prepared dilutions (unknown to the operator) of streptomycin sulphate by the two AutoAnalyzer designs and methods of calculation discussed above. In comparison with the known concentration and with results on the same samples assayed at the same time by a conventional two-level agar-diffusion method (8 × 8 quasi-latin square) with a different test organism, the Auto-Analyzer results show no bias and demonstrate that, with the duplicate recording of samples recommended, an appreciably lower error can be expected.

We thank all those at the Squibb Institute for Medical Research, New Brunswick, N.J., U.S.A., who generously supplied advance information of their procedures, Mr. W. K. Anslow, Dr. A. Ferrari and Mr. J. P. R. Tootill for helpful discussions and Mr. K. Clover for assistance with the practical work.

References

1. Haney, T. A., Gerke, J. R., Madigan, M. E., Pagano, J. F., and Ferrari, A., *Ann. N.Y. Acad. Sci.*, 1962, **93**, 627.
2. Gerke, J. R., Haney, T. A., and Pagano, J. R., *Ibid.*, 1962, **93**, 640.
3. Kavanagh, F., *Editor*, "Analytical Microbiology," Academic Press Inc., London and New York, 1963.

Received *April 16th*, 1963

Automatic Procedures for the Colorimetric Analysis of Iron- and Steel-making Slags

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The AutoAnalyzer system of automatic colorimetric analysis has been successfully applied to the determination of total iron and the oxides of manganese, phosphorus and aluminium in iron- and steel-making slags. The sample is decomposed in acid, silicon removed as metasilicic acid and the solution diluted to a fixed volume. From this stage analysis is completely automatic, the operator being required only to read optical-density values from a chart recorder and convert these into percentage content by reference to a calibration factor. The automated procedures are primarily intended for analysing at least ten sample solutions at a time. Each slag constituent is determined separately by using a specially designed flow system that can be assembled and calibrated in about 40 minutes. The speed of operation is either 20 or 40 samples per hour dependent on the type of chemical reaction involved.

THE use of the Technicon AutoAnalyzer is well established in clinical and industrial analysis. Typical applications of this equipment in the United Kingdom have included the determination of streptomycin in fermentation broths[1] and the determination of zinc, lead, molybdenum and nickel in soil extracts.[2]

The sample in solution form is aspirated, pumped through the apparatus and mixed with appropriate reagents to produce a coloured solution. This coloured solution is passed through a colorimeter; the optical density is recorded on a chart and related to percentage content from a calibration graph. A fuller description of the mechanics and principles of operation has been provided by Ferrari, Russo-Alesi and Kelly.[3]

As an initial study of the instrument's application to steelworks' materials, four spectrophotometric methods for analysing iron- and steel-making slags have been automated. These methods form part of an analytical scheme used in our laboratory,[4] in which the sample of slag is decomposed in acid, silica removed and the solution diluted to a fixed volume. From this solution, separate portions are taken for the determination of total iron, manganese oxide, phosphorus pentoxide and alumina by spectrophotometry, and calcium and magnesium oxides by complexometric titration. This paper described the adaptation of the spectrophotometric procedures for use with the Technicon AutoAnalyzer.

PREPARATION OF SAMPLE SOLUTION

A powdered slag sample weighing 0·5 g is partly decomposed in hydrochloric acid; the solution is then oxidised, perchloric acid is added, and the solution is evaporated until fumes of perchloric acid are evolved. Fuming is continued under reflux for 10 to 15 minutes to separate silicon as metasilicic acid. After removal by filtration, the precipitate is ignited and weighed as silica. Finally, the silica is volatilised as the fluoride, the residual oxides being fused and added to the sample solution. This solution is evaporated to fumes for a second time to remove hydrochloric acid and to oxidise chromium to the sexavalent state. The volume of perchloric acid remaining should be about 10 to 15 ml, giving an approximately 2·5 per cent. v/v acid solution on dilution to 500 ml. Full details of the preparation of the sample solution are given in a British Iron and Steel Research Association Publication.[4]

DETERMINATION OF ALUMINA

In the manual method, iron and manganese are separated from aluminium by precipitation with sodium hydroxide solution in the presence of hydrogen peroxide. Interference due to titanium and vanadium is prevented by a further addition of hydrogen peroxide. After the solution has been carefully neutralised and adjusted to pH 6·0, Eriochrome cyanine is added to produce the orange-red aluminium - dye complex; colour development is complete in 20 to 30 minutes.

Initial tests indicated that the colour reaction could be automated, provided that two time-delay coils were placed in the flow system to allow sufficient time for reproducible colour development. The colour reaction is extremely sensitive, and it was difficult to design a flow system to cover the desired percentage range (up to 15 per cent. of alumina, equivalent to 8 mg of aluminium per 100 ml of sample solution). Considerable dilution was necessary, and this was achieved by a method of "opposed dilution" introduced by Technicon Instruments Ltd. In the proposed system, a tube pumping diluent at 0·32 ml per minute in opposition to a sample line pumping at 0·42 ml per minute gives an effective sample intake of 0·1 ml per minute. (A tube of flow rate 0·32 ml per minute was the smallest available to us when these procedures were developed.) With a high over-all flow rate of 18 ml per minute, it was now possible to cover at least half the desired percentage range. For concentrations greater than about 7·5 per cent. of alumina, a diluted sample solution must be used.

Considerable difficulties were caused by excessively noisy records. This was traced to the formation of a blue deposit in the flow-through cell of the colorimeter and was probably due to the decomposition of the aluminium - dye complex. This difficulty was overcome by adding a small amount of acetone to the dye solution.

Contamination between successive samples is not a serious problem in the AutoAnalyzer system provided that a properly designed manifold of tubes is used. The problem is, however, accentuated when such strongly coloured dye solutions as Eriochrome cyanine are used. For this reason, it is preferable to use alternate plastic cups filled with dilute perchloric acid to provide a thorough washing after each sample has been analysed. An additional advantage of this technique is the improvement in resolution between successive peaks on the chart recorder. In the proposed flow system, sampler-plate operating at 40 tests per hour with alternate acid-filled cups gives an effective speed of 20 samples per hour.

INTERFERING ELEMENTS—

In an attempt to automate the procedure for removing iron, etc., in the manual method, the sample stream was mixed with dilute solutions of sodium hydroxide and hydrogen peroxide, and dialysed through a Cellophane membrane. Only partial success was attained since there was frequent rupturing of the membrane. In subsequent work, the use of ascorbic acid for complexing iron made it possible to dispense with the dialyser unit.

Ascorbic acid was first proposed by Hill[5] as a suitable complexing agent for iron and certain other elements in the aluminium - Eriochrome cyanine reaction. In our experience, the presence of this reagent during colour development in the manual procedure results in the formation of a coloured product that is not sufficiently stable with respect to time to permit optical-density measurements to be made. This latter consideration is of less importance in the AutoAnalyzer system, as all measurements are made under identical conditions, and, provided that the rate of colour development is reproducible, the production of

TABLE I

THE EFFECT OF VARIOUS ELEMENTS ON THE DETERMINATION OF ALUMINA

Interfering element	Amount of interfering element added, mg per 100 ml	Amount of aluminium found when—		
		0·2 mg of Al was added, mg per 100 ml	1·0 mg of Al was added, mg per 100 ml	3·0 mg of Al were added, mg per 100 ml
Titanium	1·0	0·25	1·00	—
	2·5	—	1·12	—
Manganese	20·0	0·25	0·98	—
	1·0	—	1·00	—
Chromium^{VI}	2·5	0·20	0·98	3·00
	10·0	—	0·89	—
	20·0	—	0·80	—
	1·0	—	1·02	—
Vanadium	2·5	0·18	1·02	—
	10·0	—	1·26	—
	20·0	—	1·49	—
Phosphorus	20·0	—	0·99	—
	50·0	0·20	1·02	—

a time-stable complex is not essential. Tests confirmed that in a flow system operating under closely controlled conditions (see Fig. 1) iron in amounts up to 25 mg per 100 ml of sample solution could be successfully complexed with ascorbic acid. There was, however, a slight positive interference, equivalent to about 0·05 mg of aluminium, when the amount of iron added was increased to 50 mg per 100 ml.

Previous experience in determining aluminium by using Eriochrome cyanine suggested that, of the other elements present in slags, titanium, vanadium, manganese, chromium and phosphorus might interfere in the colour reaction. The effect of these elements was determined at three concentration levels of aluminium, and the results are summarised in Table I. Interference was considered to be significant when the amount of aluminium found lay outside arbitrary limits of $\pm$ 0·03 mg at the 1·0 mg and 3·0 mg per 100 ml levels, and $\pm$ 0·05 mg at the 0·2 mg per 100 ml level when the analytical precision is not great owing to the sigmoid character of the calibration graph.

Titanium and vanadium in amounts of more than 1 mg and 2·5 mg per 100 ml, respectively, interfere seriously giving high results. It was not possible to mask interference of these elements with hydrogen peroxide as in the manual procedure, presumably because of the influence of the ascorbic acid present in the flow system. Chromium in the sexavalent state can be tolerated in amounts up to 2·5 mg per 100 ml, but with chromium contents above this level low results for aluminium will be obtained. Manganese up to 20 mg and phosphorus (as phosphate) up to 50 mg per 100 ml are without effect. In terms of the percentage oxide content, the limits are 2 per cent. of titania, 4 per cent. of chromium trioxide and 4 per cent. of vanadium pentoxide.

Method

Reagents—

Ascorbic acid, 0·5 per cent. w/v—Dissolve 5 g of ascorbic acid in water, add 10 drops of Teepol, and dilute to 1 litre.

Perchloric acid, diluted (1 + 3) *and* (1 + 39)—Prepare from perchloric acid, sp.gr. 1·54.

Dye solution—Dissolve 0·25 g of Merck Eriochrome cyanine in water, add 100 ml of acetone, and dilute to 1 litre in a calibrated flask. Prepare a fresh solution daily.

Buffer solution, pH 6·4—Dissolve 400 g of analytical-reagent grade hydrated sodium acetate in water, add 10 ml of glacial acetic acid, and dilute to 1 litre. The pH of this solution should be between 6·35 and 6·45. Store in a stoppered bottle.

Standard aluminium solution—Dissolve 0·5293 g of high purity aluminium metal in 20 ml of hydrochloric acid, add 15 ml of perchloric acid, and evaporate to fumes. Cool, add water, and warm, if necessary, to redissolve the salts. Dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

$$1 \text{ ml} \equiv 1 \text{ mg of alumina.}$$

Procedure—

Assemble the AutoAnalyzer as shown in Fig. 1. Set the sampler-plate at the rate of 40 samples per hour, and fill alternate sample-cups with dilute perchloric acid (1 + 39). Prepare a series of calibration solutions by adding up to 10 ml of standard aluminium solution to 10 ml of diluted perchloric acid (1 + 3). Dilute each solution to 100 ml in a calibrated flask, and store in a tightly stoppered polythene bottle. Prepare a calibration curve by running the calibration solutions in duplicate. Draw the base line on the chart recorder by joining together the small peaks due to the intermediate acid cups, and record the peak height of each calibration solution in terms of optical density. The base line drifts slightly with time owing to fading of the dye solution, but the drift should not exceed 0·005 optical-density units per hour. Deduct the optical density of the reagent blank solution in each determination.

The graph relating aluminium content to optical density is sigmoid in character (see Fig. 2); it is, however, rectilinear in the range 1·5 to 7·5 mg of alumina per 100 ml, and the curve between these two points can be expressed as $y = mx + c$. For contents greater than 7·5 mg of alumina per 100 ml, the graph is curved and not reproducible. In routine use, a graph should be plotted with each batch of tests, but if the alumina content of the samples is known to exceed 2 per cent., *i.e.*, falling on the linear part of the graph, it is only necessary

to run, say, three calibration solutions each day to establish its slope. At least one calibration solution should be measured with each sampler-plate of test solutions as a check on calibration drift.

Run the test samples through the apparatus, and calculate the percentage of alumina by reference to the calibration graph. Deduct the apparent percentage of alumina found in the reagent blank solution.

For samples that give optical-density values on the upper curved part of the graph, repeat the tests on a diluted sample solution containing sufficient added dilute perchloric acid to maintain the acid concentration at (1 + 39), *e.g.*, for a (1 + 1) dilution, take 50 ml of sample solution, add 5 ml of diluted perchloric (1 + 3), and dilute to 100 ml in a calibrated flask.

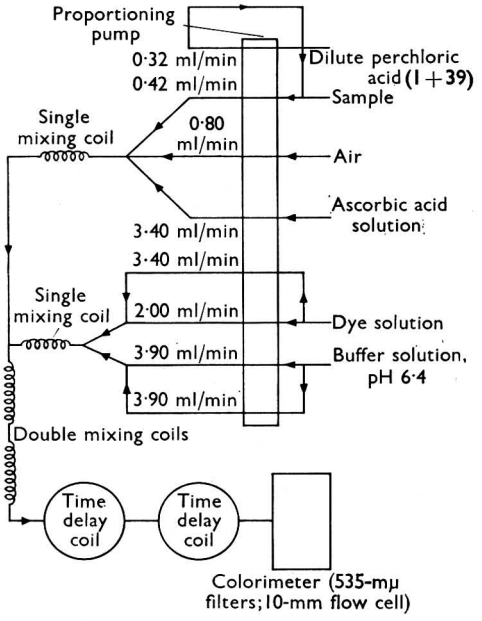

Fig. 1. AutoAnalyzer flow system for determining alumina in steel-making slags

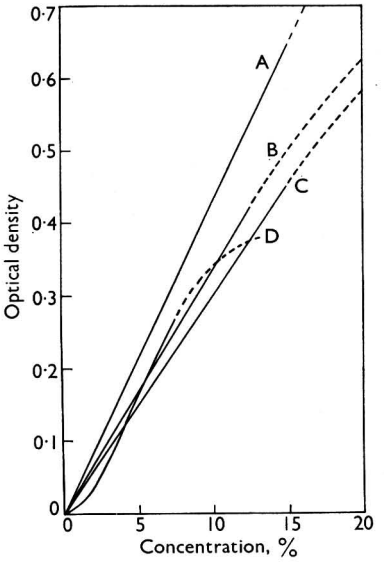

Fig. 2. Calibration graph for the analysis of steel-making slags with the AutoAnalyzer : curve A, total iron; curve B, manganese oxide; curve C, phosphorus pentoxide; curve D, alumina

DETERMINATION OF PHOSPHORUS PENTOXIDE

In the manual method, a solution containing ammonium vanadate, ammonium molybdate and dilute nitric acid is added to a portion of the sample solution to form the yellow molybdovanadophosphoric acid complex. This simple procedure proved easy to automate, but it was necessary to give some consideration to the choice of light filter and to possible interference from iron present in the sample solution. Initial tests indicated a slight positive interference from iron when the 440-mμ filter (corresponding to the wavelength used in the manual method) was used. At this wavelength the optical-density readings were rather low, *e.g.*, 0·21 optical-density units for solutions containing 5 mg of phosphorus per 100 ml, and for this reason the more sensitive 420-mμ filter was preferred (0·35 optical-density units for 5 mg of phosphorus per 100 ml). Unfortunately, interference from iron is more pronounced as the ultraviolet part of the spectrum is approached. A series of tests in which iron was added to solutions containing different amounts of phosphorus showed that interference was roughly proportional to the amount of iron present, up to phosphorus contents of 8 mg per 100 ml of test solution. Thus it is possible to make a small deduction from the apparent phosphorus content to allow for interference from iron; the deduction is 0·002 mg of phosphorus per mg of iron present.

METHOD

REAGENTS—

Molybdovanadate solution—Transfer 0·31 g of ammonium metavanadate to a beaker containing 50 ml of water and slowly add 40 ml of nitric acid, sp.gr. 1·42, while swirling the solution. Heat below the boiling-point until dissolution is complete. Dissolve 12·5 g of ammonium molybdate in 100 ml of water, warming if necessary. Cool both solutions thoroughly, mix, and dilute to 1 litre. Store in a stoppered polythene bottle; the solution is stable for about 3 days.

Standard phosphorus solution—Dissolve 1·917 g of potassium dihydrogen orthophosphate in water, dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 1 mg of phosphorus pentoxide.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 3, and set the sampler-plate at the rate of 40 samples per hour. Prepare a standard calibration graph from a series of solutions containing 10 ml of diluted perchloric acid (1 + 3) and up to 20 ml of standard phosphorus solution, diluted to 100 ml to cover a range of contents up to 20 per cent. of phosphorus pentoxide.

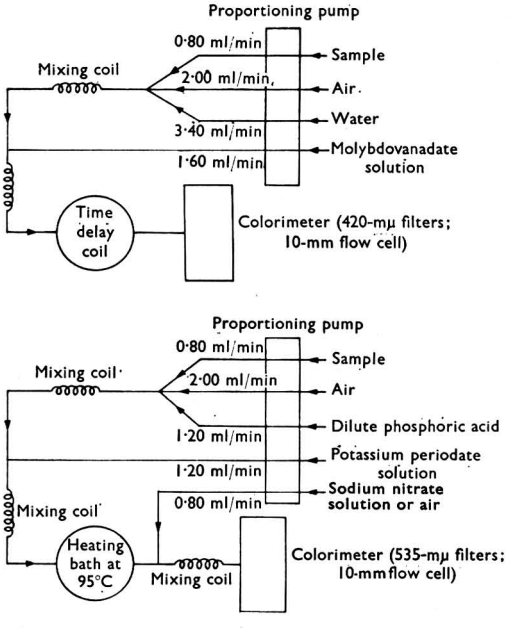

Fig. 3. AutoAnalyzer flow systems for determining (*a*) phosphorus pentoxide and (*b*) manganese oxide in steel-making slags

With the instrument used by us the calibration graph was linear up to 15 per cent. of phosphorus pentoxide (see Fig. 2); a diluted sample solution was used when the content exceeded this limit.

