[The Telephone number of the Office of the Society and "The Analyst" is now Mansion House 6608.]

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THE ANALYST

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CITY OF LIVERPOOL

Appointment of City Analyst

The Council of the City of Liverpool invite applications from qualified persons for the appointment of City Analyst at a salary of £1,750 per annum, increasing to £2,000 per annum after 12 months' service.

Applicants, whose age must not exceed 50 years, must possess the qualifications prescribed in the Public Analyst's Regulations, 1939, and the Fertilisers and Feeding Stuffs Regulations, 1932.

The duties of the person appointed will include those of Public Analyst and Agricultural Analyst for the City. As the Council have arrangements with certain other Local Authorities, he will also be required to act in a similar capacity for those Authorities, without additional remuneration.

The appointment will be subject to the provisions of the Local Government Superannuation Act, 1937, and the Standing Orders of the City Council, and will be determinable by three calendar months' notice on either side.

Forms of Application and particulars of the Duties and Conditions of Appointment may be obtained from me, and applications, accompanied by copies of three recent testimonials, must be addressed to me, endorsed "City Analyst," and received on or before Monday, the 6th day of May, 1946.

Candidates serving in H.M. Forces abroad need not complete the official form of application, but may submit direct applications within the date specified, giving particulars of age, qualifications and experience, and the number of their Release Groups, together with probable date of release.

Canvassing of members of the City Council, either directly or indirectly, will be a disqualification.

W. H. BAINES,
Town clerk.

MUNICIPAL BUILDINGS,
DALE STREET,
LIVERPOOL, 2.

University College of Swansea

SUMMER SCHOOL

IN

Optical-Crystallographic Methods

SEPTEMBER 2—13, 1946

The Course will cover the theory and practice of the use of the polarising microscope in chemistry and other branches of science and technology. The special requirements of individual students will be taken into account as far as possible.

Further particulars may be obtained from the Registrar, University College, Singleton Park, Swansea.

GOVERNMENT ANALYST of Crown Colony retiring at age of 50 requires post in England. Highest qualifications, including F.R.I.C., Branch E. Wide experience all branches analytical work. Please write Box 472, ANALYST, 47, Gresham Street, London, E.C.2.

Geological Survey Department: Southern Rhodesia

VACANCY FOR ANALYTICAL CHEMIST

Applications are invited for appointment as Analytical Chemist in the Geological Survey Department of the Government of Southern Rhodesia.

The applicant must possess an Honours Degree of a recognised university and, in addition, must have had experience in the analysis of rocks, minerals, ores, etc., and have a knowledge of metallurgy. Experience of spectrographic work will be an advantage.

The appointment will be for an initial probationary period of two years, and, subject to satisfactory service, the successful candidate will be eligible to be placed on the Fixed Establishment of the Government Service, and will also be subject to the Rules and Regulations of the Public Service. The salary scale will be £650 × £25 to £850 per annum, and the commencing rate will be according to qualifications and previous experience. Cost of living Allowance will be payable.

The successful candidate will be required to pass a Medical Examination by a Government Medical Officer.

Applications, stating age, qualifications, experience, nationality and marital condition accompanied by testimonials, should be forwarded direct to:

The Director,
Southern Rhodesia Geological Survey,
P.O. Box 366,
Salisbury, S. Rhodesia,

from whom further particulars may be obtained if required.

Preference will be given to an ex-serviceman.

Canvassing in any form will disqualify applicants.

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE Annual General Meeting of the Society was held at 5.15 p.m. on Friday, March 8th, 1946, at the Chemical Society's Rooms, Burlington House, London, W.1. The chair was taken by the President, Dr. G. W. Monier-Williams. The Financial Statement for 1945 was presented by the Hon. Treasurer and approved and the Auditors for 1946 were appointed. The Report of the Council for the year ending March, 1946, was presented by the Hon. Secretary and adopted. The following were elected Officers and Council for the coming year:

President—G. W. Monier-Williams.

Past Presidents serving on the Council—F. W. F. Arnaud, Bernard Dyer, Edward Hinks, E. B. Hughes, G. Roche Lynch, S. Ernest Melling, W. H. Roberts.

Vice-Presidents—R. C. Chirnside, (Mrs.) J. W. Matthews, E. Voelcker and, *ex-officio*, H. M. Mason (Chairman, North of England Section) and J. B. McKean (Chairman, Scottish Section).

Hon. Treasurer—George Taylor.

Hon. Secretary—Lewis Eynon.

Other Members of Council—C. A. Adams, A. L. Bacharach, R. Belcher, H. Childs, A. A. D. Comrie, H. E. Cox, W. F. Elvidge, J. Haslam, D. W. Kent-Jones, A. D. Mitchell, N. Strafford, E. C. Wood and, *ex officio*, Arnold Lees (Hon. Secretary, North of England Section) and R. S. Watson (Hon. Secretary, Scottish Section).

The Annual General Meeting was followed at 6 p.m. by a Lecture by Dr. J. H. Quastel, F.R.S., on "Biochemistry of Soil."

NEW MEMBERS OF THE SOCIETY

John Allen; Professor Hans Baggesgaard-Rasmussen, Ph.D.; Charles Stanley Bass, B.Sc. (Lond.); Dennis Ernest Hawkins, B.Sc., A.R.C.S., A.R.I.C.; Kenneth Hillman, B.Sc. (Lond.); Harold Wright Hodgson, A.R.I.C.; William Hutchinson, A.R.I.C.; Arthur George Jones, B.Sc. (Lond.), F.R.I.C.; Walter George Leach, B.Sc. (Lond.), F.R.I.C.; Reginald Frederick Looney, A.R.I.C.; Donald Vivian Richmond, B.Sc. (Lond.); Wilfred Parr Robinson, A.R.I.C.

DEATHS

WE record with regret the deaths of the following members:

Herbert Firth — John Francis Liverseege — George Henry Warburton

Annual Report of Council: March, 1946

THE roll of the Society numbers 1270, an increase of 73 over the membership a year ago.

The Council regrets to have to record the death of the following members:

Sir G. C. Clayton	H. Firth	F. H. Newington
J. Kear Colwell	Sir M. O. Forster	A. P. Platt
C. E. Corfield	E. Gabriel Jones	R. W. Richardson
John Evans	A. Lucas	J. F. Tocher

Sir Christopher Clayton, who died in his 77th year, was an Honorary Member of the Society. On leaving University College, Liverpool, in 1896, he joined the United Alkali Co., Widnes, was elected to the Board of that Company in 1907 and subsequently became a Director of Imperial Chemical Industries, Ltd. He represented Widnes in Parliament from 1922 to 1929 and the Wirral Division from 1931 to 1935. He was President of the Institute of Chemistry from 1930 to 1933, and was awarded the honour of Knighthood in 1933.

Colwell, who died at the age of 79, had been a member of the Society for 52 years. He studied at the Royal College of Science and for a year was assistant to Sir Edward Frankland. He subsequently became assistant to A. Wynter Blyth and later Public Analyst for Bedfordshire, Bedford, and the Metropolitan Boroughs of Finsbury, Holborn and St. Pancras.

Corfield, who died in his 54th year, was elected a member of the Society in 1927. He studied at King's College, London, and at the School of Pharmacy, where he subsequently served on the staff and became head of the chemistry department. In 1925 he joined the analytical and consulting practice of Harrison and Self and later became head of the firm.

John Evans, who died in his 70th year, was elected a member of the Society in 1906. In 1901 he became assistant to A. H. Allen, in 1909 a partner and in 1919 sole principal of the firm. He held a number of appointments as Public Analyst, Agricultural Analyst, Gas Examiner and Water Examiner. He served for several periods on the Council of the Society and was President from 1934 to 1936. He was appointed High Sheriff for the County of Cardigan for 1926. (Obituary, ANALYST, 1945, **70**, 440.)

Sir Martin Forster, who died at the age of 72, was elected a member of the Society in 1915. He had a distinguished record in the fields both of academic and applied chemistry. He was elected a Fellow of the Royal Society in 1905. From 1922 to 1933 he was Director of the Indian Institute of Science and in the latter year he received the honour of Knighthood.

Gabriel Jones, who died at the age of 62, was elected a member of the Society in 1912. He studied at University College, Liverpool, and obtained the B.Sc. degree with honours in chemistry. After serving as assistant to Professor Campbell Brown and W. Collingwood Williams, Public Analysts, he worked in the City Analyst's Laboratories, Liverpool, of which city he became Deputy City Analyst.

Lucas, who died at the age of 76, was elected a member of the Society in 1899. At the outset of his professional career he worked in the Government Laboratory and subsequently joined the Egyptian Government Service in which he continued until his retirement. He was awarded the O.B.E. for his services during the war of 1914–18.

Newington, who died at the age of 56, was elected a member of the Society in 1930. Most of his professional career was spent in the service of the Admiralty Chemist's Department at Portsmouth, where he began as a junior analyst in 1907, and was appointed Assistant Admiralty Chemist in 1935, Principal Chemist in 1940 and Superintendent Scientist in 1944. In 1937 he received the M.B.E.

Platt, who died at the age of 30, was only recently elected a member of the Society. He studied at Liverpool University and obtained the B.Sc. degree with honours in Biochemistry in 1936. In 1940 he was appointed Scientific Assistant in the Scientific Adviser's Division of the Ministry of Food, where he organised the anti-gas protection of food stocks and was Chief Instructor of anti-gas food personnel.

Tocher, who died at the age of 81, was elected a member of the Society in 1913. He served on the Council for three periods as an Ordinary Member, and as Vice-President in 1937–38. He began an analytical practice in 1886 at Peterhead and later in Aberdeen, and for many years was Public Analyst and Official Agricultural Analyst for a number of Scottish Authorities. He was particularly interested in statistics and published many papers on this and other subjects, and his work was recognised by the award of the Honorary degree of LL.D. by Aberdeen University.

ORDINARY MEETINGS—Five meetings of the Society were held during the year and the following papers were communicated:

"The Freezing Point of Milk." By F. J. MacDonald.

"The Electrometric Determination of Ascorbic Acid." By H. Liebmann, Ph.D., and A. D. Ayres, B.Sc.

"Magnetic Stirring in the Electro-deposition of Metals." By H. W. Webb.

"A Rapid and Simple Method for the Determination of Calcium in Presence of Strontium and Barium." By G. W. Osborn.

"Lead Printing of Ferrous and Non-ferrous Metals." By W. B. Wragge.

"Reaction of Diazotised *p*-Nitraniline with Phenols: Detection of Tricresyl Phosphate in Edible Oil." By E. Collins, M.A., F.R.I.C.

"A Simple Apparatus for Handling Standard Solutions of Bromine in Potassium Bromide." By A. J. Henry, B.Sc., Ph.D.

"The Theory of Certain Analytical Procedures, with Special Reference to Microbiological Assays." By E. C. Wood, B.Sc., A.R.C.S., F.R.I.C.

"The Determination of Carotene and Vitamin A in Butter and Margarine." By T. W. Goodwin, M.Sc., A.R.I.C., and R. A. Morton, Ph.D., D.Sc., F.R.I.C.

"A Photoelectric Method of Assaying Vitamin A in Margarine." By J. L. Bowen, N. T. Gridgeman, B.Sc., and G. T. Longman, B.Sc., F.R.I.C.

"Notes on the Selective Oxidation of Vinegar." By F. Lyne, B.Sc., F.R.I.C., and T. McLachlan, D.C.M., A.C.G.F.C., F.R.I.C.

"Application of the Intermittant A.C. Arc Technique of Spectrographic Analysis." By J. A. C. McClelland, B.Sc., Ph.D., A.R.I.C.

"The Determination of Auxins in Soils; including a Note on Synthetic Growth Substances." By J. Hubert Hamence, M.Sc., Ph.D., F.R.I.C.

The December Meeting was a Joint Meeting with the Food Group of the Society of Chemical Industry. The subject was "New Routine Tests and their Application in Modern Food Industry," and the following papers were read and discussed:

"New Routine Tests in Examining Wheaten Products." By A. J. Amos, B.Sc., Ph.D., F.R.I.C.

"New Routine Tests in the Dairy Industry." By J. G. Davis, Ph.D., D.Sc., F.R.I.C.

"The Analysis of Cured Meats and 'Curing Brines'." By J. C. Morpeth, B.Sc., A.R.I.C.

"Physical and Chemical Methods for Moisture Determination." By D. W. Grover, B.Sc., F.R.I.C.

EXTRAORDINARY GENERAL MEETING—At an Extraordinary General Meeting held on October 3rd, 1945, the following Special Resolution, moved by the President and seconded by the Honorary Treasurer, was carried unanimously.

> "That Article 10 of the Articles of Association of the Society be amended by deleting therefrom the following words:
>
> > 'Provided that no entrance fee shall be payable by any member who, being eligible for membership of, is nominated by, any area Section of the Society and pays the appropriate subscription for membership of that Section.' "

GROUPS FOR SPECIAL SUBJECTS—During the past year a third Group—The Biological Methods Group—was formed. The Inaugural Meeting of the Group was held on October 17th. Mr. A. L. Bacharach was elected Chairman and Mr. E. C. Wood, Honorary Secretary of the Group, and an address, "Biological Assay and Chemical Analysis," was given by Mr. Bacharach. The Group now numbers 84 members.

MICROCHEMISTRY GROUP—Since the Inaugural Meeting of the Group on October 4th, 1944, three further meetings have been held and the following papers read:

"The Development of Micro Methods in Analytical Chemistry." By Janet W. Matthews.

"A Scheme for the Photometric Determination of Minute Amounts of Arsenic, Copper, Lead, Zinc, Iron and Certain Other Metals in Organic Compounds, *e.g.*, Medicinals." By N. Strafford, P. F. Wyatt and F. C. Kershaw.

"Microchemistry and its Forensic Applications." By C. G. Daubney.

"Recent Advances in the Application of Micro-analysis to Medical Chemistry." By E. J. King.

"Physical Methods used in Microchemistry." By Cecil L. Wilson.

"The Determination of Trace Amounts of Sulphur Dioxide with Special Reference to the Determination of Sulphur in Ferro-alloys." By G. Ingram.

"Micro-methods used in the Analysis of Cotton." By Miss M. Corner.

"Quantitative Inorganic Micro-analysis for University Students." By Miss Christina C. Miller.

"A Review of Methods for Micro-filtration." By G. H. Wyatt.

"Some Aspects of the Micro-chemical Analysis of Ferrous Alloys." By C. Whalley.

The number of Group members is now 169, an increase of 49 over the membership at the time of the Inaugural Meeting.

The Committee has met six times during the year. At its first meeting it was decided that three meetings of the Group should be held each year, including the annual general meeting. The latter is to be held in London during January of each year and the other two meetings at suitable centres in the provinces. In pursuance of this policy two provincial meetings have been held during 1945 at Manchester and Newcastle. The provincial meetings for 1946 are to be held at Cardiff and Birmingham. As far as possible all provincial meetings are to be held in conjunction with the Local Sections of the Royal Institute of Chemistry, or other local Societies desiring to co-operate.

At the request of the Committee, Dr. Cecil L. Wilson has taken a census of all educational institutions in the country to ascertain the facilities available for (*a*) the teaching of general analytical chemistry and (*b*) the teaching of microchemical methods. An account of this

census has been given in *Nature* and, when available, reprints will be circulated to all members of the Group.

The Committee made two distinct requests to the Council. Firstly, that it should take the steps best calculated to lead to the institution of Chairs of Analytical Chemistry and to increase the number of Lectureships in Analytical Chemistry; and, secondly, that it should approach the Royal Institute of Chemistry, asking this body to encourage, by means of its examinations, the study of microchemical methods. As a result the Council has approached the Royal Institute of Chemistry, which now has both matters under consideration.

The Committee has also arranged for a census of industrial laboratories using microchemical methods. Dr. Cecil L. Wilson has kindly agreed to undertake this on behalf of the Committee and a questionnaire will be submitted to all Group members when the reprints of the *Nature* article are circulated.

The draft rules were approved at an ordinary meeting of the Group on January 23rd, 1945. The final form has yet to be approved by the Council since two new Groups have been formed and it is desirable that a standard form be agreed on between officers of the three Groups.

In conclusion the Hon. Secretary wishes to express his appreciation to the officers and members of the Committee for their support and co-operation during the year.

PHYSICAL METHODS GROUP—This Group was the second to be formed to deal with special fields of activity in which members of the Society are particularly interested. The Microchemistry Group, the first to be formed, was, however, built up on the existing foundation of the Microchemical Club, whilst the Physical Methods Group had to start entirely *de novo*. The present report can only be a provisional sketch of a rapidly developing organisation.

The Inaugural Meeting of the Group was held on February 7th, 1945, with the President of the Society in the chair. Mr. R. C. Chirnside was elected Chairman, and gave a stimulating address on "Physics and the Analyst," surveying the more important physical methods which are being employed by the analytical chemist.

At the meeting on May 3rd Dr. H. W. Thompson, of the University of Oxford, read a paper on "Infra-red Spectrography in Relation to Chemical Analysis," in which he described important war-time developments in this rapidly advancing field.

On October 20th the Group held a Joint Meeting with the North of England Section in Manchester on the subject of "Polarographic Analysis." Dr. W. Cule Davies gave "An Outline of the General Principles," Dr. J. E. Page dealt with biochemical applications, Mr. A. S. Nickelson dealt with applications to Aluminium, Magnesium and Zinc, and Mr. R. H. Jones dealt with applications to Selenium, Nickel and Cobalt. The Group is greatly indebted to the Committee of the North of England Section for making the local arrangements for this meeting and for its cordial hospitality to members coming from a distance.

All these meetings have been well attended and have provoked interesting discussions.

At the time of its inauguration the Group numbered 104 members; by April this had increased to 118, and in September it was 128. As about three-quarters of the number are within reasonable distance of London, most of the meetings are being held at present in London. The meeting in Manchester was, however, very well supported, and it is hoped to have an equally successful meeting in Scotland early next year.

A questionnaire circulated amongst members and completed by the majority of them showed that the physical methods they most frequently employ are colorimetric (visual or photoelectric). However, electrometric, chromatographic, fluorimetric, spectrographic and polarographic methods are being largely used and a certain proportion of members are using the more specialised techniques of infra-red spectrography, X-ray analysis and electrophoresis. The Committee has also considered and reported on various technical matters such as suitable definitions in spectroscopy and the application of crystallographic methods, with particular reference to the Barker Index, in the publication of which the Group has been invited by I.C.I. to co-operate with the Institute of Physics and other bodies.

SOCIETY'S OFFICES AND STAFF—The Society has acquired the lease of a room at 7/8, Idol Lane, E.C.3, as Registered Office, for the accommodation of the Editor and Assistant Secretary. Miss D. V. Wilson was appointed Assistant Secretary as from October 1st, 1945.

EDITORSHIP OF "THE ANALYST"—Dr. C. A. Mitchell retired on September 30th from Editorship of THE ANALYST, an appointment that he had held for 25 years. The Council has appointed Dr. Mitchell Honorary Librarian, and desires to place on record its high

appreciation of the distinguished service to the Society rendered by Dr. Mitchell in enhancing the value and reputation of THE ANALYST.

Mr. J. H. Lane, Assistant Editor since 1936, was appointed Editor in succession to Dr. Mitchell.

THE ANALYST—In the past year there has been some easement in the restrictions on paper supply for THE ANALYST, and in spite of continually increasing circulation it has been possible to increase the size of the journal. The total number of pages in the 1945 volume was 486, about 100 more than in the previous volume. That corresponds to an average of 40·5 pages per issue, and the year 1946 is starting with about 48 pages per issue. The extra space is urgently needed, for the number of papers and notes submitted for publication is continuously increasing. The number of papers published in 1945 was 56, compared with 40 in 1944, and the number of notes 45, compared with 39 in 1944. In view of the prospect of much greater expansion of THE ANALYST in the near future the Publication Committee is considering what modifications may be desirable in its constitution and mode of working to enable it to cope efficiently with the work of the journal.

HON. TREASURER'S REPORT—The Hon. Treasurer reports that despite the increase in expenditure due to the increase in the Society's activities, the financial position continues to be satisfactory.

ANALYTICAL METHODS COMMITTEE—With the end of the war some of the sub-committees have already resumed their activities, and others are expected to do so shortly. Two Reports from the Committee have been published during the year, *viz.*

"Determination of Total Solids in Fresh Liquid Milk." ANALYST, 1945, p. 105.

"Determination of the Solubility of Ceylon Citronella Oil." ANALYST, 1945, p. 442.

also an addendum to the report on "Determination of Small Amounts of Fluorine in Foods." ANALYST, 1945, p. 442.

These are all available separately as reprints.

The Poisons Sub-Committee has resumed its work.

The Metallic Impurities in Food Colours Sub-Committee has been reconstituted, under the Chairmanship of Dr. A. D. Mitchell, with wider terms of reference to include determination of small amounts of metallic impurities in foodstuffs generally, and two new sub-committees have been appointed, one on the Estimation of Vitamins, with Dr. E. B. Hughes as Chairman, and the other at the request of the British Standards Institution, on the Standardisation of Methods for the Examination of Gum Tragacanth, with Dr. N. Evers as Chairman.

PRIVATE WORK BY PROFESSORS AND STAFFS AT UNIVERSITIES, ETC.—At the request of the Joint Council of Professional Scientists the Council submitted a memorandum on the conditions which should govern the undertaking of private work by Professors and staffs at Universities, etc.

A memorandum, approved by the Royal Institute of Chemistry and the Institute of Physics and subsequently issued by the Joint Council, is in close accord with the memorandum submitted by the Council of the Society.

CONDITIONS OF APPOINTMENT OF PUBLIC ANALYSTS—The Council has issued to Public Analyst members of the Society two memoranda: (1) Remuneration of Part-time Public Analysts, (2) Salaries and Terms of Appointment of Whole-time Public Analysts, and is co-operating with the Royal Institute of Chemistry as to further action.

NORTH OF ENGLAND SECTION—Four meetings have been held during the year, including a Joint Meeting with the Physical Methods Group. The following papers have been read:

"A Note on the Determination of the Specific Gravity of Sterilised Milk." By Dr. G. H. Walker, B.Sc., F.R.I.C.

"Our Society." By W. Gordon Carey, F.R.I.C.

"The Peroxide Value of Oils and Fats." By H. Weatherall, F.R.I.C., and C. B. Stuffins, A.R.I.C.

"Bread." By S. W. Butterworth, B.Sc., F.R.I.C.

At the Joint Meeting the papers given were:

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

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

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

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

There have been good attendances at the meetings. The Section now numbers 205, an increase of 18 on the previous year.

The Hon. Secretary wishes to express his appreciation of the loyal support and assistance accorded to him by the Chairman and members of the Committee during the year.

SCOTTISH SECTION—Three meetings were held in the course of the year at which the following papers were presented and discussed:

"Notes on Plumbo-solvent Waters." By M. J. Robb, B.Sc., F.R.I.C.

"Some Observations on the Micro-determination of Iron." By W. N. M. Ramsay, B.Sc., F.R.I.C.

"Experimental Methods Used in the Study of Gastric Secretions in Certain Animals." By F. J. Elliot, B.Sc., F.R.I.C.

"Factors Affecting the Maltose Figure of Wheat and Flour." By J. Sword, M.A., B.Sc., Ph.D., F.R.I.C.

"Brown Bread." By H. C. Moir, B.Sc., F.R.I.C.

The Committee record with regret the death of Dr. J. F. Tocher, one of the founder members of the Scottish Section.

Five new members joined the Parent Society through the Section and three members resigned on taking up residence outwith the Scottish Area, leaving a total membership of sixty-four.

BRITISH STANDARDS INSTITUTION—Dr. E. B. Hughes was appointed representative of the Society on the Chemical Divisional Council. Mr. K. A. Williams was appointed representative of the Society on the Panel for Pipettes and Burettes of the Chemical Glassware Committee.

Dr. E. B. Hughes was appointed representative of the Society on the Committee dealing with "Methods of Test for Gum Tragacanth." Mr. W. H. Simmons was appointed representative of the Society on the Committee dealing with Vegetable Oils.

PARLIAMENTARY AND SCIENTIFIC COMMITTEE—Dr. H. E. Cox continues to represent the Society on the Executive Committee of the Parliamentary and Scientific Committee.

INSTITUTE OF PETROLEUM—Mr. W. Samuel was appointed representative of the Society on a Panel of the Lubricants Sub-Committee.

CHEMICAL SOCIETY JOINT LIBRARY COMMITTEE—Mr. J. H. Lane, Editor of THE ANALYST, was appointed representative of the Society.

The Office of the Society is available for the meetings of small Committees. The Council again expresses its thanks to organisations and to members of the Society for the accommodation of the larger Committees.

G. W. MONIER-WILLIAMS, *President*
LEWIS EYNON, *Hon. Secretary*

The Determination of Theobromine in Cocoa Residues—An Examination of the Wadsworth Method

BY J. KAY AND P. J. C. HAYWOOD

SOME years ago a method was required for the routine determination of theobromine in cocoa residues which had been partly or almost entirely defatted. The method of the late R. V. Wadsworth[1] was chosen, partly on the score of convenience and partly because of the good opinion of the method suggested by Whymper's[2] observations on the available procedures. In this method a partly dried mixture of water, magnesia and the ground sample is subjected to a series of four extractions with boiling tetrachloroethane, and after removal of the bulk of the extracting solvent by distillation the residual extract is digested and washed with 0·720 sp.gr. ether before being dried and weighed as theobromine. A correction is applied to allow for the solubility of theobromine in ether.

