THE ANALYST

The Journal of The Society of Public Analysts and Other Analytical Chemists

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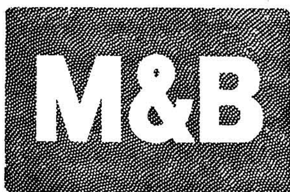

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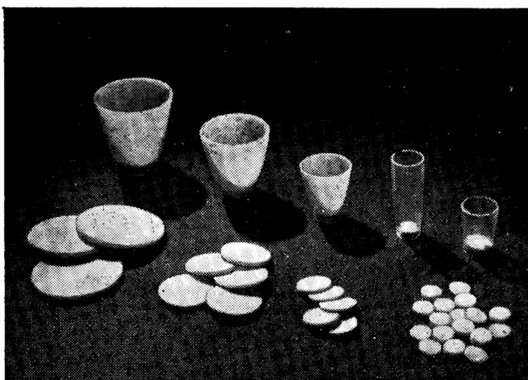

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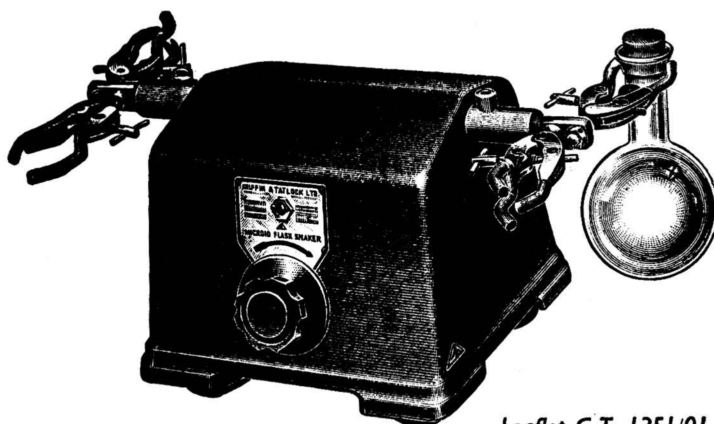

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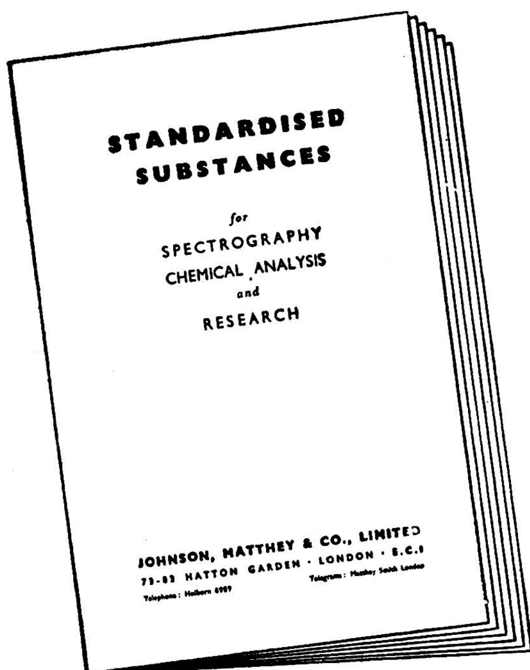

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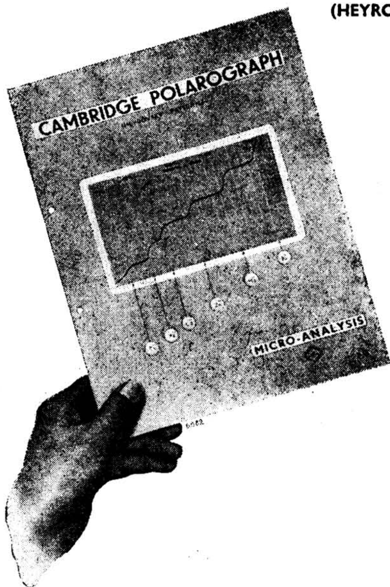

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE Annual General Meeting of the Society was held at 5 p.m. on Friday, March 5th, 1948, in the meeting room of the Royal Society, Burlington House, London, W.1. The chair was taken by the President, Mr. Lewis Eynon. The Financial Statement for 1947 was presented by the Hon. Treasurer, who explained that, as printed copies were not yet available, the proposal for its formal adoption would be deferred to an Extraordinary Meeting later, at which the appointment of Auditors for the forthcoming year would also be made. The Report of the Council for the year ending March, 1948 (see pp. 186–191), was presented by the Hon. Secretary and adopted. The following were elected Officers and Council for the coming year.

President—Lewis Eynon, B.Sc., F.R.I.C.

Past Presidents serving on the Council—F. W. F. Arnaud, E. B. Hughes, G. Roche Lynch, S. E. Melling and G. W. Monier-Williams.

Vice-Presidents—C. A. Adams, H. E. Cox, D. W. Kent-Jones and, *ex officio*, C. H. Manley (Chairman, North of England Section) and H. Dryerre (Chairman, Scottish Section).

Hon. Treasurer—G. Taylor.