For routine use plot three calibration points in duplicate to establish the slope of the graph. At least one calibration solution should be measured with each sampler-plate of test solutions as a check on the calibration.

For sample solutions containing coloured ions, repeat the determinations substituting dilute nitric acid (4 + 96) for the molybdovanadate solution. Deduct these readings to give the net optical density due to phosphorus pentoxide.

DETERMINATION OF MANGANESE OXIDE

Manganese is determined manually by oxidising it to permanganic acid by heating with potassium periodate in a strongly acid solution. In the automated procedure, development of the manganese colour is effected by passing the solution through a 40 ft length of glass tubing in the form of a coil immersed in a heating bath maintained at 95° C. Provision is made for reducing the manganese colour with sodium nitrite in order to determine the optical density of any coloured ions, such as chromium, present in solution.

METHOD

REAGENTS—

Phosphoric acid, diluted (1 + 3).

Sodium nitrite solution, 0·5 per cent. w/v, aqueous.

Potassium periodate solution, 2 per cent. w/v—Transfer 20 g of potassium periodate to a 1-litre beaker, add 800 ml of diluted phosphoric acid (1 + 3), heat almost to the boiling-point, and stir until dissolved. Cool, and dilute to 1 litre with diluted phosphoric acid.

Standard manganese solution—Dissolve 1·1144 g of potassium permanganate in 500 ml of water, reduce with a slight excess of sulphurous acid, dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 0·5 mg of manganese oxide.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 3, and set the sampler-plate at the rate of 40 samples per hour. Prepare a standard calibration graph as described under "Determination of Phosphorus Pentoxide," by using suitable volumes of standard manganese solution to cover a range of up to 20 per cent. of manganese oxide.

With our instrument the calibration graph was linear up to 12·5 per cent. of manganese oxide (see Fig. 2); a diluted sample solution was used when the content exceeded this limit.

For a sample solution containing coloured ions repeat the determinations, substituting 0·5 per cent. w/v solution of sodium nitrite for the air-line immediately after the heating bath. Deduct these readings to give the net optical density due to manganese oxide.

DETERMINATION OF TOTAL IRON

In the manual procedure, iron is reduced to the bivalent state with hydroxyammonium chloride; the solution is buffered to about pH 4 with sodium citrate solution, and 1,10-phenanthroline hydrate is added to form the orange-red complex. Colour development is complete in about 20 minutes.

The two principal difficulties encountered in automating the manual method were contamination and lack of resolution between samples. The problem of contamination was overcome by introducing intermediate acid-filled cups between successive samples as in the method for alumina. In addition, an extra large-diameter air-line was included in the flow system to scavenge contaminants from the time-delay coil used for colour development. These modifications also considerably improved resolution between successive peaks on the chart recorder.

It was established that minor variations in the amount of perchloric acid in the main sample solution would not affect the optical density of the coloured solution provided that the acid content was between 2 per cent. v/v and 3 per cent. v/v; acid contents in excess of this range would lead to low results.

The slope of the calibration graph was found to depend on the concentration of 1,10-phenanthroline hydrate present in the system, but at all concentration levels the graph was linear up to 15 mg of iron per 100 ml of test solution. Above this value the graph is curved and non-reproducible (see Fig. 2).

METHOD

REAGENTS—

Hydroxyammonium chloride solution, 0·5 per cent. w/v, aqueous.

Sodium citrate solution, 3 per cent. w/v, aqueous.

1,10-*Phenanthroline hydrate solution, 0·2 per cent. w/v, aqueous.*

Standard iron solution—Dissolve exactly 1 g of Matthey iron sponge in 20 ml of hydrochloric acid, oxidise with nitric acid, add 10 ml of perchloric acid, and evaporate to fumes. Cool, add water, and warm, if necessary, to dissolve salts. Dilute to 1 litre in a calibrated flask, and store in a stoppered polythene bottle.

1 ml ≡ 1 mg of iron.

PROCEDURE—

Assemble the AutoAnalyzer as shown in Fig. 4. Set the sampler-plate at 40 samples per hour, and fill alternate cups with dilute perchloric acid (1 + 39). Prepare a standard graph as described under "Determination of Phosphorus Pentoxide"; use suitable volumes of a standard iron solution to cover a range of contents up to 20 per cent. of total iron.

With our instrument, the calibration graph was linear up to 15 per cent. total iron (see Fig. 2); a diluted sample solution was used when the content exceeded this limit.

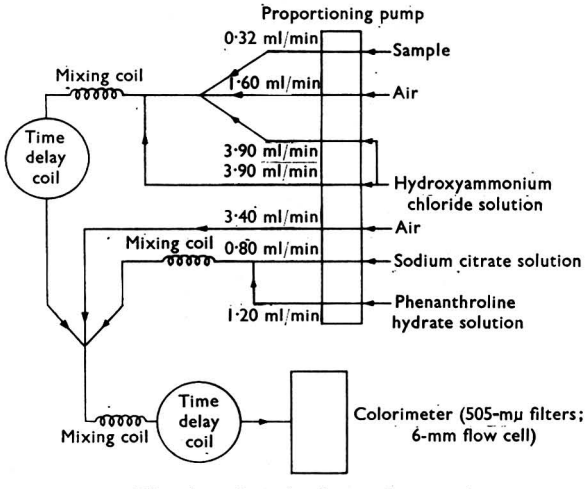

Fig. 4. AutoAnalyzer flow system for determining total iron in steel-making slags

RESULTS, PRECISION AND ACCURACY

The instrumental precision of the AutoAnalyzer system was determined by making 12 successive determinations on three solutions prepared from samples selected to give different optical-density values. Table II indicates that instrumental precision is related to optical density and varies from about 2 per cent. (as coefficient of variation) at the 0·05 level of optical density to about 0·3 per cent. at the 0·5 level. These errors do not include minor calibration errors that might be experienced in day-to-day operation or chemical errors involved in the preparation of the sample solution.

To measure the over-all analytical precision, six samples of British Chemical Standard No. 174/1 basic slag were analysed six times over a period of 3 months by the AutoAnalyzer procedures. These same sample solutions were also analysed by the manual methods; the individual results obtained are shown in part of Table IV, and the precision obtained with both manual and automatic procedures is compared in Table III. Over-all precision with the AutoAnalyzer is at least as good as that obtained by carefully operated manual processes and in the determination of the alumina it is noticeably better. This is probably due to the simpler procedure developed for automatic operation, which avoids the need for chemical separation to remove interfering elements.

The results obtained for the sample of standard slag (see Table III) are in close agreement with Certificate values. As an additional check on the accuracy of the automated methods, results obtained on a series of 10 duplicated sample solutions were compared with results obtained by the manual methods. The results are presented in Table IV together with results for the sample of standard slag mentioned above.

TABLE II

PRECISION OF THE INSTRUMENT

Sample	Mean optical density	Percentage equivalent, %	Standard deviation, %	Coefficient of variation, %
Manganese oxide—				
Slag "10"* (~ 1·75 per cent.) ..	0·0495	1·81	0·015	0·84
B.C.S. 174/1 (5·11 per cent.) ..	0·165	4·96	0·025	0·50
Slag "6" (~ 12·5 per cent.) ..	0·380	12·61	0·059	0·47
Alumina—				
B.C.S. 174/1† (1·82 per cent.) ..	0·035	1·71	0·050	2·9
Slag "10"† (~ 5·7 per cent.) ..	0·195	5·68	0·063	1·1
Slag "6" (~ 7 per cent.)	0·255	7·16	0·073	1·0
Total iron—				
Slag "6" (~ 2·5 per cent.)	0·112	2·54	0·014	0·56
B.C.S. 174/1 (8·45 per cent.) ..	0·400	8·48	0·023	0·27
Slag "10" (~ 15 per cent.)	0·659	14·91	0·034	0·23
Phosphorus pentoxide—				
Slag "6" (~ 1 per cent.)	0·031	1·00	0·020	2·00
B.C.S. 174/1 (12·3 per cent.) ..	0·374	12·20	0·060	0·49
Slag "10" (~ 15 per cent.)	0·457	14·89	0·054	0·36

* Range expansion × 4.
† Range expansion × 2.

TABLE III

COMPARISON OF PRECISION OBTAINED WITH AUTOANALYZER AND MANUAL METHODS FOR ANALYSING B.C.S. 174/1 BASIC SLAG

	Certificate value and range, %	Precision obtained with AutoAnalyzer: Mean of results, %	Standard deviation, %	Coefficient of variation, %	Precision of manual method: Mean of results, %	Standard deviation, %	Coefficient of variation, %
Manganese oxide	5·11 (5·00 to 5·15)	4·93	0·025	0·51	4·95	0·045	0·91
Total iron	8·45*	8·55	0·10	1·2	8·41	0·12	1·4
Phosphorus pentoxide	12·30 (12·16 to 12·50)	12·17	0·10	0·98	12·23	0·10	0·82
Alumina	1·82 (1·78 to 1·92)†	1·76	0·07	4·0	1·69	0·12	7·1

* Sum of mean results quoted for FeO and Fe_2O_3.
† B.I.S.R.A. Methods of Analysis Committee's results.

To establish whether or not a bias existed between the two procedures, the average discrepancy between the results was calculated, together with standard deviation and 95 per cent. confidence limits.

There is evidence of a positive bias in the determination of total iron, which is confirmed by the slightly higher results obtained for B.C.S. 174/1 standard slag (see Table III). Consideration of the manual method suggests that this bias and the high standard deviation of the average discrepancy may be due to errors introduced into the manual method during the

TABLE IV

COMPARATIVE RESULTS OBTAINED BY THE MANUAL AND AUTOMATIC COLORIMETRIC PROCEDURES

Sample number	Phosphorus pentoxide found			Total iron found			Manganese oxide found			Alumina found		
	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %
Blast furnace slags—												
M.G.S. 408 ..	0·20	0·15	−0·05	0·40	0·25	−0·15	1·30	1·35	+0·05	14·5	14·2	−0·3
	0·25	0·15	−0·10	0·40	0·30	−0·10	1·40	1·30	−0·10	14·5	14·4	−0·1
M.G.S. 409 ..	0·10	<0·1	—	0·40	0·30	−0·10	0·95	0·95	0·00	14·8	14·7	−0·1
	0·05	<0·1	—	0·40	0·30	−0·10	0·95	0·95	0·00	15·0	14·7	−0·3
Basic arc furnace slags—												
M.G.S. 406 ..	1·00	1·05	+0·05	20·8	20·6	−0·20	9·15	9·30	+0·15	5·70	5·35	−0·35
	0·95	1·00	+0·05	20·6	20·6	0·00	9·00	9·20	+0·20	5·75	5·45	−0·30
B.I.S.R.A. 1 ..	0·35	0·40	+0·05	7·40	7·35	−0·05	4·45	4·35	−0·10	3·65	3·85	+0·20
	0·40	0·45	+0·05	7·40	7·55	+0·15	4·55	4·35	−0·20	3·65	3·75	+0·10
B.I.S.R.A. 2 ..	0·55	0·50	−0·05	7·45	7·60	+0·15	4·80	4·70	−0·10	3·45	3·40	−0·05
	0·60	0·50	−0·10	7·55	7·70	+0·15	4·75	4·65	−0·10	3·50	3·45	−0·05
B.I.S.R.A. 3 ..	0·85	1·00	+0·15	16·8	17·2	+0·40	10·9	10·8	−0·10	2·70	2·80	+0·10
	1·00	1·05	+0·05	16·8	17·2	+0·40	10·8	10·7	−0·10	2·65	2·75	+0·10
B.I.S.R.A. 4 ..	1·15	1·10	−0·05	20·2	20·6	+0·40	12·4	12·1	−0·30	2·50	2·15	−0·35
	1·15	1·10	−0·05	20·1	20·5	+0·40	12·4	12·4	0·00	2·35	2·05	−0·30
B.I.S.R.A. 5 ..	1·10	1·05	−0·05	24·0	24·2	+0·20	13·1	12·9	−0·20	1·65	2·00	+0·35
	1·20	1·10	−0·10	24·4	24·5	+0·10	13·2	12·9	−0·30	1·75	2·00	+0·25
B.I.S.R.A. 6 ..	1·40	1·35	−0·05	18·7	19·1	+0·40	12·5	12·6	+0·10	2·00	2·10	+0·10
	1·40	1·40	0·00	18·9	19·3	+0·40	12·4	12·3	−0·10	1·95	2·05	+0·10
B.I.S.R.A. 7 ..	0·15	0·15	0·00	1·05	1·10	+0·05	0·80	0·60	−0·20	1·45	1·55	+0·10
	0·15	0·15	0·00	1·05	1·00	−0·05	0·85	0·55	−0·30	1·55	1·75	+0·20
B.I.S.R.A. 8 ..	0·25	0·20	−0·05	0·85	1·10	+0·25	0·95	1·00	+0·05	1·55	1·90	+0·35
	0·25	0·20	−0·05	0·85	1·00	+0·15	0·95	1·05	+0·10	1·65	1·90	+0·25
B.I.S.R.A. 9 ..	0·15	0·15	0·00	2·35	2·25	−0·10	0·65	0·60	−0·05	4·10	4·40	+0·30
	0·15	0·15	0·00	2·30	2·15	−0·15	0·65	0·70	+0·05	4·25	4·45	+0·20

TABLE IV—*continued*

Sample number	Phosphorus pentoxide found			Total iron found			Manganese oxide found			Alumina found		
	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %	By manual procedure, %	By Auto-Analyzer, %	Difference, %
Basic open hearth furnace slags—												
M.G.S. 401 ..	17·1	17·0	−0·10	11·7	11·5	−0·20	3·40	3·40	0·00	0·45	0·50	+0·05
	17·1	17·1	0·00	11·8	11·8	0·00	3·45	3·40	−0·05	0·50	0·50	0·00
M.G.S. 402 ..	4·35	4·60	+0·25	24·9	25·1	+0·20	5·05	5·00	−0·05	1·70	1·70	0·00
	4·30	4·55	+0·25	24·9	25·2	+0·10	5·05	5·00	−0·05	1·80	1·85	+0·05
M.G.S. 403 ..	5·90	5·85	−0·05	11·3	11·0	−0·30	5·40	5·45	+0·05	2·95	2·75	−0·20
	5·85	5·75	−0·10	11·4	11·2	−0·20	5·40	5·45	+0·05	2·95	2·75	−0·20
M.G.S. 410 ..	9·90	9·85	−0·05	14·4	14·2	−0·20	14·3	14·2	−0·10	1·80	1·55	−0·25
	10·0	9·90	−0·10	14·5	14·3	−0·20	14·3	14·2	−0·10	1·90	1·60	−0·30
M.G.S. 411 ..	6·75	6·80	+0·05	12·6	12·6	0·00	10·5	10·4	−0·10	3·15	3·15	0·00
	6·85	6·80	−0·05	12·6	12·4	−0·20	10·5	10·4	−0·10	3·10	3·10	0·00
M.G.S. 412 ..	4·35	4·65	+0·30	29·4	29·1	−0·30	2·00	2·05	+0·05	0·75	0·75	0·00
	4·40	4·70	+0·30	29·4	29·3	−0·10	2·00	2·00	0·00	0·80	0·80	0·00
M.G.S. 413 ..	7·35	7·55	+0·20	4·50	4·60	+0·10	4·65	4·70	+0·05	1·80	1·80	0·00
	7·25	7·50	+0·25	4·50	4·55	+0·05	4·70	4·70	0·00	1·75	1·80	+0·05
B.C.S. 174/1	12·4	12·2	−0·02	8·35	8·60	+0·25	5·00	4·95	−0·05	1·80	1·85	+0·05
	12·2	12·2	0·00	8·40	8·55	+0·15	5·00	4·95	−0·05	1·75	1·85	+0·10
	12·1	12·0	−0·10	8·25	8·45	+0·20	4·90	4·90	0·00	1·70	1·70	0·00
	12·2	12·2	0·00	8·40	8·65	+0·25	4·90	4·95	+0·05	1·55	1·70	+0·15
	12·3	12·1	−0·20	8·60	8·60	0·00	4·95	4·90	−0·05	1·55	1·75	+0·20
	12·2	12·3	+0·10	8·45	8·40	−0·05	4·95	4·90	−0·05	1·80	1·70	−0·10
Average discrepancy and confidence limits ..		+0·01 ± 0·04			+0·05 ± 0·06			−0·05 ± 0·03			0·00 ± 0·06	
Standard deviation of average discrepancy ..		0·12			0·20			0·12			0·19	

transfer of small portions (1 or 2 ml) of the sample solution when the iron is present in high concentrations, *i.e.*, more than 10 per cent. Errors involved in volumetric transfer are eliminated in the automated procedure.