Since Wadsworth's paper appeared, several observers have given attention to the matter, as indicated by the following résumé. Goryainowa[3] recommends a procedure which seems to combine the essentials of the methods of Kunze[4] and of Dekker,[5] while avoiding the precipitation of the phosphomolybdate complex involved in Kunze's method. Moir and

Hinks[6] describe a process involving extraction of the alkaloids with aqueous ethanol and magnesia, evaporation of the filtered extract to remove ethanol, clarification with zinc ferrocyanide, filtration, extraction with chloroform, evaporation of the chloroform extract and determination of the total nitrogen in the residue, after addition of sucrose, by Kjeldahl digestion, the total alkaloids being calculated from the nitrogen by use of an appropriate factor. Their method of determining the nitrogen content of the extracted alkaloids is of considerable interest, in view of the fact that Wadsworth's paper tends to prejudice other workers against the use of the Kjeldahl method as a means of assaying the purity of the extracted theobromine. Parkes and Parkes[7] suggest the use of a mixture of phenol and chloroform in place of the chloroform used in Moir and Hinks's method. The use of phenol - chloroform mixture in connection with the extraction of cocoa alkaloids was first suggested by Maupy.[8] Macdonald[9] employs Soxhlet extraction of the damp magnesia - cocoa mass prepared as in the Wadsworth method, for three hours with tetrachloroethane, and Humphries[10] recommends this procedure in preference to the four-stage extraction with tetrachloroethane involved in the original Wadsworth method. Humphries[11] also puts forward a method, based on that of Wadsworth, in which the damp magnesia - cocoa mass is extracted in a Soxhlet apparatus for twenty hours with chloroform, the crude extract is washed free from fat with light petroleum and the caffeine is removed by leaching with benzene.

STUDY OF THE WADSWORTH METHOD—Four samples of cocoa residues were examined for theobromine content by the Wadsworth method and the same samples were submitted to a referee analyst; the results are given in Table I.

TABLE I

Sample of cocoa residue	Theobromine, % by Wadsworth method	
	Authors	Referee analyst
No. 16A	3·30; 3·27	3·2
,, 16B	3·13; 3·05	3·0
,, 10	2·40; 2·26	2·3
,, 20	3·24; 3·25	2·7

The extracts obtained by the authors were bulked together and examined, as follows:

(1) Solubility in 0·720 sp.gr. ether at 20° C.: 0·005 g. per 100 ml., sufficiently near to the published figure of 0·004 g. per 100 ml. (Beilstein) to confirm that the extracts were substantially free from caffeine.
(2) Water insoluble matter: 0·6%.
(3) Ash: 2·2% (MgO).
(4) Assay: 80·0% by the modified Kjeldahl procedure described on p. 164. This figure for the purity of the extracted theobromine is in decided contrast to that reported by Wadsworth (99·5% to 99·9%), using the methods of analysis of the silver salt and Dumas nitrogen determination. When the figures given by Wadsworth for the insoluble matter and ash in the extracts he obtained are considered, it is obvious that the assay figures he returns are slightly on the high side.

Since washing with light petroleum, to remove fat, was found not to affect the yield of extract obtained by the Wadsworth method, the examination reported above proves that, in the ordinary way, some water soluble material tends to find its way into the extract, in spite of rigorous adherence to Wadsworth's instructions.

Sample No. 20 (Table I) was subjected to further examination: two theobromine estimations were carried out under the original conditions and two after drying the wet cocoa residue - magnesia mass to what appeared to be a dangerously dry condition. In all the determinations the extracts were assayed by the modified Kjeldahl method. The results of this investigation are given in Table II.

TABLE II

Conditions of drying of cocoa residue-MgO-water mixture		Yield of extract, % of sample	N % in extract, (modified Kjeldahl)	Theobromine, % of sample, corrected by N assay
As suggested by Wadsworth and interpreted by authors.	(*a*)	2·78	27·4	2·44
	(*b*)	3·29	22·1	2·34
Drying to what appeared a dangerously dry condition.	(*a*)	2·50	29·5	2·37
	(*b*)	2·65	28·1	2·39
Average of values		2·81	27·3	2·39

The results given in Table II show that much depends upon personal opinion as to the degree of hydration of the cocoa - magnesia mixture, and it is not to be expected that the analyst would be able to decide exactly to what stage the drying should be taken to yield a reasonably pure extract. The results also show, however, that quite consistent results can be obtained if the yield of extract is corrected by the nitrogen assay, using the modified Kjeldahl procedure (see below).

Over fifty samples of cocoa residues from different sources were examined by the Wadsworth - Kjeldahl method, each test being run in duplicate and, although on occasions rather different yields of total extract were obtained from the same sample, the widest difference in a pair of values corrected by nitrogen assay was 0·10% of theobromine. Table III includes a fairly typical selection of the results.

TABLE III

Description of cocoa residue	Cocoa fat, %	Theobromine, %		
		Wadsworth (referee analyst)	Wadsworth (authors)	Wadsworth-Kjeldahl (authors)
No. 20 Cocoa residue	0·6	—	2·72	2·29
Cocoa expeller cake	9·5	—	2·83	2·56
,, ,, ,, ...	15·5	—	2·32	1·98
,, residue	7·9	—	3·10	2·68
,, ,, RMS	2·4	—	2·91	2·31
Cocoa expeller cake	8·0	2·73	2·83	2·55
No. 10 Cocoa meal	0·9	2·14	2·17	1·98
,, 20 Cocoa residue	0·5	2·50	2·88	2·38
Solvent extracted rock "X" smalls ..	1·9	2·76	2·57	2·46
Cocoa residue RE	2·31	3·28	2·59	2·36

Modified Kjeldahl Method—The procedure followed by us in the assay of the extracts obtained by the Wadsworth method is as follows.

Weigh a piece of Whatman No. 40 filter paper (about 0·4 g.) accurately, and weigh on to this paper 0·08 to 0·1 g. of the extract obtained by the Wadsworth procedure. Fold the extract in the paper and drop it into a long-necked round-bottomed flask of 500-ml. capacity containing 20 ml. of conc. H_2SO_4 and 0·1 g. of copper wire and digest for 5 to 7 hr. at the boiling point, *i.e.*, until a clear, colourless liquor remains. Allow to cool and proceed as in the conventional Kjeldahl method, distilling the ammonia into 50 ml. of $N/10$ hydrochloric acid.

On a sample of theobromine of reasonable purity a figure of 30·9% of nitrogen, equivalent to 99·0% of theobromine, was obtained by this method.

A MODIFICATION OF THE WADSWORTH METHOD—The busy analyst may not like the complication of the procedure involved by the addition of a Kjeldahl determination to the original method, and it was found that a trifling modification of the original Wadsworth method yielded an extract of a fairly high and consistent standard of purity. All that is required is to distil the combined tetrachloroethane extracts, obtained in the usual manner, from a 1-litre flask until the volume is reduced to about 100 ml.; the nearly boiling residual liquor is then filtered through an 11-cm. Whatman No. 54 filter paper into a 250-ml. round-bottomed flask and the 1-litre flask and the filter are washed with 100 ml. of nearly boiling, water-free, tetrachloroethane. The filtrate and washings in the 250-ml. flask are distilled down to small bulk and the test is continued according to Wadsworth. (The authors prefer to use a sintered glass filter crucible for the filtration and washing of the final extract, rather than the tared filter paper suggested by Wadsworth.) Some results for the purities of extracts obtained by the modified procedure suggested above are given in the last column of Table IV; they were determined by the modified Kjeldahl method already described.

Probably no great error would be involved if it were assumed that this modified Wadsworth method gave an extract containing 98% of theobromine, and allowance were made in the final calculation for this degree of purity.

MACDONALD'S MODIFICATION—The modified method suggested by Macdonald and preferred by Humphries was given a short trial, in view of the claim that the method gave higher figures for the theobromine content of cocoa materials than the original Wadsworth method.

The mixture of 10 g. of cocoa residue, 2·5 g. of magnesia and 14 ml. of water, dried to the damp, not moist, stage as directed in the Wadsworth method, was transferred to an

extraction thimble and extracted in a Soxhlet apparatus, using a 250-ml. round-bottomed flask as boiler containing 130 ml. of tetrachloroethane. Extraction was carried out for 3 hr. with all the samples examined, and with one sample a second test was run in which extraction was prolonged for 9 hr. The hot extract was filtered into a second 250-ml. round-bottomed flask and the boiler and filter were washed with 100 ml. of nearly boiling tetrachloroethane The combined filtrate and washings in the second flask were distilled until the residue began to sputter. The flask and contents were cooled and 70 ml. of 0·720 sp.gr. ether were added before stoppering and allowing to stand overnight. The theobromine was filtered off, using a 2G3 sintered glass filter crucible, and a total of 30 ml. of 0·720 sp.gr. ether was employed to rinse out the flask and wash the filter. The residue on the filter was given a final wash with 20 ml. of light petroleum (b.pt. 40°–60° C.) before drying at 100° C., cooling and weighing in the crucible. The usual solubility correction of +0·004 g. was made to the weight of extract.

TABLE IV

Description of cocoa residue	Cocoa fat, %	Purity of extract, % obtained by modified Wadsworth method
Cocoa residue RH ..	2·7	97·7
,, residues	10·2 10·2 10·2	97·7 97·3 97·5 } Av. = 97·5
No. 20 Cocoa residue ..	0·6	97·3
Cocoa expeller cake ..	9·5	98·8
,, residue	7·9	97·9

It was found that the extracts obtained by the above method were not pure theobromine, being of a buff colour and averaging 92% of theobromine when assayed by the modified Kjeldahl method, in spite of refiltration of the extract. There are obvious practical advantages offered by the Macdonald method as regards the extraction stage of the procedure, but a final assay of purity of the extract obtained is still necessary.

In Table V the results obtained on four samples of cocoa residues, by the following procedures, are given.

(*a*) Original Wadsworth method.
(*b*) Wadsworth method and modified Kjeldahl assay of the purity of the extract.
(*c*) Modified Wadsworth method proposed by authors.
(*d*) Macdonald's method.
(*e*) Macdonald's method and modified Kjeldahl assay of the purity of the extract.

TABLE V

Cocoa residue	Theobromine, % determined by method (*a*)	(*b*)	(*c*)	(*d*)	(*e*)
A	1·50	1·24	1·24	1·47	1·28
B	3·37	2·95	2·97	3·27	2·98
C	3·13	2·60	2·58	2·85*	2·60*
D	2·97	2·71	2·70	2·92	2·72

* After 9-hr. extraction the figures were:
2·87% by method (*d*) and 2·60% by method (*e*).

SUMMARY—A study of the Wadsworth method for the determination of theobromine in cocoa residues has been made and modifications of the original procedure suggested for the purpose of rendering the method more generally reliable. It has been shown that, contrary to the opinion expressed in Wadsworth's paper, slight alteration of the conditions renders the Kjeldahl method suitable for the assay of purity of theobromine. The most reliable method of determining theobromine in cocoa residues was found to be Macdonald's variation of Wadsworth's extraction procedure, followed by assay of purity of the extracts by the modified Kjeldahl method, since this method gave, on the average, more complete extraction than Wadsworth's method. The authors have also proposed a modification of the Wadsworth method which avoids the necessity of assaying the purity of the extract, without involving any great sacrifice of accuracy; this should prove of value where time is a factor of importance.

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A Fluorimetric Method for the Estimation of Riboflavine in Egg White and Egg Yolk

BY T. BARTON MANN

SEVERAL workers have suggested methods for the extraction and subsequent fluorimetric estimation of riboflavine in biological materials. Hodson and Norris[1] proposed hydrolysis with sulphuric acid and Van Duyne[2] has proposed pepsin digest. For whey products, Supplee, Bender and Jensen[3] proposed the use of 80% acetone acidified with H_2SO_4, and for carotenoid-containing material the extraction of the solvent with light petroleum to remove light-absorbing pigments. For dried milk products, Sullivan and Norris[4] found a 75% solution of acetone in water provided the purest extract. Hand[5] extracted whole milk with acetone and made allowances in his final volume for contraction, casein and fat. Weisburg and Levin[6] used methanol in presence of carbon dioxide for reflux extraction and adsorbed the riboflavine on British fuller's earth, subsequently recovering the riboflavine by elution with a mixture of pyridine and methanol.

Most workers recognise that there is no universal method of extracting riboflavine; each material presents a problem which necessitates a different approach.

The main problems presented in the extraction and fluorimetric estimation of the vitamin in eggs are as follows:

(*a*) The size of sample must be related to the sensitivity of the fluorimeter and the capacity of the cell in which the riboflavine solution is to be estimated.

(*b*) It is desirable that riboflavine extracts should be free from light-absorbing impurities such as carotenoids and also from extraneous fluorescent substances.

(*c*) Riboflavine shows different intensities of fluorescence at different *p*H values.

With regard to the first problem, it is not proposed in this paper to discuss the relative merits of instruments. Broadly, they fall into two classes, of which the first, the thermionic valve type, has a single photocell and robust galvanometer and is suitable for low concentrations or small volumes of solution. This type depends for accuracy on freedom from voltage fluctuation. The second, the null point type of instrument, has opposing photocells and is independent of supply voltage fluctuations, but depends upon a highly sensitive galvanometer and requires more concentrated solutions or solutions of larger volume to effect a response.

The technique described in this paper has been evolved for use with the "Spekker" fluorimeter, an instrument of the null point type; for instruments of the single photocell amplified current type, samples may be reduced to one-fourth of those suggested or less if regard be had to the principles of the method, or dilutions may be appropriately adjusted.

With either type of instrument the wavelength of the light used for exciting fluorescence is an important factor. Lothian[7] has pointed out that the relative intensities of fluorescence of riboflavine are in the ratio, 6·0 : 1·0 : 1·8, when the wavelengths of the exciting light are 3650A, 4050A and 4360A, respectively. He suggests that the longer wavelengths should be used if extraneous light-absorbing substances are present in the solution to be measured. The technique here described, however, produces solutions free from such substances, either as carotenoid pigments or as the blue or white fluorescing substances which frequently

accompany extracts produced by acid hydrolysis, and all estimations have been performed with light of 3650A by filtering the emission from the mercury vapour lamp through a Wood's glass filter. The green fluorescence of the riboflavine extracts is filtered by means of the Hilger No. 5 green filter, which transmits light mainly at the spectral wavelength of riboflavine fluorescence. Two neutral glass filters are inserted before the left-hand photocell to act as compensators.

Principles of Method

Extraction—The egg white is extracted with neutral absolute ethyl alcohol, aldehyde-free, to yield riboflavine solutions free from blue or white fluorescence; due allowance is made for the loss in volume by contraction when alcohol is mixed with egg white, which contains a high proportion of water.

The egg yolk is extracted with 55% neutral ethyl alcohol to yield solutions free from carotenoid pigments or extraneous fluorescence; an allowance is made for increased volume of the extract due to the moisture of the egg yolk.

In this laboratory it has been found impossible to extract riboflavine from whole egg, that is, a mixture of white and yolk, by the use of alcohol of any specific strength, for the reason that if the alcohol is diluted to the point at which interfering carotenoid pigments of yolk are not extracted, then such alcohol is not of sufficient strength to coagulate the proteins of egg white. Conversely, alcohol of a strength sufficient to coagulate egg white extracts interfering pigments from the yolk.

Preparation of Extracts—Break the egg and separate the white and the yolk into two tared beakers, each containing a glass stirring rod tared with it.

Egg White—Weigh the white and beat its several layers with the stirring rod to a thin homogeneous consistency free from clots, avoiding froth as much as possible. Pour approximately 26 ml. into a 100 ml. graduated cylinder and ascertain the weight of the sample thus transferred by difference. Add to it by pipette or burette 75 ml. of absolute ethyl alcohol, which will coagulate the proteins; stopper the cylinder with a rubber bung, shake vigorously and allow to stand for a few minutes. Note the contraction and record the volume of the alcohol used and the moisture content of the sample. Egg white contains 87% of moisture (Winton[8]). Then add ten glass beads, diam. $\frac{3}{8}$ in., and shake the cylinder for two hours on an end-over-end shaker. Filter the contents through a dry 15 cm. No. 2 Whatman filter paper and estimate the riboflavine in the filtrate.

Example (a)

Wt. of white	35·788 gm.
,, ,, sample, about 26 ml.	26·148 ,,
Moisture of sample, 87%	22·7 ml.
Absolute alcohol added	75·0 ,,
	97·7 ml.
Contraction	2·5 ,,
Volume for estimation	95·2 ,,

Egg Yolk—Weigh the yolk, beat it to a homogeneous consistency with the appropriate stirring rod and pour about 8 gm. (8 ml.) into a 100 ml. graduated cylinder already containing 50 ml. of 55% ethyl alcohol, accurately measured from a pipette or burette, and ten glass beads, diam. $\frac{3}{8}$ in. Ascertain the weight of sample thus transferred by difference. If the sample of yolk is gently poured into the alcohol it will coagulate in fine threads which will not stick to the glass beads or cylinder; rapid pouring should be avoided, as it will cause the formation of large particles of coagulated yolk which will need very vigorous shaking to break. Add a further 50 ml. of 55% ethyl alcohol, stopper the cylinder, and shake vigorously for two minutes by hand and then for two hours on an end-over-end shaking machine. Then filter the contents through a dry 15 cm. No. 2 Whatman filter paper, and estimate the riboflavine in the filtrate.

Example (b)

Wt. of yolk	17·787 gm.
,, ,, sample, about 8 ml.	8·513 ,,
Moisture of sample, 50%	4·25 ml.
55% ethyl alcohol added	100·00 ,,
Volume of liquid for estimation	104·25 ,,

Contraction negligible.

Egg yolk contains 50% of moisture (Winton[8]), and no significant contraction occurs when 55% ethyl alcohol is used in conjunction with the size of sample suggested.

Fifty-five per cent. ethyl alcohol has been found to be the minimum strength required to coagulate the proteins of egg yolk and is of insufficient strength to extract carotenoid pigments, fat or other substances which cause interference either because of light absorption or extraneous fluorescence.

ESTIMATION—The estimation of the riboflavine requires a standard graph prepared by means of pure solutions of the vitamin. No detectable difference has been found in the graph, whether it is prepared from riboflavine dissolved in water or in alcohol of various strengths. Hence the graph may conveniently be prepared from aqueous solutions and used for the alcoholic extracts obtained in the present method.

According to Rosenberg[9] the solubility of pure riboflavine in water is 12 mg. in 100 ml. at 27·5° C. and 19 mg. at 40° C.; in ethyl alcohol it is 4·5 mg. per 100 ml. at 27·5° C.

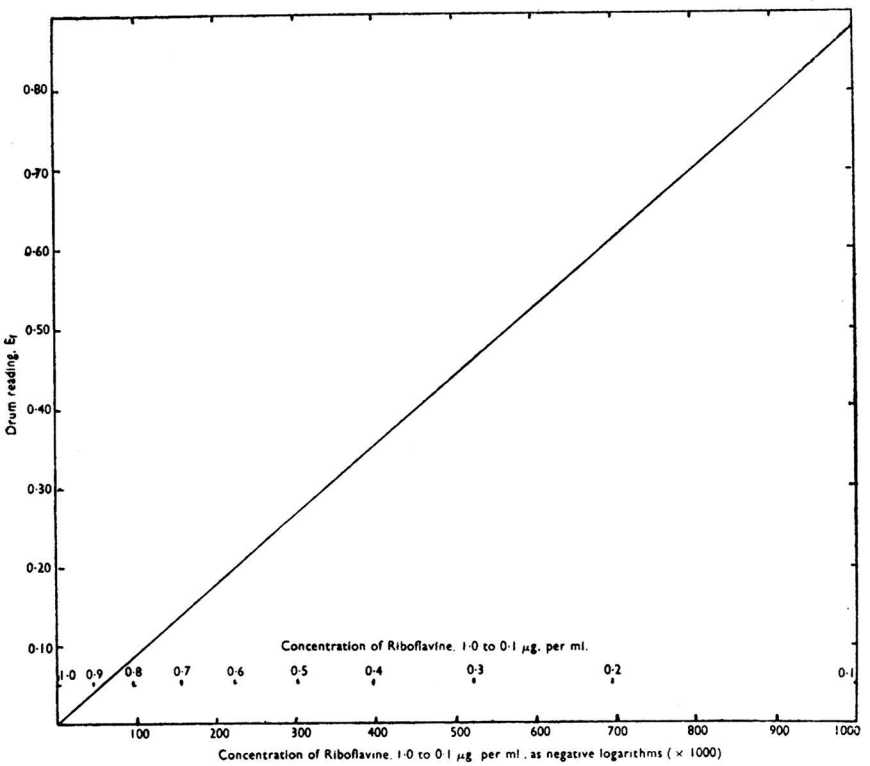

Fig. 1.

A convenient solution for graph preparation and for the comparison cell for null point determination is 1 μg. per ml. and may be prepared from a stock solution containing 5 mg. of riboflavine per 100 ml. of distilled water. This stock solution complies with the above solubilities at normal temperatures. The riboflavine should be weighed on a microbalance and the distilled water should be acidified with two drops of glacial acetic acid. Such a stock solution will serve without appreciable deterioration for about four weeks if stored in the dark at a temperature not above 4° C. Two ml. diluted to 100 ml. with distilled water will furnish 1 μg. per ml., from which other dilutions are made as necessary.

In alcoholic or aqueous solutions, within concentrations of 1·0 to 0·2 μg. per ml., and with standard emission, the fluorescence of riboflavine solutions is a linear function of the concentration. In the "Spekker" fluorimeter the variable aperture which governs emission is calibrated logarithmically, so to give full effect to this linear function in the preparation of the graph the concentration (c) of riboflavine is best plotted as a negative logarithm against drum readings (E_f). By this means the highest concentration, namely, 1 μg. per ml. coincides

with the reading 0·00 on the drum of the variable aperture. Hence by adopting negative logarithmic notation for the abscissa both null point and E_f 0·00 fall at the origin. Therefore, if the average of several readings of the lowest concentration of pure riboflavine solution (*e.g.*, 0·2 μg. per ml.) is made and this point is plotted, then a straight line from this point to the origin becomes the graph of the linear function of all dilutions falling within the range of concentration from 1·0 to 0·2 μg. per ml. The standard graph as used in this laboratory is set out in Fig. 1, in which the minus log readings of concentration along the abscissa have been multiplied by 1000 to avoid decimals. These are the "graph readings" given in the Tables and the corresponding concentrations can be found from them by reference to a table of antilogarithms of negative logs. But if the graph is drawn on semi-log. paper it will give the riboflavine concentrations directly, as indicated by the values above the base line in Fig. 1.

CALCULATION—The calculations of riboflavine content of egg white and egg yolk respectively are shown in the following examples:

Example: Egg white

Wt. of white .. .. 35·788 gm.
,, ,, sample .. .. 26·148 ,,
Volume of liquid for estimation .. 95·2 ml.

Extract read	Drum reading, E_f	Graph reading	Riboflavine μg./ml. of soln. read	Riboflavine μg./g. of white*	Riboflavine μg. in whole white	Average
(*a*) Undiluted ..	0·080	91	0·8110	2·950	105·5	106·7
(*b*) Diluted 1:2 ..	0·335	384	0·4130	3·010	107·5	
(*c*) ,, 1:3 ..	0·490	562	0·2742	2·995	107·2	

* Computation, *e.g.*, for test (*c*), $\frac{95{\cdot}2 \times 0{\cdot}2742 \times 3}{26{\cdot}148} = 2{\cdot}995$ μg. per g.

Example: Egg yolk

Wt. of yolk .. .. 17·787 gm.
,, ,, sample .. .. 8·513 ,,
Volume of liquid for estimation .. 104·25 ml.

Extract read	Drum reading, E_f	Graph reading	Riboflavine μg./ml. of soln. read	Riboflavine μg./g. of yolk*	Riboflavine μg. in whole yolk	Average
(*a*) Undiluted ..	0·317	362	0·4345	5·32	94·6	94·9
(*b*) Diluted 1:2 ..	0·576	660	0·2188	5·36	95·2	

* Computation, *e.g.*, for test (*b*), $\frac{104{\cdot}25 \times 0{\cdot}2188 \times 2}{8{\cdot}513} = 5{\cdot}36$ μg. per g.

The completeness of extraction and the accuracy of the determination are shown in Tables I and II, which give recoveries of riboflavine added to egg white and egg yolk respectively.