Hon. Secretary—K. A. Williams.

Other Members of Council—N. L. Allport, J. F. Clark, D. C. Garratt, J. G. A. Griffiths, (Miss) I. H. Hadfield, J. H. Hamence, J. R. Nicholls, J. E. Page, F. A. Robinson, R. W. Sutton, A. M. Ward, H. N. Wilson and, *ex officio*, Arnold Lees (Hon. Secretary, North of England Section) and R. S. Watson (Hon. Secretary, Scottish Section).

After the business outlined above had been completed the meeting was opened to visitors, and a lecture on "The Proximate Analysis of Mixtures by Methods depending on Differential Solubility and Saturation" was given by Dr. G. M. Bennett, C.B., M.A., F.R.I.C., F.R.S., Government Chemist (see pp. 191–196).

NEW MEMBERS

Kirk Baird, A.R.I.C.; Stanley Bance, B.Sc. (Lond.), A.R.I.C.; Edmund Bishop, B.Sc. (Glas.), A.R.T.C., A.R.I.C.; Leslie Brealey, B.Sc. (Lond.); Herbert James Bridger, B.Sc. (Lond.), A.R.I.C.; Lawrence Cyril Chadwick, M.A. (Cantab.), A.R.I.C.; Bernard Handel Cossham, A.R.I.C.; Herbert Joseph Cluley, B.Sc. (Lond.), A.R.I.C.; George Stephen Crouch; Louis Ellis, B.Sc., Ph.D. (Leeds), F.R.I.C.; Donald Anthony Elvidge, B.Sc. (Lond.); Maurice Freiman, B.Sc. (Rand); Harry Howard Gething, B.Sc. (Lond.), A.R.I.C.; Norman Hackney, B.Sc. (Lond.), F.R.I.C.; Cyril Garforth Hampson, B.Sc., F.R.I.C.; Percy John Hardwick, M.Sc., Ph.D. (Lond.), F.R.I.C.; Frederick Reginald Harris, B.Sc. (Lond.), Ph.D. (Sheff.), F.R.I.C.; John Holley Harwood, B.Sc. (Lond.), A.R.I.C.; Jean Muriel Hubbard; Frank Edmund Humphreys, B.Sc. (Lond.), Ph.D., F.R.I.C., A.R.C.S.; Gerald Charles Hutton, B.Sc. (Lond.), A.R.I.C.; Stanley Jacobs, M.Sc., Ph.D. (Lond.), F.R.I.C.; Gilbert Jessop, M.Sc., Ph.D. (Sheff.), Ph.D. (Cantab.); Alan Jewsbury, B.Sc. (Lond.), A.R.I.C.; John Dewi Jones, B.Sc. (Wales); Louis Klein, M.Sc., Ph.D. (Lond.), F.R.I.C., M.Inst.S.P.; Albert Edward Laxton, M.Sc. (Lond.), F.R.I.C.; John Augustus Lewis; Albert John Nutten, B.Sc.; Cecil Allen Parker, B.Sc., Ph.D. (Lond.), A.R.I.C.; John Paterson Paterson, B.Sc. (Lond.), A.R.I.C.; Albert Edward Ross, B.Sc., A.R.I.C.; Douglas Macintosh Ross, B.Sc. (Glas.), A.R.I.C.; Beno Schneidmesser, M.Sc., Ph.D. (Jerus.); Edwin John Schorn, F.R.I.C., Ph.C.;

Francis Leslie Selfe, F.R.I.C.; Alan Mayer Selway; Joseph Henry Skellon, B.Sc. (Bristol), M.Sc., Ph.D. (Lond.), F.R.I.C.; Dorothy Joan Taylor, B.Sc. (Birm.); Alexander Francis Thomson, B.Sc. (Aber.), A.R.I.C.; John Todd, B.Sc., Ph.D.; Ralph Edward Weston, B.Sc. (Lond.), A.R.I.C.; Stephen Matthew Austin Whettem, B.Sc. (Lond.), F.R.I.C.; Thomas White; Arthur Charles Wiggins, B.Sc. (Lond.), A.R.I.C.; Cyril Mason Willcox, B.Sc. (Lond.), M.A. (Cantab.), F.R.I.C.; Ernest Philip Underwood, B.Sc. (Lond.), A.R.I.C.; John Terence Yardley, B.Sc. (Lond.), A.R.I.C.

DEATHS

WE regret to have to record the deaths of

Albert Henry Mitchell.
Samuel Russell Trotman.

Annual Report of Council: March, 1948

THE roll of the Society numbers 1389, a net increase of 44 over the membership a year ago.

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

Bernard Dyer	S. R. Naidu	A. Sherlock
R. Ellison	E. Oddy	Sir Bernard Spilsbury
Sir F. Gowland Hopkins	J. W. Pooley	H. P. Smith
F. A. Mason	W. R. Schoeller	J. Smith
C. A. Mitchell		

Dr. Bernard Dyer, who died within a few days of his 92nd birthday, was elected an Associate Member of the Society in 1875 and had a remarkable record of service to the Society. He was elected a Member of Council in 1880, Honorary Secretary in 1883, an office which he held for 14 years, and President in 1897–1899, and served as a Past President on the Council until his death. He held numerous appointments as Official Agricultural Analyst and Public Analyst. He published many papers in agricultural chemistry and on subjects arising from his work as Public Analyst. (Obituary, ANALYST, 1948, 73, 123.)