Results for manganese oxide show a small negative bias, but it is difficult to determine whether or not this is of practical significance.

There is no evidence of significant bias in the determination of phosphorus pentoxide and alumina. A high standard deviation of the average discrepancy was observed in the comparison of the alumina results, and this may possibly reflect errors introduced into the manual procedure by the chemical separation, as mentioned above.

Conclusions

The AutoAnalyzer system of automatic colorimetric analysis can be used for determining the total iron and the oxides of phosphorus, manganese and aluminium in iron- and steel-making slag. Accuracy and precision are equal to and possibly better than those obtained by carefully operated manual procedures.

After sample preparation and manual determination of silica, the time required for each of the automated procedures is 1 hour for the analysis of either 20 or 40 samples, depending on the type of chemical reaction involved. To this time must be added a period of about 30 to 40 minutes for assembling the apparatus, the passage of the first test solution through the flow system and the measurement of calibration and reagent blank solutions. After assembly and calibration of the apparatus, operation is completely automatic apart from the filling of sample-cups. While the instrument is in automatic operation, the operator can prepare additional sample solutions and convert optical-density readings to percentage concentration.

Recent developments in the AutoAnalyzer system have now made it possible to perform multiple analytical determinations. One sampler unit is coupled to a series of separate flow systems, each with its own colorimeter and chart recorder. Thus, if a combined system of this type were used, it would be possible to determine the four slag constituents simultaneously with a further saving in time.

The helpful co-operation and advice of Dr. J. Marten, Chief Chemist of Technicon Instruments Ltd., is gratefully acknowledged. We also thank Mr. P. Haycox for the manual test results reported here.

References

1. Shaw, W. H. C., and Fortune, I., *Analyst*, **1962**, **87**, 187.
2. Stanton, R. E., and McDonald, A. J., *Chem. & Ind.*, **1961**, 1406.
3. Ferrari, A., Russo-Alesi, F. M., and Kelley, J. M., *Anal. Chem.*, **1959**, **31**, 1710.
4. Scholes, P. H., Speight, K., and Loxley, R., BISRA Restricted Report MG/D/245/63.
5. Hill, U. T., *Anal. Chem.*, **1959**, **31**, 429.

Received *February 18th*, **1963**

Analysis of Aluminium Alkyls

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Organo-aluminium compounds, containing alkyl groups between methyl and butyl, are now used extensively as catalysts in processes for the manufacture of polyethylene and polypropylene, and are also of interest as intermediates in the manufacture of other chemicals. Trialkylaluminium compounds, dialkylaluminium chlorides or dialkylaluminium alkoxides containing these alkyl groups are all used as polymerisation catalysts. As well as alkyl groups these compounds frequently contain different proportions of aluminium-bound hydride groups produced during manufacture. Procedures are described for determining such hydride and alkyl groups in organo-aluminium compounds.

Bonitz[1] and Ziegler *et al.*[2] have developed gasometric procedures for the analysis of the lower aluminium alkyls, such as triethyl-, tripropyl- or tributylaluminium. In these procedures a known weight of sample reacts at a low temperature with an alcohol of high boiling-point, such as 2-ethylhexanol, under an atmosphere of nitrogen or helium. On alcoholysis each alkyl group liberates one mole of gaseous alkane and each hydride group liberates one mole of hydrogen.

$$>\mathrm{Al}\boxed{C_nH_{2n+1}} + \mathrm{H}\ \mathrm{OCH_2-CH(C_2H_5).CH_2.CH_3} \longrightarrow >\mathrm{Al-OCH_2-CH(C_2H_5).CH_2.CH_3} + C_nH_{2n+2}$$

$$>\mathrm{Al}\boxed{\mathrm{H}} + \mathrm{H}\ \mathrm{OCH_2-CH(C_2H_5).CH_2.CH_3} \longrightarrow >\mathrm{Al-OCH_2-CH(C_2H_5).CH_2.CH_3} + \mathrm{H_2}$$

The alkyl and hydride contents of the samples are then calculated from the amount of the gas evolved and its composition. Ziegler *et al.*[2] employed mass spectrometric and other methods of gas analysis for determining the composition of the gas mixture evolved.

Ziegler *et al.*[2] state that recoveries of gas by their procedure were lower than expected from the composition of the samples analysed. They attributed low gas yields to a partial dissolution of the evolved paraffin - hydrogen mixture in the 2-ethylhexanol reagent. We analysed several carefully purified samples of triethylaluminium and tri-isobutylaluminium and confirmed that recoveries of gas were lower than expected when 2-ethylhexanol or n-hexanol was used as the alcoholysis reagent.

From the results obtained in this work we considered it probable, however, that the low recoveries of gas obtained were due to incomplete reaction of alkyl and hydride groups with the alcoholic reagent, rather than dissolution of gas in the reagent. Thus, appreciably higher gas yields were obtained when sample decomposition was effected with a mixture of n-hexanol and monoethylene glycol or a mixture of water and monoethylene glycol than when 2-ethylhexanol was used alone. We therefore studied the reaction of lower alkyl groups (up to butyl) and hydride groups with a range of hydroxylic compounds (alcohols, glycol, water) in order to discover suitable reagents for the quantitative decomposition of each type of organo-aluminium compound.

Gas - liquid chromatography offered a simple and rapid method for analysing the gaseous mixture of alkane, hydrogen and nitrogen withdrawn from the gasometric apparatus after decomposition of the sample. We used this method of analysis throughout the investigation.

Work carried out to permit selection of suitable reagents for the determination of alkyl and hydride groups in trimethylaluminium, triethylaluminium, tri-n-propylaluminium, tri-isobutylaluminium and some of their chloro- and alkoxide derivatives is described below. A description is also given of the apparatus and procedure used in carrying out the analysis.

Selection of hydroxylic reagent

Methylaluminium and ethylaluminium compounds—

It has been stated already that incomplete decomposition of the alkyl and hydride groups in triethylaluminium samples appeared to occur on reaction with 2-ethylhexanol or n-hexanol. Alternative reagents that would react quantitatively and smoothly with triethylaluminium were therefore sought.

We found that higher gas yields were obtained when a 4 + 1 mixture of n-hexanol and monoethylene glycol was used for decomposing triethylaluminium instead of anhydrous n-hexanol alone. It was decided, therefore, to see if a still higher yield of gas was obtained when an aqueous reagent was used for decomposing the sample.

It is not practicable to add water directly to triethylaluminium, diethylaluminium chloride or diethylaluminium ethoxide, as the ensuing reaction is extremely vigorous, even when carried out at —70° C. Also, we showed that an undesirable "fissioning" side reaction, which converts alkyl groups to ethylene and hydrogen instead of ethane, occurs to some extent when aqueous reagents or aqueous monoethylene glycol reagents are added directly to neat organo-aluminium compounds of low molecular weight.

Normal reaction— $> AlC_2H_5 + H_2O \longrightarrow C_2H_6 + >AlOH$

"Fissioning" reaction— $> AlC_2H_5 + H_2O \longrightarrow C_2H_4 + H_2 + >AlOH$

We found it possible to obtain complete reaction without any "fissioning" side reaction by using a combination of n-hexanol and aqueous reagents. The major decomposition of the alkyl is effected by an initial reaction with n-hexanol. An aqueous solution, containing 20 per cent. of sulphuric acid, is then added, and complete reaction occurs without "fissioning." No ethylene was detected in the gases liberated in these reactions.

Results obtained on typical samples of distilled trimethylaluminium and triethylaluminium are shown in Table I; it can be seen that yields of gas were good.

Table I

Analysis of freshly distilled methyl and ethyl compounds

Trimethylaluminium—	Content found, % w/w		*Triethylaluminium*—	Content found, % w/w	
$Al(CH_3)_3$	99·5	99·2	$Al(C_2H_5)_3$	94·4	94·6
$Al(CH_3)_2H$	Nil	Nil	$Al(C_2H_5)_2H$	2·2	2·2
			$Al(C_2H_5)_2(C_4H_9)$ ..	2·0	2·1
Total	99·5	99·2	Total	98·6	98·9

Hydrolysis of chloro- or alkoxide derivatives results in the formation of hydrochloric acid or the corresponding alcohol; however, these compounds dissolve in the reagent, and we have shown that they do not interfere in the determination of alkyl groups.

Propylaluminium compounds—

Propylaluminium compounds are less reactive than are the ethyl compounds, and it was considered possible that the "fissioning" side reaction might not occur when a reagent containing water was added directly to the propyl compounds to which hexanol had not been added.

The first reagent tried was a 3 + 7 v/v mixture of monoethylene glycol and water; it was added to a sample of di-n-propylaluminium isopropoxide cooled to —30° C. Analysis of the gas obtained, however, showed the presence of considerable amounts of hydrogen and propylene, indicating that extensive "fissioning" of the propyl groups to hydrogen and propylene had occurred under these conditions.

$$>Al\text{–}CH_2\text{–}CH_2\text{–}CH_3 + H_2O \longrightarrow >AlOH + CH_3\text{–}CH{=}CH_2 + H_2.$$

We next tried a 3 + 7 v/v mixture of monoethylene glycol and 20 per cent. aqueous sulphuric acid. Rather surprisingly, propylene was completely absent from the gas generated in these experiments, indicating that "fissioning" did not occur when the aqueous reagent used was acidic. This decomposition procedure was then applied to the analysis of a sample of di-n-propylaluminium isopropoxide and also to a sample of tri-n-propylaluminium. The

results obtained in the determination of propyl and hydride groups, together with separate determination of aluminium and n-propoxide[3] are shown in Table II.

TABLE II

ANALYSIS OF PROPYLALUMINIUM COMPOUNDS

Sample No.	n-Propyl found, % w/w	Hydride found, % w/w	n-Propoxide found, % w/w	Aluminium found, % w/w	Total of components, %	Empirical formula	Sum of subscripts in empirical formula	Departure of sum from 3·00 (*i.e.*, stoicheiometric AlR_3), %
Di-n-propylaluminium isopropoxide—								
1	48·8	<0·01	30·1	14·7	93·6	$Al_{1\cdot00}Pr_{2\cdot08}(OPr)_{0\cdot94}$	3·02	+1
2	43·3	<0·01	38·1	14·8	96·2	$Al_{1\cdot00}Pr_{1\cdot84}(OPr)_{1\cdot18}$	3·02	+1
Tri-n-propylaluminium—								
3	78·7	0·01	0·7	16·7	96·1	$Al_{1\cdot00}Pr_{2\cdot96}H_{0\cdot02}(OPr)_{0\cdot02}$	3·00	0
4	78·0	0·01	0·6	16·5	95·1	$Al_{1\cdot00}Pr_{2\cdot97}H_{0\cdot01}(OPr)_{0\cdot02}$	3·00	0

The total of the constituents determined was about 95 per cent. As it was known that these samples contained a few per cent. of hydrocarbon solvent, it was not possible to check the recovery of gas directly. Aluminium is always tervalent, however, and a test of the reliability of the analytical results is obtained when the calculated valencies, based on the analytical results, are compared with the value of 3. Values for the valency of aluminium calculated in this way are shown in Table II; it can be seen that they are within 1 per cent. of the theoretical value of 3. The recoveries of gas were therefore good.

A 3 + 7 v/v mixture of monoethylene glycol and 20 per cent. aqueous sulphuric acid was therefore adopted as a suitable reagent for the direct decomposition of propylaluminium compounds.

BUTYLALUMINIUM COMPOUNDS—

The two types of reagent developed for the ethylaluminium and the propylaluminium compounds were then applied to the analysis of butylaluminium compounds. The acidic glycol reagent was found to produce some "fissioning" of the butyl groups, which was overcome when the decomposition was carried out at −65° C, and recoveries of gas were slightly higher than with the n-hexanol - aqueous sulphuric acid reagent.

The results obtained on a sample of tri-isobutylaluminium are shown in Table III. In this sample the isobutoxide group content was determined by the method previously described.[3] It can be seen that the total of the components determined is close to 100 per cent., indicating good recovery of gas in the decomposition procedure.

TABLE III

ANALYSIS OF TRI-ISOBUTYLALUMINIUM

Constituent determined	Content found, % w/w	
$Al(C_4H_9)_3$	87·6	87·1
$Al(C_4H_9)_2H$	6·6	6·6
$Al(C_4H_9)_2(OC_4H_9)$	6·0	6·0
Total	100·2	99·7

LOWER ALKYL AND HYDRIDE GROUPS IN HIGHER ALKYLALUMINIUM COMPOUNDS—

It is sometimes necessary to determine alkyl groups, up to butyl, and hydride groups in organo-aluminium compounds containing alkyl groups higher than butyl. Higher molecular-weight organo-aluminium compounds can be prepared by the displacement of butyl groups in tri-isobutylaluminium with the appropriate higher olefin. These higher molecular-weight aluminium alkyls may still contain small amounts of hydride and lower alkyl groups.

The aqueous sulphuric acid - monoethylene glycol reagent, as used for the analysis of butylaluminiums, was found to be suitable for the analysis of these compounds. These materials were often highly viscous, and good recoveries of gas were obtained only when good mixing during the reaction was maintained by means of magnetic stirring.

METHOD FOR DECOMPOSING ALKYLS AND DETERMINING THE GASES EVOLVED

APPARATUS—

The apparatus, which is similar to that described by Ziegler *et al.*,[2] is shown in Fig. 1. It consists of a reaction vessel attached by means of a flexible coupling to a gas manifold

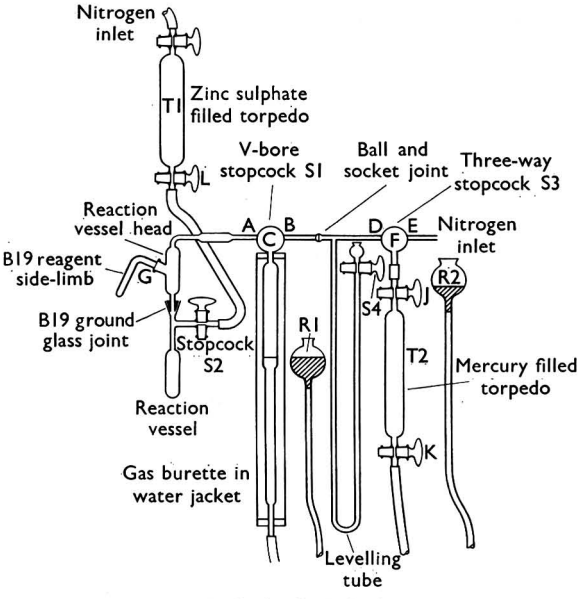

Fig. 1. Hydrolysis alcoholysis apparatus

system, incorporating a gas burette for volumetric measurement of the gas evolved, and leads to a gas sampling torpedo for transfer of the gas to the gas - liquid chromatograph for subsequent analysis. Mercury is used as the confining liquid in both the gas burette and in the sampling torpedo. The manifold system connects to a supply of pure nitrogen (white spot), which is dried by passage through a drying tower packed with Linde 4A molecular sieve.

The reaction vessel connects, via a stopcock, to a supply of aqueous zinc sulphate solution, which is used for discharging the reaction gases into the sampling torpedo.

The apparatus should be cleaned and dried thoroughly before each determination, and the cones at G and H and stopcocks S_1, S_2 and S_3 should be lubricated with silicone grease.

REAGENTS—

Decomposition reagent A—Mix 30 ml of 20 per cent. v/v aqueous sulphuric acid with 70 ml of monoethylene glycol. To 100 ml of mixed reagent add 1 ml of a non-ionic surface-active agent, *e.g.*, Nonidet P42, a condensation product of dioctyl phenol and ethylene oxide obtainable from the British Drug Houses Limited.

Decomposition reagent B—n-Hexanol.

Decomposition reagent C—Aqueous sulphuric acid (20 per cent. v/v) containing 0·05 per cent. of water-soluble methyl orange.

SAMPLING—

Organo-aluminium compounds received for analysis may contain a hydrocarbon diluent. Hydrocarbon diluents that boil below 180° C can be removed by vacuum distillation at a

pressure of 0·1 mm of mercury at a maximum temperature of 65° C. Avoid heating above 65° C, as, at this pressure, many organo-aluminium compounds are somewhat volatile or may decompose above this temperature.

Transfer the appropriate weight of sample to the weighed reaction vessel by means of a safety pipette. Purge the reaction vessel and sampling pipette with dry nitrogen during transfer of the sample, as described by Crompton.[3]

The weight of an organo-aluminium compound required for a determination is that from which approximately 70 ml of gas at S.T.P. will be evolved. Calculate the weight of neat sample required from—

$$\text{Weight of sample} = \frac{70 \times 1000}{22400} \times \frac{M}{n} \text{ mg}$$

where M = molecular weight of the compound and
n = number of alkyl groups per molecule of compound.

Samples should be free from metallic sediment, such as free aluminium, as reaction of metal with the acid decomposition reagents would occur with the evolution of hydrogen, which would cause high results for aluminium-bound hydride. Metallic sediments can usually be completely removed from the sample by centrifugation.