TABLE I

RECOVERY OF RIBOFLAVINE ADDED TO BULKED EGG WHITE

Added riboflavine	Sample wt., g.	Vol. of extract, ml.	Dilution	Drum reading, E_f	Graph reading	Riboflavine Concn. in extract μg./ml.	Riboflavine Average μg./ml.	Riboflavine μg./g. white
I None	25·209	94·45	—	0·035	39	0·9141 = 0·9141	0·9496	3·56
			1 : 2	0·275	315	0·4842 × 2 = 0·9684		
			1 : 3	0·430	492	0·3221 × 3 = 0·9663		
II 50 μg.	24·850	95·10	1 : 2	0·112	128	0·7447 × 2 = 1·4894	1·4981	5·74
			1 : 4	0·370	424	0·3767 × 4 = 1·5068		
III 100 μg.	24·838	96·10	1 : 4	0·269	309	0·4909 × 4 = 1·9636	1·9415	7·51
			1 : 6	0·432	495	0·3199 × 6 = 1·9194		
IV 150 μg.	25·444	97·65	1 : 6	0·350	400	0·3981 × 6 = 2·3886	2·3999	9·21
			1 : 9	0·500	572	0·2679 × 9 = 2·4111		

	Riboflavine found in sample plus quantities added	Total riboflavine found, μg.	% Loss or gain
I	3·56 μg. per gm.		
II	88·5 + 50 μg. added = 138·5 μg.	142·7	+3·03
III	88·5 + 100 ,, ,, = 188·5 ,,	186·5	−1·06
IV	90·6 + 150 ,, ,, = 240·6 ,,	234·0	−2·75

TABLE II

RECOVERY OF RIBOFLAVINE ADDED TO BULKED EGG YOLK

Added riboflavine	Sample wt., g.	Vol. of extract, ml.	Dilution	Drum reading, E_f	Graph reading	Riboflavine: Concn. in extract µg./ml.	Riboflavine: Average µg./ml.	µg./g. yolk
I None	8·812	104·4	—	0·330	378	0·4188 =0·4188	0·4263	5·05
			2 : 3	0·470	539	0·2891 × 3/2 =0·4337		
II 10 µg.	8·837	105·4	—	0·243	278	0·5275 =0·5275	0·5285	6·30
			2 : 3	0·395	452	0·3532 × 3/2 =0·5298		
III 20 µg.	8·154	106·1	—	0·197	226	0·5943 =0·5943	0·5978	7·79
			1 : 2	0·456	522	0·3006 × 2 =0·6012		
IV 30 µg.	8·365	107·2	—	0·146	166	0·6823 =0·6823	0·6769	8·66
			1 : 2	0·414	474	0·3357 × 2 =0·6714		

	Riboflavine found in sample plus quantities added	Total riboflavine found, µg.	% Loss or gain
I	5·05 µg. per gm.		
II	44·6 + 10 µg. added = 54·6 µg.	55·7	+2·02
III	41·1 + 20 ,, ,, = 61·1 ,,	63·5	+3·93
IV	42·4 + 30 ,, ,, = 72·4 ,,	72·5	+0·14

The accuracy of determination is also demonstrated in Table III, which shows results for the determination of riboflavine in duplicate bulk samples of egg white and egg yolk, respectively.

TABLE III

RESULTS OF ANALYSES ON DUPLICATE BULK SAMPLES OF EGG WHITE AND EGG YOLK

Wt. of sample, g.	Vol. of extract, ml.	Dilution	Drum reading, E_f	Graph reading	Riboflavine: Concn. in extract µg./ml.	Riboflavine: Average µg./ml.	µg./g. sample
			EGG WHITE				
27·341	96·25	1 : 2	0·220	252	0·5598 × 2	1·1158	3·93
		1 : 4	0·486	556	0·2780 × 4		
25·996	95·12	1 : 2	0·243	280	0·5248 × 2	1·0558	3·86
		1 : 3	0·394	451	0·3540 × 3		
			EGG YOLK				
10·048	105·0	—	0·308	352	0·4446	0·4437	4·65
		2 : 3	0·462	530	0·2951 × 3/2		
9·370	104·7	—	0·355	406	0·3926	0·3973	4·44
		2 : 3	0·500	572	0·2679 × 3/2		

FREE AND BOUND RIBOFLAVINE—The question arises whether riboflavine in egg white and egg yolk exists in the bound form in addition to free riboflavine extracted by this method. Rosenberg[9] states: "In milk, in urine, in the retina, or generally speaking in places where no respiration or fermentation takes place the free riboflavine is found. In tissues, riboflavine occurs as such, as riboflavine-phosphoric acid and in the form of riboflavine-phosphoric-acid-adenine-dinucleotide. Each of these forms occurs in the free state as well as combined with specific proteins."

He quotes Lantz for the statement that chemically bound riboflavine can be liberated by heating. Attempts in this laboratory to analyse egg white and egg yolk for total riboflavine by methods involving heat have furnished lower riboflavine contents than the method proposed in this paper.

It is well known that optimum fluorescence of riboflavine obtains at a *p*H closely approaching neutrality, and Rosenberg[9] gives *p*H 3·0 to 9·0 for optimum fluorescence. In this laboratory methods of estimating riboflavine by acid hydrolysis have resulted in lower results than by the alcohol extraction method proposed in this paper. It is found that acid hydrolysis tends to produce extraneous fluorescence and the *p*H adjustment is troublesome; in addition, egg white is difficult to hydrolyse because of frothing.

The author has been unable to apply the pepsin digest method of Van Duyne[2] to egg white and egg yolk, first because the excessive time taken to produce complete digestion

entails a great risk of bacterial and mould contamination, with possibility of production of riboflavine by these organisms; and secondly, one cannot produce clear filtrates of the digest.

It would appear that riboflavine exists in egg white and egg yolk in the free form.

PRECAUTIONS—As riboflavine is slowly destroyed by exposure to light, all manipulation should be performed in a dim light. The results of analyses given in this paper have been obtained when the shaking has been performed in the dark, and the extracts have not been brought closer to light than 15 ft. from a 100 watt gas-filled bulb.

Caution must be exercised in the choice of alcohol and apparatus. The author has found some consignments of alcohol yield blue fluorescence on receipt. As alkali destroys riboflavine, glassware should be cleansed from free alkali. Synthetic rubber bungs should not be used as these have been found to introduce troublesome extraneous fluorescence into the extracts.

To avoid errors in volume due to contraction, dilutions of the extracts when necessary should be performed with alcohols of appropriate strengths, 77·5% for egg white and 55% for egg yolk extracts, respectively. These are prepared by adding 77·5 ml. of aldehyde-free absolute alcohol to 22·5 ml. of distilled water, and 55 ml. of alcohol to 45 ml. of water, all quantities being measured separately.

SUMMARY

A fluorimetric method is presented for the estimation of riboflavine in egg white and egg yolk. Egg white is extracted with absolute ethyl alcohol and an allowance is made for contraction in volume. Egg yolk is extracted with 55% ethyl alcohol to yield a solution free from carotenoids. The riboflavine is estimated in the extracts by means of the Spekker fluorimeter.

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An Absorptiometric Method for the Determination of Inorganic Fluorides in Organic Products

BY T. C. J. OVENSTON AND C. A. PARKER

EXPERIENCE with the rapid routine determination of inorganic fluorides of the cryolite type present in organic products in amounts ranging from about 0·1% to over 1·0% in terms of fluorine suggests that the highest accuracy may be obtained by using a method which involves preliminary ashing in presence of magnesium acetate followed by the familiar distillation of fluorine as hydrofluosilicic acid and titration with thorium nitrate.[1,2] However, precise determination of the end-point of the titration, which depends on the appearance of a purple lake, requires considerable practice on the part of the operator. Moreover, if the distillation is carried out in presence of sulphuric acid, the temperature has to be carefully controlled to prevent introduction into the distillate of traces of sulphate, which would interfere with the subsequent titration.[3] On the other hand, although small amounts of perchlorate do not affect the titration, the use of perchloric acid for the decomposition of the fluoride is accompanied by a certain amount of risk where organic matter may be present. Furthermore, the cost of perchloric acid is much greater than that of sulphuric acid.

A search for an alternative method which would call for no particular skill led to a study of the Steiger–Merwin[4,5] method of estimating fluoride by its action on peroxidised titanium solution. It was anticipated that by measuring the degree of bleaching of the colour with the

Spekker Photoelectric Absorptiometer a higher accuracy would be obtained than has hitherto been associated with this method.

Attempts were also made to eliminate the distillation stage in the procedure, but these were not successful.

Effect of *p*H on the Steiger–Merwin Reaction and its Significance in Quantitative Measurements—Wichmann and Dahle,[6] who were concerned with minute traces of fluorine, found the original Steiger–Merwin procedure not sufficiently sensitive. They observed that the bleaching action of fluorides on peroxidised titanium solutions increased

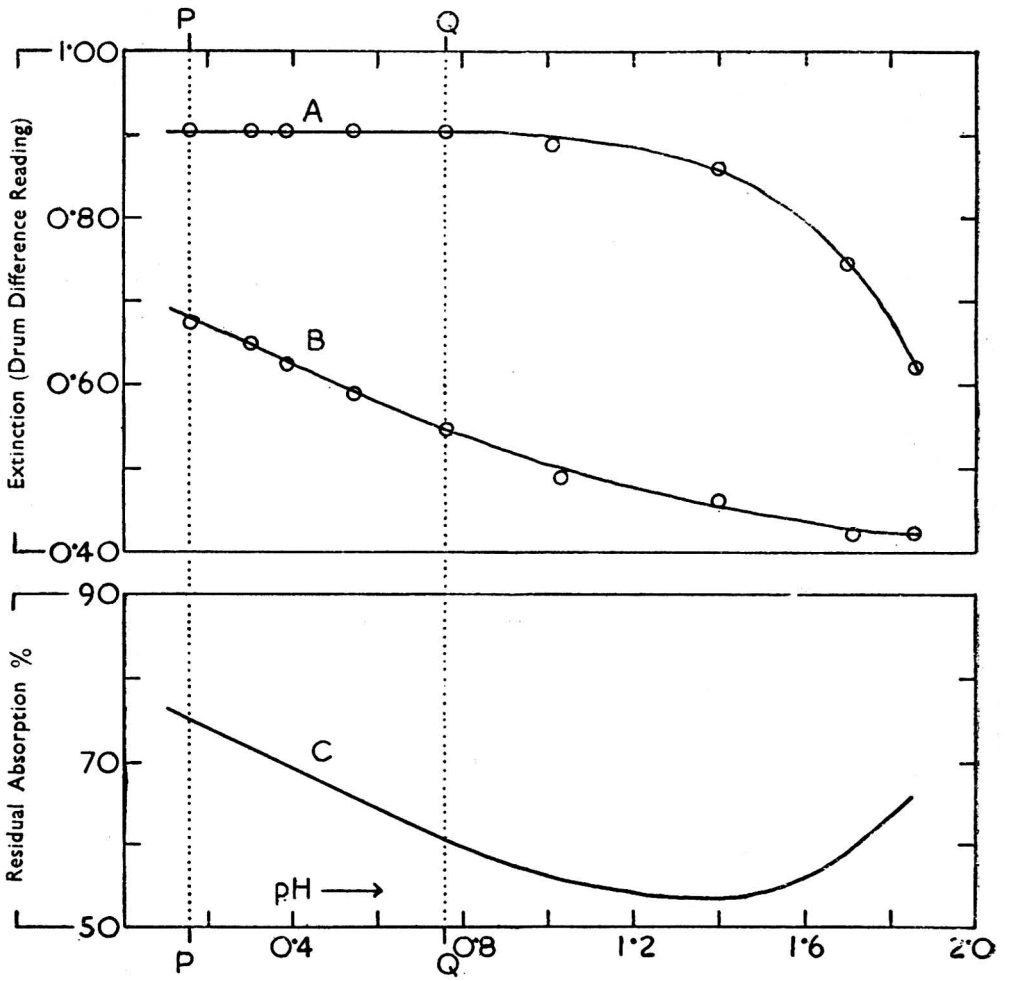

Fig. 1. Effect of *p*H on Extinction and Residual Absorption of F-Ti Solution.
A = Extinction of Ti solution alone.
B = Extinction of Ti solution containing fixed amount of F.
C = Residual Absorption.

with increasing *p*H to an optimum value at about *p*H 1·5, above which it fell rapidly. By measuring the degree of bleaching at this optimum *p*H and suitably adjusting the concentrations of the solutions the method was made extremely sensitive. The *p*H had to be controlled to within 0·02, however, which made the method tedious. These investigators used a Nutting Polarising Photometer in conjunction with a Corning 53 Violet Glass Filter, and the mean of ten readings was taken for each solution. The *p*H was measured colorimetrically with a block comparator.

Similar curves have been obtained by us (Fig. 1) based on measurements obtained with the "Spekker," and *p*H values determined potentiometrically. Curve A shows how the extinction

(as measured by the drum difference reading) of a peroxidised solution of 13·5 mg. of titanium dioxide per 100 ml. (containing no fluoride) varies with *p*H, the latter being adjusted by varying the concentration of sulphuric acid. It may be seen that the extinction remains constant over a wide range of strong acidity, and begins to fall in the region of *p*H 1·0; above this value it falls with increasing rapidity. Curve B was obtained in the same way except that the solutions contained in addition fluoride equivalent to 8·0 mg. of cryolite (4·34 mg. of F). In this case the colour density decreased gradually over the whole range.

Curve C represents the "residual absorption" (*i.e.*, the absorption of the bleached solution expressed as a percentage of that of the unbleached standard) plotted against *p*H. From this it may be seen that the maximum bleaching occurs at about *p*H 1·4, which in view of the difference in the methods used for measuring *p*H is in fair agreement with the figure obtained by Wichmann and Dahle. The fact that these investigators worked with hydrochloric acid solutions instead of the more usual sulphuric acid solutions does not appear to affect the *p*H relationships. Chlorides have practically no influence on the extent of bleaching, whereas sulphates tend to reduce it. The use of sulphuric acid was preferred in the present work because the effect of small variations in sulphate content (which might originate from the use of sulphuric acid as the distilling medium) would thus be damped out.

From an examination of Curves A and B it becomes obvious why Wichmann and Dahle found it necessary to exert such careful control over *p*H (±0·02) at their chosen value of 1·5. Small changes in *p*H will appreciably alter the colour density not only of the bleached solution but also (to an even greater extent) that of the blank.

The effect of variation in acid concentration will, of course, diminish rapidly with decrease of *p*H, and for this reason it was decided to work with fairly strong acid solutions. Two concentrations were tried; these corresponded to *p*H values of 0·16 and 0·76 respectively, indicated by P and Q in Fig. 1. As can be seen, Q allows about 50% greater range of drum reading than P. However, the expected corresponding increase in accuracy at *p*H 0·76 was not altogether realised, for the optimum sensitivity in the two cases was obtained at different fluorine concentrations, that for P corresponding to about 8 mg. of cryolite and that for Q to about 5 mg. of cryolite. At the same time, small variations in acid concentration were found to have over three times as much effect at the lower acid concentration Q. The degree of accuracy obtained with the higher acid concentration P was sufficient for the present purpose, and in view of the comparative insensitiveness to experimental fluctuations in the quantity of acid this concentration was adopted as being the most suitable for a routine method.

ADAPTATION OF THE METHOD FOR USE WITH THE SPEKKER PHOTOELECTRIC ABSORPTIOMETER—With the 1 cm. cell and Ilford Violet Filters No. 601, a concentration of 13·5 mg. of titanium dioxide in 100 ml of sulphuric acid solution was found to give a drum difference reading of 0·9 divisions, using a water-to-water setting of 1·0. Under these conditions the useful range of fluorine concentration extends from zero to about 7 mg. per 100 ml. of solution. Increases in fluorine concentration above this did not produce sufficient additional bleaching to maintain a satisfactory degree of accuracy. Within this practical range the residual absorption extends from 100% (for zero concentration) to about 67% (for a concentration of 7 mg. of F per 100 ml.) at the chosen *p*H of 0·16. This corresponds to an effective range of 0·30 drum divisions on the "Spekker."

An alternative method involving the use of 4 cm. cells and Ilford Blue Filters No. 602 was also investigated. Owing to the large absorptions involved, readings had to be taken by setting the unbleached titanium solution (13·5 mg. per 100 ml.) to zero drum reading. By this means the effective drum range was increased, but only at the expense of the sensitivity of the electrical circuit of the "Spekker," owing to the much reduced intensity of the light reaching the photocells. Thus no net advantage was gained.

METHOD OF APPLICATION—After ashing from 5 to 10 g. of the sample, the fluorine is steam-distilled as hydrofluosilicic acid from 65% w/w sulphuric acid, using the assembly recommended by the Analytical Methods Committee of the Society of Public Analysts and Other Analytical Chemists.[7] The temperature should be maintained above 140° C. during distillation, but the usual careful control is not essential so long as there is no danger of priming. At least 150 ml. of distillate should be collected and this can then be made up to an appropriate volume. The best results are obtained when the aliquot taken for a test contains about 4 mg. of fluorine.

The subsequent procedure is as follows. To the aliquot of the distillate, contained in a

100 ml. graduated flask, add by pipette 25 ml. of a standard titanium sulphate solution (containing 150 ml. of concentrated sulphuric acid and the equivalent of 0·135 g. of TiO_2 per litre) and 5 ml. of 10 vol. hydrogen peroxide. Mix and place in a thermostat at 20° C. for half an hour. Then dilute to 100 ml. with water.

Take the "Spekker" drum reading, using a 1 cm. cell and Ilford Spectrum Violet Filters No. 601, both for the sample solution and for a blank made up from the reagents, with a water-to-water setting of 1·00. Obtain the residual absorption from the following formula:

$$\text{Residual absorption} = \frac{\text{Drum difference reading of sample}}{\text{Drum reading of blank}} \times 100.$$

TEMPERATURE CORRECTION—A 10° C. rise in temperature of the solution (under the prescribed conditions of measurements) was found to result in a decrease in residual absorption equivalent to an 8% fall in fluorine concentration. If, therefore, the temperature of the solution at the time of measurement differs significantly from 20° C. a correction is required as follows:

$$F = F_t \times (0{\cdot}84 + 0{\cdot}008t)$$

where F = true fluorine content and F_t = apparent fluorine content at t° C.

CONSTRUCTION OF CALIBRATION CURVE—The fluorine content is conveniently read off from a curve prepared from values of residual absorption obtained with known amounts of pure sodium fluoride. In the construction of this curve it is essential to treat the sodium fluoride by the same distillation procedure, in order to obtain the necessary concentration of combined silica in the final solution. Silicates, of course, have an appreciable influence on the extent of bleaching.

STABILITY OF SOLUTIONS—The bleached peroxidised titanium solutions are very stable, and no change in depth of colour was detected after standing over a long period. The inclusion of the distillation stage in the method, by converting the fluorine to hydrofluosilicic acid, guards against attack on the optical glass surfaces of the cells.

SOME COMPARATIVE RESULTS—A comparison of the thorium nitrate and absorptiometric methods was made in the following way. A sample reputed to contain 2·05% of an insoluble inorganic fluoride was analysed 12 times by both methods, the same distillate being used for each pair of determinations. The amount of sample taken for each analysis was varied slightly, and was unknown to the analyst. In the first six analyses the distillation was carefully controlled at a temperature of 140° to 145° C., to eliminate the danger of sulphuric acid distilling over. In the last six analyses no particular care was taken with the distillation, and in the last two of these the distillation temperature was abnormally high. The results obtained by the two methods are given in Table I.

TABLE I

Analysis No.	Distillation Temp., °C.	Fluoride found, %	
		Absorptiometric method	Thorium nitrate method
1	140 to 145	2·06	2·09
2	,,	2·04	2·06
3	,,	2·03	2·10
4	,,	2·10	2·16
5	,,	2·00	2·12
6	,,	1·95	1·99
7	160 to 170	1·98	2·06
8	150 to 160	2·01	2·00
9	,,	2·02	2·04
10	160 to 170	2·01	2·08
11	about 170	1·96	2·34
12	,, 180	2·11	2·21

It is evident that in the last two analyses (Nos. 11 and 12) an appreciable amount of sulphuric acid had distilled over, and the thorium nitrate titration has here given high results. On the other hand, the absorptiometric method gives normal figures in all cases, indicating that this extra sulphuric acid does not affect the extent of bleaching under the prescribed conditions. (Large additions of acid would tend to give lower figures by this method.)

CONCLUSION—The absorptiometric procedure here developed is a suitable alternative to the thorium nitrate titration for the rapid routine estimation of inorganic fluorides in

organic products when present to the extent of about 0·1% or more of fluorine. It offers the following advantages over the thorium nitrate method:

(*a*) Sulphuric acid may be used for the preliminary distillation without the need of particular care in controlling the temperature of the distilling liquid.

(*b*) No particular skill is required and personal errors are eliminated in the absorptiometric method. (The thorium nitrate titration requires considerable practice, and the determination of the end-point depends to a certain extent on personal judgment which makes it necessary for each operator to carry out his own standardisation of the thorium nitrate solution).

We are indebted to the Director of Scientific Research, Admiralty, for permission to publish this paper.

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The Titration of Microgram Quantities of Fluorides

BY H. AMPHLETT WILLIAMS

ONE of the principal sources of error in any colorimetric or titrimetric method for the determination of fluorine arises from the influence of the foreign ions which usually accompany this element. By means of a perchloric acid distillation fluorine can be separated from the great majority of interfering substances, but traces of these may occur in the distillate, together with perchloric acid, in proportions still sufficient to affect the determination of microgram quantities of fluorine. That serious interference may be caused by other halides and such anions as ClO_4', NO_3', SO_3'', SO_4'', PO_4''', AsO_3''', and AsO_4''' has been shown by numerous investigators, and the individual quantitative effect of each on the estimation of large and small amounts of fluorine may be found in the literature. To make appropriate corrections in the calculation of the fluorine, however, involves a separate determination of each impurity and complicated computations where more than one is present.

Many improvements in distillation apparatus and technique have been evolved with a view to reducing the distillation of other anions, but their complete suppression would not appear to be possible without causing retention of the fluosilicic acid.

In the analysis of materials from which the distillation of interfering bodies is anticipated, or detected, the distillate may be evaporated in a platinum dish with a fixative, treated with silver perchlorate or sulphate if other halides are present, and redistilled. By these means the majority of foreign anions may be eliminated almost entirely; but apart from the additional time required, losses of fluorine have been observed upon redistillation,[1] and the appearance of an appreciable degree of acidity, chiefly due to perchloric acid, in the final distillate cannot be obviated.

The magnitude of interference errors naturally varies according to the procedure used for the determination of fluorine in the distillate. The thorium nitrate titration method, originated by Winter,[2] has been widely adopted for this purpose on account of its accuracy, scope and convenience, and is considered below. For this titration careful adjustment of the distillate to a definite *p*H, usually at a point between 2·6 and 3·5, is of the utmost importance. These limits are defined at the lower level by the incidence of an unduly high "blank" titration and at the higher level by an increasingly indistinct end-point, whilst within the range the lower the initial *p*H the higher the thorium titre per unit of fluorine.

Interference caused by substances which tend to precipitate either thorium or fluorine would appear to be insuperable except by eliminating them; interference caused by anions such as Cl', ClO_4', NO_3' is of a different nature and has been shown by Dahle *et al.*[3]

and by Reynolds and Hill[4] to be mainly due to the salts formed when the distillate is neutralised with sodium hydroxide prior to the necessary *p*H adjustment. Much attention has recently been directed towards compensating titrimetric technique, and Dahle, Wichmann and Bonnar's "acid-substitution" modification[3] of Allen's "back-titration" method represents a valuable contribution which with further improvements has greatly reduced this source of error.

Present technique, however, necessitates three titrations for each fluorine determination, *viz.*

(1) Titration of the acidity in an aliquot of the distillate so that either (*a*) a balancing quantity of a corresponding "salt" may be added to the "blank" solution,[5] or (*b*) a second aliquot, to be used for the fluorine titration, may be adjusted to the correct *p*H without neutralisation;[6]
(2) Titration of the fluorine in the prepared aliquot with a solution of thorium nitrate of convenient strength, and addition of a similar volume to the "blank"; and
(3) Back-titration of the thorium in the "blank" with a standard solution of sodium fluoride or potassium silicofluoride.

The third titration constitutes a standardisation of the thorium nitrate solution under conditions very nearly (but not quite) the same as those obtaining in the titration of the fluoride distillate. The conditions, however, are still not quite identical and the end-points, which depend upon the dispersion of a lake in a delicately balanced ionic system, may be influenced by the different reaction-products formed during the titration of the potassium silicofluoride in the distillate with thorium nitrate on the one hand, and the titration of the thorium nitrate in the "blank" with sodium fluoride or potassium silicofluoride on the other hand, as well as by the presence of undetected distillation products, slight errors in *p*H or salt adjustment, and any difference in the volumes when the titrations are completed.

Object of Investigation—The present investigation, which was undertaken in the hope of simplifying the titration of the fluorine and reducing interference, was directed towards the preparation of a standard thorium nitrate solution having a definite fluorine equivalence and the suppression of the effects of accompanying substances by controlling the degree of ionisation of the thorium fluoride and of the indicator.