Sir F. Gowland Hopkins, who died in his 86th year, was an Honorary Member of the Society. He studied at the Royal School of Mines and became private assistant to Dr. P. F. Frankland. Later, he joined the Medical School at Guy's Hospital, where he became the first Sir William Gull research student. He went to Cambridge in 1898 to develop teaching and research on the chemical aspects of physiology, and in 1901 isolated and identified tryptophan in collaboration with S. W. Cole. The creation of a University Readership in Chemical Physiology for him in that year enabled him to pursue allied researches culminating in 1906 in the announcement of the discovery of vitamins. From 1921 to 1943 he was Sir William Dunn Professor of Biochemistry. He was at one time an Official Analyst to the Home Office, and later played a prominent part in the direction of State research. He was President of the Royal Society from 1930 to 1935 and the recipient of honorary degrees from many universities; he was knighted in 1925, and received the Order of Merit in 1935.

F. A. Mason died in his 60th year. He was educated at the Merchant Taylors' School and St. John's College, Oxford, and at the University of Munich. He carried out investigations under W. H. Perkin at Manchester and Oxford and, while demonstrating at the Royal College of Science, London, undertook investigations for the British and French Governments. After a period devoted to the study of the chemistry of dyestuffs, he was appointed to the Inspectorate of the Ministry of Education, and became Staff Inspector of Chemistry in 1936.

C. A. Mitchell, who joined the Society in 1894, was elected a member of the Council in 1899. He became Editor of THE ANALYST in 1921, and retained the post until his retirement in 1945. He was Secretary of the Society from 1925 to 1937. He graduated from Exeter College, Oxford, in 1889, and after a short time became assistant to Otto Hehner. He was made a Fellow of the Institute of Chemistry in 1897, and received the degree of Doctor of Science from the University of Oxford in 1929. His early interest in oils and fats is reflected in numerous papers in THE ANALYST. He became interested in the chemistry of vinegar, and worked on this subject throughout his life. His knowledge of inks and handwriting was often brought out in evidence given in the Courts.

He was the first chemist to be President of the Medico-Legal Society (in 1935–37), having previously been a Vice-President of the Society and a Joint Editor of the *Medico-Legal Journal.* He was a Vice-President of the Institute of Chemistry in 1937–40. (Obituary, ANALYST, 1948, **73**, 55.)

S. R. Naidu, who died at the age of 53, was elected a member in 1939. He was trained at Madras, where he graduated B.A. as well as M.B. and B.S. After holding appointments as Assistant Chemical Examiner to the Government of Madras and Assistant Professor to the Medical College there, he came to England and obtained the Diploma of Imperial College, the F.R.I.C. (Branch E) and the M.Sc. of the University of London. He returned to India, where he was appointed Chemical Examiner to the Government of Madras. He was awarded the M.B.E.

J. W. Pooley died at the age of 51. He was educated at Reigate Grammar School and King's College, London. He became Assistant Chemist at Kingsnorth Airship Station, and in 1919, after a few months with Alexander Duckham & Co., joined the staff of the Government Laboratory. At the time of his death he was one of the Senior Chemists and in charge of the *Ad Valorem* Duties Section.

W. R. Schoeller joined the Society in 1921, and was a member of Council in 1922–23. He was an abstractor for THE ANALYST from 1921 to 1946. With numerous collaborators he published a long series of papers on the analytical chemistry of metals, culminating in his book, published under the auspices of the Society in 1937, on the Analytical Chemistry of Tantalum and Niobium. With A. R. Powell he published his book on the Analysis of Minerals and Ores of the Rarer Elements, the second edition appearing in 1940. He graduated Ph.D. of Greifswald, and became a Fellow of the Institute of Chemistry in 1935.

A. Sherlock was 53 when he died. He entered the laboratory of Pilkington Bros., St. Helens, in 1908, and remained with the company until his death in September, 1947, becoming Head Analyst and Manager of the Central Laboratory. He lectured for many years on inorganic and physical chemistry and on the chemistry of glass manufacture at St. Helen's Municipal Technical College.

H. P. Smith, who died in his 71st year, was elected a member in 1908. He studied at the Leeds School of Science and Technology; after holding appointments with a number of companies, including Armstrong, Whitworth & Co., he became Chief Chemist to John Summers & Sons, of Shotton, and he held this position until 1929. He was for many years Lecturer in Chemistry, Metallurgy and Iron and Steel Manufacture under the Lanarkshire County Council and the Flint County Council.

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

"A Note on the Chapman and McFarlane Method for the Estimation of Reducing Groups in Milk Powder." By C. H. Lea.

"The Determination of Carotene in Dried Grass." By W. A. G. Nelson.