PROCEDURE FOR PURGING THE APPARATUS—

At this stage the reagent side-limb, the reaction vessel and sampling torpedo T_2 are not connected to the apparatus. Open the gas burette to atmosphere, and raise mercury reservoir R_1 until the burette is filled with mercury to the barrel of stopcock S_1. Connect A to B with stopcock S_1, and purge with nitrogen through inlet I. Connect B and C, and allow the nitrogen pressure to depress the mercury until about 15 ml of nitrogen have entered the gas burette. Cut off the nitrogen pressure by connecting E to F with stopcock S_3. Lower reservoir R_1 until the mercury levels in the burette and reservoir are the same. Connect D to F with stopcock S_3, and then slowly raise reservoir R_1 until 5 ml of nitrogen remain in the burette. Connect A to B with stopcock S_1, and then D to E with stopcock S_3. Continue purging with nitrogen through inlet I.

DECOMPOSITION OF METHYL- AND ETHYLALUMINIUM—

By pipette place 1·5 ml of reagent C in the reagent side-limb, ensuring that no drops of this aqueous reagent remain above the liquid level. Then place from a pipette 1·5 ml of immiscible reagent B in the side-limb on top of the aqueous phase. This reagent, n-hexanol, will float on top of the aqueous phase, and no globules of aqueous reagent should be present in the upper hexanol layer. Connect the side-limb to the reaction vessel head, and connect springs across the glass lugs. Nitrogen now leaves via socket H.

Purge with nitrogen the interior of a clean oven-dried reaction vessel; use a glass inlet tube. Remove the inlet, and apply a gentle purge to the side-arm of the loosely stoppered reaction vessel. Discontinue the purge with nitrogen, close stopcock S_2, and closely stopper the vessel. Weigh the reaction vessel accurately. Purge the reaction vessel gently with nitrogen, remove the stopper, and, by pipette, place the required volume of sample in the reaction vessel. Stopper the vessel, close stopcock S_2, and re-weigh.

Remove the nitrogen supply line from inlet I, and immediately transfer it to the side-arm of the reaction vessel. Open stopcock S_2 on the reaction vessel, and then remove the stopper. Clamp the reaction vessel to the head of the apparatus, and close stopcock S_2. Fasten the springs connecting the reaction vessel to the head. Connect D to E with stopcock S_3, and rotate stopcock S_1 through one complete revolution to bring the internal pressure of the system to atmospheric pressure; connect A to C with stopcock S_1.

Adjust the height of mercury reservoir R_1 until the mercury level in both limbs of the U-levelling tube are the same, with stopcock S_4 open. Measure the volume of nitrogen in the burette, and record the atmospheric pressure and ambient temperature. The temperature of the water-jacket surrounding the gas burette should not differ by more than 1° C from room temperature when gas volumes are being read.

Immerse the lower bulb of the reaction vessel in a cooling bath maintained at −60° C. Leave for 5 minutes to cool, and level off the mercury in the gas burette and reservoir R_1 when necessary.

Slowly rotate the reagent side-limb until about half of the n-hexanol layer has flowed into the reaction vessel; ensure that none of the aqueous phase enters the reaction vessel at this stage. As generation of gas proceeds, equalise the mercury levels in the burette and reservoir R_1. When evolution of gas appears to be complete, remove the cooling bath, and allow the reaction vessel to attain room temperature. Immerse the reaction vessel in a cold-water bath, and heat up to 50° C. Add the aqueous reagent C by again slowly rotating the side-limb. Further evolution of gas occurs when this reagent is added, so continuously equalise the mercury levels in the gas burette and reservoir R_1. Heat the water-bath surrounding the reaction vessel to the boiling-point, and maintain at the boil for 30 minutes. Remove the water-bath, and again equalise the mercury levels as the gas contracts.

Allow the system to come to equilibrium overnight. Adjust the reservoir R_1 until the mercury levels in both limbs of the U levelling tube are the same, stopcock S_4 being open. Measure the volume of gas in the burette, and record the atmospheric pressure and room temperature.

Displace all the gas in the reaction vessel, etc., by first connecting mercury-filled sampling torpedo T_2, fitted with a reservoir, R_2, to inlet F on stopcock S_3. Connect E to F, and raise the mercury level to the barrel of stopcock S_3. Now connect D to F with stopcock S_3 and A to B with stopcock S_1. Close stopcock S_4 on the U levelling tube.

Now displace reaction gases by attaching a source of saturated zinc sulphate, supplied from a torpedo, T_1, to stopcock S_2. Open stopcocks S_2, L and M, and apply a gentle pressure of nitrogen at stopcock M. The zinc sulphate solution will now expel the gas contained in the reaction vessel and reagent side-limb. When the level of the zinc sulphate solution reaches A on stopcock S_1 close stopcock S_2, and connect B to C. Raise reservoir R_1 to displace the gas from the burette into torpedo T_2. Keep the mercury in torpedo T_2 and reservoir R_2 at the same level during transfer of the gas. Close stopcocks J and K, and disconnect the torpedo from the manifold. Shake the torpedo to mix the gas sample, and analyse the gas by gas - liquid chromatography.

TABLE IV

GAS - LIQUID CHROMATOGRAPHIC CONDITIONS

	Stage 1	Stage 2
	Hydrogen, methane and C_2 hydrocarbons	C_3 and C_4 hydrocarbons
Column	3 feet × $\frac{3}{16}$-inch i.d. glass	18 feet × $\frac{1}{8}$-inch stainless steel
Column packing	Davison silica gel 912, 28- to 60-mesh	12 feet of dimethylsulpholane + 6 feet of dinonyl phthalate (both 20 per cent. on 44- to 60-mesh Celite)
Column and detector temperature	30° C	30° C
Sample size	5 ml	0·25 ml
Detector	Katharometer	Katharometer
Bridge current	150 mA	150 mA
Carrier gas	Nitrogen (white spot)	Hydrogen
Carrier gas flow	2 litres per hour	3 litres per hour

DECOMPOSITION OF PROPYL- AND BUTYLALUMINIUM—

Carry out the decomposition of propyl- and butylaluminium compounds in the same way as described for the methyl- and ethylaluminiums, with the exception of the reagent composition. Use only one reagent, 2·5 ml of reagent A, in the reagent side-limb. Add this reagent, as before, by slowly rotating the reagent side-limb to give dropwise addition. During the warm-up period from −60° C to room temperature, however, the reaction may become vigorous. Should this happen, control it by temporarily returning the reaction vessel to the cooling bath. After reaction is complete, heat the water-bath surrounding the reaction vessel to the boiling-point, cool, and expel the gas into the mercury-filled torpedo, T_2, as previously described.

ANALYSIS OF EVOLVED GAS—

The gas-chromatographic analysis of the evolved gas is carried out in two stages. In the first stage hydrogen, methane and C_2 hydrocarbons are determined on a column of silica gel with nitrogen as carrier gas. In the second stage a combination of dimethylsulpholane and dinonyl phthalate columns is used for determining the C_3 and C_4 hydrocarbons.

Suitable gas - chromatographic conditions are shown in Table IV; any conventional gas chromatograph adapted for the analysis of gases can be used.

Calibrate the apparatus for hydrogen, methane and C_2 hydrocarbons by analysing accurately prepared mixtures of each gas with nitrogen. Determine the peak areas by electrical integration or from the product peak height × width at half peak height × attenuation factor.

The preparation of these calibration mixtures can be simplified by using a Wösthoff gas-blending pump (type A18/2a, obtainable from H. Wösthoff, O.H.G., Apparatbau, Bochum, Germany). Calibrate the gas - liquid chromatograph in terms of peak area against volume per cent. for hydrogen, methane and C_2 hydrocarbons; check this calibration periodically.

For the C_3 and C_4 hydrocarbons, use the gas-mixing pump to prepare mixtures of the gases with hydrogen, and determine the response factors for each gas relative to n-butane. This should be done accurately once every 4 months. Carry out a daily calibration check on a mixture of n-butane and hydrogen, and determine the area responses; the relative area response for each gas can then be determined for that day from the relative response factors. Calculate the percentage, by volume, of each gas from the product peak area × relative area response factor.

Calculate the total gas composition by normalising the individual percentage volumes so that their sum is 100 per cent.

CALCULATION OF RESULTS

CALCULATION OF GAS YIELD—

The volume of gas (V ml), corrected to S.T.P., generated during the reaction is given by—

$$V = \frac{273}{760} \cdot \left\{ \frac{(P_2 - P)\,(D + V_2)}{T_2} - \frac{(P_1)\,(D + V_1)}{T_1} \right\}$$

where D = volume (in millilitres) of dead space in apparatus, *i.e.*, the combined volume of the reaction vessel, side-limb and reaction vessel head. Determine D by weighing the amount of mercury needed to fill these three vessels.

P_1, P_2 = atmospheric pressure in mm of mercury when measuring initial and final gas volumes, respectively.

T_1, T_2 = ambient temperatures in degrees absolute when measuring initial and final gas volumes, respectively.

V_1, V_2 = volume of gas in millilitres in burette before and after evolution of gas, respectively.

P = a small correction term in mm of mercury allowing for the saturation vapour pressure exerted by the aqueous sulphuric acid - monoethylene glycol Reagent A. This correction term is sufficiently small to be ignored when the two phase n-hexanol - aqueous sulphuric acid reagent is used. The values of P at different temperatures are—

Temperature, °C	15	20	25	30
P, mm of mercury	6·9	9·4	12·7	16·9

CALCULATION OF ALKYL AND HYDRIDE CONTENTS—

These are given by—

$$\text{Hydride in sample} = \frac{a \times V \times 1{\cdot}008}{W \times 22{,}400}\ \%\ \text{w/w}$$

$$\text{Alkyl in sample} = \frac{b \times V \times M}{W \times C}\ \%\ \text{w/w}$$

where a = percentage by volume of hydrogen in generated gas.
b = percentage by volume of alkane in generated gas.
M = group weight of alkyl group being determined.
V = volume in millilitres of S.T.P. of gas generated in analysis.
W = weight in grams of organo-aluminium sample.
C = volume in millilitres per mole (at S.T.P.), relationship of the alkane gas involved, *e.g.*, for methane and ethane C = 22,400, for propane C = 21,970 and for butane C = 21,830.

CALCULATION OF COMPOUND COMPOSITION OF SAMPLE—

The method of calculating the compound composition of an organo-aluminium sample from its determined hydride, alkyl and alkoxide[3] contents is shown below. A, B, C and D are the percentage w/w contents of ethyl, hydride, ethoxide and butyl groups determined, respectively.

Then—

$$Al(C_2H_5)_2H = \frac{B \times (\text{mol. wt. of } Al(C_2H_5)_2H = 86{\cdot}1)}{(\text{atomic wt. of hydrogen} = 1{\cdot}008)} \%\ w/w$$

$$Al(C_2H_5)_2(OC_2H_5) = \frac{C \times (\text{mol. wt. of } Al(C_2H_5)_2(OC_2H_5) = 130{\cdot}15)}{(\text{group wt. of } (OC_2H_5) \text{ group} = 45{\cdot}06)} \%w/w$$

$$Al(C_2H_5)_2(C_4H_9) = \frac{D \times (\text{mol. wt. of } Al(C_2H_5)_2(C_4H_9) = 142{\cdot}20)}{(\text{group wt. of butyl group} = 57{\cdot}11)} \%\ w/w$$

$$Al(C_2H_5)_3 = \left\{A - \left(\frac{B}{1{\cdot}008} + \frac{C}{45{\cdot}06} + \frac{D}{57{\cdot}11}\right) 2 \times 29{\cdot}06\right\} \frac{114{\cdot}15}{3 \times 29{\cdot}06} \%\ w/w$$

(where group weight of ethyl groups = 29·06 and molecular weight of triethylaluminium = 114·15).

We thank the Directors of Shell Chemical Company Ltd. for permission to publish this paper.

REFERENCES

1. Bonitz, E., *Chem. Ber.*, 1955, **88**, 742.
2. Ziegler, K., Gellert, H. G., Martin, H., Nagel, K., and Schneider, J., *Annalen*, 1954, **589**, 91.
3. Crompton, T. R., *Analyst*, 1961, **86**, 652.

Received *April 8th*, 1963

The Determination of Selenium in Biological Material by Radioactivation

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Neutron-activation analysis has been applied to the determination of selenium in biological material. Samples were activated in a flux of 10^{12} neutrons per sq. cm per second for 18 minutes, and a rapid chemical separation was developed for selenium-81. Under these conditions the practical limit of sensitivity was found to be 5×10^{-9} g. Decontamination from arsenic, bromine, manganese, sodium and zinc was tested and found to be satisfactory. Selenium contents of fertilisers, tomato tissue and human blood were measured by the procedure.

Our knowledge of the behaviour of selenium in plants and animals has undoubtedly been hampered by the absence of sufficiently sensitive methods for determining the element. Until recently the only microanalytical method was that described by Robinson *et al.*,[1] which measures the red colour of elementary selenium after a chemical separation. With the discovery that trace amounts of selenium are important and possibly essential to mammals[2,3] there has been a revival of interest in other methods for determining the element. It appears that levels in normal biological tissue range from 0·005 to 0·5 μg per g so that only ultra-micromethods are suitable. Such methods include the combined spectrophotometric and isotope-dilution method proposed by Kelleher and Johnson[4] and Watkinson's fluorimetric method,[5] but most colorimetric methods[1,6,7] and X-ray fluorescence methods[8] are not yet sufficiently sensitive. To avoid errors due to reagent contamination, several workers have used neutron-activation techniques, and one such technique is described below.

Table I lists the radionuclides of selenium produced by thermal neutron activation and some of their relevant properties. It can be seen that only three of the nuclides can be made in high specific activities, and of these the best (selenium-77*m*) is exceedingly short-lived, with a half-life of only 17·5 seconds. Nevertheless, it has been used by several workers[9,10,11,12] for determining the element, with the use of an activation period of a few seconds and a multi-channel analyser focussed on the 0·16-MeV gamma-ray peak. This can be a satisfactory method provided the half-life of the peak is measured, since many other short-lived nuclides have a gamma-ray of about the same energy.

TABLE I

RADIONUCLIDES PRODUCED BY A THERMAL NEUTRON FLUX OF 10^{12} neutrons per sq. cm per second ON SELENIUM

Radionuclide	Activity of selenium after activation for one half-life, mC per g	Half-life	Maximum beta energy, MeV	Gamma energies, MeV
Selenium-75	25	120 days	—	0·27 and 0·14
Selenium-77*m*	97	17·5 seconds	—	0·16
Selenium-79*m*	2·9	3·9 minutes	—	0·096
Selenium-81*m*	1·5	57 minutes	—	0·103
Selenium-81	25	18·6 minutes	1·60	none
Selenium-83*m*	0·46	69 seconds	3·40	1·01 and 2·02
Selenium-83	0·04	25 minutes	1·70	0·36 and 2·34

Most workers have used selenium-75 to determine the element.[13 to 19] This nuclide has a convenient gamma-ray for counting, and its half-life is more than adequate to allow complete chemical separation from other activities. Its main disadvantage is the long activation time needed. Most analysts have activated their samples for only 7 to 14 days, which gives them only 5 to 10 per cent. of the specific activity quoted in Table I. This results in an equivalent loss of sensitivity, which is undesirable for biological samples.

The use of shorter lived selenium isotopes for activation analysis has also been suggested. The group at the University of Michigan[20,21] have used a rapid chemical separation method coupled with gamma-ray spectrometry for detecting 3·9-minute selenium-79*m*, but the limit of detection is only 10 μg. Yajima *et al.*[22] have employed 57-minute selenium-81*m* for determining selenium in tellurium, but the sensitivity is also poor. In this work we have used 18-minute selenium-81, which has the advantage of giving a theoretical sensitivity equal to that obtainable by using selenium-75 and requiring a much shorter activation time. Selenium-81 is virtually a pure beta-emitter, which may explain why it has not previously been considered for activation work, though its use is mentioned by Leddicotte.[17]

Method

Activation—

Samples were collected under the cleanest possible conditions and sealed into clean polythene ampules. Standards were prepared by first dissolving selenium dioxide in water spotted on to weighed 1-cm × 1-cm squares of Whatman No. 541 filter-paper, which were then re-weighed. When dry, the standards were sealed in small polythene bags. The selenium content of these filter-papers was found to be <0·01 μg per square. For each run, four samples and two standards were packed into a plastic "Rabbit" and activated for 18 minutes in a flux of about 10^{12} neutrons per sq. cm per second in the Harwell reactor BEPO.

Reagents—

All reagents were of recognised analytical grade.

Ashing mixture—A (1 + 1) mixture, by volume, of 16 N nitric acid and 70 per cent. perchloric acid.

Nitric acid, 2 N.

Hydrochloric acid, 12 N.

Hydrobromic acid, 48 *per cent. w/v.*

Hydrogen peroxide, 30 *per cent. w/v.*

Teepol solution, 1 *per cent. v/v.*

Acetone.

Sulphur dioxide, liquefied.

Nitrogen, 99·9 *per cent. pure.*

Selenium carrier solution—Prepared by dissolving 2·8106 g of selenium dioxide in distilled water and diluting to 100 ml.

1 ml ≡ 20 mg of selenium.

Arsenic carrier solution—Sodium arsenate solution, 10 per cent. w/v.

Manganese carrier solution—Manganese nitrate solution, 50 per cent. w/v.

Phosphorus carrier solution—Ammonium dihydrogen orthophosphate solution, 10 per cent. w/v.

Tellurium carrier solution—Sodium tellurate, 10 per cent. w/v in N hydrochloric acid.