Factors Influencing the Reaction—A study of the reaction between thorium nitrate and a fluoride indicates that in dilute aqueous systems the simple compound ThF_4 is never formed in stoicheiometric equivalence to the fluorine present, even when an excess of thorium nitrate is added, owing to ionisation factors. The solubility product of ThF_4, which increases with an excess of thorium nitrate, is sufficiently great to admit of a high ionic concentration in the dilutions involved in determinations of fluorine in food; and it is probable that the ions $ThF^{\cdots}$, $ThF_2^{\cdot\cdot}$, $ThF_3^{\cdot}$, together with free F' or HF_2' ions, are produced in proportions depending upon (1) the excess of thorium, and (2) the influence of $H^{\cdot}$ and other ions in the solution. Calculations of the degree of such ionisation and of the influence of accompanying salts afford little assistance in arriving at a formula for ascertaining the amount of fluorine, owing to a simultaneous dissociation of the thorium-alizarin-S lake. The facility with which this lake dissociates in aqueous solutions is shown by the fact that its characteristic colour is banished not only by fluorides and other substances forming undissociated compounds with thorium but also by acids and neutral salts. As a *p*H indicator, alizarin-S has been shown by Kolthoff[7] to be peculiarly sensitive to neutral salt effects.

When thorium and fluorine occur in correct stoicheiometric proportion, F' ions remain free owing to the dissociation of the ThF_4; these compete with the alizarin-S for the $Th^{\cdots\cdot}$ ions in the lake, causing the latter to dissociate further, and the yellow colour of acid alizarin-S to predominate. This dissociation is increased by the low *p*H obtaining and by the nitric acid or sodium nitrate formed as the titration proceeds. An excess of $Th^{\cdots\cdot}$ ions must therefore be added in order to suppress the F' ions sufficiently to permit the formation of enough lake to give the end-point colour.

For very small amounts of fluorine, *e.g.*, a few μg. in 50 ml. of water, more than twice the theoretical equivalent of thorium is necessary before the end-point is attained, but as the titration becomes larger the necessary excess is progressively diminished, through reduction of the percentage dissociated, owing to the following causes: (1) an increase in *p*H owing to dilution; (2) a decrease in the concentration of neutral salts originally present; and (3) a limitation in the number of free ions owing to the approaching saturation. A point is, indeed, reached when further increments of F and Th become stoicheiometric; but with

dilute solutions of approximately 0·001 *N* the colour change becomes too gradual before this point is reached, owing to buffering phenomena, to be of any practical use. These considerations indicate the importance of ensuring a definite, controlled degree of dissociation before any given Th:F equivalence can be relied upon at differing fluoride concentrations.

CONTROL OF DISSOCIATION—1. *At a Minimum*—By addition of an equal volume of alcohol, as in the original method,[2] dissociation can be greatly reduced, and Hammond and MacIntire[8] showed that application of the stoicheiometric value of thorium nitrate will give accurate results in aqueous-alcoholic systems of adjusted *p*H, when free from interfering salts. Armstrong,[9] however, and Reynolds and Hill[4] have shown that the influence of salts, such as alkali halides, nitrates, perchlorates and sulphates, is considerably greater in aqueous-alcoholic than in aqueous systems. In so far as the sensitivity of the indicator is concerned, little is to be gained when alcohol is added; indeed, the 100% dilution involved appears to cause a distinct loss in sensitivity at low titrations. Experiments indicated that the substitution of acetone or glycerol for the alcohol afforded no advantage.

In attempts to reduce the "salt effect," the use of alternative alkalies for the neutralisation of the distillate was considered; but it was found that potassium hydroxide, ammonia and triethanolamine showed very similar effects to those of sodium hydroxide. Neither did the use of alternative acids, such as perchloric, nitric, acetic or monochloroacetic, show any advantage over hydrochloric acid for the subsequent adjustment of the *p*H.

In the absence of any satisfactory means of reducing dissociation to a negligible level, experiments were directed towards controlling it at an optimum.

2. *At an Optimum*—The explanations (1) and (2), offered above for the falling-off in the Th : F ratio as the titration increases suggested the possibility of maintaining a constant degree of dissociation, either by buffering or by compensating additions of acid or salt. It has been found, moreover, that a lower *p*H is desirable for the titration of larger quantities of fluorine, to counteract the loss of sensitivity shown by the indicator, and proportionate increases in acidity offered advantages in this respect also.

The most suitable buffer available appeared to be that of Hoskins and Ferris,[10] but for titrations of 0–100 μg. F in 50 ml. the "blanks" were too high and the colour change too protracted. Addition of monochloroacetic acid or sodium chloride to the thorium nitrate solution proved unsatisfactory, but it was found that a constant Th : F equivalence could be arranged by addition of suitable proportions of either hydrochloric or nitric acid. The use of nitric acid was subsequently abandoned as the end-point at high titrations proved to be rather less sharp than with hydrochloric acid. The effects of varying additions of hydrochloric acid are shown in Graph I.

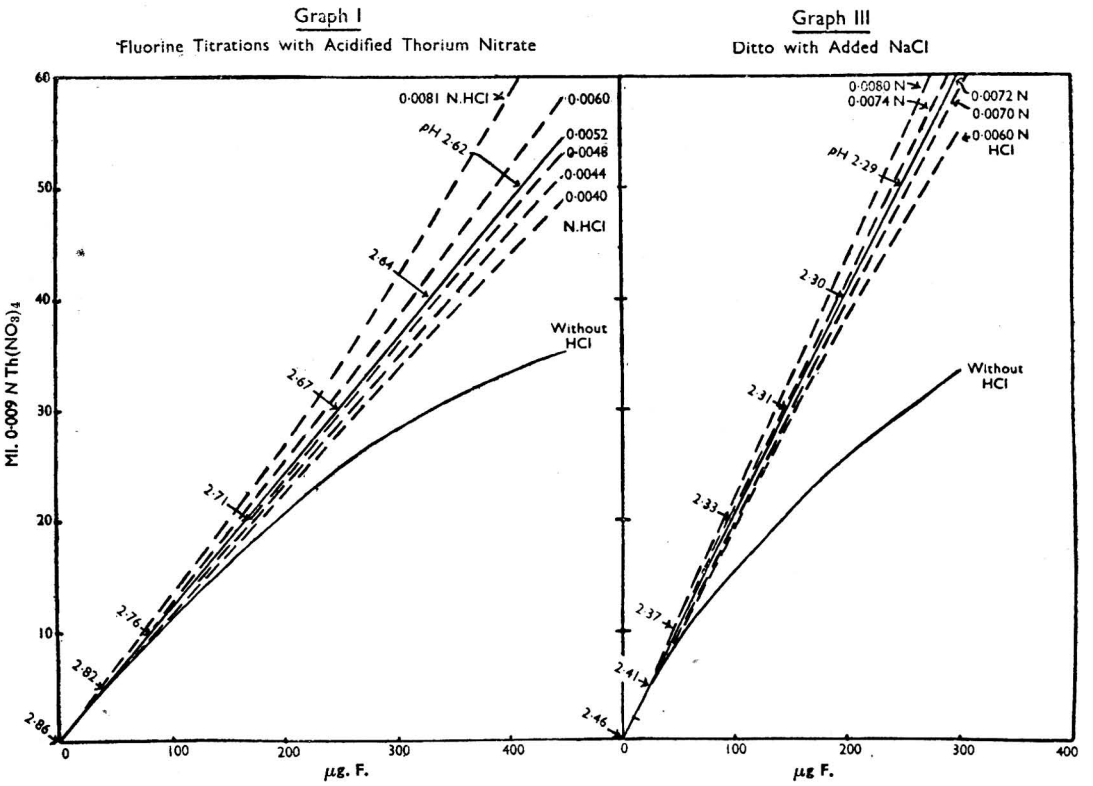
Weak standard solutions of thorium nitrate had formerly been found somewhat unreliable, owing to undue alterations in the fluorine equivalent upon dilution or storage. But addition of hydrochloric acid appeared to have a stabilising effect, enabling 0·1 *N* solutions to be kept indefinitely and 0·001 *N* solutions for about a week, after which an apparent increase in strength was observed, resulting from partial neutralisation of the very dilute acid by alkali absorbed from a glass container. Compared as to keeping qualities, however, with dilute solutions of sodium fluoride, the adoption of acid thorium nitrate as a primary standard showed definite advantages.

INDICATOR SOLUTION—If 50 ml. of water, with the usual quantities of acid and alizarin-S indicator, but without any fluorine, are titrated, a certain amount of thorium solution is required to form sufficient lake to provide the optimum end-point. This amounts to about 0·3–0·4 ml. of 0·001 *N* $Th(NO_3)_4$ and constitutes an important "blank" which must be deducted from the fluorine titration. To avoid this "indicator blank," a solution of alizarin-S was made containing sufficient thorium nitrate to form the desired proportion of lake; this expedient also enabled the strength of the alizarin-S to be doubled (*i.e.*, increased to 0·02%), thus facilitating recognition of the end-point without the disadvantage of a doubled "blank." Sufficient acid was also included in the indicator solution to avoid its separate addition to the distillate. Nitric acid was found unsatisfactory for this purpose owing to its oxidising effect upon the indicator (which became almost colourless in a month), but hydrochloric acid did not appear to be incompatible and the combined acid-indicator-lake solution possessed good keeping qualities. The solution was so formulated that the addition of from 2 ml. to 50 ml. of F-free distilled water gave the correct end-point, and the curves shown therefore proceed from the origin.

SUPPRESSION OF "SALT ERRORS"—If a salt such as sodium chloride is added to the

fluoride solution, it will be anticipated from the influence on the ionic strength of the solution that the effect of successive additions on the dissociation of the ThF_4 and the alizarin-S lake would progressively diminish as the concentration of the salt increases.

To ascertain experimentally the quantitative effect of sodium chloride on thorium-fluorine titrations, a large number of titrations were carried out with varying amounts of fluoride and sodium chloride (1 to 250 μg. F; 1 ml. of 0·1 *N* to 40 ml. of 2 *N* NaCl). To simplify interpretation the *p*H was controlled by use of a suitably acidified thorium nitrate solution giving a linear Th : F relationship at each sodium chloride level. The factor by which the thorium required exceeded the stoicheiometric equivalence for ThF_4 is shown in Graph II, and the diminishing effect in actual fluoride titrations of progressive increases in salt concentration will be observed. Thus, whilst an increment in the amount of sodium chloride (or perchlorate) from nil to 2 ml. of 0·1 *N*, corresponding to an acidity sometimes observed in 50 ml.



of fluoride distillates, increases the thorium titre by >2%, a similar increment from 20 to 20·2 ml. of *N* sodium chloride increases the titre by <0·2%—a deviation within the limits of error in judging the end-point.

In a similar way the influence of any errors in neutralising or acidifying the distillate can be reduced to a minimum by maintaining the *p*H at the lowest suitable level. Thus *p*H and salt errors can both be reduced by the use of an acidified thorium nitrate solution in conjunction with added sodium chloride.

It will be seen from Graph II that in order to obtain the maximum advantage from the addition of salt in titrations of 0–200 μg. F in 50 ml. of distillate, about 1 g. should be added; larger amounts offer little advantage and are inconvenient. The addition of this amount necessitated an increase in the acidity of the thorium nitrate solution to maintain linear Th:F relationship, and the influence of variations of acidity in a standard thorium solution, in the presence of added salt, is shown in Graph III.

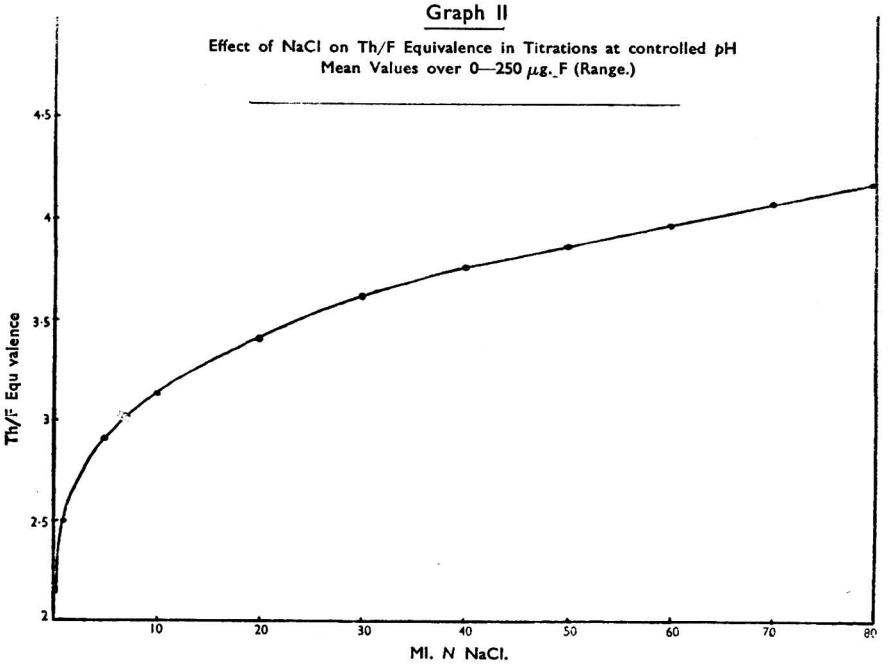
Adding 10 ml. of 2 *N* sodium chloride solution to 50 ml. of the fluoride solution, and increasing the acidity of the indicator solution by one-fifth and the thorium nitrate sufficiently to eliminate the "salt blank," a formula for a standard thorium solution of a

convenient strength of 1 ml. ≡ 5 μg. F was arrived at by series of trials. Measurements of *p*H with and without salt were made with a potentiometer (glass electrodes), and results are included at various points on the final curves to show the decrease involved as the fluorine increases.

SENSITIVITY OF INDICATOR—One of the chief factors limiting the accuracy of thorium-fluorine titrations is the increasing lack of sensitivity exhibited by the indicator at higher titrations. Owing no doubt to a proportionate ionisation of the ThF_4, the indicator, which responds readily to 1 drop of an acidified 0·001 *N* $Th(NO_3)_4$ solution for titrations of 1 to 2 ml., shows barely a discernible change for a 1 ml. increment in a 40–50 ml. titration. It is therefore particularly important to avoid the introduction of any step in the titration procedure likely to cause further loss of sensitivity; and, as it has been stated[9] that presence of sodium chloride hindered recognition of the end-point in micro-titrations of fluorine, the behaviour of the alizarin-S lake indicator under various conditions, including established and experimental methods, was carefully studied.

Graph II

Effect of NaCl on Th/F Equivalence in Titrations at controlled *p*H
Mean Values over 0—250 μg. F (Range.)



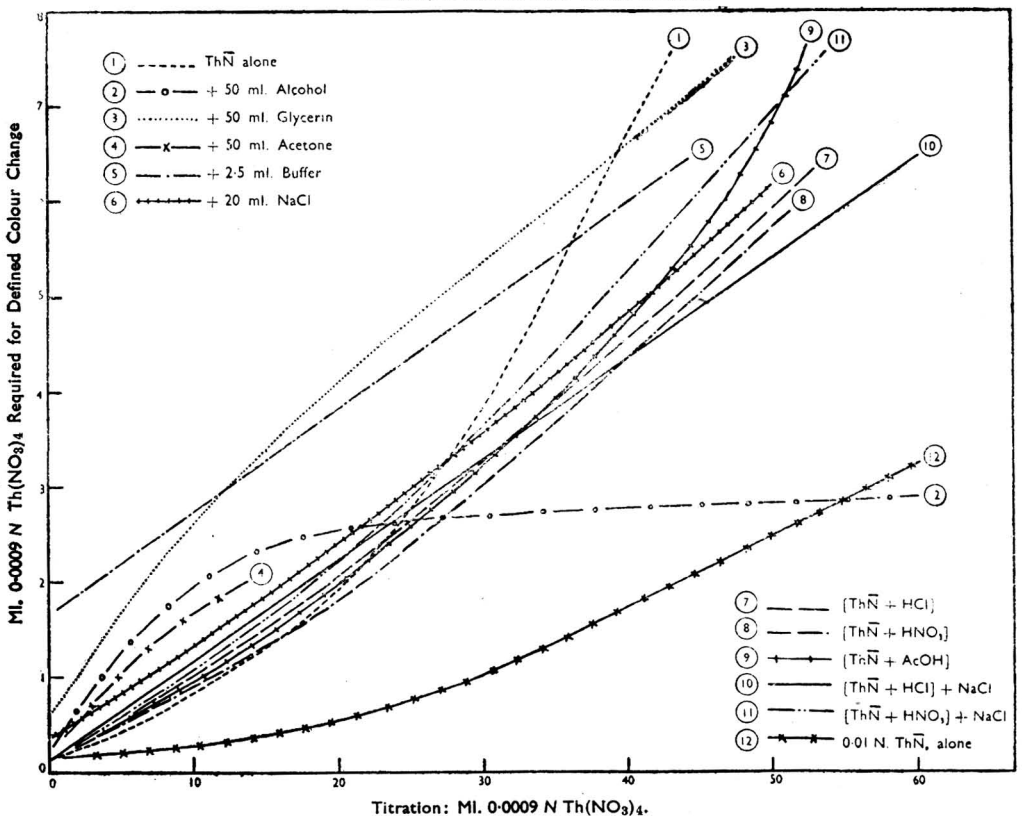
In order to obtain comparable sensitivity data the volume of thorium nitrate solution required to bring about the change-over in the indicator was measured for a series of titrations of various quantities of fluorine under each set of conditions. The range of sharpest response for the thorium lake indicator being from $\frac{1}{3}$ to $\frac{2}{3}$ of its full colour range, three coloured solutions were prepared from suitable mixtures of cobalt nitrate and potassium chromate of shades similar to those assumed by the indicator in a fluoride titration at $\frac{1}{3}$, $\frac{1}{2}$ and $\frac{2}{3}$ of its colour change. The solution corresponding to the $\frac{1}{2}$ colour change, on which the acid-indicator-lake mixtures were formulated, provided a convenient permanent end-point standard for all subsequent titrations, and the $\frac{1}{3}$ and $\frac{2}{3}$ colour change solutions were used for measuring sensitivities.

Titrations were carried out with quantities of added fluoride increasing from zero to the equivalent of about 50 ml. of thorium nitrate solution, so as to bring out tendencies which might be unnoticed at the lower levels. The colour change at high titrations is so gradual that precise measurements could not be made, but by taking means of replicates it was possible to construct representative curves showing clearly the influence of various factors on the sensitivity. In each instance the fluoride solution was made up to 50 ml. with distilled water, and 2·5 ml. of 0·05 *N* HCl (except where a buffer was used) and 1 ml. of 0·02% alizarin-S solution were added, other additions being as specified.

The results, reproduced in Graph IV, show that the addition of sodium chloride does not seriously affect the sensitivity under the conditions adopted. The use of an acidified thorium nitrate solution gives distinctly sharper end-points at the higher fluorine levels than thorium nitrate alone, and its use, in conjunction with the added sodium chloride, gives a sensitivity which compares favourably with that afforded by the other methods examined. The use of the sodium monochloroacetate buffer proposed by Hoskins and Ferris[10] for *p*H control in titrating larger quantities of fluorine, resulted in high blanks and poor sensitivity, as did the addition of an equal volume of glycerol.[11,4] The addition of alcohol, to reduce dissociation, afforded a different type of curve, showing poor sensitivity with small amounts of fluorine but an improvement with larger amounts. With acetone the quality of the colour altered as the titrations increased and the sensitivity at the higher levels could not be assessed by the same colour standards; no advantage was observed.

Graph IV

Sensitivity of Indicator under Various Conditions



As the present investigation was nearing completion a method was published by Matuszak and Brown[12] in which a thorium nitrate solution of 0·05 *N* strength, acidified with acetic acid to the extent of 1·2 *N*, was used to give a linear Th:F equivalence for the titration of larger amounts of fluorine in the analysis of volatile organic compounds. By modifying the strength of the acetic acid to 0·2 *N* I found that a 0·001 *N* $Th(NO_3)_4$ solution could be made, having a linear Th:F equivalence, for use with μg. quantities of fluorine. The indicator sensitivity with this solution, after preliminary *p*H adjustment with hydrochloric acid as usual, was found, as shown in Graph IV, to be similar for titrations up to 30 ml. to that obtained with the thorium nitrate solution acidified with hydrochloric acid. The use of acetic acid, however, for the preliminary *p*H adjustment of the fluoride solution, as recommended by Matuszak and Brown, appeared to be contra-indicated by the large quantity (14 ml. of *N* acetic acid) necessary to produce the lower *p*H now advocated for μg. titrations, with correspondingly high blanks (20 ml. of 0·001 *N* $Th(NO_3)_4$) and reduced sensitivity.

The greatly enhanced sensitivity afforded when larger amounts of fluoride are present

and 0·01 *N* $Th(NO_3)_4$ solution can be used is also shown in Graph IV. In these circumstances it is suggested that the use of sodium chloride still offers advantages in suppressing "salt errors," but that the addition of acid to the thorium solution is unnecessary since after the initial 2 or 3 ml. of a titration (for which an allowance may be made) further increments cause proportional precipitation of thorium fluoride until the end-point is reached, and the Th:F equivalence becomes stoicheiometric.

STANDARDISATION—The formula for the thorium nitrate solution was based upon the following substances which were taken as standards:

(1) *Sodium fluoride*—Prepared from purest obtainable sodium carbonate and hydrofluoric acid (SiO_2, SO_3 and P_2O_5, <0·002% in each case); neutralised, and twice recrystallised in platinum basins, moistened with HF and heated to 450° C. Assay: by Travers' method, 98·8% NaF; by CaF_2 precipitation, 99·4% NaF; by conversion to sulphate, 99·8% of the theoretical increase in weight, corresponding to 99·0% NaF + 1·0% Na_2CO_3. Taken as 99·0% NaF.

(2) *Potassium silicofluoride*—Prepared by passing SiF_4 into KCl solution, separating the precipitate, washing and drying at 60° C. *in vacuo*.[5] Assay: by Travers' titration, 88·3% K_2SiF_6. Free from Cl. Impurity probably silicic acid. "Analytical Reagent" quality was unobtainable, and "technical" supplies of silicofluorides were inferior. Taken as 88·3% K_2SiF_6.

(3) *Thorium nitrate*—Pure crystals. Assay: from residue on ignition, 99·0% $Th(NO_3)_4.4H_2O$; by oxalic acid precipitation (in 0·05 *N* HCl solution), 98·7% $Th(NO_3)_4.4H_2O$. Taken as 98·7%.

(4) *Sodium chloride*—Three supplies were used, of ordinary "Analytical Reagent" quality, from different manufacturers. Analyses: F <0·3 p.p.m.; P_2O_5 <2 p.p.m.; SO_3 <30 p.p.m.; Ca <50 p.p.m.

The thorium nitrate solution was standardised against solutions containing 0·001% F prepared from the sodium fluoride and from the potassium silicofluoride; no difference was observed in the titre, nor in the behaviour of the indicator, between the fluoride and the silicofluoride solutions when titrated by the procedure described below.

PROPOSED METHOD

REAGENTS—(1) *Acidified standard thorium nitrate solution*—(*a*) Stock solution: contains thorium nitrate equivalent to 1·27 g. of $Th(NO_3)_4.4H_2O$, and 72 ml. of *N* HCl, in 100 ml. (*b*) Dilute solution: dilute 5 ml. of (*a*) to 500 ml. with F-free distilled water. 1 ml.≡5 μg. F.

(2) *Acid-indicator solution*—Dissolve 0·020 g. of sodium alizarin monosulphonate (Alizarin-S) in distilled water, add 100 ml. of the dilute acidified thorium solution (1) (*b*), and 14·3 ml. of *N* HCl and make up to 200 ml. Two ml. of this solution added to 50 ml. of F-free distilled water and 10 ml. of 2 *N* NaCl in a Nessler glass should give the correct end-point colour; if not, impurities in the salt or other chemicals may be responsible and the proportion of (1) (*b*) should be modified accordingly. The colour should be judged at once, as it is liable to alter on standing.

(3) *Salt solution*—2 *N* NaCl (accurate), of "Analytical Reagent" quality.

(4) *Colour standards*—(*a*) Temporary: dilute a quantity of a standard fluoride solution containing 100 μg. F to 50 ml. with distilled water, add 10 ml. of (3), 2 ml. of (2), 20 ml. of (1) (*b*) and mix. Being effectually buffered, the colour is stable for a few hours, after which it may fade owing to precipitation. (*b*) Permanent: mix 3 ml. of 10% HCl with 50 ml. of a solution containing 1% of $CoCl_2$, add 30 ml. of 0·1% K_2CrO_4 solution and dilute to 100 ml. Three ml. of this stock mixture are diluted with distilled water to a volume approximately equal to the anticipated volume of the test solution when titrated. The colour should be identical with that of (*a*); if it is not, owing perhaps to a difference in the quality of the thorium nitrate used, the proportions of ingredients should be adjusted until a match is obtained. Alternatively, alizarin-S may be used as a colour standard in a buffered solution of suitable *p*H.

(5) 2 : 5-*Dinitrophenol indicator solution*—0·05%. Carbon dioxide in the quantities encountered in fluoride distillates does not appreciably interfere at the lower limit of the *p*H range of this indicator; and being colourless at the required *p*H the indicator may be used in the aliquot to be titrated with thorium nitrate.

(6) *Sodium hydroxide solution*—0·05 to 0·1 *N*.

(7) *Hydrochloric acid*—0·01 to 0·02 *N*.