"A Micro-chromatographic Method for the Detection and Approximate Determination of the Different Penicillins in a Mixture." By R. R. Goodall and A. A. Levi.

"The Determination of Small Amounts of Hexachlorocyclohexane (Benzene Hexachloride)." By Bernard H. Howard.

"Iodimetric Methods of Estimating Peroxide Oxygen." By J. H. Skellon and E. D. Wills.

"The Determination of Arsenic Pentoxide in White Arsenic." By D. A. Lambie.

"A Semi-micro Combustion Method for the Determination of Organic Carbon." By J. B. Rickson.

"Some Observations on the Semi-micro Determination of Carbon and Hydrogen by the Sucharda and Bobranski Method, using a Macro-Balance." By Frank Goulden.

"The Micro-analytical Test for Purity in Food, with Special Reference to Cereals." By D. W. Kent-Jones, A. J. Amos, P. S. Elias, R. C. A. Bradshaw and G. B. Thackray.

The paper by Dr. Goodall and Dr. Levi was preceded by a brief account of the chemical nature of the Penicillins by F. A. Robinson.

JOINT MEETING—The December Meeting was, as usual, a Joint Meeting with the Food Group of the Society of Chemical Industry. The subject was "The Occurrence, Physiological

Importance and Estimation of Metallic Contaminants in Foodstuffs," and the following papers were read and discussed:—

"The Occurrence of Metallic Contaminants in Foodstuffs." By G. E. Forstner.

"The Public Health Aspect of Metallic Contaminants in Foodstuffs." By G. W. Monier-Williams.

"Trace Metals in Human Nutrition." By W. F. J. Cuthbertson.

"The Estimation of Metallic Contaminants in Foodstuffs." By N. L. Allport and D. C. Garratt.

NORTH OF ENGLAND SECTION—Four meetings have been held during the year and the following papers have been presented:—

"The Chemist in the Colonies." By J. F. Clark.

"Ice Cream." By E. L. E. Humphries.

"The Determination of Organic Phosphorus." By C. H. Manley.

"Colour—A Neglected Chapter in Food Analysis." By H. E. Cox.

"Recent Advances in Soil Biochemistry." By H. Lees.

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

"Methods of Removing 'Free' Iron Oxides from Clays." By R. C. Mackenzie.

"The Diagnosis of Deficiences in Plants." By J. G. Hunter.

"The Determination of Steroids in Urine." By S. L. Tompsett.

"Some Notes with Demonstrations on the Application of the Precipitin Test in the Determination of Proteins." By H. Dryerre.

Three new members joined the Section during the year, making a total membership of 66.

MICROCHEMISTRY GROUP—Three meetings have been held during 1947, in London, Sheffield and Cambridge. The Sheffield meeting was held jointly with the Sheffield Section of the Royal Institute of Chemistry and the Sheffield Metallurgical Association, and the Cambridge meeting was held jointly with the Physical Methods Group.

The following papers have been read:—

"A Review of Electrolytic Methods of Microchemical Analysis." By A. J. Lindsey.

"The Oxidation of Nitrogen during the Micro-combustion of Organic Substances." By A. E. Heron.

"The Separation of Crystals and 'Gums' on the Micro and Semi-micro Scale." By A. L. Bacharach.

"The Microchemical Determination of Molybdenum in Steel." By J. E. Wells and R. Pemberton.

"The Determination of Carbon, Hydrogen and Nitrogen in Aliphatic Nitro-Compounds." By A. E. Heron.

"A Review of Micro-methods for the Determination of Oxygen in Organic Compounds." By C. E. Spooner.

"A New Spot-test for the Detection of Sulphites and Sulphur Dioxide." By R. Belcher and G. Ingram.

"The Microchemical Analysis of Aluminium Base Alloys." By J. Townsend and C. Whalley.

"Micro-methods for Molecular Weight Determination." By Cecil L. Wilson.

"Turbidimetric Methods used in Agricultural Analysis." By J. Tinsley.

"Microchemical Applications of Potentiometric Methods." By J. T. Stock.

"Microanalysis using X-ray Diffraction Technique." By H. P. Rooksby.

The number of Group members is now 210, an increase of 29 since the last report.

The Committee has met three times during the year. The census of industrial laboratories having been completed, a report has been prepared by Dr. Cecil L. Wilson and published in *Chemistry and Industry*. Reprints of this report have been circulated to all members of the Group. The Association of British Chemical Manufacturers has also taken 250 copies and circulated them amongst constituent firms with the object of stimulating comment.

A team, sponsored by D.S.I.R., has visited Germany to investigate progress in microchemistry. The findings are described in a B.I.O.S. report now in press. The team consisted of two representatives of the Microchemistry Group and one representative from the Association of British Chemical Manufacturers.

PHYSICAL METHODS GROUP—During the past year the Physical Methods Group has held two meetings in London and one each in Newcastle and Cambridge. The Cambridge meeting was held jointly with the Microchemistry Group. The meetings had an average attendance of over 80 members and visitors. The following papers were read at meetings of the Group—

Polarographic Analysis Meeting in London on November 26th, 1946.