Chemical separation of selenium—

Activated samples containing organic matter were placed in 150-ml beakers together with 10 ml of ashing mixture, 1 ml of selenium carrier solution and 2 drops each of arsenic, manganese, phosphorus and tellurium carrier solutions. They were boiled until the organic matter was destroyed and all nitric acid had volatilised, so that white fumes of perchloric acid were visible. They were then cooled and transferred to 50-ml round-bottomed flasks containing 5 ml of hydrochloric acid and 5 ml of hydrobromic acid. Distillation heads[23] were fitted immediately, and a current of nitrogen was passed through the flasks for approximately 4 minutes while they were heated with a small bunsen burner. The distillates were collected in 50-ml centrifuge tubes containing 5 ml of hydrochloric acid, 10 ml of water, 0·1 ml of Teepol solution and 2 drops of manganese carrier solution. Then sulphur dioxide was immediately passed into the centrifuge tubes through a glass capillary until a dark red precipitate of selenium was seen. This precipitate was separated by centrifugation, and dissolved in about 0·25 ml of nitric acid. Hydrogen peroxide (0·1 ml), 10 ml of hot water, and 5 ml of hydrochloric acid were added, and sulphur dioxide was again passed through the hot solution to precipitate selenium. This selenium was separated by centrifugation, washed with water and acetone and finally transferred to a weighed aluminium counting tray as a

slurry with acetone. When dry, it was counted with the minimum of delay and was subsequently weighed. The mean chemical yield was 70 per cent., and most of the loss was mechanical.

Table II lists the time taken by these operations. In practice it was possible to begin counting two half-lives (36 minutes) after removal of six samples from the reactor, with two analysts performing the chemical manipulations.

TABLE II

TIME REQUIRED FOR UNIT PROCESSES IN THE CHEMICAL SEPARATION

Operation	Time required, minutes
Opening samples	1
Ashing	3
Distillation	4
First precipitation by sulphur dioxide	1
Centrifugation	1
Dissolution of selenium	0·5
Second precipitation by sulphur dioxide*	1
Centrifugation and washing	4
Transfer to counting tray	0·5
Drying	2

* This was the least reproducible step, and sometimes took up to 3 minutes.

TREATMENT OF STANDARDS—

The filter-paper standards were boiled for 4 minutes with 5 ml of hydrochloric acid, 10 ml of water, 0·1 ml of Teepol solution and 2 drops of manganese carrier solution. (Tracer experiments with selenium-75 showed that 99·7 per cent. of the selenium activity was recovered in this step.) The solutions were decanted into 40-ml centrifuge tubes, and sulphur dioxide was passed through the solutions. The selenium precipitates were washed and plated out as described above for the samples.

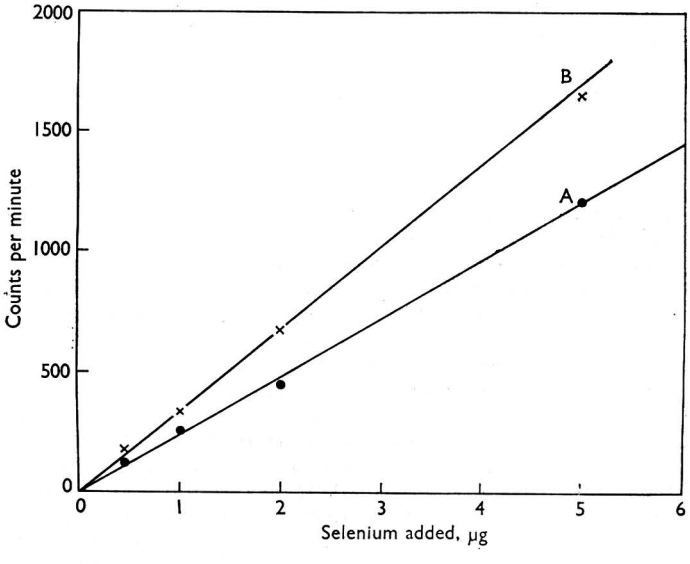

Fig. 1. Recovery of known amounts of selenium spotted on to: curve A, filter-paper; curve B, tomato seeds

DETERMINATION OF RADIOACTIVITY—

The beta-activity of the precipitates was counted with a 2B2 end-window Geiger counter of approximately 40 per cent. efficiency. Radiochemical purity was checked by comparing the decay curves of samples and standards over several half-lives. The decay curve of neutron-activated selenium is complex, since after the selenium-81 has largely decayed there are still counts from selenium-83, selenium-81*m* and selenium-75, which have longer half-lives (see Table I). Gamma spectrometry was also used for detecting possible impurities in precipitates having high count-rates.

DISCUSSION OF THE METHOD

Fig. 1 shows a recovery curve for known amounts of selenium spotted on to (*a*) filter-paper and (*b*) tomato seeds. Each point represents the mean of four determinations agreeing to ± 5 per cent. It can be seen that the count rate is directly proportional to selenium content in the range 0·5 to 5 μg of selenium.

The theoretical sensitivity of the method is determined both by the flux available and by the background of the counter used. In our experiments, assuming a chemical separation time of 40 minutes and a background of 30 counts per minute, 5 μg of selenium in the sample would double this background. This amount can be taken as the practical limit of sensitivity when BEPO is used.

TESTING THE RADIOCHEMICAL PROCEDURES—

The chemical procedures described above were tested with portions of radiochemically pure arsenic-76, bromine-82, manganese-56, selenium-75, sodium-24 and zinc-65. The percentages of these nuclides contaminating the final precipitate and other fractions were determined by scintillation counting, and the results shown in Table III were obtained.

As might be expected, the volatile element bromine is largely eliminated during the initial ashing step, whereas the four metals remain in the residue after distillation. The amounts of arsenic, manganese, sodium and zinc found in the distillate must be a measure of the amount of spray carried over in our distillation procedure.

ACCURACY OF THE METHOD—

Errors may occur in the activation process, in the chemical processes, or during counting. The flux gradient in BEPO is less than 2 per cent. over the Rabbit volume. The high flux of fast neutrons in BEPO could give rise to the production of selenium-81 by the reactions—

(*i*) $^{81}Br(n,p)^{81}Se$.
(*ii*) $^{84}Kr(n,\alpha)^{81}Se$.
(*iii*) $^{235}U(n,f)^{81}Se$.

Little is known about reaction (*i*), though the cross-section for 14-MeV neutrons has been calculated to be 0·023 barns.[24] By activating ammonium bromide in BEPO we have shown that 1 g of bromine gives an apparent content of 22 μg of selenium. This is an upper limit since the ammonium bromide may have contained some selenium as an impurity. Since bromine is present in biological tissue in amounts ranging from 1 to 10 μg per g, the (*n*,*p*) reaction (*i*) should not give rise to a significant error.

Reaction (*ii*) can be neglected in biological material because of the extreme rarity of krypton and its low cross-section. For example, the normal concentration of krypton in blood is estimated to be only 0·0002 μg per ml, which is far below the concentration of selenium.[25]

Reaction (*iii*) can also be neglected since the fission yield of selenium-81 is only 0·13 per cent., and uranium is present in extremely small amounts in biological material, *e.g.*, 0·014 μg per ml of whole blood.[25]

As regards the chemical stages, the elements that are likely to distil under the conditions described here include antimony, arsenic, bromine, chlorine, germanium, iodine, mercury, tellurium and tin. Leddicotte[17] obtained excellent decontamination from antimony, arsenic, sodium, tellurium and tin, by using a similar but slightly simpler separation. The nuclides of arsenic, germanium, mercury and tin have very different half-lives from that of selenium-81, but interference could be serious from 21-minute antimony-124, 25-minute tellurium-131, 37-minute chlorine-138 or 18-minute bromine-80. In biological material we regard

bromine-80 as the most serious impurity since it has the same half-life as selenium-81, and bromine is much more abundant than either antimony or tellurium. Fortunately, decontamination from the halogens is reasonably good, as shown in Table III. The only long-lived impurity we have been able to find in our samples was manganese-56. Because of the high cross-section of manganese, this nuclide constitutes a major source of radioactivity in activated vegetable material, and even after a distillation step traces may contaminate the final selenium precipitate. If hydrogen peroxide is added when the selenium precipitate is dissolved in nitric acid, we find that decontamination from manganese is markedly improved; acidified hydrogen peroxide is a well-known solvent for manganese dioxide.

Table III

Percentages of six elements found in radiochemical fractions

Fraction	Arsenic found, %	Bromine found, %	Manganese found, %	Sodium found, %	Zinc found, %	Selenium found, %
Volatilised during ashing ..	0	95·41	0	0	0	0·25
Distillation residue ..	99·30	0·04	99·88	99·74	99·78	0·95
First selenium supernate ..	0·69	4·54	0·12	0·26	0·14	0·55
Second selenium supernate	0·019	0·013	0·00005	0·0015	$\leqslant$0·05	0·55
Final selenium precipitate	0·00037	0·0027	0·00019	0·0000081	$\leqslant$0·03	97·70

Table IV

Selenium content of various materials

Sample	Selenium content
N. African phosphate rock	10·6 μg per g
Superphosphate	3·77 μg per g
Tomato leaf	0·088 μg per g
Tomato seed	0·025 μg per g
Tomato leaf*	1·25 μg per g
Tomato fruit*	0·24 μg per g
Human blood, mean	0·32 μg per ml
Human blood, range of 8 samples ..	0·26 to 0·37 μg per ml

* Grown in soil rich in selenium.

A small error is introduced in weighing the final precipitate, since this only weighs about 15 mg and ordinary balances are reliable to $\pm$0·2 mg.

When counting long-lived nuclides it is possible to obtain counting accuracy of within 1 per cent., by registering 10,000 counts. This may not be possible for rapidly decaying nuclides; a count rate of 10,000 counts per minute is given from samples containing about 1·5 μg of selenium, and many biological samples contain much less than this. Care must also be taken to record the exact time at which a count is begun, since an error of 1 minute corresponds to a 3·5 per cent. correction for radioactivity decay.

Results

Some results obtained by this method of analysis are shown in Table IV. The results for plant tissues and blood are of the same order of magnitude as those determined by earlier workers.[5,10,25] The relatively high figures for the widely used fertilisers, phosphate rock and superphosphate, are a little disquieting in view of the toxicity of the element. Tagwerker[3] states that any soil containing more than 0·5 μg per g of selenium is potentially dangerous and that chronic selenium toxicity may be caused by rations containing more than 5 μg per g of selenium.

We thank Miss M. J. Dick for carrying out most of the counting involved in this work.

References

1. Robinson, W. O., Dudley, H. C., Williams, K. T., and Byers, H. G., *Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 274.
2. Schwarz, K., and Foltz, C. M., *J. Amer. Chem. Soc.*, 1957, **79**, 3292.
3. Tagwerker, F., *J. Agric. Vet. Chem.*, 1960, **1**, 23 and 78.
4. Kelleher, W. J., and Johnson, M. J., *Anal. Chem.*, 1961, **33**, 1429.

5. Watkinson, J. H., *Ibid.*, 1960, **32**, 981.
6. Bonhorst, C. W., and Mattice, J. J., *Ibid.*, 1959, **31**, 2106.
7. Ray, E. M., Goodyear Atomic Company Report GAT-372, 1961.
8. Handley, R., *Anal. Chem.*, 1960, **32**, 1719.
9. Okada, M., *Nature*, 1960, **187**, 594.
10. McConnell, K. P., in "Modern Trends in Activation Analysis," College Station, Texas, 1961, p. 137.
11. Anders, O. U., *Anal. Chem.*, 1961, **33**, 1706.
12. Gibbons, D., and Simpson, H., paper SM32/34 presented at the Conference on Short-lived Radioisotopes, International Atomic Energy Agency, Vienna, 1962.
13. Fineman, I., Ljunggren, K., Forsberg, H. G., and Erwall, L. G., *Int. J. Appl. Rad. Isotopes*, 1959, **5**, 280.
14. Erion, W. E., Mott, W. E., and Shedlovsky, J. P., *Trans. Amer. Nucl. Soc.*, 1960, **3**, No. 1, 252.
15. Schindewolf, U., *Geochim. Cosmochim. Acta*, 1960, **19**, 134.
16. Gaittet, J., *Ann. Chim., Paris*, 1960, **77**, 1219.
17. Leddicotte, G. W., National Academy of Sciences Report NAS–NS 3030, 1961.
18. Koch, R. C., and Roesmer, J., *J. Food Sci.*, 1962, **27**, 309.
19. Morris, D. F. C., and Killick, R. A., *Talanta*, 1963, **10**, 279.
20. Maddock, R. S., and Meinke, W. W., University of Michigan Progress Report 1959, **8**, 94.
21. ——, ——, *Ibid.*, 1962, **11**, 68.
22. Yajima, S., Kamemoto, Y., Shiba, K., and Onoda, Y., *J. Chem. Soc. Japan, Pure Chem. Sect.*, 1961, **82**, 343.
23. Bowen, H. J. M., and Cawse, P. A., U.K. Atomic Energy Research Establishment Report R2925, Harwell, 1959.
24. Gardner, D. G., *Nuclear Phys.*, 1962, **29**, 373.
25. Bowen, H. J. M., U.K. Atomic Energy Research Establishment Report R4196, Harwell, 1963.

Received *April 8th*, 1963

Determination of Thorium and Phosphorus Pentoxide in Solution and in Insoluble Thorium Phosphate

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Methods are described for the gravimetric determination of thorium in the presence of substantial amounts of phosphate, and of phosphate in the presence of thorium. The application of these methods to the analysis of insoluble thorium phosphates is described.

In the course of studies on the preparation of thorium phosphates[1] by solid-phase and precipitation reactions, a method for determining thorium and phosphate present together in solution or in an insoluble compound was needed. Accurate methods for determining thorium in its pure compounds or in the absence of interfering substances such as phosphate are readily found, but procedures for use in the presence of relatively high concentrations of phosphate have not been described.

Some excellent reviews[2,3,4] on the determination of thorium are too voluminous to be summarised here. Although thorium can be precipitated as thorium iodate[5,6] from nitric acid solution, and the separation can be achieved in the presence of phosphoric acid, the well-known method of determining thorium oxalate deserved attention in view of its simplicity. Accordingly, a procedure was established by which thorium could be determined by this method even in presence of a fairly large amount of phosphate.

In preliminary work an attempt was made to separate thorium from phosphate by adsorbing the thorium ions on a cation-exchange resin (Zeo-Karb 225), initially in the hydrogen form. Thorium was strongly adsorbed by the resin, but all attempts to elute it afterwards, even by strong mineral acids, failed. This was not unexpected in view of the high charge on the thorium ion, Th^{4+}, and hence the process was abandoned. The separation of thorium from phosphate by ion-exchange was apparently successful, but the quantitative recovery aimed at could not be achieved. Determination of phosphate was also attempted after separation of thorium on the same cation-exchange resin. The results were not reproducible, and about 3 per cent. of the total phosphate was lost on the resin column. Evidently some phosphate was retained in the form of a cation complex.

Schoeller and Powell's method[7] for determining phosphoric acid in monazite sand was examined to see whether or not it could be used in presence of large amounts of thorium. Twenty-five millilitres of phosphoric acid solution (0·0908 g of phosphorus pentoxide) and 25 ml of thorium nitrate solution (0·1081 g of thorium dioxide) were placed in a 250-ml beaker. The thorium phosphate jelly was dissolved by adding 2 to 3 ml of concentrated sulphuric acid. The solution was saturated with potassium sulphate and after thorough mixing was set aside overnight; the bulk of the thorium was precipitated as thorium sulphate.[7] After a single precipitation of thorium by this method the amount of phosphate eventually recovered was 3·9 to 4·1 per cent. less than that originally taken; after double precipitation the amount of phosphate recovered was 1·2 to 1·7 per cent. less than that taken. Schoeller and Powell's method, designed for use when only small amounts of thorium are involved, is evidently not successful when major amounts of thorium are present.

Method

Reagents—

Standard thorium nitrate solution—Prepared from AnalaR thorium nitrate. Thorium was determined as thorium oxide after precipitation by oxalate solution.[6] The standard solution gave 0·1081, 0·1083 and 0·1079 g of thorium dioxide in 25-ml portions.

Standard phosphoric acid solution—Prepared from AnalaR grade phosphoric acid.

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Phosphorus pentoxide was determined by double precipitation with magnesia mixture and ignition of the magnesium ammonium phosphate to magnesium pyrophosphate. Treatment of two 25-ml portions of standard solution gave 0·1424 and 0·1424 g of magnesium pyrophosphate (0·0908 and 0·0908 g of phosphorus pentoxide).

Oxalic acid wash liquid—A 2 per cent. aqueous solution of oxalic acid containing 1 ml of concentrated nitric acid per 100 ml of solution.

DETERMINATION OF THORIUM IN THE PRESENCE OF PHOSPHATE IN A SOLUTION—

Mix 25 ml of thorium nitrate solution and 25 ml of phosphoric acid solution in a 400-ml beaker. Dissolve the jelly formed by adding 10 ml of concentrated nitric acid and warming. Add about 25 ml of water, and then add, dropwise, to the boiling solution 30 to 40 ml of a saturated solution of oxalic acid, with constant stirring. Finally, dilute the solution with water to about 300 ml to reduce the acid concentration to the admissible maximum of 3·5 ml of concentrated nitric acid per 100 ml of solution. Never dilute the solution before adding oxalic acid solution; in the absence of oxalic acid the insoluble phosphate jelly reappears. Boil the diluted solution for about 5 minutes, and set aside overnight.