PROCEDURE—Add 3 drops of 2 : 5-dinitrophenol indicator (5) to a convenient quantity of the distillate or fluoride solution in a Nessler glass, and the dilute NaOH (6), if necessary, until the solution when mixed assumes a faint yellow colour; then add a drop, or sufficient, of the dilute HCl (7) just to discharge the colour. It is an assistance to have a Nessler glass containing distilled water for comparison, as the colour becomes very pale near the end-point. If the presence of free halogen is suspected, 1 ml. of a 1% solution of hydroxylamine hydrochloride may be added, preferably before neutralisation.

Measure 50 ml. (±0·5 ml.) of the neutralised solution, containing between 0·5 and about 150μg. F, into another Nessler glass, add 10 ml. (±0·05 ml.) of the salt solution (3), 2 ml. (±0·01 ml.) of the acid-indicator solution (2), and mix. Titrate with the dilute thorium solution (1) (*b*) until the colour exactly matches that of the standard colour solution (4) (*a*) or (4) (*b*). For a 5 g. sample, 1 ml. of dilute thorium solution ≡ 1 p.p.m. F (subject to fixative and distillation blanks, if any).

COMPARISON OF RESULTS—The efficacy of the method evolved was tested by adding reputedly interfering salts to solutions containing a known amount of fluorine and determining the fluorine, both as described above and by the "back-titration" method. Ten ml. of standard sodium fluoride solution containing 100 μg. F were made up with the added substance and q.s. distilled water to 50 ml. for each test. Results obtained are shown in Table I.

TABLE I

INTERFERENCE FROM FOREIGN SALTS IN BACK-TITRATION AND SALT-ACID-THORIUM METHODS

100 μg. F in each test

	Back-titration method		Salt-acid-thorium method	
Added substance	Titrations	Error as % of the F	Titrations	Error as % of the F
None	10·0	0	20·0	0
1 ml. 0·1 *N* $HClO_4$, neutralised	10·1	+ 1	20·0	0
5 ,, ,, ,, ,,	10·6, 10·7	+ 6	20·1	+ 0·5
1 ,, 0·1 *N* HCl ,,	10·2	+ 2	20·0	0
5 ,, ,, ,, ,,	10·8, 10·9	+ 8	20·1	+ 0·5
0·1 ml. 0·1 *N* H_2SO_4 ,,	10·7	+ 7	20·4	+ 2
0·4 ,, ,, ,, ,,	12·9, 12·6	+27	21·9, 22·1	+10
50 μg. P_2O_5 ,,	End-point indeterminate		End-point indeterminate	

SUMMARY—The thorium nitrate titration of fluorides has been studied and a simplified technique is proposed, comprising:

(1) The use of an acidified standard thorium nitrate solution of a convenient strength with a constant fluorine equivalence;

(2) Dissociation control by addition of sodium chloride, as a means of reducing interference;

(3) A single titration against a permanent colour standard in place of the three titrations involved in current methods, and an extension of the titratable range.

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February 6th, 1946

Notes

THE EVALUATION OF UNSATURATION IN MIXTURES OF HYDROCARBONS (MOTOR SPIRITS) BY THE USE OF PYRIDINE SULPHATE BROMIDE

THE present method of determining the degree of unsaturation of a motor spirit is that introduced by McIlhiney[1] and appears in a modified form in the Institute of Petroleum's Standard Methods for Testing Petroleum and its Products (5th Ed.).

The method consists essentially in the measurement of the unsaturation of the hydrocarbon mixture by means of a solution of bromine in carbon tetrachloride, but, owing to the unselective action of this reagent, substitution to some extent also takes place. The fraction of bromine absorbed in this reaction depends on a number of factors, such as the structure of the hydrocarbons present and particularly on the position of the unsaturated linkages in the molecules. As a result, although repeatable values are obtainable, the Bromine Numbers so found are, in some cases, unsatisfactory as a measure of unsaturation in hydrocarbon mixtures.

This substitution reaction appears to have been largely eliminated by the bromide-bromate method of Francis,[2] although, according to Green,[3] a modified Lewis and Bradstreet method[4] yields more consistent results. Cortese[5] showed, however, that the bromide-bromate method was unreliable for certain ring structures and for substances of unknown constitution.

For the determination of the iodine values of fatty oils Rosenmund and Kuhnhenn[6] suggested the use of pyridine sulphate bromide as a reagent capable of providing a more active halogen than the iodine monochloride used in the Wijs method. With pyridine sulphate bromide, moreover, the possibility of substitution appears to be practically negligible and the effective excess of reagent, therefore, less critical. Bolton and Williams[7] have applied this reagent to the measurement of the unsaturation of the unsaponifiable fraction of olive oil, later shown by Drummond and others[8] to consist mainly of the unsaturated hydrocarbon squalene, and pyridine sulphate bromide is now the standard reagent for measuring the unsaturation of the unsaponifiable fraction of fatty oils and fats.

Consideration does not appear to have been given, however, to the use of this reagent for the determination of the unsaturation in other hydrocarbon mixtures such as motor spirits. Its use for this purpose has now been examined and the first results indicate that the use of pyridine sulphate bromide in this determination avoids the variable substitution reaction encountered in the McIlhiney method and the trial and error technique of the Francis process and gives a rapid and reliable measure of unsaturation.

Reagents—(1) Approximately $N/10$ pyridine sulphate bromide in glacial acetic acid. (2) Aqueous 10% potassium iodide solution. (3) Standard $N/10$ sodium thiosulphate solution.

Procedure—The appropriate quantity of motor spirit may be measured with a calibrated pipette, but the following weighing technique was found to give excellent results. A glass vessel is constructed from the bottom 1½ in. portion of a thin walled test-tube drawn out to a thick capillary, and then redrawn to a hair capillary 1 in. in length. After weighing, warm the tube in the flame of a bunsen, and immerse the capillary in the sample. On cooling somewhat, the requisite amount of liquid is sucked into the tube, which then is allowed to cool completely before inverting to allow the liquid to flow into the wider section. In this way, the loss of high vapour pressure constituents which occurs with a pipette is avoided. After the second weighing, drop the tube containing the sample into a bottle of the type used for iodine value determination, containing 25 ml. of carbon tetrachloride and a glass marble. Add the pyridine sulphate bromide reagent from a burette, replace the bottle stopper and break the weighing tube by the impact of the marble on shaking the bottle. After an interval of five minutes, determine the excess reagent by addition of potassium iodide solution and titration of the liberated iodine with thiosulphate. The Bromine Number is computed from the formula $(A - B) \times 8 \times N/w$, in which A = ml. of standard thiosulphate equivalent to the pyridine sulphate bromide used in the blank, B = ml. of thiosulphate equivalent to excess of bromide reagent, N = normality of standard thiosulphate solution and w = weight of sample in grams.

The results given in Table I form an interesting comparison between the methods of McIlhiney (A) and Francis (B) and the Rosenmund and Kuhnhenn modification here described (C).

TABLE I

COMPARISON BETWEEN THE METHODS OF MCILHINEY (A), FRANCIS (B) AND THE ROSENMUND AND KUHNHENN MODIFICATION (C)

		Bromine numbers		
		Method A	Method B	Method C
Shale oil fractions:				
No. 1		103·1	102·1; 103·0	100·6; 102·0
,, 2		83·0; 83·2	83·1; 83·1	83·5; 82·6
,, 3		65·1; 67·9	74·5; 74·7	73·2; 72·6
,, 4		57·9; 56·9	71·4; 71·1	71·4; 72·2
,, 5		51·3; 51·8	63·4	62·8; 62·9
,, 6		36·5; 37·6	49·7; 50·1	50·9; 50·6
,, 7		51·9; 52·0	62·5; 62·7	59·3
Old cracked spirit			47·9; 48·2	47·9; 47·2
Straight hydrocarbons	Theory			
Benzene (redistilled)	nil	nil	nil	nil
Cyclohexane (redistilled)	,,	,,	,,	,,
Cyclohexene (commercial, redistilled)	—	182·2	—	182·1
Cyclohexene*	190·5	—	182·9	191·2
Octene†	142·7	—	140·0	142·2

* *Cf.* "*Organic Synthesis*," V, XII, 33. † *Cf.* "Preparation of a Mixture of 1-Octene and 2-Octene," F. C. Whitmore and I. M. Herndon, *J. Amer. Chem. Soc.*, 1933, **55**, 3428.

Table II gives the results of applying method C to the analysis of synthetic mixtures of saturated and unsaturated hydrocarbons of known Bromine Numbers.

TABLE II

ANALYSIS OF SYNTHETIC MIXTURES BY METHOD C

Mixture	Bromine number		Percentage Olefines		Excess reagent
	Theory	Found	Actual	Found	(calculated)
Octene in benzene					
No. 1	35·5	35·7	24·9	25·0	36
„ 2	35·9	36·1; 36·2; 36·0	25·3	25·4; 25·4; 25·3	167; 194; 643
„ 3	71·2	71·6; 71·6; 71·5	50·1	50·4; 50·3; 50·3	428; 242; 412
„ 4	95·9	95·7; 96·8; 95·7	67·7	67·3; 67·8; 67·3	359; 333; 275
Cyclohexene in benzene					
No. 5	61·6	60·7; 61·3	33·9	33·4; 33·7	273; 200
„ 6	48·2	48·9; 49·7	26·5	27·3; 26·9	296; 436
Cyclohexene in cyclohexane					
No. 7	37·5	37·9	20·6	20·8; 20·8	348; 258

SUMMARY—A modified Rosenmund and Kuhnhenn method for the estimation of unsaturation has been applied to hydrocarbon mixtures and found to offer certain advantages over the existing methods of McIlhiney and Francis. The reagent employed, pyridine sulphate bromide, is prepared easily by the method of Rowe, Furnas and Bliss,[9] and remains stable for long periods if kept in a dark coloured bottle. The substitution reaction which occurs in the McIlhiney method, and the uncertainty of the excess reagent required in the Francis method are both eliminated, and the weighing technique described avoids the loss of low boiling point fractions which occurs when the sample is measured by volume.

This investigation is being continued to determine whether or not theoretical addition occurs with a wide variety of unsaturated hydrocarbons when pyridine sulphate bromide is used as reagent.

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We wish to thank Scottish Oils, Ltd., for supplying samples of shale oil distillate used in this investigation, and the Governors of the Heriot Watt College for laboratory facilities.

HERIOT WATT COLLEGE
EDINBURGH

G. E. WILSON
H. B. NISBET

October, 1945

Ministry of Food

STATUTORY RULES AND ORDERS*

1946—No. 312. Order dated March 5, 1946, amending the Flour Order, 1945. Price 1d.

This amending Order provides for the rate of extraction of national flour to be increased from 82½% to 85% and of "W" flour from over 82½% to over 85%, as from Sunday, 10th March, 1946.

— **No. 384. The Table Jellies (Maximum Prices) Order, 1946. Dated March 23, 1946.** Price 2d. with No. 385.

The Manufactured and Pre-packed Foods (Control) Order, 1942, prohibits the production and sale of table jellies and related products except under licence. Licences having now been issued to permit the resumption of manufacture of table jelly tablets, table jelly crystals and table jelly compounds, this Order prescribes maximum prices therefor at each stage of distribution.

In this Order

"Table jellies" means table jelly tablets, table jelly crystals and table jelly compounds.

"Table jelly compound" means a product consisting of sugar and gelatin or other jelly-forming material, with other ingredients, intended to be made up with milk so as to produce a jelly table sweet.

"Table jelly crystals" means crystals consisting of sugar and gelatin or other jelly-forming materials, with or without other ingredients (but not including farinaceous products) intended to be made up with water so as to produce a jelly table sweet.

"Table jelly tablets" means tablets consisting of sugar and gelatin or other jelly-forming material, with other ingredients (but not including farinaceous products) intended to be made up with water so as to produce a jelly table sweet.

* Italics signify changed wording.

The Schedule to the Order sets out maximum prices applicable to first hand sale, sale by wholesale and sale by retail, of the following descriptions of table jellies.

Table jelly tablets—
1-pint packets (*i.e.*, containing not less than 5 oz. net weight),
Table jelly crystals (Group A, manufactured by certain specified makers)—
1-pint packets (*i.e.*, containing not less than 3 oz. net weight),
Containers of 7 lb. net weight or a multiple thereof.
Table jelly crystals (Group B)—
1-pint packets (*i.e.*, containing not less than 3¾ oz. net weight).
Containers of 7 lb. net weight or a multiple thereof.
Table jelly compounds—
1-pint packets (*i.e.*, containing not less than 2½ oz. net weight).

These are the only descriptions of table jellies that may be sold and they must conform with the weights specified above. Deficiencies up to ⅛ oz. may be allowed by a court at discretion.

In any prosecution it shall be a defence to prove that the seller purchased the jelly in the container in which he sold it, with a written warranty that the weight of contents was not less than that now specified, a statement of net weight on the label being deemed to be a written warranty and a statement that a packet is of "1-pint size" being construed as a statement that the net weight conforms with the requirement of the Schedule for that particular description of table jelly.

1946—No. 385. General Licence under the Labelling of Food (No. 2) Order, 1944. Dated March 23, 1946. Price 2d. with No. 384.

This General licence permits for a limited period the sale of table jellies, *within the meaning of S.R. & O., 1946, No. 384 (above)*, under labels which do not conform to the requirements of the Labelling of Food (No. 2) Order, 1944.

It came into force on March 31, 1946, *and will cease to have effect*

(*a*) as respects the sale by retail or display for sale by retail of table jellies, on June 30, 1947; and

(*b*) as respects the delivery of table jellies pursuant to a sale otherwise than by retail—

(i) on December 31, 1946, where delivery is pursuant to a sale by a packer or labeller of the table jelly; and

(ii) on March 31, 1947, where delivery is pursuant to any other sale.

British Standards Institution

DENSITY HYDROMETERS FOR USE IN MILK

AMENDMENT NO. 1: FEBRUARY, 1946, TO B.S. 734 : 1937

Page 5, *Foreword, line* 36. For "Appendix D" substitute "Appendix C."

Page 7, *Foreword, lines* 25–34. Delete "The table has been . . . from Richmond's tables" and substitute the following:

"The table is based on a formula which correlates the total solids content of milk with its fat content and density at 20° C. This formula was derived from Richmond's well known formula, which correlates the composition of milk with its specific gravity $S_{60° F./60° F.}$, by adjusting for the expansion of milk and the density of water at 60° F. (for details of the formula and its derivation, see p. 27). Hence the results obtained by the use of the density hydrometers specified and Table C will agree with the results obtained by the use of a specific gravity hydrometer and Richmond's formula, when the two methods are applied under the same conditions of Recknagel contraction.

It should be noted, therefore, that laboratories which have previously used Richmond's method, without pre-warming the milk, will obtain the same results by using the density hydrometer and Table C provided that the milk is not pre-warmed, whilst if the recommendation to pre-warm milk samples to 40° C. be adopted (see pp. 5 and 20) the results obtained will be slightly lower than those obtained hitherto by an amount depending on the extent of the Recknagel contraction of the unwarmed samples. The adoption of the pre-warming procedure is recommended as it has been found by practical tests that it ensures reproducible results and when used in conjunction with Table C yields results for total solids which are in satisfactory agreement with those obtained by direct gravimetric determinations."

Page 27, *Appendix C, line* 26. For "40° F." substitute "40° C."

Page 28, *Appendix C, lines* 4–7. Delete.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Caramel in Wine, Spirits, Vinegar and Vanilla Extract. G. E. Mallory and R. F. Love (*Ind. Eng. Chem., Anal. Ed.*, 1945, 17, 631–637)—The method was primarily developed for the analysis of wine but is applicable to distilled spirits, vinegar and vanilla extract. It precipitates the caramel from a sample completely and in a pure state, and since no colour is removed from a caramel-free sample the formation of a brown ppt. in the method is proof of the presence of caramel, the identity of which, if desired, can be confirmed by known tests. For quantitative purposes the ppt. is redissolved and the caramel determined with a Lovibond tintometer. Beyer (*J. Assoc. Off. Agr. Chem.*, 1943, **26**, 164; ANALYST, 1943, **68**, 195) has shown that caramel solns. conform with Beer's law and that readings with the Lovibond tintometer are as accurate as those made with a photometer. The measure of the depth of colour must be taken as the sum of the units of colour represented by all the glasses used in matching (*i.e.*, red and brown glasses). Unless it is colourless, all wine free from caramel contains brown colour even if it appears to the eye to be of a pure red colour. If caramel is added to wine and is recovered by the method of analysis and its colour determined, the sum of the brown and red slides of the Lovibond scale divided by the sum of the brown and red slides required for matching the colour of the original sample containing caramel multiplied by 100 is the % of caramel colour in the total colour. Investigation of the method showed that there is manipulative loss of 12·5% of caramel and the final result must, therefore, be corrected for this loss.

Method for wine—Prepare a washing soln. by dissolving 6 g. of powdered boric acid and 2 g. of powdered citric acid in 100 ml. of pure 95% alcohol, warming to 55° C. and then cooling to room temp. Prepare the precipitating soln. immediately before use by mixing 56 ml. of this soln. with 19 ml. of ether and 25 ml. of acetone. Place 25 ml. of wine containing 10–21% by vol. of alcohol and not more than 20% of solids in a 130-ml. glass-stoppered cylinder graduated to 100 ml. and add in the order named, shaking after each addition, 0·3 g. of powdered potassium bitartrate, 0·1 g. of powdered tartaric acid and 0·4 g. of powdered bisulphite (not metabisulphite; minimum 87% $NaHSO_3$). After 10 min. add 100 ml. of the precipitating soln., shake the stoppered cylinder vigorously for 1 or 2 min., removing the stopper every 15 or 20 sec. to release the pressure, and allow the mixture to stand overnight to promote complete pptn. of the caramel. Place in a Gooch crucible a mat of paper pulp of thickness 1/16th in. or less, rinse it with alcohol by suction and decant through it the supernatant liquid in the cylinder until only about 10 ml. remain. Mix this liquid and ppt. in the cylinder and pour it rapidly through the crucible, rinsing the cylinder several times with 5–10 ml. of the alcoholic boric and citric acid soln. and pouring the rinsings through the crucible. Wash the ppt. with 25 ml. of this alcoholic washing solution which has been heated to boiling on a hot plate, then with 10 ml. of 95% alcohol and with 10 ml. of 4% alcoholic sodium hydroxide soln. and finally with 10 ml. of 95% alcohol. Transfer the paper and ppt. into a 150-ml. beaker and rinse the crucible into the beaker, first with 5 ml. of 0·5 *N* sodium hydroxide and then with 15 ml. of water and confirm the alkalinity of the liquid with litmus paper. Boil and stir the liquid for several min. to dissolve caramel. Filter the liquid through moistened paper, wash the paper with water until the filtrate measures 23 ml., make the filtrate faintly acid with *N* hydrochloric acid and complete the vol. to 25 ml. Read the colour of the caramel soln. in a 0·5-in. cell in the Lovibond tintometer, correct the reading for 12·5% manipulative loss, divide it by the colour reading of the original wine and multiply by 100 to obtain the % of caramel colour in the total colour.

Method for spirits—Shake 25 ml. of spirits with 50 ml. of ether in a separating funnel for 1 min. to remove alcohol and after 15 min. separate the aqueous layer and discard the ethereal layer. Shake the aqueous layer vigorously with 25–35 ml. of ethyl acetate to remove uncharred oak tannin and after 15 min. separate the lower layer into a glass-stoppered cylinder, add 3 g. of caramel-free brown sugar, fill to the 21 ml. mark with water and complete the vol. to 25 ml. with 95% alcohol. When the sugar has dissolved proceed as directed for wine. After acidifying the final soln. add 0·07 g. of ammonium chloride, mix and allow to stand for 30 min. to remove the last traces of tannin from redwood or uncharred oak. Filter and read the colour as described for wine.

Method for vinegar—To 22 ml. of vinegar add 3 ml. of 95% alcohol and proceed as directed for wine, making the final vol. up to 22 ml.

Method for vanilla extract—Treat 25 ml. of the extract with 25 ml. of water and 1 g. of purified talc and filter the mixture through paper pulp in a Gooch crucible, returning the first portion of the filtrate to the crucible until a layer of talc has formed and the filtrate is clear. Place 25 ml. of the filtrate in a glass-stoppered cylinder and proceed as for wine. Make the final vol. up to 12·5 ml. and read the colour directly, or to 25 ml. and multiply the reading by 2.

In addition to the chemical reagents used another substance is necessary for pptn. of caramel, the nature of which is unknown. This substance occurs in wine, vinegar and vanilla extract but not in distilled spirits or standard solns. of caramel. The only material found capable of replacing the unknown substance was brown sugar, and hence the necessity for the addition of brown sugar in the procedure described for spirits. Pure sucrose or dextrose is ineffective; the authors used a light brown granulated cane sugar. The brown sugar used must be free from caramel and should be tested by dissolving 3 g. in enough 10 to 21% alcohol to make 25 ml. and subjecting the soln. to the method described. The ratio of the concns. of reagents used in the method is important and volumes should be measured accurately. The isolated caramel is free from sugar and does not reduce Fehling's soln. This is important, since in the final stage of the method the caramel can be boiled with alkali without formation of more caramel. All readings should be made in a slightly acid soln. and if it is desired to evaporate a caramel soln. after separation it should first be rendered alkaline because non-acid proof caramel may be pptd. in a liquid that is more than slightly acid. The total solids in samples subjected to the method should not exceed 20%. The average composition by wt. of 16 samples of commercial caramel used in the investigation was 67·5% of solid matter and

32·5% of water. When 0·1 g. was dissolved in 100 ml. of water the average colour reading of the soln. in a 0·5-in. cell was 9·6 brown + 0·6 red.

A. O. J.

Spectrophotometric Procedure for the Estimation of Vitamin A in Oleomargarine. J. B. Wilkie and J. B. De Witt (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 174–186)—In preliminary expts. with commercial margarine it was found that neither the antimony trichloride method of Oser *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 724) nor the indirect spectrophotometric method of Neal and Luckman (*Id.*, 1944, **16**, 358) was universally applicable. Accordingly, a direct spectrophotometric procedure was developed involving the chromatographic fractionation of the unsaponifiable extract, precautions being taken to prevent or minimise destruction of vitamin A and visual control of the chromatographic separations being effected by the use of weak ultra-violet light. Stir 10 g. of margarine in a tall 300-ml. beaker with 50 ml. of boiling 95% ethanol until the sample has disintegrated and add 0·25 g. of anhydrous sodium sulphite. Cool to 35° C., add 25 ml. of 50% aq. potassium hydroxide, stir for 5 min. and leave at room temp., with occasional stirring, for 15 min. Transfer the soln. into a 500-ml. separating funnel, rinsing the beaker with 50 ml. of fresh 5% $Na_2S_2O_4$ solution. Add 100 ml. of light petroleum, shake vigorously and after 5 min. run the aq. layer into another funnel and extract it with 50 ml. of a mixture of 90% of light petroleum and 10% of U.S.P. ether. After 3 min. separate the aq layer and repeat the procedure, making altogether 7 extractions, and combine all the extracts with the first extract. If a three-layered system forms, continue the extraction with 50 ml. of light petroleum alone and with such systems add only the top layer to the original extract. Pour through the combined extracts two 200-ml. portions of water and separate the layers without shaking. Make 5 more washings with 20-ml. portions of 5% sodium hydrosulphite ($Na_2S_2O_4$) soln., shaking well and removing the aq. layer after 2 min. Wash the extract twice with 200-ml. portions of water without shaking or stirring, and allowing 10 min. for the second washing to separate. Filter the extract rapidly through 25 ml. of a powdered mixture of anhydrous sodium sulphate (90%) and sodium sulphite (10%). Evaporate the extract on the water-bath until the vol. can be adjusted to 10 ml. with light petroleum.

Prepare the chromatographic column in a tube of 20 mm. diam. and 10 cm. long containing a sintered filter of medium porosity, the lower end of the tube being constricted to fit into the rubber stopper of a 250-ml. suction flask. Place a 3-mm. layer of sodium hydrosulphite directly upon the sintered disc, apply suction and add the adsorbent, *viz.*, a mixture of 3 parts of purified diatomaceous earth (Celite) and 1 part of magnesium oxide. Tamp the adsorbent lightly as it is added in small amounts until its depth is 2·5 cm., then add a 5-mm layer of $Na_2S_2O_4$ and a 1-cm. layer of sodium sulphite. Wet the column with 30 ml. of light petroleum and add rapidly a 5-ml. aliquot of the extract. When the extract is about to disappear into the column add more light petroleum until the vitamin A appears as an intensely fluorescent band visible in weak ultra-violet light. Elute the band with light petroleum or with a 0·2% soln. of glacial acetic acid in light petroleum until it is *ca.* 5 mm. from the end of the column. Replace the suction flask with a clean one and complete the elution until the fluorescence disappears. Evaporate the eluate sufficiently to adjust its vol. to 10 ml. with light petroleum. Determine the optical density of this soln. at 5 $m\mu$ intervals over the range from 270 $m\mu$ to 450 $m\mu$ and calculate the vitamin A potency of the margarine by means of the formula

$$E^{1\%}_{1\,cm.}\ (340\ m\mu) \times 2500 =$$

U.S.P. units of vitamin A per g. of margarine.