"Amperometric Titration." By J. T. Stock.
"The Rotating Platinum Electrode." By C. J. O. R. Morris.
"The Application of the Cathode Ray Oscillograph to Polarography."
Part I. "Underlying Principles." By J. E. B. Randles.
Part II. "General Lay-out and Uses of the Cathode Ray Polarograph." By L. Airey.

Fluorimetric Analysis Meeting in London on February 11th, 1947.

"Apparatus Design for Fluorescence Measurement." By E. J. Bowen.
"Notes on Fluorescence Quenching." By E. J. Bowen.
"Some Applications of Fluorimetry in Vitamin Analysis." By E. Kodicek.
"The Use of Fluorimetric Analysis in the Study of Pterins." By Delia M. Simpson.

Gas Analysis Meeting in Newcastle on May 2nd, 1947.

"Gas Analysis at Low Pressures." By C. E. Ransley.
"The Analysis of Hydrocarbon Gases by Low Temperature Distillation." By J. H. D. Hooper.
"A New Apparatus for Gas Analysis by the Soap Film Method." By W. J. Gooderham.

Micro-physical Methods of Analysis Meeting in Cambridge on September 26th, 1947.

"Micro-methods for Molecular Weight Determination." By C. L. Wilson.
"Turbidimetric Methods used in Agricultural Analysis." By J. Tinsley.
"Microchemical Applications of Potentiometric Methods." By J. T. Stock.
"Micro-analysis using X-ray Diffraction Technique." By H. P. Rooksby.

The Polarographic Discussion Panel was formed at the last Annual General Meeting of the Group, and has had three very successful meetings, at Imperial College on April 25th, University College on July 25th, and Norwood Technical College on October 3rd, 1947. The discussions at these meetings were opened by Dr. W. Cule Davies, Dr. E. Jessop, Dr. E. R. Roberts, Professor J. Heyrovský, Dr. R. Brdička, Mr. A. S. Nickelson, Mr. L. Airey and Dr. F. J. Bryant. The membership of the Panel is now over 40.

The Group has been represented on the Barker Index Committee by Dr. J. G. A. Griffiths, Dr. J. H. Hamence and the Hon. Secretary.

The number of Group members is now 183, an increase of 35 since the last Annual Report.

BIOLOGICAL METHODS GROUP—The Group has held four meetings during the year under review. The Annual General Meeting was followed by an ordinary meeting, at which the following papers were presented:—

"The Assay of Anti-Thyroid Substances using Tadpoles." By Miss H. M. Bruce.
"The Computation of Microbiological Assays of Amino-Acids and other Growth Factors." By E. C. Wood.

Three meetings were held in March, April and May, 1947, which together comprised a Symposium on "The Production and Care of Laboratory Animals." The following papers were read:—

"Breeding of Animals." By A. L. Bacharach.
"Feeding of Animals." By A. S. Parkes.
"Common Diseases." By H. J. Parish.
"Records." By N. T. Gridgeman.
"Housing." By J. I. M. Jones and E. C. Wood.

Although a conference of a similar kind took place in the United States in 1945, this was the first time that an organised discussion had been held on the subject in Great Britain. The meetings attracted many visitors as well as members of the Group, and the vigorous discussions which followed the papers covered a very wide ground. The Symposium was undoubtedly found most valuable by those who attended, and led to the exchange of a great

deal of useful information and practical hints on every aspect of animal husbandry. It is hoped that the proceedings at the Symposium will be published shortly in THE ANALYST. The membership of the Group is now 98.

PUBLIC ANALYSTS AND OFFICIAL AGRICULTURAL ANALYSTS COMMITTEE—During 1947 the Committee has met on three occasions. It has considered and made recommendations on lead in spices and the freezing point of milk. It has further dealt with internal matters relating to appointments and considered a number of proposals regarding the standardisation of foodstuffs.

ANALYTICAL METHODS COMMITTEE—Sub-Committees have been very active and considerable progress is reported from those on Metallic Impurities in Foodstuffs, Vitamin Estimations, Tragacanth and Poisons; progress reports indicate that some standard methods should be ready for presentation in the near future. A Panel of the Vitamin Estimations Sub-Committee has been formed to investigate the determination of biologically active carotenoids.

A Report from the Committee on the Purity of Nicotinic Acid used in Assays has been published. (ANALYST, 1947, p. 501.)

The questionnaire recently circulated to members asking for co-operation to assist in the expected increase of work in analytical standardisation has received strong support. Already some 120 names have been forwarded from individuals and organisations willing to co-operate; these will strengthen considerably the personnel and specialised knowledge available to the Committee.

The Standard Methods Sub-Committee has made very substantial progress with the compilation of a Bibliography of Standard Methods of Analysis, having covered over 30 of the main sub-divisions of the subject. It is hoped that this Bibliography will be ready for publication in the near future.

HON. TREASURER'S REPORT—The financial position of the Society for 1947 is satisfactory, but a close watch is being kept on the finances so that the effects of increasing costs and the increasing activities of the Society may be observed.