Filter the solution through a Whatman No. 40 filter-paper; allow most of the precipitate to remain in the beaker. Wash the beaker and the precipitate on the filter-paper with about 150 ml of oxalic acid wash liquid. Transfer the precipitate, with the filter-paper, to the original beaker containing about 100 ml of concentrated nitric acid, and add about 5 ml of concentrated hydrochloric acid. Break up the filter-paper, and gently boil the solution in the covered beaker. After about 45 minutes the solution should be clear brown and much of the filter-paper should have been destroyed. Continue boiling the solution until its volume is reduced to about 25 ml. Transfer the beaker to a steam-bath, cautiously evaporate some of the remaining acid, and, when crystals begin to appear, cool the beaker. (It is important at this stage not to evaporate to dryness, otherwise the products tend to become insoluble.) Wash the sides of the beaker and the clock glass with the minimum amount of water. Add about 20 ml of concentrated nitric acid, and evaporate the solution nearly (but not completely) to dryness on a steam-bath. Cool the solution, and then add about 2 ml of concentrated nitric acid and 20 ml of a saturated solution of oxalic acid; dilute the solution as described above, and set aside overnight. Filter the solution through a Whatman No. 40 filter-paper, and thoroughly wash the precipitate of thorium oxalate with oxalic acid wash liquid. Transfer the precipitate and paper to a platinum crucible, dry, and then char the paper over a low flame. Finally, convert the precipitate into thorium dioxide by ignition over a full flame until it is white in colour and constant in weight.

Thoria was satisfactorily determined by this procedure; 25-ml portions of thorium nitrate solution (0·1081 g of thoria) in the presence of 25-ml portions of phosphoric acid (0·0908 g of phosphorus pentoxide) gave the results tabulated below—

Thoria found, g	0·1082	0·1079	0·1080
Deviation, %	+0·09	−0·18	−0·09

DETERMINATION OF PHOSPHATE IN PRESENCE OF THORIUM IN SOLUTION—

It was eventually found that the most convenient method for determining phosphorus pentoxide in the presence of thorium was that reported by Hoffman and Lundell[8] for determining phosphorus pentoxide in phosphate rock. Satisfactory results were obtained only when precautions similar to those suggested by Hoffman and Lundell were taken—

(*a*) A large amount of citric acid must be used in order to keep the thorium in solution.

(*b*) A concentrated solution of "magnesia mixture" is necessary.

(*c*) Before precipitation of ammonium magnesium phosphate, it is necessary to chill the solution in ice for at least half an hour; after precipitation it must be vigorously shaken for 1 hour to break down super-saturation.

The results of determinations of phosphorus pentoxide under different conditions of precipitation are shown in Table I. It can seen that vigorous shaking during precipitation is essential if reproducible results are to be obtained.

TABLE I

THE EFFECT OF DIFFERENT CONDITIONS OF PRECIPITATION ON THE RECOVERY OF PHOSPHORUS PENTOXIDE

Conditions of precipitation	Thoria taken, g	Phosphorus pentoxide taken, g	Phosphorus pentoxide found, g	Deviation, %
Solution chilled in ice and shaken vigorously	0·1104	0·0933	0·0933	0·0
			0·0931	−0·2
			0·0936	+0·3
Precipitation at room temperature without shaking the solution	0·1104	0·0933	0·0914	−2·0
			0·0915	−1·9
Solution chilled in ice but not shaken	0·1104	0·0933	0·0921	−1·3
			0·0916	−1·8

DETERMINATION OF THORIUM AND PHOSPHORUS PENTOXIDE IN INSOLUBLE THORIUM PHOSPHATES—

Grind the thorium phosphate to a powder in an agate mortar, and weigh into a platinum crucible. Add AnalaR sodium carbonate (5 to 6 times the weight of the sample), and mix the powders thoroughly. Fuse the mixture over a bunsen burner for about half an hour, cool, and transfer the crucible to a 150-ml beaker. Cover the crucible with water, and place the beaker on a steam-bath for about an hour, to disintegrate completely the fused mass. Filter the liquid through a 9-cm Whatman No. 40 filter-paper after adding macerated paper. Wash the crucible, the beaker and the residue on the filter-paper with about 250 ml of a 1 per cent. solution of sodium carbonate, and collect the filtrate in a 500-ml conical flask. Acidify the filtrate with hydrochloric acid, and evaporate to about 75 ml.

Transfer the residue, with the filter-paper, to a platinum crucible; burn off the paper, and then ignite the residue gently for 10 minutes. Mix with AnalaR sodium carbonate (about five times the weight of the original specimen), and fuse the mixture for half an hour. Disintegrate the fused cake in water as described, and filter the solution through a 9-cm Whatman No. 40 filter-paper after adding macerated paper. Collect the filtrate and washings (about 200 ml of 1 per cent. ammonium chloride solution) in the original conical flask. Add more hydrochloric acid to keep the solution acidic, and reduce the volume to about 100 ml by boiling. Then determine phosphorus pentoxide by double precipitation with magnesia mixture by the conventional procedure.

Transfer the filter-paper containing the residue to a weighed silica crucible, ignite, and heat until the weight of ignition residue is constant. Then add powdered potassium pyrosulphate (ten times the weight of the residue), and fuse the mixture over a small bunsen flame for 15 to 20 minutes, or until the thoria has completely dissolved in the pyrosulphate melt.

Transfer the crucible and fused cake to a 400-ml beaker, and cover the crucible with about 150 ml of water and 5 ml of concentrated nitric acid. Heat on the steam-bath for half an hour, until the fused cake has disintegrated and much of it has dissolved. Stir the solution at frequent intervals while heating for a further half hour to complete dissolution. Remove and wash the crucible, and adjust the volume of the solution to about 200 ml. (Whenever an attempt was made to dissolve the fused cake by *boiling* with water, an insoluble crystalline precipitate resulted.) Boil the solution, and precipitate thorium oxalate by adding a saturated solution of oxalic acid, as described previously. From this stage the procedure corresponds exactly with that described for determining thorium in the presence of phosphate in solution. The results of several determinations on different synthetic specimens of thorium phosphate are presented in Table II.

It is noteworthy that the percentage of thorium oxide found after pyrosulphate fusion is always considerably less than the percentage of "ignited residue," and that the thoria *plus* phosphorus pentoxide percentage is substantially less than 100; the thoria *plus* ignited residue percentages, however, are closer to the expected values of very nearly 100. This suggested that the high concentrations of alkali-metal ions inevitably introduced by the fusion with pyrosulphate had interfered with the precipitation of thorium oxalate, and that the ignited residue values more nearly represent the thoria content of the samples. To elucidate this point and to exclude other sources of error (such as retention of thoria by slight

reaction with the silica crucible), test determinations were carried out on pure thoria. Sodium pyrosulphate was used for some of the fusions, because of the possibility that sodium might cause less interference in the oxalate precipitation than potassium.

TABLE II

RESULTS

Weight of thorium phosphate taken, g	Ignited residue, %	Thoria found after pyrosulphate fusion, %	Phosphorus pentoxide found, %	Thoria + phosphorus pentoxide found, %	Ignited residue + phosphorus pentoxide found, %
0·5328	58·90	56·68	40·37	97·05	99·27
0·5100	74·27	73·04	25·43	98·47	99·70
0·4836	73·20	71·53	26·55	98·08	99·75
0·5362	71·54	70·53	27·83	98·36	99·37
0·3399	69·13	67·46	30·34	98·80	99·47
0·5464	66·86	65·57	32·41	97·88	99·27

The entire fusion process, starting with the double fusion with sodium carbonate, was carried out on pure thorium oxide, prepared by igniting AnalaR thorium nitrate at 600° C in a platinum dish for 5 to 6 hours. Determinations were carried out in which both silica and platinum crucibles were employed for the pyrosulphate fusion. The results are presented in Tables III and IV.

TABLE III

FUSION WITH POTASSIUM PYROSULPHATE IN SILICA CRUCIBLES

Weight of thoria taken, g	Weight of ignited residue, g	Thoria found after potassium pyrosulphate fusion, %	Loss in weight of silica crucible during fusion, g
0·3050	0·3054	97·34	0·0013
0·2924	0·2927	97·50	0·0019
0·3284	0·3282	97·75	0·0018
0·2953	0·2950	97·49	0·0016

TABLE IV

FUSION IN PLATINUM CRUCIBLES

Fusion reagent	Weight of thoria taken, g	Weight of ignited residue, g	Thoria found after pyrosulphate fusion, %
Potassium pyrosulphate	0·3232	0·3230	97·58
	0·2960	0·2963	97·09
	0·3046	0·3044	97·31
Sodium pyrosulphate	0·3023	0·3025	97·32
	0·3139	0·3142	97·74

These results show that the weight of thoria obtained after fusion with sodium or potassium pyrosulphate is always less by about 2·5 per cent. than the weight of thoria taken. Both sodium and potassium ions evidently have an adverse effect on the precipitation of thorium oxalate. The ignited residue, however, corresponds accurately in weight with the thoria initially taken.

Attempts to dissolve the ignited residue by heating with concentrated sulphuric acid, so that the introduction of alkali metals was avoided, were unsuccessful.

The insoluble thorium phosphates were therefore fused twice with sodium carbonate, as described, and phosphorus pentoxide was determined in the water extract by double precipitation with magnesia mixture. The ignited residue was taken to represent the thoria present in the original sample.

I thank the Ministry of Education, Government of Pakistan, for an Overseas Scholarship. I also thank Dr. A. J. E. Welch for supervising the work and Mr. L. S. Theobald for advice.

References

1. Welch, A. J. E., and Rahman, A., in the press.
2. Moeller, T., Schweitzer, G. K., and Starr, D. D., *Chem. Rev.*, 1948, **42**, 63.
3. Rodden, C. J., *Editor*, "Analytical Chemistry of Manhattan Project," McGraw-Hill Book Co. Inc., New York and London, 1950.
4. Fonseka, J. P. R., D.I.C. Thesis (Inorganic), 1955.
5. Meyer, R. J., and Speter, M., *Chem. Zeit.*, 1910, **34**, 306.
6. Vogel, A. I., "A Textbook of Quantitative Inorganic Analysis," Second Edition, Longmans Green & Co. Ltd., London, 1953.
7. Schoeller, W. R., and Powell, A. R., "The Analysis of the Minerals of the Rare Elements," Third Edition, Charles Griffin, London, 1955, p. 111.
8. Hoffman, J. I., and Lundell, G. E. F., *Bur. Stand. J. Res.*, 1937, **19**, 59.

First received *February 28th*, 1962
Amended, *December 19th*, 1962

SHORT PAPERS

A Field Method for determining 2,4-Tolylene Di-isocyanate Vapour in Air

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Tolylene di-isocyanate is widely used in the production of urethane products such as flexible foams, lacquers and synthetic rubbers. During the manufacture of these materials the vapour of tolylene di-isocyanate may be evolved, and this can constitute a danger to health if proper care in storage, handling and ventilation is not taken. The effectiveness of precautionary measures can best be assessed by measuring atmospheric concentrations of tolylene di-isocyanate at appropriate times and places.

The recent reduction in the maximum permissible concentration of tolylene di-isocyanate from 0·1 to 0·02 p.p.m. v/v in air[1,2] has meant that the widely used M.S.A. T.D.I. Detector is insufficiently sensitive for the determination of concentrations in the region of the new limit. With this detector, based on the work of Marcali,[3] a 3-litre sample of air is drawn through 15 ml of dilute hydrochloric acid, when tolylene di-isocyanate is hydrolysed to the corresponding diamine; this is then diazotised with a sodium nitrite - sodium bromide solution, the excess of nitrite removed by treatment with sulphamic acid and the diazonium compound coupled with *N*-1-naphthylethylenediamine to give a pink coloured solution. The colour of the solution is then matched visually in turn against three strips of pink coloured plastic material representing concentrations of 0·05, 0·10 and 0·20 p.p.m. of tolylene di-isocyanate, respectively. This method is claimed to determine primarily the 2,4-isomer, as the 2,6-isomer is stated to react much more slowly.

Concentrations in the region of 0·02 p.p.m. can be determined with the existing method by increasing the sample size from 3 to 15 litres, but this involves either an inconveniently long sampling time of 15 minutes or the use of a larger (but less portable) pump to sample at a faster rate. This paper describes a modification of the earlier method in which a 3-litre sample of air is drawn through a specially designed absorber containing 3 ml of a mixture of dilute hydrochloric acid and *N*,*N*di-methylformamide.[4] The pink colour is developed as described above and matched visually against inorganic colour standard solutions.

Method

Apparatus—

Sampling pump—Capable of drawing air at a rate of 1 litre per minute through the absorber. The M.S.A. 4-cylinder hand-cranked pump has been found convenient for this purpose. Alternatively, when convenient, a water-filled aspirator may be used.

All-glass absorber—Of the type shown in Fig. 1. The dimensions of the narrow lower part are important and should be adhered to; the upper part should be sufficiently large to prevent loss of the contents of the absorber by splashing during sampling.

Test-tubes—100 mm × 10 mm.

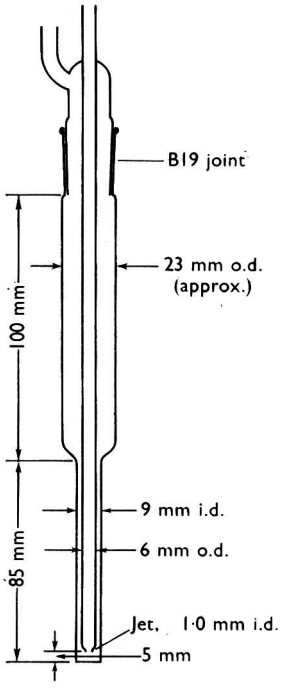

Fig. 1. All-glass absorber

Reagents—

Sodium nitrite - sodium bromide solution—Dissolve 3·0 g of sodium nitrite and 5·0 g of sodium bromide in about 80 ml of water, and dilute to 100 ml.

Sulphamic acid solution, 10 per cent. w/v.

Hydrochloric acid, dilute—Dilute 25 ml of concentrated hydrochloric acid, sp.gr. 1·18, to 1 litre with water.

N-1-*Naphthylethylenediamine dihydrochloride solution*—Dissolve 50 mg of the dry salt in about 25 ml of water, add 1 ml of concentrated hydrochloric acid, and dilute to 50 ml with water. Use within 48 hours.

N,N-*Dimethylformamide*—Distil through a water-cooled all-glass condenser, rejecting the first and last 10 per cent. of the distillate.

Absorber solution—Add 25 ml of concentrated hydrochloric acid, sp.gr. 1·18, to 500 ml of water. To this solution add 250 ml of *N,N*-dimethylformamide, and dilute to 1 litre with water.

Cobaltous chloride colorimetric solution—A solution in dilute hydrochloric acid containing 59·5 mg of the analytical-reagent grade salt, $CoCl_2.6H_2O$ (purity not less than 97·5 per cent.) per ml.[5]

Cupric sulphate colorimetric solution—A solution in dilute hydrochloric acid containing 62·4 mg of the analytical-reagent grade salt $CuSO_4.5H_2O$ (purity not less than 99·0 per cent.) per ml.[5]

Colour standard solutions—Mix the volumes of the cobaltous chloride and cupric sulphate colorimetric solutions specified in Table I, and dilute to 500 ml with dilute hydrochloric acid. These solutions have the same quality and depth of shade as the test solutions (containing the equivalent amounts of tolylene di-isocyanate) prepared as described below. Store in clean glass bottles having tightly fitting glass stoppers.

TABLE I

COMPOSITION OF COLOUR STANDARD SOLUTIONS

Cobaltous chloride solution, ml	Cupric sulphate solution, ml	Tolylene di-isocyanate, p.p.m.
8·75	13·75	0·01
17·50	27·50	0·02
35·00	55·00	0·04

PROCEDURE—

Draw 3 litres of the air sample at a rate of about 1 litre per minute through the absorber containing 3·0 ml of absorber solution. Disconnect and lift the inlet tube, allowing the liquid in it to drain into the body of the absorber. Expel the last drop by blowing gently on the inlet tube, and then withdraw this completely. Add 0·1 ml (3 drops) of sodium nitrite solution, close the absorber body with a glass stopper, and mix the solution by gentle shaking; set aside for 1½ minutes. Add 0·1 ml (3 drops) of sulphamic acid solution, stopper the absorber, mix the solution by gentle shaking, and set aside for 1½ minutes. Add 0·1 ml (3 drops) of *N*-1-naphthylethylenediamine dihydrochloride solution, stopper the absorber, and mix the solution by gentle shaking. Fill 100-mm × 10-mm test-tubes with the three colour standard solutions to the same depth as that of liquid in the absorber. Between 1½ and 2 minutes after adding the last reagent to the contents of the absorber, compare the colour of the test solution with each of the colour standard solutions in turn by looking downwards through the solutions towards a sheet of white paper held a few inches below the bottom of the tubes. Report the concentration of tolylene di-isocyanate as less than, equal to or more than that of the nearest colour standard.

BASIS OF METHOD

During the development of this method, a number of relevant factors were investigated.