Since margarine is variable in composition and all the limitations of the method are not known, it is sometimes desirable to make recovery expts. to ascertain the possible loss of vitamin in the procedure. To a duplicate sample add 1 ml. of light petroleum containing 150 to 200 units of vitamin A immediately before adding the potassium hydroxide for saponification. Assay both samples as described and calculate the % recovery of added vitamin from the formula $2(D_{340}$ standard + unknown $- D_{340}$ unknown) $= D'_{340}$ the density of the standard, *i.e.*, equivalent to 1 ml. of the standard made up to 10 ml.

$$\text{Recovery } \% = \frac{D'_{340}}{D_{s340}} \times 100.$$

To confirm the results by the antimony trichloride method set the galvanometer of a rapid direct reading photoelectric colorimeter (with standard Corning filters 245 and 978) to read 100 with 1 ml. of petroleum spirit and 9 ml. of the reagent (¼ lb. of antimony trichloride in 500 ml. of dry alcohol free chloroform). Place 1 ml. of the 10-ml. sample prepared for chromatographing into a colorimeter tube, add 1 ml. of light petroleum, mix quickly and transfer 1 ml. into another tube. Run 9 ml. of the antimony trichloride reagent into each tube in turn and take the max. reading of the galvanometer (usually in *ca.* 4 sec.). From the average G_a obtain L_a from transmittance-density tables. To 1 ml. of the original 10 ml. sample in a colorimeter tube add 1 ml of a 1 : 10 dilution of the standard vitamin A soln. Mix and transfer 1 ml. of this mixture into another tube, add 9 ml. of the reagent as previously described, and from the average of the readings G_b obtain L_b. From these data the vitamin A content of the original sample can be calculated. Some specimens of light petroleum spirit fluoresce excessively and show marked light absorption below 300 $m\mu$, the effect being intensified with concentration. Expts. showed that whilst commercial light petroleum can be purified by distillation from lime, reagent grade spirit is pure enough for routine work with margarine. As an arbitrary test place the petroleum in a 1-cm. quartz cell and determine its transmission at 300 $m\mu$ without use of a blank cell. Light petroleum having a transmission of more than 85% is satisfactory. The green fluorescence of vitamin A sometimes becomes blue in the chromatographic column or the green band develops a blue halo, and some samples of margarine showed only the blue fluorescence. Expts. showed that this change has probably occurred in the sample. It is considered not to have any practical effect upon the methods described.

A. O. J.

Biochemical

Determination of Ascorbic Acid. Application of the Indophenol-Xylene Extraction Method to Large Numbers of Tomato and Tomato Juice Samples. W. L. Nelson and G. F. Somers (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 754–756)—To prepare the 2,6-dichlorophenol-indophenol soln. dissolve 40 mg. of the crystals in hot water, filter, cool and dilute to 100 ml. Store

this soln. at 3°–5° C. and dilute about 15 ml. to 100 ml. before use. To prepare the acetate buffer soln. dissolve 500 g. of sodium acetate trihydrate in water, dilute to 1 litre and mix with 1 litre of glacial acetic acid. When a 5-ml. aliquot of the diluted dye soln. is mixed with 2 ml. of the buffer soln. and 5 ml. of the extracting acid (3% metaphosphoric acid) and extracted with 15 ml. of xylene, the xylene extract should give 30% transmission in the Evelyn colorimeter with filter No. 520. Prepare and filter the extract of the sample as described by Morell (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 793),* repeating the filtration of the first portion, if necessary, until the filtrate is clear. Treat an aliquot of the filtrate (1 to 10 ml., according to the amount of ascorbic acid present) in a large test tube with 2 ml. of the buffer soln. followed immediately by 5 ml. of the dye soln., mixing rapidly but thoroughly after each addition. After 15 sec. add 15 ml. of xylene, close the tube with a rubber stopper and shake vigorously for 15 sec. Draw the xylene layer into a colorimeter tube by suction through a cottonwool plug and read its colour in an Evelyn colorimeter with a No. 520 or No. 515 filter. The reagents should be added from automatic pipettes without rubber connections. The xylene layer may be clarified by standing or by centrifuging, but the method described with a cottonwool plug is more convenient, a new plug being used for each aliquot. The intensity of colour of the dye after extraction with xylene is constant for 3 hr. but not overnight, and readings should preferably be made within 1 hr. The xylene may be dried with calcium chloride and recovered for subsequent use by distillation in an all-glass still. The ascorbic acid content of the aliquot is ascertained from a standard curve constructed by plotting on semi-logarithmic paper the % transmission of dye - xylene solns. from known amounts of ascorbic acid against the ascorbic acid concn. Values obtained between 35% and 90% transmission conform excellently with Beer's law.

With tomato samples duplicate determinations seldom differ by more than 1% transmission ($\equiv$ 2–4 μg. of ascorbic acid). With tomato and tomato juice samples clear filtration is essential in order to avoid the appreciable blank value given by suspended carotenoids soluble in xylene. With dehydrated cabbage, potatoes and sweet potatoes clear filtrates are not necessary, since xylene-soluble pigments do not occur. It is essential to add the dye soln. as soon as possible after the buffer soln. to avoid loss of ascorbic acid. The xylene used should be tested for freedom from oxidising agents. Completely decolorise a small amount of the dye soln. with ascorbic acid and shake vigorously with the xylene. If no colour develops in the xylene layer in 10 min. the xylene is suitable for use, otherwise it must be distilled from an all-glass still. The method is particularly adaptable to the determination of ascorbic acid in large numbers of samples and requires less experience than the titrimetric method. A. O. J.

Use of Enzyme in Riboflavine Determination. Free and Combined Riboflavine. L. Rosner, E. Lerner and H. J. Cannon (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 778–779)—In the fluorimetric determination of riboflavine by a method using a Florisil column the authors found that, with many natural products, higher results are obtained when incubation with enzyme is included than when this step is omitted and that the higher values are supported by microbiological results. There is some evidence that the higher values are due not to improved extraction in presence of the enzyme (although this may occur in some products), but to release of riboflavine from a combined form. The method used is similar to that of Andrews (*Cereal Chem.*, 1943, **20**, 3). Extract the sample in a volumetric flask with 0·1 *N* sulphuric acid for 1 hr. on a boiling water-bath and then incubate for 1 hr. with 0·3 g. of polidase at 48° C. and *p*H 4·5. If a significant amount of pigment is present, treat the mixture at this stage with potassium permanganate and hydrogen peroxide. Dilute to volume, filter and pass an aliquot of the filtrate through a Florisil column (12 cm. by 8 mm.). Wash the column with water and elute the riboflavine with 20% pyridine in 2% acetic acid. Measure the fluorescence of the eluate and compare it with that of a known amount of riboflavine added to the sample. Obtain a blank reading by addition of solid sodium hydrosulphite. To determine whether or not the higher values obtained with enzyme treatment are due to more effective extraction, samples of dried brewers' yeast, enriched white bread and dried skim milk were extracted with 0·1 *N* sulphuric acid and the filtered extract was treated with enzyme and carried through the usual procedure. The values obtained with enzyme treatment of the filtered extract compared with those obtained by enzyme treatment of the unfiltered sample showed that extraction by enzyme is not a contributing factor to the increased values. Either part of the riboflavine fluoresces to a different degree from the remainder or it is lost on the Florisil column. The similarity of the results obtained when enzyme treatment and column were omitted to those obtained with enzyme treatment and column indicated no change of fluorescence with enzyme treatment, and it thus appeared that riboflavine was lost at the column either by non-adsorption or by non-elution. An aliquot of the extract obtained without enzyme treatment was put through the column, the drippings and washings (which would contain any riboflavine that escaped adsorption) were collected and the riboflavin adsorbed on the column was eluted with the pyridine mixture. It was found that the fluorescence of the unadsorbed drippings and washings represents the difference between values obtained with and without enzyme treatment. The effect of the extent of washing was studied by determining the amount of unadsorbed riboflavine in seven successive 25-ml. portions of the washings with boiling water with omission of the enzyme treatment and with enzyme treatment for 1, 2 and 18 hr. With this severe washing a significant amount of unadsorbed riboflavine was found even after enzyme treatment for 2 hr. Quantitative recovery of riboflavine added to extracts of natural products proved that the adsorptive power of the Florisil is not impaired by any constituents of these extracts. The most plausible explanation of these results is that the riboflavine that is not adsorbed is different from free riboflavine. Rubin and De Ritter (*J. Biol. Chem.*, 1945, **158**, 639) tested the adsorption of riboflavine phosphate and succinate on Florisil and concluded that these forms are fairly completely adsorbed. However, their data show that only 86% of the phosphate is adsorbed on a single column compared with 100%

* Comminute 25 g. of sample with 100 ml. of 3% metaphosphoric acid in a blendor running at high speed for 2 min. and filter through a dry fluted Whatman No. 12 filter paper, discarding the first 10 ml. of filtrate, which is cloudy.—Ed.

of free riboflavine, and since their column was washed only once with 25 ml. of hot water, their results cannot be regarded as casting doubt upon the existence of combined riboflavine in extracts of natural products. On the other hand their results do indicate that even in pure soln. the adsorptive behaviour of riboflavine phosphate does differ from that of free riboflavine. It appears clear, therefore, that when a Florisil column is used in the determination of riboflavine, enzyme treatment is necessary to avoid the risk of losing the combined riboflavine. When the hydrosulphite blank value is low the same results are obtained whether fluorescence of the extract is read directly or whether enzyme treatment and the column are included, but when the extract is highly coloured poor results are obtained without the use of the column. Yeast was found to contain a larger proportion of combined riboflavine (up to 80% of the total riboflavine) than any other material tested. A. O. J.

Frozen Vitamin Standards. O. E. Stamberg and D. W. Bolin (*Ind. Eng. Chem., Anal. Ed.*, **1945, 17**, 673)—In vitamin determinations the frequent preparation of accurate standards is time-consuming, and standards stored at low temperatures rapidly become unreliable. The use of frozen vitamin standards is possible. Aneurine hydrochloride and riboflavine standards were prepared in 2% acetic acid containing 10 μg. per ml., the solns. being prepared and subsequently handled in the dark. Aliquots (5 ml.) were pipetted into 15-ml. vials tightly stoppered with cork stoppers which had previously been soaked in 2% acetic acid and then in distilled water before drying. The vials were immediately placed in a refrigerator at $-15°$ C. in a box to exclude light and at an angle of 45°. Incompletely filled vials will not break on freezing if they are placed at an angle. The frozen standards were tested at various intervals of time and were compared with freshly prepared standards and found to be perfectly satisfactory after 6 months' storage. The Coleman photofluorometer was used for the measurements, and aneurine was oxidised by the method of Conner and Straub (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 385; ANALYST, 1941, **66**, 504). The frozen standards were thawed in water at room temp. and immediately a 2-ml. aliquot was diluted with 2% acetic acid to give the desired concn. This was done in a dark room—a condition specially important with riboflavine. The method is perhaps applicable to other vitamins. A. O. J.

Biological Assay of Vitamin D_3. Effect of the Calcium and Phosphorus Content of the Diet. J. I. M. Jones (*Biochem. J.*, **1945, 39**, 324–328)—The effect of varying the calcium and phosphorus contents of a standard diet on the responses of chicks to graded doses of vitamin D_3 was studied, the criteria utilised being gain in weight, tarso-metatarsal distance (T.M.T.) and percentage of bone ash of tibiae. It was found that the gain in weight and T.M.T. varied with the Ca : P ratio of the diet, the former rising and the latter falling as the ratio increased. The percentage of ash in the bones did not vary with the Ca : P ratio within the same range. The effect of vitamin D_3 on weight increase diminished as the Ca : P ratio increased. With an adequate dosage of vitamin D_3, the differences produced by variations in the Ca and P contents of the diet tended to disappear when the Ca : P ratio was greater than 1. The optimal increase of response with graded amounts of vitamin D_3 was obtained with 1·5% Ca and 1·5% P in the diet. With a Ca : P ration of 1, the gain in weight increased with increases in the dietary level of calcium and phosphorus up to 1·5%, after which it decreased; this occurred at all dosage levels of vitamin D_3. The minimum T.M.T. response was reached at 1·5% Ca and 1·5% P, beyond which it increased again. No such reversal occurred with the percentage of bone ash. The food utilisation (gain in weight per 100 g. of food consumed) ran parallel with the absolute gain in weight of the chicks, and the increased food consumption following an increase in the vitamin D_3 content of the diet was very marked. The regression of gain in weight, T.M.T. and bone ash on log vitamin intake for Ca/P = 1·5/1·5 was statistically linear over the range 10 to 22·5 B.S.I. units of vitamin D_3 per 100 g. of food. The bone ash criterion of response constitutes the most reliable basis for assaying vitamin D_3 in foodstuffs, but the gain in weight and T.M.T. methods are simpler and more economical. As the gain in weight method requires no special apparatus, it is recommended for routine assays. F. A. R.

Methods for the Estimation of Alloxan. R. M. Archibald (*J. Biol. Chem.*, **1945, 158**, 347–373)—No adequate methods appear to have been published for the estimation of alloxan in tissues, blood or urine, and only five methods appear to have been described for its detection. In this paper, six methods of estimating alloxan are described; the choice of method will depend partly on the nature of interfering substances present, partly on the concn. of alloxan and partly on the apparatus available. Methods 1 and 2 require about 2 mg. of alloxan, are very accurate and are of especial value in determining the purity of alloxan preparations and estimating the amount of alloxan present as an impurity in preparations of other oxidation products of uric acid. Methods 3, 4 and 5 are more sensitive and will estimate 0·02 to 0·2 mg. of alloxan. Method 4 is more complicated than the other two, but, as it measures a product of the potassium cyanide reaction (oxaluric acid) not measured by any other method, it may be of value when relatively high concns. of reducing substances are present; it cannot be used for the estimation of alloxan in presence of relatively high concns. of urea. Method 6 is the most sensitive of all and measures 0·02 to 0·2 μg. of alloxan; it cannot be used with solns. which are fluorescent before addition of the reagent.

The first four methods depend on the reaction of alloxan with potassium cyanide in weakly acid or alkaline solns. Method 1 involves the quantitative measurement of the carbon dioxide liberated in the reaction: 2 alloxan + KOH $\longrightarrow$ dialuric acid + $NH_2.CO.NH.CO.COOK + CO_2$. Method 2 involves the estimation by titration with ceric sulphate soln. of the dialuric acid formed in this reaction, whilst method 3 measures the same substance colorimetrically by means of phosphotungstic acid, which is reduced, giving a blue colour. Method 4 depends on the measurement of the other product of this reaction (oxaluric acid), which reacts much more rapidly than alloxan on heating with diacetyl monoxime in acid soln., yielding a yellow colour. Methods 5 and 6 depend on the reaction of alloxan with *o*-phenylenediamine, to give a product, the constitution of which is not established with certainty; in method 5 the product is estimated colorimetrically and in method 6 fluorimetrically.

Solns. of alloxan are unstable at a *p*H higher than 3·5, but solns. of *p*H 2·5 to 3·4 are stable for 24 hr.

at room temp. even at low concns. Standard solns. should therefore be prepared in 0·002 *N* sulphuric acid. Alloxan is rapidly destroyed in whole blood, plasma and urine, and therefore blood proteins should be pptd. *immediately* (within a matter of secs.) after drawing the samples. Since glutathione destroys alloxan and is present in blood almost entirely in the red cells, it is an advantage to ppt. plasma proteins without preliminary haemolysis of the solns. It is also necessary to lower the *p*H of the medium as soon as possible after drawing the blood sample. The choice of protein precipitant will depend on the selected method of estimation, but zinc hydroxide should not be used, as it ppts. alloxan quantitatively.

The following method gave satisfactory results. Add 1 vol. of whole blood to 8 vols. of 3% Na_2SO_4, $10H_2O$ soln., followed by 0·5 vol. of 10% sodium tungstate soln. and 0·5 vol. of 0·75 *N* sulphuric acid. By means of this procedure rupturing of the cell membrane is prevented, the acidity is increased to *p*H 3 and the supernatant liquid is almost free from thiol-compounds.

Method 1—*Gasometric method*—Transfer 2 ml of soln., containing 0·2 to 12 mg. of alloxan, from a rubber-tipped stopcock pipette into the chamber of a van Slyke-Neill blood gas apparatus and extract dissolved gases by lowering the mercury to the 50-ml. mark and shaking the apparatus for. 1 min. Eject the extracted gases and introduce 1 ml. of 0·05 *M* potassium cyanide by means of another pipette. Seal the bore of the stopcock with mercury and mix the solns. The *p*H must be 7·0 or higher at this point. After 2 min., introduce 0·5 ml of (approx.) *N* lactic acid, again seal the stopcock with mercury and extract the carbon dioxide by shaking after lowering the mercury to the 50-ml. mark. Record the pressure p_1 with the gas at the 0·5-ml. mark if the sample contains 3 mg of alloxan or less, or at the 2-ml. mark if it contains more than 3 mg of alloxan. Also record the temperature. Add 0·5 ml. of 5 *N* sodium hydroxide to absorb the carbon dioxide and take the reading (p_2) at the mark used for the p_1 reading. Correct for the pre-formed carbon dioxide in the soln. and reagents by introducing into the chamber another 2-ml. portion of the alloxan soln. followed by 0·5 ml. of *N* lactic acid and 1 ml. of cyanide soln. Measure the pressures before and after absorption of the carbon dioxide; the blank reading, *c*, is the difference between the two pressures. The amount (mg.) of alloxan in the sample $= F \times (p_1 - p_2 - c)$, where *F* is a factor obtained from a table in the original paper, of which a skeleton is given below.

Temp. °C.	*F* for gas vol. 0·05 ml.	*F* for gas vol. 2·0 ml.
10	0·01032	0·04051
15	0·00992	0·03932
20	0·00975	0·03827
25	0·00951	0·03729
30	0·00929	0·03642
35	0·00907	0·03564

These factors are obtained from those given by van Slyke and Sendroy for the determination of carbon dioxide (*J. Biol. Chem.*, 1927, **73**, 127, Table IX, liquid vol. (*S*) 3·5 ml. and gas vols (*a*) 0·05 and 2·0 ml.; see also Peters and van Slyke, "*Quantitative Clinical Chemistry Methods*," Baltimore, 1932, p. 277) by multiplying by 3·637 × 2; 3·637 is the ratio of the mol. wt. of alloxan to that of carbon dioxide and 1 mol. of the latter is liberated from 2 mols. of alloxan. Where the sample contains pre-formed carbon dioxide comparable in amount with that formed in the reaction with potassium cyanide the procedure should be modified as follows. To 2 ml. of sample in the blood gas apparatus add 0·5 ml. of *N* lactic acid, shake out the carbon dioxide and eject as described by van Slyke for the estimation of urea in whole blood. Repeat the extraction twice, add 0·5 ml. of 0·1 *M* alkaline potassium cyanide (dissolve 0·65 g. of potassium cyanide in 90 ml. of water and add 12 ml. of 18 *N* sodium hydroxide free from carbonate) and proceed as described above.

Method 2—*Titrimetric method*—Put 1 to 10 ml. of the sample, containing 0·5 to 3·0 mg. of alloxan into a 20–40 ml. reaction tube capable of being evacuated and displace the air in the tube by leading in carbon dioxide or nitrogen just above the surface of the liquid. Add 1 ml. of 0·05 *M* potassium cyanide, insert the lubricated stopper and immediately evacuate through the side-arm. The *p*H of the mixture should be about 6·5. After 5 min. release the vacuum with the side-arm attached to the CO_2-delivery tube. Add 1 ml. of 18 *N* sulphuric acid and 1 drop of 0·005 *M* *o*-phenanthroline ferrous complex (*J. Amer. Chem. Soc.*, 1931, **53**, 3908) and titrate with 0·001 *N* ceric sulphate (1 ml. of 0·1 *N* ceric sulphate and 1 ml. of 18 *N* sulphuric acid diluted to 100 ml.) until the golden-brown colour changes to a very faint blue. Pass a current of CO_2 over the surface of the liquid during the titration to preserve an oxygen-free atmosphere. Run a blank with water in place of the potassium cyanide soln. One mg. of alloxan monohydrate ≡ 6·25 ml. of 0·001 *N* ceric sulphate, so that the amount (mg.) of alloxan monohydrate in the sample $= (T_s - T_b) \times 0{\cdot}16$, where T_s and T_b are the titres for the sample and blank respectively.

Method 3—*Photometric method with phosphotungstic acid*—Mix a 5-ml. sample, containing 0·015 to 0·15 mg. of alloxan, and also 5 ml. of water with 1-ml. portions of the reagent mixture with cyanide. To prepare this reagent, mix 3 vols. of 0·67 *M* Na_2HPO_4 (240 g. of $Na_2HPO_4.12H_2O$ in 1 litre), 0·05 vol. of 0·5 *M* potassium cyanide (6·5 g. in 200 ml.) and 1 vol. of phosphotungstic acid reagent (reflux 50 g. of sodium tungstate with 400 ml. of water and 40 ml. of syrupy phosphoric acid for 2 hr. and dilute to 500 ml.); the phosphotungstic acid should be added to the alkaline buffer not more than 1 min. before the reagent is to be used. To other 5-ml. aliquots of the sample and water, add 1 ml. of the above reagent mixture in which 0·05 vol. of water replaces the cyanide soln. Dilute 1, 2 and 3 ml. of a standard soln. of alloxan monohydrate (0·02 mg. per ml.) in 0·002 *N* sulphuric acid to 5 ml. and leave all the solns. for 30 min. in the dark. Evaluate the colours at 700 *mμ* against a water blank in a photoelectric colorimeter or compare with the standards in a visual colorimeter. The difference between the optical densities with and without cyanide is proportional to the alloxan present.

Method 4—*Photometric method with diacetyl monoxime*—Pipette duplicate 5-ml aliquots of a soln. containing 0·025 to 0·25 mg. of alloxan into test-tubes of *ca.* 30 ml. capacity. Pipette 1, 2, 4 and 5 ml. of standard solns. of alloxan monohydrate (0·04 mg. per ml.) in 0·002 *N* sulphuric acid into similar tubes and dilute standards and unknowns to 7 ml. with water. In one tube of each pair (standards and unknowns) put about 0·5 mg. of potassium cyanide and, if the solns. are acid, add anhydrous potassium carbonate until effervescence ceases. Displace the carbon dioxide in the tube by blowing in air and mix. Put 7 ml. of water into

another tube to serve as a blank. Then to each tube add 5 ml. of a mixture of sulphuric acid (1 vol.), phosphoric acid (3 vol.) and water (1 vol.) and 0·5 ml. of 3% diacetyl monoxime soln. Mix, stopper and heat the tubes for exactly 15 min. in a boiling water-bath. After this procedure protect the tubes from light. Cool the tubes in a cold water-bath and evaluate the colour at 470 $m\mu$ in a spectrophotometer; if the tubes are kept in absolute darkness this can be done at any time within 12 hr. Read the samples, both with and without cyanide, against the reagent blank set at zero optical density, and then read the standards to which potassium cyanide was added against the corresponding standards without potassium cyanide set at zero optical density. Plot the optical densities, read for each standard, against the amount (mg.) of alloxan monohydrate, and from the resulting curve read off the amount (mg.) of alloxan equivalent to (*a*) optical density of the sample tube treated with cyanide (*A*) and (*b*) the corresponding sample tube untreated with cyanide (*B*). The amount (mg.) of alloxan monohydrate in the aliquot of soln. heated $= A - B$. This corrects for the small amount of colour formed from alloxan itself in absence of cyanide as well as for the colour due to substances other than alloxan.

Method 5—*Photometric method with o-phenylenediamine*—Mix 5-ml. portions of a sample containing 0·02 to 0·2 mg. of alloxan and also 5-ml. portions of water for the blank, and 2, 4 and 5 ml. of a standard soln. of alloxan monohydrate (0·04 mg. per ml.) in 0·002 *N* sulphuric acid with 1-ml. portions of *o*-phenylenediamine soln. (50 mg. in 100 ml. of glycerol stored in the dark and 5 ml. diluted to 10 ml. with *M*.NaH_2PO_4 within an hour before use). After 20 min. measure the optical densities at 390 $m\mu$ of the standards and samples against the blank set at zero optical density. Plot the optical densities of the standards against the corresponding weights of alloxan and calculate the amount present in the unknowns from these curves. To obtain accurate results, the standards should have approx. the same concn. as the unknowns

Method 6—*Fluorimetric method with o-phenylenediamine*—To 5 ml. of the sample add 0·5 ml. of *o*-phenylenediamine soln. prepared as in method 5 and leave in a dark place. After 1 hr. evaluate the fluorescence in a fluorimeter against the reagent blank in comparison with a standard soln. of approx. the same conc. The amount (mg.) of alloxan in sample $= \frac{\text{reading of sample}}{\text{reading of standard}} \times$ mg. of alloxan in standard. As the colour is unstable to light, the tubes should not be allowed to stand near the path of light from the fluorimeter before taking the readings.