THE ANALYST—The paper situation following the fuel crisis early in 1947 checked the continued expansion of THE ANALYST, and the 1947 volume has only 558 pages compared with 600 for 1946. The position is now more normal and some expansion is possible, but for 1948 it has been considered advisable to save space by adopting smaller type for notes and reviews and closer setting for abstracts. The number of original papers and notes published in 1947 were 61 and 19 respectively, compared with 80 and 31 in 1946, in which year, however, the amount of space occupied by abstracts was abnormally low.

The reorganisation of the abstracting work of the journal undertaken by the Associate Editor, Mr. L. S. Theobald, forecast in last year's Report (ANALYST, 1947, 72, 137), has resulted in a considerable increase in the number of abstractors and the number of journals "covered," and a plentiful supply of abstracts. As hitherto, the abstracts give working details, as far as possible. In view of the wide variety of analytical methods dealt with and the necessity for adapting the number of abstracts made to the capacity of THE ANALYST to publish them, a panel of referees has been formed to whom, individually, the Associate Editor can refer papers on a particular subject for an expert opinion as to their suitability for abstracting.

HONORARY MEMBERSHIP OF THE SOCIETY—The Council is very pleased to record that Professor Sir Robert Robinson, M.A., D.Sc., LL.D., D.Pharm., F.R.I.C., President of the Royal Society, has been elected an Honorary Member of the Society. The Council has congratulated him on the award of the Nobel Prize for Chemistry made to him during the year.

The Council has also congratulated Dr. G. M. Bennett, Government Chemist, on his election as a Fellow of the Royal Society.

SPECIAL COMMITTEES—Three Special Committees have held meetings during the year. Of these one is concerned with the possibility of making a change in the name of the Society, with the scope and wording of the Memorandum and Articles of Association, with the constitution and method of election of members of the Society and with the method of election of members of Council.

The second is considering the question of improving training in analysis, and the third has dealt with the relations of the Society with other bodies who require assistance in selecting, improving and adopting methods of analysis.

JOINT COMMITTEE OF THE SOCIETY AND THE ROYAL INSTITUTE OF CHEMISTRY—A new Joint Committee has been appointed to deal with all matters of common interest to the Society and the Royal Institute of Chemistry and to take over the functions of the two Committees which formerly undertook this work.

CHEMICAL COUNCIL—The Society has entered the Joint Subscription Scheme of the Chemical Council. Representations have been made to the Council by the Society for assistance in improving the supply of chemicals and apparatus, and active co-operation has been given to us by the Council in this matter.

CENTENARY OF THE CHEMICAL SOCIETY—The President presented an Address on behalf of the Society to the Chemical Society at the opening ceremony of the Centenary Celebrations in July.

XITH INTERNATIONAL CONGRESS OF PURE AND APPLIED CHEMISTRY—A delegation of the Society attended the Congress.

BARKER INDEX—The Committee considering the publication of the Barker Index has included representatives of the Physical Methods Group of the Society. The Society has made a donation towards the cost of publication.

WHITE PAPER ON THE POST-WAR LOAF—The Government White Paper on the nature of the post-war loaf has made reference to the desirability of obtaining the advice of the Society in certain directions. A representative of the Society has attended meetings at the Ministry of Food and discussions are proceeding.

LEWIS EYNON, *President*
K. A. WILLIAMS, *Hon. Secretary*

The Proximate Analysis of Mixtures by Methods depending on Differential Solubility and Saturation

BY DR. G. M. BENNETT, C.B., F.R.S.

(Lecture delivered at the Annual General Meeting of the Society, March 5th, 1948)

THE particular mixtures here discussed are of closely related organic compounds, although the methods to be described are not in fact limited in their application to such substances, and inorganic materials could be examined by similar methods.

The purpose of the investigation may be the complete proximate analysis of a mixture the components of which are already known, or on the other hand it may be to discover the presence of unknown components or simply to make a test as to the homogeneity of a single specimen. Solubility methods are particularly useful in the latter case if the substance to be dealt with has no sharp melting point to serve as a criterion of purity. It may be noted that the melting point and the solubility test are closely related from the physico-chemical point of view.

If one component of a mixture is soluble in a solvent in which the others are insoluble that component can readily be determined by extraction and such examples are familiar to any analyst. The mixtures now in question, however, are of substances that differ in their solubility in various solvents, but only in degree, none being insoluble.

The principle underlying the methods to be considered is the simple and self-evident one that a substance does not dissolve in a solvent already saturated with it. But as soon as this principle is applied in practice a complication arises, because the passage of other compounds into solution may, and in general does, alter the solubility of the substance in question. This mutual solubility effect may be positive or negative, but with closely related organic substances it will in general involve an increase of solubility, and though the effect in dilute solution may be small, it should not be ignored.

One or two general considerations may be mentioned at the outset. It will be clear that in applying solubility methods the exact control of the working temperature is essential. In general, too, the formation of solid solutions of polymorphic forms or of complexes in solution may lead to anomalous results.