STRENGTH OF COLOUR STANDARD SOLUTIONS—

The composition of these solutions was arrived at by a process of trial and error. The quality of shade was first matched by mixing cupric sulphate and cobaltous chloride solutions in different proportions until the shade of the mixture was judged to be correct by two people. Solutions of different strengths, but with the two components in the same proportion, were next prepared and matched with test solutions prepared from 0·21, 0·42 and 0·84 μg of tolylene di-isocyanate (corre-

sponding to the weights present in 3 litres of test samples containing 0·01, 0·02 and 0·04 p.p.m. of tolylene di-isocyanate). When the approximate strengths of the inorganic standards had been ascertained, further solutions were prepared covering narrower concentration ranges, and the final concentrations were decided on.

In preparing coloured solutions from tolylene di-isocyanate, a standard solution containing 7·0 μg per ml was prepared in aqueous acetic acid as described by Marcali[3]; 0·5-, 1·0- and 2·0-ml portions of this solution were diluted to 50 ml with dilute hydrochloric acid, and 3·0-ml portions of these diluted solutions were then treated as described above.

Reaction with the 2,6-isomer—

Under the conditions described, the conclusion[2] that the reaction was specific for the 2,4-isomer was largely confirmed. There was a slow reaction with the 2,6-isomer, which produced a colour much bluer than that produced by the 2,4-isomer. The inorganic colour standards were matched against colours produced from a mixture containing 80 per cent. of the 2,4-isomer and 20 per cent. of the 2,6-isomer. The error when these standards were used for determining other commercial tolylene di-isocyanates, whose 2,4-isomer content might range from 60 to 100 per cent., would not be a serious one.

Time required for colour development—

In the procedure it is recommended that the matching of the colour of the test solutions with that of the inorganic colour standards be done between 1½ and 2 minutes after the addition of the *N*-1-naphthylethylenediamine dihydrochloride. This time interval was adopted because, on further standing, the colour of the test solutions deepened slightly and became bluer in shade, presumably owing to slow reaction of the 2,6-isomer.

Recovery from a sample of air—

No attempts were made to produce accurately known atmospheric concentrations of tolylene di-isocyanate. It was found, however, that concentrations in the range 0·01 to 0·10 p.p.m. were produced when a stream of nitrogen was bubbled through 20 ml of 2,4-tolylene di-isocyanate in a 15-cm × 2·5-cm test-tube at 20° C, at speeds from 10 to 100 ml per minute, and diluted with 10 litres of air per minute. Tests were then carried out by sampling through two absorbers in series and observing the ratio of isocyanate found in the first absorber to that found in the second, by measuring the optical densities at 544 mμ with a Unicam SP600 spectrophotometer. Initial tests were carried out by using dilute hydrochloric acid without added *N*,*N*-dimethylformamide as the absorber solution; the results were—

Amount of tolylene di-isocyanate found in 2 absorbers, p.p.m. v/v	0·040	0·040	0·035	0·035	0·035	0·025
Amount found in 1st absorber, as percentage of total	66	66	67	69	69	63

In later tests an absorber solution containing *N*,*N*-dimethylformamide was used; the results of these tests were—

Amount of tolylene di-isocyanate found in 2 absorbers, p.p.m. v/v	0·100	0·055	0·045	0·035	0·025	0·025
Amount found in 1st absorber, as percentage of total	93	80	81	90	87	95

Conclusions

The sensitivity of the test has been increased five-fold by reducing the volume of absorber solution used from 15 ml[2] to 3 ml. To ensure the absorption of a sufficiently high proportion of the total isocyanate in only 3 ml of liquid, an absorber giving a high ratio of depth to volume has been designed. The retaining power of the liquid was increased by incorporating 25 per cent. of *N*,*N*-dimethylformamide in the absorber solution, resulting in the retention in the first absorber of 88 ± 8 per cent. of the total amount of isocyanate trapped from a dry nitrogen stream. As this was intended as a rapid field method the accuracy and precision of these results was considered to be adequate.

REFERENCES

1. Ministry of Labour, *Safety, Health and Welfare Series* No. 8, Toxic Substances in Factory Atmospheres, 1962 Amendment, H.M. Stationery Office, London.
2. "Threshold Limits for 1961," *Arch. Environ. Health*, 1961, **3**, 489.
3. Marcali, K., *Anal. Chem.*, 1957, **29**, 552.
4. Funke, W., and Hamann, F., *Farbe u. Lack.*, 1958, **64**, 120.
5. United States Pharmacopoeia, Fourteenth Revision, pp. 929 to 931.

Received *April 1st*, 1963

The Determination of Small Amounts of Sulphur in Toluene by Reduction with Raney Nickel

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GRANATELLI'S method,[1] in which sulphur compounds are reduced to sulphides by Raney nickel and the hydrogen sulphide produced on acidification is determined, is potentially attractive for the determination of total sulphur in certain types of material, particularly in highly refined hydrocarbons containing extremely small amounts of sulphur. However, when the method was tried in this laboratory on toluene solutions of known sulphur content the recoveries varied between 70 and 85 per cent. of theory.

Modifications in the mode of preparation and use of the Raney nickel catalyst resulted in an increase in the percentage recovery from elementary sulphur, carbon disulphide and 2- and 3-methylthiophen to 95 $\pm$ 5 per cent. at a level of 1 to 10 p.p.m. of sulphur.

The modifications deal with the temperature at which the Raney nickel is activated, the detailed instructions for washing the activated catalyst and the immediate use of the catalyst after its preparation.

METHOD

REAGENT—

Nickel - aluminium alloy—50 per cent. of nickel, 50 per cent. of aluminium, for preparing catalytic nickel. Obtainable from British Drug Houses Ltd.

ACTIVATION OF NICKEL CATALYST—

Assemble an apparatus similar in dimensions to that described by Granatelli,[1] and grease the joints lightly with silicone grease. Remove the reduction flask, measure into it 10 ml of 2·5 M sodium hydroxide solution, and raise the temperature to 75° to 80° C. Add, in one portion, 0·5 $\pm$ 0·05 g of nickel - aluminium alloy; a vigorous reaction ensues, and precautions should be taken against the mist of caustic liquor ejected from the flask. Set aside the flask for 10 minutes. Decant the supernatant liquid as completely as possible from the flask, and wash down the neck of the flask with 10 to 15 ml of water from (for convenience) a polythene wash bottle. Swirl the water with moderate vigour to disturb the nickel residue slightly, but avoid entrainment of air. With the minimum delay required for the residue to settle, decant off the water as completely as possible. Repeat the washing of the neck of the flask and the nickel residue twice with water, and then with 10 ml of isopropanol. Decant off the isopropanol, and add a further 10 ml of isopropanol to the flask. Without delay transfer by pipette 10 or 25 ml of a sample expected to contain 1 to 10 p.p.m. of total sulphur, and replace the flask in the apparatus.

PROCEDURE—

Pass nitrogen through the sample solution at a slow rate (about 2 bubbles per second) for 10 minutes before heating is commenced, and then continue as described by Granatelli.[1] The absorber may be connected to the apparatus at the start of the procedure; this de-aerates the contents of the absorber without the necessity of manipulating the gas flow and absorber later in the procedure.

Discussion of the method

Treatment of the nickel - aluminium alloy with hot sodium hydroxide solution and the use of freshly prepared catalyst give improved efficiency of reduction of sulphur compounds in general. Certain compounds are affected by the alkalinity of the catalyst; if, for example, the nickel is washed just once with water, only about 20 per cent. recovery of sulphur is obtained from 2- or 3-methylthiophen. However, for the complete reduction of phenylvinylsulphone it is necessary to limit the number of washings with water to one. Elementary sulphur is completely reduced, irrespective of variations in the washing of the nickel.

Limited experiments have shown that a short reduction with triple washed Raney nickel, with subsequent addition of a small amount of sodium hydroxide solution and a further period of heating will decompose mixtures of compounds requiring different alkalinities for their reduction; this may serve as a satisfactory arbitrary procedure that will eliminate the need for knowing what sulphur compounds are present in a new type of sample.

The modified method is eminently suitable for the routine determination of total sulphur in toluene in the 1 to 10 p.p.m. range and should also be applicable to other aromatic and aliphatic hydrocarbons and to alcohols. The process is simple, quiet and relatively free from hazard, and is such that a routine operator can supervise two sets of apparatus at the same time.

Reference

1. Granatelli, L., *Anal. Chem.*, 1959, **31**, 434.

Received *April 1st*, 1963

The Determination of Phosphorus in Different Leathers by Oxygen-flask Combustion

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A history of the development and uses of the oxygen-flask combustion technique of analysis is presented in the review article by Schöniger.[1] This type of analysis is popular because of its simplicity, ease of operation and the comparatively short time involved in making the determination. It is advisable, however, to observe all safety precautions recommended for combustions of this type. Combustions should be conducted behind a safety glass shield with protective covering for the hands. All combustions in connection with this work were noted to be safe and without incident.

The primary objective of this work was to find a suitable rapid method for determining phosphorus, applicable to the analysis of leather. Initial work showed that when nitric acid was used as the absorbent in the combustion flask, as proposed by Fleischer *et al.*[2] or by Barney, Bergmann and Tuskan,[3] the burning sample produced an objectionable amount of sparking with consequent incomplete combustion and loss of sample. Also, the presence of nitric acid caused erratic colour development with the Association of Official Agricultural Chemists (AOAC) colour reagents.[4] The absorbents suggested by Kirsten and Carlsson[5] were found effectively to suppress spark formation during combustion so that samples were rarely lost by sparking.

The combustion chamber consisted of a regular 500-ml short-necked flat-bottomed flask having a 24/40 ground-glass joint with glass stopper. A hole was drilled in the end of the glass stopper to anchor the wire supporting the sample carrier. Wires extending from the glass stopper and the sample carrier were inter-connected so that they could be disengaged by rotation of the stoppered flask. The sample carrier could thus be completely immersed in the absorbent solution for more efficient dissolution of combustion residues.

Flasks were prepared for the combustion by placing in them 5 ml of 0·8 N sodium hydroxide and flushing with oxygen for about 3 minutes. Seven millilitres of saturated bromine water were then quickly added to the contents of the flask from a pre-calibrated transfer pipette having the tip removed for fast delivery. The fuse of the sample was then ignited and the sample assembly plunged into the flask to ensure a closed seal during and after combustion. After combustion, the sample carrier was demounted into the absorbent and the stoppered flask held for 30 minutes

with intermittent shaking. The stopper with its wire stem was then washed and removed. Five millilitres of 22 N sulphuric acid were next added, and the contents of the flask with the sample carrier were boiled for 4 minutes over a gas flame. Boiling provided supplementary oxidation conditions, complete bromine removal, and conversion of phosphate complexes to orthophosphates suitable for analysis by the selected colour reagents.

Dependent on the expected phosphorus content in the sample, the entire contents of the combustion flask were transferred quantitatively into a 50-, 100- or 250-ml calibrated flask and made up to the mark. Portions (2, 5 or 10 ml) considered most likely to give full colour development with the colour reagents were placed in separate 25-ml calibrated flasks. Two millilitres of each of the three AOAC colour producing reagents[4] were added to each portion, and the contents of the flasks were then made up to the mark. It was found that 25-ml burettes were satisfactory for adding the reagents, with advantages in speed and convenience over the use of pipettes. However, the AOAC colour reagents are sensitive to high concentrations of acid, and the acidity should be reduced to 21 milli-equivalents or less per 100 ml before the AOAC colour reagents for full colour development are added. Because of the small amounts of phosphorus in the combustion residues of the last three leather samples analysed (see Table I) the acidity was reduced in the combustion flask, the contents were transferred to a 100-ml calibrated flask, 3 ml of each AOAC colour reagent added, and the contents of the flask were made to the mark for colour development. Smaller amounts of colour reagents were found satisfactory for these low phosphorus values and thus a greater volume of water could be used for rinsing and added during transfer of the sample to the calibrated flask.

Readings of the colour intensity developed were made on a Beckman DB spectrophotometer at 650 mμ against a blank solution treated in the same manner as the sample solution. A standard 1-cm glass cell was used, but the cell was sealed and equipped with conduits to allow it to be operated by a GME Automatic Transferator. Satisfactory conduits were made of intramedic polythene tubing of internal diameter 0·055 inch, external diameter 0·075 inch. About 4·6 ml of sample were found to be a convenient amount for transfer. Flushing between samples was not necessary except when there were wide differences in colour intensities.

It was found convenient and desirable to allow the AOAC reagents to react with the sample for a period of about 2 hours to develop full colour and minimise slight differences in reading times. All readings were compared with a phosphate standard treated under the same conditions as the sample portions, *i.e.*, temperature, size of vessel, milli-equivalents of acid present, reaction time, etc.

Table I lists phosphorus values for three reference standards, *viz.*, Syntropan (amprotropine phosphate; 3-diethylamino-2,2-dimethylpropyl tropate phosphate) triphenylphosphine and triphenyl phosphate, a commercial grade dioctadecyl phosphite and five different sheepskin leathers. These leathers were tanned with a phosphorus-containing compound, tetrakis(hydroxymethyl)-phosphonium chloride, in combination with resorcinol, by Windus, Filachione and Happich's

TABLE I

PHOSPHORUS DETERMINED IN CHEMICALS AND LEATHERS BY THE CARIUS AND PROPOSED METHODS

	Weights of sample used in proposed method,	Phosphorus content		
		Theoretical,	Found by— Carius method,	Found by— proposed method,*
Chemicals—	mg	%	%	%
Syntropan	4·38 to 7·00	7·64	7·59	7·65
Triphenylphosphine	3·96 to 5·49	11·83	11·78	11·85
Triphenyl phosphate	6·01 to 6·22	9·51	9·28	9·69
Dioctadecyl phosphite, commercial grade	7·85 to 12·21	5·28	4·63	4·65
Origin of leather—				
United States sheepskin	10·40 to 12·00	—	2·68	2·71
Iran sheepskin	10·30 to 32·30	—	2·15	2·16
New Zealand sheepskin	23·52 to 25·41	—	0·40	0·46
Iran sheepskin	27·92 to 29·70	—	0·48	0·48
Iran sheepskin	26·46 to 32·73	—	0·42	0·47

* Standard deviation of the method was 0·025.

method.[6] Phosphorus values obtained by the Standard Carius method[7] correlate with the proposed method. The results obtained on the test substances illustrate the reliability of the proposed method for determining phosphorus content, although the phosphorus is bound in various different molecular combinations.

I thank Miss Oksana Panasiuk for the Carius analyses and Dr. Clyde L. Ogg for his constant interest and helpful advice.

REFERENCES

1. Schöniger, W., "Facts and Methods for Scientific Research 1," No. 2, F. & M. Scientific Corporation, New Castle, Delaware, 1960.
2. Fleischer, K. D., Southworth, B. C., Hodecker, J. H., and Tuckerman, M. M., *Anal. Chem.*, 1958, **30**, 152.
3. Barney, J. E., II, Bergmann, J. G., and Tuskan, W. G., *Ibid.*, 1959, **31**, 1394.
4. Association of Official Agricultural Chemists, "Official Methods of Analysis of the AOAC," Ninth Edition, Washington, 1960, Section 6.062, p. 84.
5. Kirsten, W. J., and Carlsson, M. E., *Microchem. J.*, 1960, **4**, 3.
6. Windus, W., Filachione, E. M., and Happich, W. F., U.S. Patent No. 2,992,879, July 18th, 1961.
7. Association of Official Agricultural Chemists, *op. cit.*, Section 38.012, p. 644.

Received *January 7th*, 1963

Inexpensive Sampling Device for Gas Chomatography

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INVESTIGATIONS in progress in this laboratory necessitated the use of a gas-sampling device suitable for "on stream" sampling. Commercially available valves were relatively expensive and the delivery dates quoted were unsatisfactory, so the possibility of constructing a suitable valve was investigated.

A simple method has been described[1] in which were used three glass stop-cocks requiring simultaneous operation for optimum performance. It was realised that a multiple valve performing these basic operations should be available commercially. Drallim Industries Ltd. (Bourne Works, Station Approach, Whyteleaf, Surrey) were consulted, and a valve to our specification was quickly supplied from their range of "custom built" valves. It was ready for use and required only the addition of a calibrated sampling loop. The valve was completely satisfactory and so the temporary sampling loop was replaced by a turret device that permitted the size of sample to be changed readily.

Minimum sample volume was limited by the internal volume of the valve (4·7 ml), and the turret volumes were arranged to deliver samples of, nominally, 5, 10, 15 and 20 ml. For convenience, these volumes were achieved by using twin bores connected by grooves and sealed by a rubber gasket secured by the top cap; the lower end of the bores were sealed with neoprene "O" rings. This simple adaptation, made in our workshops, is shown in Fig. 1. The valve was perfect in operation, requiring a simple turn through 90° to effect sample injection.