The reactions vary in specificity. The gasometric method estimates alloxan and compounds which dissociate into it (*e.g.*, alloxantin). The only other substance that reacts is ninhydrin, but it reacts much more slowly. In the titrimetric method, an increase in reducing action on treatment with cyanide, only occurs with alloxan, alloxantin and ninhydrin, but dialuric acid (and therefore also alloxantin), uric acid, esculin and ascorbic acid reduce ceric sulphate rapidly whether cyanide is present or not. Glutathione, cysteine and ergothioneine reduce ceric sulphate slowly. In method 3 alloxan and ninhydrin do not reduce phosphotungstic acid in absence of cyanide, but dialuric acid and alloxantin reduce the reagent either in presence or absence of cyanide. Cyanide intensifies the colour produced from alloxantin but not from dialuric acid. Ascorbic acid rapidly reduces phosphotungstic acid, and addition of cyanide doubles the intensity of the resulting blue colour. Weakly acid solns. of glutathione and cysteine reduce phosphotungstic acid rapidly, but addition of cyanide decreases the amount of reduction. Ergothioneine reduces phosphotungstate slowly in absence, and very slowly in presence, of cyanide. Only alloxan and compounds dissociating into it react in method 4. Only alloxan and alloxantin give a yellow colour in method 5, whilst ninhydrin gives a yellow ppt. Alloxan and compounds which dissociate into it give a green fluorescence with *o*-phenylenediamine, whilst ninhydrin, which is itself fluorescent, yields a product with a stronger fluorescence; ascorbic acid gives a product with a blue fluorescence. F. A. R.

Modification of Current Micro-methods for the Estimation of Diodone (3-5-di-iodopyridone-N-acetic Acid). J. A. Barclay and R. A. Kenney (*Biochem. J.*, 1945, **39**, 375–377)—Put 2 ml. of a tungstic acid filtrate of plasma or 2 ml. of diluted urine into a hard glass test-tube and add 1 drop of bromide-bromate soln. (dissolve 10 g. of potassium bromate and 80 g. of anhydrous sodium bromide in 100 ml. of water, add 40 ml. of 85% phosphoric acid, shake and leave for 24 hrs. before use; store in the refrigerator until required). Shake until the solution has an even yellow colour, and immerse in a boiling water-bath for 3 mins. Cool in ice-water and add 2 ml. of a 10% soln. of sodium formate in 80% ethanol from a blow-out pipette in such a manner as to rinse the sides of the tubes. Shake for a few secs., and leave at room temperature for 15 mins. with occasional shaking. Add 2 drops of 85% phosphoric acid, shake until mixed, and add 1 drop of a 50% soln. of potassium iodide in *N* sodium hydroxide. After 10 mins., evaluate the colour in a photoelectric colorimeter, using a filter of Chance blue glass OB 2. Prepare a calibration curve over the range of 0–10 mg. per 100 ml., using pure Diodone. The recovery of Diodone by this method was almost theoretical, whereas most of the published methods gave results up to 20% higher. F. A. R.

Colorimetric Determination of Paraldehyde. W. W. Westfield (*J. Lab. Clin. Med.*, 1945, **30**, 1076–1077)—The *p*-hydroxydiphenyl reaction for paraldehyde in biological fluids (Hitchcock and Nelson, *J. Pharm. Exp. Therap.*, 1943, **79**, 281) has been applied to the determination of acetaldehyde in blood by Stotz (*J. Biol. Chem.*, 1943, **148**, 585). It is shown that the convenient procedure of Stotz may be used with little modification for the determination of paraldehyde in blood.

Method—To 1 ml. of a solution expected to contain between 0·2 and 0·5 μg. of paraldehyde add 0·5 ml. of 5% copper sulphate solution, cool in ice and add 8 ml. of sulphuric acid (sp.gr. 1·84) slowly with constant agitation. Add 0·2 ml. of a 1% solution of *p*-hydroxydiphenyl in 0·5 *N* sodium hydroxide, disperse the precipitate by shaking and leave at room temperature for 1 hr., shaking occasionally. Heat in a boiling water bath for 90 secs., cool and measure the intensity of the resultant colour by means of a photoelectric colorimeter with a 565 mμ filter. The instrument should be adjusted to read 100 for a blank test conducted simultaneously on 1 ml. of water. The reading obtained is correlated with the quantity of paraldehyde by reference to a curve prepared from the data obtained by treating a series of standard solutions

of paraldehyde as described above. Possible sources of error in the use of this reagent have been described by Barker and Summerson (*J. Biol. Chem.*, 1941, **138**, 535); it is emphasised that pure reagents must be used and that to obviate interference from lactic acid the solution must be adequately cooled during the addition of the sulphuric acid. The usual 1 to 10 tungstic acid blood filtrate further diluted tenfold does not give any colour in absence of paraldehyde. Larger amounts of paraldehyde than 25 mg. per 100 ml. may be determined by using more-diluted filtrates. For smaller quantities of paraldehyde than 2 mg. per 100 ml. the distillation procedure of Stotz (*loc. cit.*) must be used, since the method described above is inapplicable to an undiluted 1 to 10 blood filtrate; by this means 0·5 mg. of paraldehyde per 100 ml. can be determined, but smaller quantities are not determinable owing to interference caused by traces of acetaldehyde. Figures are quoted which indicate that satisfactory recoveries of added paraldehyde can be obtained.

J. A.

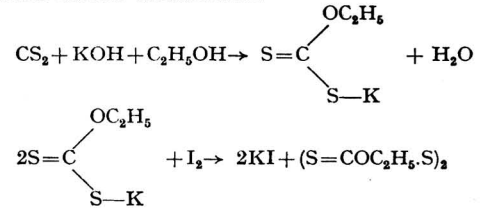
Photoelectric Determination of Blood Thiocyanates without Precipitation of Proteins. W. N. Powell (*J. Lab. Clin. Med.*, 1945, **30**, 1071–1075)—The method using a ferric salt as reagent, originally adapted by Schreiber (*Biochem. Z.*, 1925, **163**, 241) from the procedure of Leared (*Proc. Roy. Soc.*, 1869, **18**, 16), has been widely modified. In the present method precipitation of the proteins is unnecessary.

Method—Dilute 0·5 ml. of serum with 4 ml. of water, mix, add 0·5 ml. of a 5% solution of ferric nitrate, $Fe(NO_3)_3.6H_2O$, containing 25 ml. of conc. nitric acid per litre, slowly with shaking, mix well and leave for 5 min. Determine the intensity of the colour produced in a Klett-Summerson photoelectric colorimeter with a No. 54 (500–570 mμ) filter. A blank prepared in the same manner and at the same time, except that 0·5 ml. diluted nitric acid (1 in 40) is substituted for the ferric nitrate solution, is used and the blank reading subtracted from that of the unknown. The instrument should be calibrated against a pure thiocyanate solution prepared by dissolving 2·0 g. of potassium thiocyanate in a litre of water, standardising it against a silver nitrate solution containing 2·294 g. $AgNO_3$ per litre and diluting so that 1 ml. of thiocyanate solution ≡ 1 ml. of silver nitrate solution. This dilution contains 1 mg. of thiocyanate ion per ml. and for use should be diluted tenfold. Any turbidity produced in the test solution is compensated for by the blank and it is shown that such turbidity does not increase within 1 hr. Recoveries are satisfactory (av. error ± 1%) and a comparison of the method with one employing preliminary precipitation of the proteins indicates equal, if not superior, accuracy for the proposed procedure.

J. A.

Organic

Determination of Carbon Disulphide. R. L. Bishop and E. L. Wallace (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 563–564)—As several methods for the determination of carbon disulphide fail when applied to small amounts in carbon tetrachloride a satisfactory modification of the iodimetric method of Matuszak (*id.*, 1932, **4**, 98) has been worked out. Alcoholic potassium hydroxde is added and converts the disulphide to xanthate which is titrated with iodine soln., using the dead-stop end-point (Pring and Spencer, ANALYST, 1930, **55**, 375). The starch end-point is unsatisfactory in presence of much carbon tetrachloride.



The apparatus used is like that of Wernimont and Hopkinson (*id.*, 1940, **12**, 308–310, and 1943, **15**, 273; ANALYST, 1940, **65**, 534) except that an ordinary 50 ml. burette is used for the iodine soln. Some trouble was experienced with the mercury connections to the platinum electrodes and it is preferred to use 6 in. lengths of platinum wire which can be connected directly to the copper leads.

Reagents—Sodium thiosulphate solns., 0·1 *N* and 0·001 *N*—Dissolve 25 g. of pure crystalline sodium thiosulphate in freshly boiled and cooled distilled water, making 1 litre of soln., and standardise against pure potassium dichromate. In a glass-stoppered bottle this soln. remains constant for several weeks. Prepare 0·001 *N* soln. just before use by diluting the 0·1 *N* soln. with boiled cooled water. *Iodine solns.*, 0·1 *N* and 0·001 *N*—Dissolve 12·7 g. of pure iodine and 20 g. of potassium iodide and dilute to 1 litre. Prepare 0·001 *N* soln. daily by dilution and standardise by titrating a measured vol. of 0·001 *N* sodium thiosulphate to a dead-stop end point at 100 millivolts electrode potential. *Alcoholic potassium hydroxide soln.*—Dissolve 6 g. of pure hydroxide in 100 ml. of absolute alcohol and store in a bottle with a paraffined glass stopper in a refrigerator. *Acetic acid*—Dilute 60 g. of glacial acid to 1 litre.

Method—Take 25 ml. of sample, or less if the carbon disulphide content exceeds 50 p.p.m., in a 250 ml. glass-stoppered conical flask and add 2 ml of alcoholic potassium hydroxide soln. Allow to stand, with occasional shaking, for 30 min. Add 1 drop of phenolphthalein soln., neutralise with acetic acid and add 3 or 4 drops in excess. Add immediately 50 ml. of ethyl alcohol (described as 3A specially denatured), connect the flask to the titration apparatus and titrate with 0·001 *N* iodine. Treat a blank similarly, omitting only the sample. During the titration the galvanometer at first remains undeflected, then as the end-point is approached each drop of iodine soln. causes a temporary deflection of about 3 scale divisions. At the true end-point a permanent deflection of 3–5 divisions occurs, but the titration may be made quicker by choosing an arbitrary deflection of 10 or 15 divisions as the end-point and finishing all titrations at the same point.

1 ml. of 0·001 *N* iodine ≡ 76 μg. of carbon disulphide.

L. A. D.

Chlorite Holocellulose, its Fractionation and Bearing on Summative Wood Analysis and on Studies of the Hemicelluloses. L. E. Wise, M. Murphy and A. A. D'Addieco (*Paper Trade J.*, 1946, **122**, Jan. 10, T.A.P.P.I. Sect., 11–19)—With coniferous woods, the sum of the % of ash, acetyl, extractives, lignin, hemicelluloses and α-cellulose is 98–100, and this is regarded as satisfactory. With hardwoods, however, the summation is less satisfactory (less than 98%); reasons for these divergencies are discussed, together with the possibility of the existence of balancing errors.

Errors may arise from ash in the hemicellulose fraction, the accurate determination of which in hardwoods presents difficulty; from the retention of small amounts of lignin in the hemicellulose (which would tend to balance the ash error); and from the discrepancy between the uronic acid contents as determined on the wood and on the hemicellulose, the latter being fictitiously high owing to catalytic liberation of carbon dioxide during the hydrolysis to glucose. The chief value of the chlorite method for the quantitative isolation of holocellulose is as a research tool, rather than for analytical purposes.

J. G.

Direct Oxidation Tests on Soap. E. J. Better and A. Davidsohn (*Oil. and Soap*, 1945, **22**, 325–327)—A modification of the simplified method of Lea (Rancidity in Edible Fats, Food Investigation Special Report, No. 46, p. 107) is proposed for determining oxidative rancidity in soaps and cosmetics. *Method*—Weigh 0·5 g. to 1·0 g. of soap or 1·0 g. to 3·0 g. of cosmetic emulsion, depending on the expected peroxide value and the water content, into a 40 ml. test tube. Add 1·0 g. of potassium iodide and 20 ml. of a 2 : 1 mixture of glacial acetic acid and chloroform. Boil for about 10 sec. until the soap is dissolved and continue boiling for exactly 30 sec. longer. Cool the tube under running water, dilute the contents with 30 ml. of freshly-boiled water, and titrate with 0·002 *N* thiosulphate. Conduct a blank test omitting the test material. Calculate the peroxide value by subtracting the blank titration from the test titration and dividing by the weight of test material employed.*

The following tentative conclusions are drawn. The peroxide value of a soap is less than that of the fatty matter from which it is made, but depends on the composition of this. It rises rapidly on exposure of the soap to air or light, and on heating in the dark. Free alkali protects soap from oxidation; unsaponified oil promotes it. Fat antioxidants protect soap.

Inorganic

Determination of Bases by Means of Calomel. Application to Lime in Commercial Calcium Arsenate. L. N. Markwood, H. D. Mann and R. H. Carter (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 570–571)—Bases, except those, like ammonia, which form complex mercury compounds, react with excess of calomel and liberate chloride ion quantitatively. The reaction is probably complicated but may be represented as follows:

$$Ca(OH)_2 + Hg_2Cl_2 \rightarrow Hg_2O + H_2O + CaCl_2$$
$$Hg_2O \rightarrow HgO + Hg.$$

Mercuric ions are found in the solution, probably because the mercuric oxide dissolves in presence of soluble chlorides, and they are removed by adding zinc dust. The chloride ion may then be determined by direct titration with silver nitrate or by the Volhard method. Tests on pure lime gave good results, and the following method was evolved for the determination of free lime in commercial calcium arsenate. The arsenate itself hydrolysis and produces some additional lime, but if the sample weight and liquid volume mentioned below are used the results agree with those found by the acidimetric method.

Weigh 1 g. of sample into a 250 ml. glass-stoppered conical flask and mix well with 3 g. of calomel. Add 200 ml. of freshly boiled and cooled distilled water and agitate mechanically for ½ hr. (With lime alone the reaction is very much quicker than when the arsenate is present.) Filter twice through a dry paper, stir 3 g. of zinc dust into the filtrate, filter and collect a 150 ml. aliquot portion. Titrate by the Volhard method, as silver arsenate would be precipitated in neutral soln.

1 ml. of 0·1 *N* $AgNO_3 \equiv 3{\cdot}705$ mg. of $Ca(OH)_2$.

Calcium carbonate has a negligible effect on the result, while magnesium hydroxide reacts as lime. The results of the new method agree well with those of the Smith and Hendricks acidimetric method for all types of commercial calcium arsenate. The end-point is sharp and unaffected by the presence of dyes in the sample.

The reaction may be employed to determine other bases, and, by using excess of base, to determine calomel.

L. A. D.

Determination of Small Amounts of Silica. M. F. Adams (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 542–543)—The determination of small quantities of silica in technically important materials is often required, *e.g.*, in alumina produced from clay when the silica content may be about 0·03%. As the amounts are too low for the standard gravimetric method the colorimetric method based on the yellow colour produced with ammonium molybdate is considered. This method is satisfactory in many special cases, but is not considered generally applicable without special precautions. Errors may be caused by the tendency of the colloidal silica to polymerise to a form in which it does not give a colour with molybdate; by the presence of iron or fluorine, which interfere with the formation of a complex; by the presence of phosphate or arsenate, which give similar colours; and by many neutral salts which influence the colour if they are present in relatively large concentration. The method as now described is of general application because in it the silica is first isolated and then dissolved in such a way as to make a soln. in which it has a definite and suitable molecular state.

Method.—Digest a sample containing about 1 mg. of silica in a platinum crucible with not more than twice as much perchloric acid as is equivalent to the basic constituents. (If more than 10 mg.-equivalents of cations are present per mg. of silica, sulphuric acid must be used, as described below.) Heat to fuming for 5 min., cool, dilute to 10 ml. and filter through a 5 cm. No. 40 Whatman paper. Wash with cold water to remove the perchloric acid. Wipe the sides of the crucible with the paper and then burn it off in the tilted crucible. Place 0·5 g. of sodium carbonate on the residue, fuse, turn the crucible upright so that the melt spreads over the bottom and quench in water. Dissolve by heating with 5 ml. of water and transfer the soln. to a 50 ml. graduated flask. Add a drop of phenolphthalein soln. and titrate with approx. 10 *N* sulphuric acid from a micro-burette. Add a further equal amount of acid and 0·5 ml. in excess. Add 2 ml. of ammonium molybdate soln. (10%), dilute to 50 ml. and take colorimetric readings after 5 min. The colour is constant for more than 1 hour. Colorimetric comparison may conveniently be with a buffered soln. of potassium chromate (Swank and Mellon, *id.*, 1934, **6**, 348,

* The blank test is not mentioned in the original paper but its inclusion is presumably taken for granted, for it is part of the Lea method.

—Abstractor.

ANALYST, 1934, **59**, 773) using a soln. of 0·602 g. of chromate per litre as the test soln. is 0·1 *N* in acid. The comparison solns. should be equivalent to <40 mg. of silica per litre (preferably 5 to 20 mg.), and should contain 0·5 g. of borax. Blank determinations on the reagents are necessary and it is pointed out that once-distilled water may not be free from silica.

If the cation content exceeds 5 mg.-equivalents per sample or 10 per mg. of silica, evaporate almost to dryness with sulphuric acid and extract the soluble salts with ammonium chloride soln. (4%). In presence of substances such as nickel and chromium whose sulphates are difficult to dissolve a sufficiently small sample must be taken to permit the use of perchloric acid, with some sacrifice in precision. High results occur when samples containing both titanium and phosphate are treated with perchloric acid; the trouble is avoided by using a mixture of perchloric and sulphuric acids. When sulphuric acid is used, any lead sulphate must be removed before evaporation, to avoid damage to the crucible. Barium sulphate interferes with the colorimetric comparison and must be removed.

Calcined alumina is fused with soda and boric acid and treated with sulphuric acid; aluminium metal is dissolved in sodium hydroxide in presence of hydrogen peroxide. If the sample contains appreciable amounts of fluorine the standard procedure as used in the gravimetric method is employed to remove it (Hillebrand and Lundell, "*Applied Inorganic Analysis*," John Wiley, New York, 1929).

The precision of the method is about ± 2% on 0·5 mg. of silica and ± 10 μg. on smaller amounts.

L. A. D.

Colorimetric Determination of Molybdenum in Iron and Steel. M. Kapron and P. L. Hehman (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 573–576)—By adding water-soluble solvents of low volatility of the glycol ether type to molybdenum-containing solns. stable colours may be developed with thiocyanate which are suitable for absorptiometric measurement. The two solvents considered most satisfactory are butyl Cellosolve (ethylene glycol monobutyl ether) and butyl Carbitol (diethylene glycol monobutyl ether).

Reagents.—Potassium thiocyanate soln.—50 g. of pure potassium thiocyanate made up to 1 litre with distilled water. *Stannous chloride soln.*—Dissolve 350 g. of stannous chloride dihydrate in 250 ml. of conc. hydrochloric acid, without heating to more than 50° C. Cool, add 250 ml. of water and dilute to 1 litre with diluted hydrochloric acid (1 + 1). If clear, add 3 to 5 g. of pure tin and leave for 24 hr. If not clear, leave for 24 hr., filter and add the tin. *Phosphoric-perchloric acid mixture.*—To 500 ml. of water add 333 ml. of phosphoric acid (85%) and 167 ml. of perchloric acid (70–72%). Mix and cool.

Method—A. Carbon and low-alloy steels—Dissolve 1 g. in 5 ml. of diluted hydrochloric acid (1 + 1) and 15 ml. of perchloric acid (70–72%) in a covered 300 ml. tall-form beaker. If the steel contains more than 0·5% of carbon or 0·05% of sulphur dissolve the sample in 20 ml. of diluted hydrochloric acid (1 + 1), oxidise by adding conc. nitric acid drop by drop, cool slightly and add 15 ml. of perchloric acid. Heat gently to dense fuming and continue the fuming for 5 to 7 min. Cool, add 20 ml. of water and boil for 3 to 5 min. Cool and dilute to 200 ml. in a graduated flask. Pipette 25 ml. into a 100 ml. graduated flask and add from burettes, with swirling, in the given order, 15 ml. of solvent, 5 ml. of potassium thiocyanate soln. and 5 ml. of stannous chloride soln. Dilute to the mark immediately, mix, leave for 10 min. and make the absorption measurement, using a filter with maximum transmission at about 470 mμ.

B. High chromium and stainless steels—Dissolve 1 g. in 5 ml. of diluted hydrochloric acid (1 + 1) and 20 ml. of perchloric acid (70–72%) in a 500 ml. conical flask or a covered 300 ml. tall-form beaker. Heat gently to dense fuming and fume for 5 to 7 min. Cool slightly and add 1 to 2 ml. of conc. hydrochloric acid while swirling the vessel. Heat again until dense fumes are evolved, and repeat the addition of hydrochloric acid and heating until yellow-orange fumes of chromyl chloride cease to be evolved. It may be necessary to add more perchloric acid to prevent crystallisation. Cool the soln., add 20 ml. of water and complete as under *A* above. If the steel dissolves with difficulty in the above acid mixture use the procedure for high carbon or sulphur steels under *A*. Sodium chloride may be used instead of hydrochloric acid in the removal of the bulk of the chromium, but the higher salt concentration may cause troublesome crystallisation.

C. Tungsten steels—Dissolve 0·5 g. in 20 ml. of phosphoric-perchloric acid mixture in a covered 300 ml. tall-form beaker. Heat gently until the sample is completely dissolved and the soln. begins to fume gently (175° to 190° C. approx.). Cool slightly, add 10 ml. of perchloric acid (70–72%) and heat to dense fuming. Remove much of the chromium by repeated dropwise addition of 2 to 3 ml of conc. hydrochloric acid, alternating with heating until dense fumes of perchloric acid are evolved. Cool slightly before adding each portion of hydrochloric acid, and replace the perchloric acid lost by cooling and adding 5 ml. portions. Then heat to fuming for 3 to 4 min. to remove all the hydrochloric acid, cool, dilute to about 100 ml., boil for 2 to 4 min., cool and dilute exactly to 200 ml. Pipette 25 ml. into a 100 ml. graduated flask, add 10 ml. of diluted sulphuric acid (1 + 1), cool, develop the colour as under *A* and leave for 20 min. before measurement. Care must be taken to prevent pptn. of the tungsten during the analysis. The use of more perchloric acid than specified should be avoided. An excessive loss of phosphoric acid during fuming may cause turbidity of the final soln., which can be prevented by adding 10 ml. of 20% ammonium citrate or tartrate soln. after the sulphuric acid. Addition of extra sulphuric acid also helps to prevent pptn. If desired, 5 ml. of a sulphuric-perchloric acid mixture may be substituted for the final 5 ml. of perchloric acid before fuming. The temp. of the soln. is thus raised to 203° to 205° C. and complete oxidation is assured.

D. Cast iron—Dissolve 1 g. in 25 ml. of diluted hydrochloric acid (1 + 1) in a covered 300 ml. tall-form beaker. Add conc. nitric acid drop by drop and then add 10 ml. more of nitric acid and heat for 3 to 5 min. to complete the oxidation. Cool, filter through a No. 41 Whatman paper or its equivalent and wash the paper and residue alternately with hot diluted hydrochloric acid (1 + 1) and hot water, 6 times or more if necessary. Wash finally with hot water until the filtrate is clear. Evaporate the filtrate to 15 ml., cool, add 15 ml. of perchloric acid (70–72%) and mix thoroughly. Boil until dense fumes are evolved for 5 to 7 min. Cool and proceed as under *A*.

Notes—The 1 g. samples are taken so that other elements (nickel, chromium, manganese) may also be determined. The amounts specified are chosen for a working range of 0 to 0·3% of molybdenum. Higher concentrations may be dealt with either by taking a smaller sample and making it up to 1 g. with molybdenum-free steel, or by using a smaller sample or more dilute solns. and making a new calibration curve. If, for example, 0·125 g. samples are taken, 5 ml. of diluted hydrochloric acid (1 + 1) and 10 ml. of perchloric acid are used. If the solvent is added after the thiocyanate soln. no effect is observed, but if it is added after the stannous chloride soln. the colour fades somewhat. The precision and accuracy of the method are satisfactory. The effect of some other elements is as follows. Aluminium, vanadium, titanium and nickel in the amounts usually found have a negligible effect. Ten per cent. of chromium causes an error of about 0·06% of molybdenum, but if as much as possible is volatilised as chromyl chloride the error will be less than 0·002%. If the sample contains less than 1% of chromium no steps to remove it are required. More than 0·3% of copper causes precipitation of cuprous thiocyanate. This turbidity may be prevented by adding, as 10% soln., 0·1 g. of gum-arabic per 0·1% of copper to the 25 ml. aliquot portion before adding other reagents. Photometric readings are then most accurate 5 to 15 min. after colour development. Up to 5% of cobalt does not interfere, but 15% leads to an error of + 0·011% of molybdenum. Using the procedure under *C* above, up to 20% of tungsten did not cause a difference from the accepted value of the molybdenum exceeding 0·009%. In general the colours are stable for 24 hr. L. A. D.

Determination of Zinc in Corrosion-Resistant Steels. L. G. Bricker, S. Weinberg and K. L. Proctor (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 661–663)—Difficulties are encountered in the determination of small amounts of zinc in corrosion-resistant alloy steels and a dithizone extraction method has therefore been developed in which the final measurement is made in a spectrophotometer or absorptiometer. An account of some of the procedures which were examined and rejected is included in the paper.