In dealing with a mixture of n components it would be logically possible to make use of a solvent saturated with 1 or with 2 or with any number of the components up to $(n - 1)$,

but it is apparent that saturation of the solvent with either 1 or n − 1 of the components will most readily give useful information.

The first case to be considered is therefore the extraction of such a mixture with a solvent saturated with one component. An example of this is provided by a method for determining DDT in commercial samples described by Cristol, Hayes and Haller.[1] In this procedure 2·000 g. of the sample were dissolved in 150 ml. of 75 per cent. aqueous ethanol previously saturated at 25·0° C. with DDT. The crystals deposited at 25·0° C. were collected, washed with the saturated solution and dried at 80° C. The results were low by 1·4 per cent. and this was added as an empirical correction.

This small consistent defect arises from the increased solubility of the DDT owing to the presence in solution of the other components of the mixture. Provided that a series of similar mixtures are to be examined the application of such an empirical correction should be satisfactory.

The importance of conducting the crystallisation at precisely the same temperature as the saturation is obvious. An instance of the use of this method recently came to the notice of the present writer where results had been obtained which were consistently high instead of low. The explanation was undoubtedly the use by the operator of a thermostatic air-oven for temperature control. If the saturated solution was kept at the back and the working vessel at the front of the oven, a small difference of temperature might be introduced which would account for the result. It may be pointed out that although this analysis was carried out by crystallisation it could equally well be conducted by extracting the finely divided sample with the saturated solvent at the fixed temperature.

The second method to be considered involves the extraction of one component from an n-component mixture by using a solvent saturated with all the (n − 1) other components. This is the "*Auslaugungsmethod*" of the Dutch chemist Holleman,[2] who used it to determine the proportions of the isomerides formed in aromatic substitution. Holleman from the first realised that mutual solubility effects must be taken into account.

Consider first the simple case of the mixture of two isomeric nitrophthalic acids formed by nitrating phthalic acid.[3] Water was used as solvent and the amounts in solution were determined by titration. 100 g. of water were agitated at 25° C. with an excess of the α-acid and successive known quantities of β-acid were added, the total dissolved acid being determined after each addition as shown below.

β-Acid added g.	Total acid in solution g. per 100 g. of water
nil	2·048
0·100	2·138
0·200	2·261
0·300	2·387
0·500	2·605
1·000	3·211
2·000	4·428

These figures give a linear graph, which was used as a reference curve. The mutual solubility effect is very evident, *e.g.*, 1·0 g. of β-acid added corresponds to 3·211 g. in solution instead of the calculated figure of 3·048 g.

By agitating a known weight of the mixture to be analysed with a measured quantity of water and an excess of α-acid, and determining the concentration of acid in solution at equilibrium, reference to the curve gave the weight of β-acid which must have been dissolved and so the percentage of the β-acid in the mixture. This was found to be 50·2, 50·8, 50·8, 50·4 in successive experiments, mean 50·5 per cent. of β-acid. Hence α-acid = 49·5 per cent. (by difference). Reliance was placed on specific tests to ascertain that no third substance was present.

Any suitable chemical or physical measurement may be used to determine the concentration in solution, and Holleman, when dealing with neutral organic substances as components, commonly used the density of the solution as a measure, the reference curve then being a graph of density of the resulting solution against the amount of the particular component dissolved (in each instance in presence of an excess of the others). The method is theoretically applicable to any number of components, but Holleman did not have more

than three to deal with. Saturation required agitation for at least several hours and sometimes 24 hours.

The product from the nitration of nitrobenzene contained the three isomeric dinitrobenzenes and this was a case examined by density measurement.[4] Two reference curves were used, the values of d_{25} for alcohol saturated with two of the isomerides being plotted against added weights of the third. A test analysis gave the following result:—

Isomeride	*ortho*	*para*	*meta*
Percentage in mixture taken	9·97	1·48	88·55
Percentage found	9·8	1·6	88·6 (by difference)

The product of nitrating nitrobenzene at 30° C. was found to contain: *ortho* 8·1, *para* 1·0 and *meta* 90·9 per cent. Here again the amount of *m*-isomeride was determined by difference.

This method proved very valuable, although it is laborious. A few years ago the present writer and Dr. P. V. Youle[5] made use of it, and their experiences are described here because they illustrate both the strength and the weakness of the method.

The problem was to find the proportions of the three isomerides formed in the nitration of benzenesulphonyl chloride, the most important datum being the exact percentage of *m*-isomeride. As the sulphonyl chlorides are somewhat sensitive to hydrolysis they were converted at once by the action of aniline into nitrobenzenesulphonanilides, which are stable. A separate test showed that any small losses at this stage did not affect the relative proportions of the components.