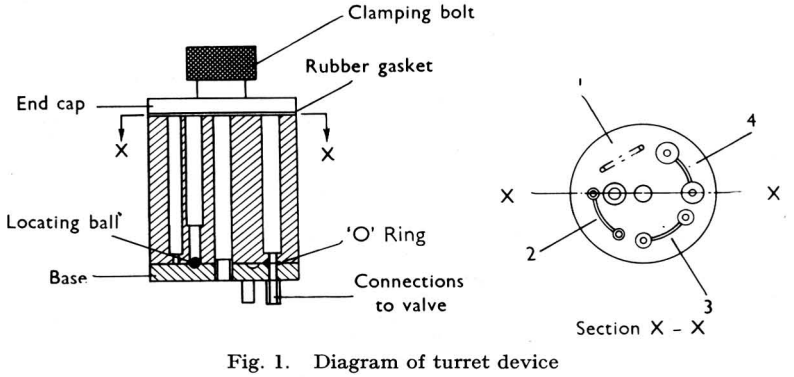


Fig. 1. Diagram of turret device

*Present address: Energy Conversion Ltd., Chertsey Road, Sunbury-on-Thames, Middlesex.
†Present address: Messrs. Nash & Thompson, Instrument Makers, Hook Rise, Tolworth, Surrey.

To extend the range of sample sizes obtainable, various modifications to the basic valve were suggested. In collaboration with Drallim Industries Ltd. a modified valve was produced (supplied at £17 10s.). Although this was more costly than the standard valve (£9 5s.) it was still approximately half the cost of other commercially available sampling valves and had a similar range of application. The turret was made in our own workshop and can be produced cheaply with the minimum of engineering facilities. However, the turret is not essential, and either the standard or the improved valve can be used with fixed sampling loops, as is common with other commercial sampling valves.

The volume of the modified valve is only 1·74 ml, and samples of as small as 2 ml can be dispensed.

TABLE I

TYPICAL RESULTS OBTAINED WITH THE SAMPLING VALVE

Volume of sample dispensed, ml	Integrator counts	Mean	Standard deviation	Coefficient of variation, %
5	365, 373, 367, 368, 365, 366, 367, 368, 366, 366	367	0·8	0·25
10	828, 824, 818, 828, 826, 824, 826, 824, 824, 824	824	0·4	0·05
15	1172, 1170, 1172, 1166, 1168, 1164, 1164, 1166, 1164, 1166	1168	0·6	0·05
20	1562, 1556, 1552, 1554, 1552, 1552, 1550, 1552, 1550, 1550	1554	0·8	0·05

Some typical results obtained when the sampling valve was used are shown in Table I. The counts were obtained by Nogare, Bennett and Harden's method[2] of integration; the deviations noted were probably due to the method of integration rather than to a fault in the sampling valve.

The manufacturers claim the valve to be leak-free to 200 lb per sq. inch, and the results of our experiments show no indication to the contrary. The steadiness of the base-line and the reproducibility of results suggest that leaks, if any, are extremely small. Our investigations involve measurements of down to 1 part of nitrogen in 20,000 parts of hydrogen, and any leak to atmosphere would be immediately visible.

REFERENCES

1. "Standard Methods for Testing Petroleum and its Products," Eighteenth Edition, Institute of Petroleum, London, 1959, Method 169/59.
2. Nogare, S. D., Bennett, C. E., and Harden, J. C., "Gas Chromatography," Academic Press Inc., New York and London, 1958.

Received *February 6th*, 1963

Book Reviews

ANALYTICAL MICROBIOLOGY. Edited by FREDERICK KAVANAGH. Pp. xvi + 707. New York and London: Academic Press Inc. 1963. Price 157s.

Analytical microbiology has been defined as "that branch of microbiology in which micro-organisms are used as reagents for the quantitative determination of certain chemical compounds." As an analytical tool it has proved invaluable for vitamins, amino acids and antibiotics, many of which cannot be measured by purely chemical techniques. This book is probably the most comprehensive so far produced on the subject.

It is no ordinary laboratory manual of methods, but rather a treatise dealing fully with the fundamental aspects as well as describing many assays in detail. The longest chapter is that on antibiotics (157 pages) in which Dr. Kavanagh and eight collaborating authors have given a general survey of the field and described in detail the assay of twenty antibacterial and antifungal substances. The examples have been chosen to illustrate the principles of assaying and to indicate current practices; none of the methods described is necessarily the "best," but all, it is stated, are usable and no claim is made to have produced a compendium of recommended methods for all types of sample.

Vitamins also form the subject of a long chapter (154 pages), each section of which has been written by a specialist. The assays of biotin, folic acid, pantothenic acid and thiamine have received special attention; a section deals with methods for vitamin B_{12} and its congeners in which four different micro-organisms are used, and another describes the *Lactobacillus leichmanii* assay in detail. There is also a section on agar-plate assays with particular reference to pantothenic acid, inositol and pyridoxine.

British workers will be pleased to see the prominence given to large-plate methods, to which a whole chapter by J. S. Simpson has been devoted. As well as dealing fully with the general and statistical aspects of large-plate methods, their application to penicillin and riboflavin is discussed in detail. As Dr. Kavanagh himself points out, the Petri-plate method is inherently inefficient and the greater precision of the large-plate method makes it worthy of more consideration than it has hitherto received in the United States.

The theory of antibiotic inhibition zones is ably discussed by K. E. Cooper in a lengthy chapter. The apparent simplicity of these diffusion tests is deceptive and Dr. Cooper's mathematical treatment should have a salutary effect on those who still rely on absolute size of inhibition zones as sole indications of strain sensitivity.

In his chapter on elements of photometric assays Dr. Kavanagh discusses such questions as the physical factors affecting turbidity and the calibration of photometers. He also examines the importance of such factors as inoculum preparation, incubation temperature and the design and validity of assays. There is an interesting chapter by T. A. Haney, J. R. Gerke and J. F. Pagano on automation of microbiological assays; it will surprise many to learn how far some laboratories have proceeded in this direction.

Finally, mention must be made of G. D. Shockman's chapter on amino acids, which is probably the most extensive report on the subject for many years. In just over one hundred pages he has collected together much of the published work in this field during the last two decades and added a great deal based on his own experience.

For their success, microbiological assays depend on a better understanding of the processes involved and on meticulous attention to details of technique. This book should help from both these points of view and will surely be welcomed in any laboratory specialising in these methods. Its price, however, even by present day standards, is appallingly high, and it is to be hoped that this will not make it inaccessible to those—and they are many—who would enjoy and benefit from reading it.

S. A. PRICE

THE RADIOCHEMICAL MANUAL. Part 2. RADIOACTIVE CHEMICALS. Pp. vi + 78. Amersham, Bucks.; The Radiochemical Centre. (Also available from Her Majesty's Stationery Office.) 1963. Price 25s.

Part 1 of this manual dealt with physical data, presented largely in tabular form (see *Analyst*, 1962, **87**, 916). Part 2 is mainly in text form and deals with chemical aspects of radioisotopes and labelled compounds. Further parts of the manual are stated to be in preparation.

Part 2 begins with a section on sources of radioactive chemicals (21 pages), comprising four chapters on radiochemical manufacture, choice of target materials, separation of primary isotopes, and "a general approach to labelled compounds." A further section on characteristic features of radioactive chemicals (15 pages) consists of three chapters on the purity and instability of radioactive chemicals and on the behaviour of minute amounts and very dilute solutions. The third section (17 pages) has separate short chapters on labelled compounds of carbon, hydrogen, sulphur, phosphorus, iodine and chlorine. Definitions of terms used, 13 Tables, showing synthetic routes to labelled compounds of ^{14}C, ^{35}S, ^{32}P and ^{36}Cl, and an index complete the book.

Most of this book, therefore, provides information on the distinctive properties of radioactive chemicals and on the problems and limitations associated with their use. In addition, in the first section, the reader is quite properly acquainted with some of the problems facing the supplier of radioactive chemicals, *e.g.*, the need for ensuring radiochemical as well as chemical purity, and the stock-holding difficulties created by a variety of factors, such as short half-lives and decomposition by self-irradiation. The information provided must be considered authoritative, in view of the vast experience of the Radiochemical Centre in the preparation and handling of radioactive chemicals. As will be apparent from the length, the book aims to be a guide, rather than a textbook, and references to more detailed information are given at the end of each chapter.

The book is attractively produced and well worth buying.

H. J. CLULEY

SOME GENERAL PROBLEMS OF PAPER CHROMATOGRAPHY: RELATIONS BEWEEN PAPER CHROMATOGRAPHIC BEHAVIOUR AND CHEMICAL STRUCTURE: ATTEMPTS AT SYSTEMATIC ANALYSIS. Edited by I. M. HAIS and K. MACEK. Pp. 220. Prague: Publishing House of the Czechoslovak Academy of Sciences. 1962. Price Kcs. 22.50.

The book is a collection of 32 lectures (of which 3 are only summaries) given by well established authorities in the field of paper chromatography. These and the appropriate discussions have been excellently translated and are presented in a logical order as opposed to their chronological order; this has greatly enhanced the readability of the book.

The book, like the Symposium of which it is a record, is divided into the two sections indicated in the title. Each section is a useful account of modern ideas and techniques and covers aspects of use and interest to inorganic and organic analytical chemists as well as to those more interested in the fundamental aspects of chromatography.

Although all papers are of a high standard, the three introductory papers to the symposium and the two sections and their accompanying discussions are of the highest order, and are well worthy—as is the book in general—of a place in the library of any chemist dealing with paper chromatography.

For students of chromatography, this book is a "must," and its price puts it well within the range of all interested chemists. L. S. BARK

Publications Received

ZONE ELECTROPHORESIS IN BLOCKS AND COLUMNS. By H. BLOEMENDAL. Pp. viii + 219. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 40s.

THE STRUCTURE OF MOLECULES: AN INTRODUCTION TO MOLECULAR SPECTROSCOPY. By GORDON M. BARROW. Pp. xii + 156. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) $4.35.

HOW CHEMICAL REACTIONS OCCUR: AN INTRODUCTION TO CHEMICAL KINETICS AND REACTION MECHANISMS. By EDWARD L. KING. Pp. xii + 148. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) $4.35.

ELEMENTARY CHEMICAL THERMODYNAMICS. By BRUCE MAHAN. Pp. xii + 155. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) £4.35.

THE SHAPE OF CARBON COMPOUNDS: AN INTRODUCTION TO ORGANIC CHEMISTRY. By WERREN HERZ. Pp. xiv + 152. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $2.15; (cloth) $4.35.

TECHNIQUE OF ORGANIC CHEMISTRY. Volume VIII. Part II. INVESTIGATION OF RATES AND MECHANISMS OF REACTIONS. Edited by S. L. FRIESS, E. S. LEWIS and A. WEISSBERGER. Second Edition. Pp. xii + 703–1582 + Index (pp. 21). New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price £11.

ANALYTICAL CHEMISTRY OF THE ACTINIDE ELEMENTS. By ALFRED J. MOSES. Pp. x + 137. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 45s.

THE APPLICATION OF MATHEMATICAL STATISTICS TO CHEMICAL ANALYSIS. By V. V. Nalimov. Translated by PRESENJIT BASU. English Translation Editor: DR. M. WILLIAMS. Pp. x + 294. Oxford, London, Paris and Frankfurt: Pergamon Press. 1963. Price 84s.

THE CHEMISTRY OF NATURAL PRODUCTS. Special and Introductory Lectures presented at the Second International Symposium on the Chemistry of Natural Products, Prague, Czechoslovakia. Pp. vi + 215. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 60s.

Reprinted from Pure and Applied Chemistry, *Vol.* 6, *No.* 4. *Pp.* 493–717.

A DICTIONARY OF SCIENTIFIC UNITS: INCLUDING DIMENSIONLESS NUMBERS AND SCALES. By H. G. JERRARD, B.Sc., Ph.D., F.Inst.P., and D. B. MCNEILL, T.D., M.Sc., PH.D., F.INST.P. Pp. 197. London: Chapman & Hall. 1963. Price 21s.

MOLECULAR VIB-ROTORS: THE THEORY AND INTERPRETATION OF HIGH RESOLUTION INFRARED SPECTRA. By HARRY C. ALLEN, JUN. and PAUL C. CROSS. Pp. viii + 324. New York and London: John Wiley & Sons, Inc. 1963. Price 105s.

THE QUANTUM THEORY OF MOLECULAR ELECTRONIC STRUCTURE: A lecture-note and reprint volume. By ROBERT G. PARR. Pp. xvi + 510. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $7.65; (cloth) $11.00.

THERMODYNAMICS OF SMALL SYSTEMS. Part I. By TERRELL L. HILL. Pp. xii + 171. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $6.55; (cloth) $9.90.

PHYSICAL INORGANIC CHEMISTRY. By M. J. SIENKO and R. A. PLANE. Pp. x + 166. New York and Amsterdam: W. A. Benjamin Inc. 1963. Price (paper) $4.35; (cloth) $7.70.

TREATISE ON ANALYTICAL CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING with the assistance of ERNEST B. SANDELL. Part II. ANALYTICAL CHEMISTRY OF THE ELEMENTS. Volume VIII. Pp. xxii + 556. New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price 150s.

KOMPLEXOMETRIE. Part 1. PRINZIPIEN UND GRUNDBESTIMMUNGEN. By Dr. rer nat. RUDOLF PŘIBIL. Second Edition. Pp. 179. Leipzig: Veb Deutscher Verlag für Grundstoffindustrie. 1963. Price DM 13.80.

ANALYTISCHE CHEMIE. Part I. MASSANALYSE. Part II. GEWICHTSANALYSE. By DIPL.-CHEM. HERBERT RODICKER. Second Edition. Pp. 166; 141. Leipzig: Veb Deutscher Verlag für Grundstoffindustrie. 1961; 1962. Price (Part I) DM 7.80; (Part II) DM 7.50.

NUCLEOPHILIC SUBSTITUTION AT A SATURATED CARBON ATOM. By C. A. BUNTON, Ph.D. Pp. x + 172. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 40s.

Volume 1 *of a series of Monographs entitled "Reaction Mechanisms in Organic Chemistry" Edited by Prof. E. D. Hughes, F.R.S.*

HANDBOOK OF LABORATORY DISTILLATION. By ERICH KRELL. Edited by E. C. LUMB. Translated from the Second German Edition by C. G. VERVER. Pp. x + 561. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 100s.

PRACTICAL MATHEMATICS FOR CHEMISTS. By F. H. C. KELLY, D.Sc., M.Sc., A.Melb.T.C., F.R.A.C.I., A.Aust.I.M.M. Pp. viii + 148. London: Butterworth & Co. (Publishers) Ltd. 1963. Price 20s.

Reports of the Analytical Methods Committee: Reprints

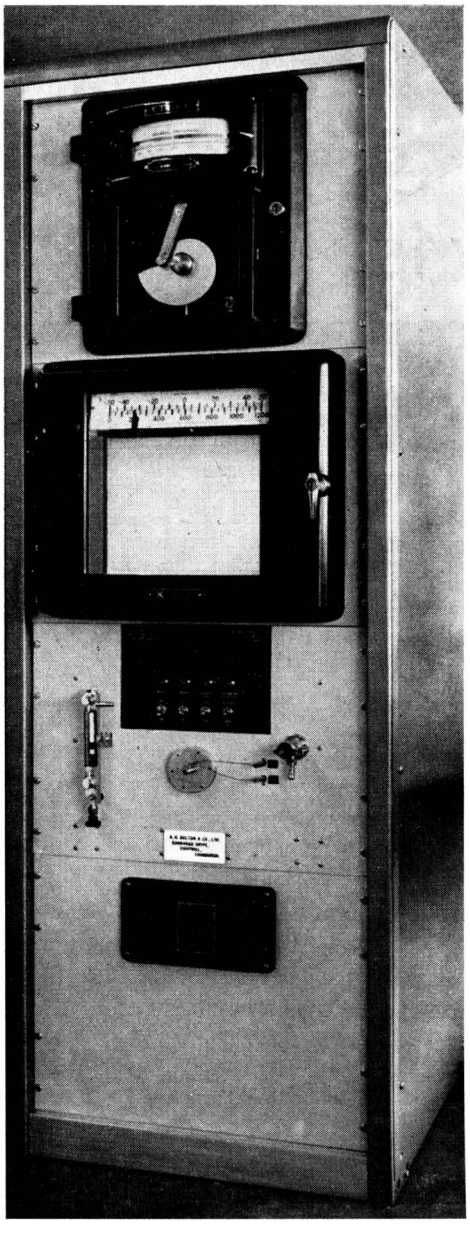
Nitrogen Factors for Beef, and The Semi-micro Determination of Chlorine in Agricultural Technical Organic Chemicals and their Formulations

THE Report prepared by the Meat Products Sub-Committee, "Nitrogen Factors for Beef," reprinted from *The Analyst*, June 1963, 88, 422–423, and the Report prepared by the Chlorine in Organic Compounds Sub-Committee, "The Semi-micro determination of Chlorine in Agricultural Technical Organic Chemicals and their Formulations," reprinted from *The Analyst*, June 1963, 88, 415–421, are now available from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1. Price to members 1s. 6d. each; to non-members, 2s. 6d. each.

Reports of the Analytical Methods Committee are only available from the Secretary (not through Trade Agents) and remittances, made out to the Society for Analytical Chemistry, MUST accompany orders.

Errata

JULY (1963) ISSUE p. 546 line 2. For "differences" read "difference." p. 547, Fig. 3. The equations "Y=0·272–0·935x" should read "Y=0·272+0·935x."



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Volume 88. No. 1050, Pages 659-742 September, 1963

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Printed and Published for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. B. Attrill, 14 Belgrave Square, London, S.W.1.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47 Gresham Street, London, E.C.2.

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