Reagents—Zinc-free distilled water—Redistil ordinary distilled water in Pyrex apparatus. This water is used throughout the method and for the reagents. *Standard zinc chloride soln.*—Dissolve 1·000 g. of pure zinc in 10 ml. of hydrochloric acid (1 + 1), cool and dilute to 1 litre. Dilute a portion of this soln. so that 1 ml. of the final soln. contains 0·01 mg. of zinc. *Citric acid soln.*—Dissolve 20 g. of the pure acid in 100 ml. of water. *Hydrochloric acid 0·1 N*—Dilute 9 ml. of the conc. acid to 1 litre. *Ammonium hydroxide 0·1 N*—Dilute 8 ml. of ammonia soln. (sp.gr. 0·90) to 1 litre. *Methyl orange soln.*—Dissolve 0·1 g. in 100 ml. of water. *Sodium thiosulphate soln.*—Dissolve 15 g. of the pure salt in 100 ml. of water. *Phthalate buffer soln.* (*p*H 6·0)—Add 79 ml. of 0·1 *N* sodium hydroxide to 2·000 g. of pure potassium hydrogen phthalate and dilute to 100 ml. exactly. *Chloroform - dithizone soln.*—Dissolve 0·010 g. of diphenylthiocarbazone in 100 ml. of pure chloroform and store in a glass-stoppered brown bottle. *Carbon tetrachloride - dithizone soln.*—Dissolve 0·010 g. in 500 ml. of pure carbon tetrachloride. Store as above. *Sodium sulphide soln.*—Dissolve 0·05 g. of pure sodium sulphide in 100 ml. of water.

Method—Dissolve 0·05 g. of sample, in a 50-ml. conical flask, in 3 ml. of conc. hydrochloric acid and 2 ml. of conc. nitric acid. Add 3 ml. of perchloric acid (70%) and 1 drop of hydrofluoric acid (48%). Evaporate until perchloric acid vapour condenses in the neck of the flask and all the chromium is oxidised. Pass dry hydrogen chloride (produced by allowing conc. hydrochloric acid to drop from a tap funnel into a suction flask containing conc. sulphuric acid) over the surface of the hot soln. to volatilize chromium and tin. Cool and dilute the soln. with 10 ml. of water. Add 5 ml. of citric acid soln., neutralise with conc. ammonia soln. (litmus paper) and add 2 or 3 drops in excess (*p*H 8 to 8·5). Cool, transfer to a 125-ml. separating funnel, add 10 ml. of chloroform-dithizone soln. and shake for 20 sec. Run the chloroform layer into another funnel. Repeat the extraction of the aqueous soln. with successive 5 ml. portions until the dithizone is greenish-purple after shaking. If the copper content of the sample is less than about 0·5% 20 to 30 ml. of chloroform-dithizone soln. should suffice. Discard the aqueous layer. Add 10 ml. of 0·1 *N* hydrochloric acid to the combined chloroform extracts and mix for at least 1 min. Withdraw and discard the chloroform. Extract the soln. twice with 5 ml. portions of chloroform to remove any remaining dithizone. Add 1 drop of methyl orange soln. to the acid soln. and add 0·1 *N* ammonium hydroxide until the soln. is yellow. Add 1 ml. of sodium thiosulphate soln. and 5 ml. of phthalate buffer soln. and then add exactly 25 ml. of carbon tetrachloride-dithizone soln., shake for 1 min. and siphon off the aqueous layer. Add 25 ml. of sodium sulphide soln., shake for 15 sec. and siphon off the aqueous layer. Shake with 25 ml. of sodium sulphide soln., drain a portion of the carbon tetrachloride layer into the absorptiometer cell and measure, using a filter with maximum transmission at 520 mμ. Blank determinations on the reagents should be made with every set of determinations, preferably using a sample of zinc-free steel. With great cleanliness and care (all glass ware should be washed with conc. hydrochloric acid and rinsed with water) the blank can be kept below 2 μg. of zinc. Reagents, if necessary, should be made slightly ammoniacal, extracted with chloroform-dithizone soln. until the dithizone remains green, and filtered. Acids should be taken from fresh bottles and redistilled if they cause high blanks. Standards for calibrating the instrument are made by adding 10 ml. of water, 1 ml. of sodium acetate soln. (25%) and measured amounts of standard zinc soln. to clean separating funnels, adding 25 ml. of carbon tetrachloride-dithizone soln. and completing as above.

If more than 0·5% of copper is present, dissolve the sample in 5 ml. of conc. hydrochloric acid and evaporate to 1 ml. Add 15 ml. of water and pass hydrogen sulphide over the surface of the soln., swirling occasionally, until it is saturated. Heat to boiling, keep warm for 15 min., filter into a 50 ml. conical flask, washing with a small amount of water, add the perchloric acid and proceed as above.

L. A. D.

Electrogravimetric Determination of Copper in Copper-Base and Tin-Base Alloys by Controlled Potential Electrolysis. J. J. Lingane (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 640–642)—Lingane recently described apparatus (*id.*, 1945, **17**, 332) which automatically keeps the potential of an electrode at any desired constant value during an electrolysis and so makes the application

of Sand's graded potential procedures more convenient. In the procedure given copper is deposited from a slightly acid tartrate soln. The results are as accurate as those of classical electrolytic methods.

Apparatus—The cell is a 250 ml. beaker containing a 5 cm. × 5 cm. diam. platinum gauze cathode which surrounds a similar 5 cm. × 2·5 cm. diam. cylindrical anode. A mechanical stirrer is used whose glass shaft passes down through the centre of the anode and carries a U-form paddle which sweeps the annular space between anode and cathode. A 6 mm. tube filled with a gel of 3% of agar in sat. potassium chloride soln. is used as a salt bridge to a saturated calomel electrode (S.C.E.). The tip of the bridge is close outside the cathode cylinder and near its middle.

Method—Dissolve 0·5 to 2 g. of sample in 8 ml. of 12 *N* hydrochloric acid, adding 2 ml. of 16 *N* nitric acid in small portions. Boil very gently for a minute or two and add 100 ml. of 0·1 *M* sodium tartrate, 1 g. of urea and 10 ml. of 5 *N* sodium hydroxide. Dilute to 200 ml., add 1 to 2 g. of hydroxylamine hydrochloride as anodic depolariser and electrolyse with a cathode potential of — 0·36 volt *versus* the S.C.E. If much lead is present (> 50 mg.) a coarse white ppt. of lead hydrogen tartrate may form, and the solution must be filtered before diluting to 200 ml. Electrolysis is usually complete in 1 hr., when the current, initially about 3 amp., has dropped to less than 0·02 amp. Stop the electrolysis by lowering the beaker quickly without disconnecting the electrodes. Wash the cathode quickly in water, dip it in 2 baths of pure acetone, dry for 3 min. at 70° C., cool for 20 min. and weigh.

Relatively large amounts of tin, antimony, lead and zinc, and small amounts of iron, nickel, arsenic and phosphorus do not interfere. The method should also be successful in presence of manganese, aluminium, chromium, cobalt, vanadium, uranium, cadmium and other metals having reduction potentials more negative than that of copper. Bismuth, gold, mercury and metals of the platinum group will interfere. Traces of silver too small to be pptd. as chloride will be deposited, but larger amounts may be removed by filtering off the chloride. The theoretical background of all the separations, and some particulars of unsuccessful attempts to separate bismuth and copper are given. It has been found that attempts to plate out the lead at — 0·56 to —0·80 volt after removing the copper give low values, apparently owing to solution of the lead while washing the deposit. This second analysis is therefore not recommended except for approximate results. L. A. D.

Determination of Small Quantities of Phosphorus in Steel. C. Rainbow (*Nature*, 1946, **157**, 268)—The application to the determination of phosphorus in steel of Berenblum and Chain's modification (*Biochem. J.*, 1938, **32**, 295) of Fiske and Subbarow's method (*J. Biol. Chem.*, 1925, **66**, 375) for the determination of phosphates is described. It is claimed that the procedure is rapid and that it requires only small samples (0·05 to 0·2 g.) of the substance under examination.

Method—To 5 ml. of the solution of the steel (expected to contain about 1 μg. of phosphorus per ml.) add 0·5 ml. of 10 *N* sulphuric acid, 2 ml. of distilled water and then 2·5 ml. of 5% ammonium molybdate solution. Add 10 ml. of *iso*-amyl alcohol ("pure for milk testing") and extract by shaking for 2 min. Allow to separate, discard the aqueous layer and wash the solvent extract three times with 5 ml. of *N* sulphuric acid, discarding each separate aqueous layer. Add 15 ml. of the dilute stannous chloride solution [ABSTRACTOR'S NOTE:—Berenblum and Chain (*loc. cit.*) specify this diluted solution as that obtained by freshly diluting a 35% solution of stannous chloride, $SnCl_2.2H_2O$, in concentrated hydrochloric acid 200-fold with *N* sulphuric acid], shake for 30 secs., discard the aqueous layer and wash the solvent three times each with 5 ml. of the diluted stannous chloride solution, discarding each separated aqueous layer. Dilute the solvent solution to 10 ml. with absolute alcohol and compare the blue colour with that produced by submitting standard solutions of phosphorus to the same procedure.

Preparation of Steels for Test—Dissolve 0·05 to 0·2 g. of steel (according to the expected phosphorus content) in 2·5 ml. of a mixture of equal parts of concentrated nitric acid and water contained in a covered beaker, warming if necessary. Add 2·5 ml. of 2·5% potassium permanganate solution, boil for a few min., cool, add 3% ferrous sulphate solution until a clear solution is obtained and a very slight excess is present and dilute to 50 ml. Use 5 ml. of this solution for the test. For certain special steels it may be necessary to use *aqua regia* to dissolve the sample, in which event all free chlorine should be removed by warming on the steam bath, and extra potassium permanganate will be required to produce a permanent precipitate.

Brass and Bronze—In absence of tin, dissolve the sample in nitric acid, boil to remove brown fumes and dilute to volume. If tin is present, dissolve the sample in *aqua regia*, evaporate almost to dryness on the water bath and dissolve the residue in hydrochloric acid. Dilute to volume, adding, if necessary, just sufficient hydrochloric acid to keep the tin in solution; an excess of hydrochloric acid must not be present, but any precipitation of tin is accompanied by loss of phosphorus. J. A.

Gas Analysis

Determination of Ether Vapour in Gaseous Mixtures containing Ethylene and Determination of Dissolved Ethylene in Ethyl Ether. C. C. Meloche and W. G. Frederick (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 795–796)—Since ethylene is formed in the manufacture of ether, a good method for determining dissolved ethylene is desirable in a complete examination of ether for all possible impurities. It was found that a method could be based upon the formation of a compound of low vapour pressure containing ether of crystallisation, in particular upon the compound formed with hydroferrocyanic acid. To prepare hydroferrocyanic acid dissolve 56 g. of potassium ferrocyanide crystals in 100 ml. of hot water, add 300 g. of crushed ice together with a piece of solid carbon dioxide, add 400 ml. of conc. hydrochloric acid and immediately collect the white ppt. on a Buchner funnel containing some dry ice, keeping the liquid at 0° C. during the operation. Discard the filtrate and add the entire yield of free unwashed acid to 150 ml. of conc. sulphuric acid under a well ventilated hood. A stable addition compoud with sulphuric acid is formed, the mixture becomes warm, evolves hydrochloric acid and possibly some carbon monoxide and part of the solid dissolves. After releasing gas by shaking and suction add 100 ml. of water and stir the mixture well. The fine white ppt. that forms remains in suspension. Store the mixture in a glass-stoppered bottle and allow it to

stand at room temp. Decant about 100 ml. of this suspension into a small single Hempel gas pipette leaving any coarse crystals behind. If the tubing of the pipette other than the capillary tubing is of large diameter (*e.g.*, 12 mm.) no clogging will occur, and the reagent is stable in the pipette without precautions to exclude oxygen. Expts. were made with gaseous mixtures containing ether, ethylene and hydrogen. Ether vapour was determined by absorption in the hydroferrocyanic acid reagent in the Hempel pipette, the reagent being shaken five times with the gaseous mixture, ethylene was determined by absorption in 100% sulphuric acid containing silver sulphate and nickel sulphate as catalysts and hydrogen was found by difference. Mercury was used as the retaining liquid. It was found that once the reagent had become saturated with the other gases it was specific for ether vapour. A measured vol. of oxygen shaken with the reagent was recovered quantitatively. After standing in the pipette for several weeks in contact with air the reagent showed little if any visible change and was still available for the quantitative absorption of ether vapour. For ether vapour concns. of less than 10% the reagent may be used continuously, but for higher concns. it must be allowed to stand in the pipette for a few hours between analyses if quantitative absorption is to be effected. The partial pressure of ether vapour in mixtures of ethylene and nitrogen saturated with ether vapour at low temp. (*e.g.*, 0° C.), when determined by means of this reagent, agreed with the published values and the proportion of ethylene in the residual gas agreed with the value obtained in the original gaseous mixture before saturation with ether. Since the reagent is more effective for smaller concns. of ether vapour there should be no difficulty in determining small amounts (*e.g.*, a few tenths of 1%) of ether vapour in ethylene. The authors made no determinations of amounts of ether vapour less than 6·6% v/v, but there were indications that 0·5 to 3% of ether vapour is satisfactorily absorbed. Solns containing ether and known amounts of dissolved ethylene and nitrogen were analysed by the boiling-out method of Meloche and Frederick (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 796). The ether vapour was removed from the recovered gases by the hydroferrocyanic acid reagent and the ethylene was determined by the method previously mentioned. With moderate partial pressures of ethylene (55% v/v or less) in the dissolved gases recovered the determination of ethylene is quantitative. The removal of ether vapour in this manner thus makes possible the subsequent determination of ethylene by the customary methods and also the determination of total dissolved gas if ethylene is present.

A. O. J.

Physical Methods, Apparatus, etc.

Instrumentation Studies. L11. Penetration of Papers by Water Vapour. VI. Institute of Paper Chemistry (*Paper Trade J.*, 1946, **122**, Jan. 3, *T.A.P.P.I. Sect.*, 1–10)—The test specimen is sealed between a space of const. high relative humidity (R.H.), and a narrow space of variable R.H. communicating with a large space (*e.g.*, a room) of known and constant R.H., through a diffusion resistance, which may be adjusted so as to obtain a standard R.H. in the small space; this is measured by a small electric hygrometer. The water-vapour permeability is expressed in terms of the setting of the diffusion resistance. Alternatively, the diffusion resistance may be set at infinity and the rate of increase of the R.H. in the narrow space (due to permeation of water vapour through the sample) measured on the hydrometer. For a given paper there is satisfactory correlation between these methods and the usual gravimetric methods, but the former are far more rapid. J. G.

Water Resistance of Shipping Containers. Anon. (*Paper Trade J.*, 1945, **121**, Dec. 13, *T.A.P.P.I. Sect.*, 225–226)—T.A.P.P.I. Suggested Method T 805 sm–45. The containers are placed on a false floor in an enclosed cabinet supplied with water showers at 73 ± 2° F.; the water is controlled thermostatically in a reservoir beneath the false floor, and re-circulated back to the showers by means of a pump. The nozzles are 6 ft. above the containers and are spaced so that 2·5 ± 0·5 gal. of water per hr. fall uniformly on each sq. ft. of floor area. The normal operating time is 168 hr. The standard tests for packagings are applied before and after the treatment, and the changes in values and general condition of the containers are noted.

J. G.

Determination of the Air Resistance of Paper. Anon. (*Paper Trade J.*, 1945, **121**, Dec. 6, *T.A.P.P.I. Sect.*, 223–224)—T.A.P.P.I. Tentative Standard, T 460 m–44. The apparatus consists of an outer vertical cylinder, partly filled with a light spindle oil, and an inner cylinder which can slide freely up and down inside it on guide tracks. The conditioned paper sample is clamped so as to close the top of the inner cylinder, the bottom of which is open. The test is made by timing the rate of fall of the inner cylinder through an appropriate height, *i.e.*, depending on the air-resistance of the paper. The greater this resistance the slower is the rate of fall. An average of 10 tests, 5 with each side of the paper uppermost, is taken. The average time (sec.) for 100 ml. of air to be displaced through 1 sq. metre of paper is recorded. The reproducibility varies from 5 to 10% for 40 to 300 sec., respectively. J. G.

Reviews

KINGZETT'S CHEMICAL ENCYCLOPAEDIA. Revised and edited by RALPH K. STRONG, Ph.D. Pp. x + 1092. Seventh Edition. London: Baillière, Tindall & Cox. 1945. Price 45s.

Kingzett has become an important reference book, always to be kept in a readily accessible place. It was originally written in 1919 by the late C.T. Kingzett, F.I.C., and five editions were published before his death. Subsequently the sixth and seventh editions have been revised and edited by Dr. Strong of the United States, and we now have a volume containing three times the number of pages in the first edition.

The subject matter of a chemical encyclopaedia is so vast that it is impossible for one volume to be exhaustive; hence it is not to be expected that everything sought for will be found here. In addition it is difficult for an editor to decide how much or how little to include on any one topic. The fact that two editors, one British and one American, have been concerned with the subject matter is evident from the use, sometimes in succeeding paragraphs, of different names or spellings for the same substance; and it cannot always be inferred whether "this country" refers to Britain or the United States.

The preface to the new edition states that it was prepared under the severe handicap of war conditions, but in spite of this over 150 changes have been made. These changes are mainly additions, and it does not seem that any attempt has been made to delete out-of-date or valueless information. Surely the time has come to omit from the article on Electricity "An accident has been reported to the effect that a basket full of cats dropped into ordinary illuminating gas generated so much electricity as to cause an explosion thereof in contact with air," and from the note on Rum that it is "credited as having a greater food value than any other spirit."

Several errors have been noted, the most important being the definition of the Polenske Value of Fats, which is given as the obsolete Polenske Difference Value (1907) instead of the Polenske New Butter Value (1904) still current. Dulcin is referred to without further description under Saccharin and obviously refers to *p*-phenetylcarbamide; in the headings, however, dulcin is given as a synonym for dulcitol. Honey does not contain "from 61 to 75% of glucose (grape sugar, dextrose)" and tetryl is not tetranitroaniline. The refractive index of Pollopas is given as "between 1·54 and 1·9."!

These and other errors, mostly of a minor character, are of small significance when compared with the wealth of information collected within nearly three inches of pages. All chemists need at times to consult a reference book covering the whole field of chemistry in order to refresh their memory or to obtain information on new materials; such a need, in handy and compact form, is filled by Kingzett. J. R. NICHOLLS

THE CHEMISTRY OF CELLULOSE. By EMIL HEUSER. Pp. iv + 660. New York: John Wiley & Sons, Inc. Price $7.50. London: Chapman & Hall, Ltd. Price 32s.

In the opinion of the reviewer the author is to be congratulated on this compendium and digest of the literature (up to the autumn of 1943) on the chemistry of cellulose. This literature is very diffuse and scattered over many inaccessible and technical journals. The modern views on cellulose have been developed during the inter-war years, not without considerable controversy, and much of the original work has been accomplished in laboratories connected with industries using cellulose in one or more of its numerous forms. A really critical digest of the literature and an orderly presentation of the chemistry of cellulose will, therefore, be welcome to the steadily increasing number of graduates who are entering the cellulose field. To such the book can be recommended; to the analyst the importance attached throughout to the concept "degree of polymerisation" will be a help with the interpretation of well-known analytical procedures.

The lengthy chapter on the microscopic and sub-microscopic structure of the cellulose fibre is well illustrated, and here the author's critical faculty has had full play, with most helpful results.

The following list of the headings of the other chapters, all dealing with the physico-organic chemistry of the subject, indicates the wide scope and chemical as distinct from technical character of the book:—Reactions of cellulose with water, with aqueous alkalies and with organic bases, ammonia and concentrated salt solutions; action of Cuprammonium Hydroxide on Cellulose; Cellulose Esters, Xanthates and Ethers; Oxidation of Cellulose, its decomposition by Acids and by Heat and Biological Processes; the Chain Structure and Molecular Weight of Cellulose. A good index, both author and subject, adds to the usefulness of the book, the excessive weight of which, composed as it is of much mineral matter and little cellulose, makes it in one sense burdensome to the reader. ROBERT H. PICKARD

QUANTITATIVE ORGANIC MICROANALYSIS BASED ON THE METHODS OF FRITZ PREGL. By JULIUS GRANT, M.Sc., Ph.D., F.R.I.C. Pp. vii + 238. Fourth English Edition. London: J. & A. Churchill, Ltd. 1945. Price 21s.

When, in 1917, Pregl published *Die quantitative organische Mikroanalyse* describing his own methods, he did not find it necessary to include a single reference to the literature. Although subsequent developments necessitated a change in this respect, the book and its English translations remained until after Pregl's death, in 1928, a personal account of this investigator's own field. In 1935 the German text was completely rewritten by H. Roth, who included the findings of other microanalytical chemists: an English translation appeared in 1937 but, like its original, this did not present adequately non-German work. For the first time we now have a thoroughly English view of the subject, paying due respect to foreign contributions, and it seems unfair to Dr. Grant to call this "the fourth English edition"—it should be regarded as *his* first edition. The new presentation omits most of Pregl's digressions, but the author wisely retains detailed descriptions of experimental procedures, attention to which is essential for successful organic microanalysis. The systematic treatment of each determination and the competent indexing are two important improvements.

The book opens with a chapter on microchemical balances of various manufacturers, their selection, erection and use: in considering possible weighing errors, the valuable paper by A. H. Corwin (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 258) on the Kuhlmann balance might well have been noted. After a short, but adequate, description of general microchemical technique there follows the largest and most important section, 96 pages on the determination of the elements. This would be improved by division into chapters, for example: carbon and hydrogen, nitrogen by Dumas and Kjeldahl methods, the halogens, sulphur, phosphorus and arsenic, metals. Similarly, separate chapters would be preferable for each of the following general groups: carbonyl, active hydrogen, primary amino, alkoxyl, alkyl-imino, acetyl and benzoyl, methyl-carbon, *iso*propylidene. Presumably paper restrictions are to blame for this feature. In this part of the book the author not only describes in detail, or gives references to, alternative methods, but he also comments on the known idiosyncrasies of those selected. In assessing the relative merits of the Pregl and other methods he is right to draw attention to "the considerable amount of work devoted to the perfection of the former." While these determinations, *i.e.*, the bulk of the subject, are otherwise admirably presented, the reviewer would have welcomed more than the half-page allotted to the determination of carbon by wet oxidation; also the electrolytic methods might have included that of B. L. Clarke and H. W. Hermance (*J. Amer. Chem. Soc.*, 1932, **54**, 877). The concluding chapters describe the determination of the usual physical constants (melting and boiling points, density, molecular weight, optical rotatory power and molecular refractivity) and give introductory accounts of absorption spectroscopy, surface tension and viscosity, colorimetry and nephelometry, gas analysis, electrometric methods, polarography and fluorescence analysis. It is evident that these and other physico-chemical measurements could with advantage be collected into a self-contained book.

This edition is well produced in the same style as the earlier ones; it contains only a few misprints, the most obvious being the inversion of Figs. 26 and 29 and the expression of an optical rotation in "°C." on page 209. The above criticisms are not intended to detract from a valuable work which all who practice microchemistry should possess, whether or not they already have an earlier edition. Those approaching the subject for the first time will find sufficient experimental detail to enable them to attain proficiency by following the instructions carefully for the first few weeks.

G. H. WYATT

MICROCHEMISTRY GROUP

A MEETING of the Microchemistry Group in conjunction with the Cardiff Sections of the Royal Institute of Chemistry and the Society of Chemical Industry will be held in Cardiff on Friday, May 17th, 1946.

The final arrangements have not been completed but it is expected that a works visit will be arranged for the afternoon and the papers will be read at an evening meeting. Members will be informed of the detailed arrangements by circular.

The following papers will be read:

"Determination of Cyanide by the Picrate Method." "A Waterbath Rack for Heating Simultaneously Many Tubes of Reactants." By Dr. J. G. A. Griffiths and Mr. J. K. Whitehead.

"Some Observations on the Kjeldahl Method for the Determination of Nitrogen." By Mr. A. E. Beet.

"Methods for the Construction of Microchemical Apparatus." By Mr. R. Belcher.

BIOLOGICAL METHODS GROUP

MEMBERS of the Biological Methods Group are asked to bring to the notice of the Hon. Secretary any original work having a bearing on the objects of the Group on which they would be prepared to read a paper at a Group Meeting. Papers presented at Group Meetings can and should also be submitted to the Publication Committee of the Society with a view to publication in THE ANALYST.

ISSUE OF NEW LIST OF MEMBERS

THE last printed List of Members of the Society with addresses was issued in 1943, and it is proposed to issue a new List this year. The Secretary would be obliged for notifications of recent changes in degrees of members and in the appointments of Public Analysts and Official Agricultural Analysts. Subject to individual notification otherwise, the addresses in the new List will be those to which THE ANALYST and other communications from the Society are now being sent.

Any notifications on the points mentioned should be sent as soon as possible to the Secretary, "Society of Public Analysts," 7–8, Idol Lane, London, E.C.3.

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