In order to reduce the time involved, and also to allow the use of small quantities, the refractive index of the solution was adopted as the quantity measured, although it was realised that some loss of accuracy might result. The extractions were conducted in open test tubes, each with its stirrer, arranged in a row in a thermostat at 20·0° ± 0·1° C., the water of which also circulated through the Abbé refractometer. Amyl valerate of b.p. 190° C. was chosen as solvent, its volatility being so low that (as found by specific tests) no loss occurred from the open tubes. It dissolves 5 per cent. of the *meta* isomeride, and its low refraction ($n = 1{\cdot}4130$) was a factor of importance. In the earlier experiments 5 ml. of solvent and 0·5 g. of sample were used, but these quantities were subsequently reduced to 1 ml. and 0·1 g. respectively.

The three nitrobenzenesulphonanilides were prepared synthetically and three refractive index - composition curves plotted, one for each isomeride, using solvent saturated with the other two. To ensure saturation it was found necessary to stir for 3 hours and to allow to stand for a further 1 hour to let the solution recover from the heat generated by stirring. One drop was sufficient for the refractive index observation.

The washed and dried reaction product appeared to be the expected mixture of isomerides, as shown by one of several sets of ultimate analyses:—

	C	H	N	S
Found, per cent.	51·6	3·7	10·1	11·3
Calculated for $C_6H_4(NO_2)SO_2.NHPh$, per cent.	51·8	3·6	10·1	11·5

A test extraction on an artificial mixture of the isomerides gave the following result:—

	ortho	*meta*	*para*
Components in mixture taken, per cent.	5·7	85·7	8·6
Found (mean of 2 values), per cent.	6·5	86·2	7·6

which supported the expectation that the proportions could be determined to within about ± 1 per cent.

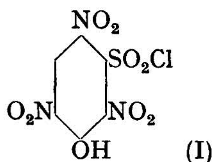
When, however, the measurements were carried out on the reaction mixture the results (mean of 2 to 5 values) were: *ortho* 6·9, *meta* 97·9 and *para* 4·55 per cent., giving a total of 109·35 per cent.

Although there was no anticipation that any substance but the three isomerides would be present, and in spite of the indication of the ultimate analyses, the total of 109 per cent. clearly showed that there was a fourth component in the mixture. Careful tests showed that neither the original substance (as anilide) nor an over-nitration product was present. If the mixture contained some other substance, X, this would be extracted with each of the isomerides, and so would appear three times in the total; but, as the sequel shows, it would not be safe to conclude that X was present to the extent of exactly one-third of the excess over 100 per cent. It was realised in retrospect that it was fortunate that Holleman's procedure of finding the proportion of the third component by difference had not been followed.

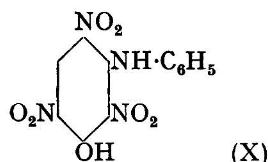
A direct test was made for the presence of a fourth substance as follows. The solvent was saturated at 20° C. with the three pure isomerides, when a refractive index of 1·4310 was found. The solution was again stirred with the further addition of a quantity of the nitration mixture, when the refractive index was found to have risen to 1·4324. The additional 0·0014 is clear evidence of the further component X.

After a number of experiments it was found that the nitration mixture when boiled with aqueous sodium acetate solution yielded a coloured solution depositing on acidification an orange-red solid, m.p. 162° C., identified as trinitrohydroxydiphenylamine. This was the substance X and it was now found that saturation of the solvent with the four known components gave a refractive index that was not increased by adding the nitration mixture. The formation of this substance, although surprising, is readily interpreted. Simultaneously with the nitration, a small quantity of *m*-hydroxybenzenesulphonyl chloride must be formed which is at once nitrated to the corresponding trinitro compound (I). Aniline converted this, not into its sulphonanilide but into X by displacement of the sulphonyl chloride group rendered labile by the three nitro-groups.

The discovery of this by-product was of considerable theoretical interest and led to further similar observations.[6]



(I)



(X)

These facts having been ascertained, the proximate analysis of the four component system was undertaken. Four reference curves were now made (Fig. 1). It may be pointed out that the slope of the X curve is greater than those of the three isomerides, so that the effect of a small weight of X on the refractive index simulates the presence of a larger weight of one of the isomerides.

Component	*ortho*	*meta*	*para*	X	
Weight of mixture, g.	0·1029	0·0518	0·1000	0·0995	
Weight of solvent, g.	1·0192	1·1697	1·0254	1·0399	
Refractive index	1·4300	$1{\cdot}4290_5$	$1{\cdot}4220_5$	1·4314	
Component per 100 g. of solvent, g.	0·58	4·03	0·28	0·19	
Component present in mixture, per cent.	5·73	91·0	2·87	1·98	
Duplicate determination	5·34	91·6	2·00	1·70	
Mean	5·5	91·3	2·5	1·8	Total 101·1 per cent.

An examination of the limits of accuracy of the actual measurements pointed to errors of the order of ±1 per cent. for the *o*- and *p*- and ±0·5 per cent. for the *m*-isomeride. The percentage of *m*-isomeride found was in satisfactory agreement with the value (91·5 per cent.) found by thermal analysis (using melting point or freezing point observations).

An application of this method as a criterion of purity was recently made by Dr. J. C. D. Brand[7] in studying the product of sulphonating the quaternary salt from *p*-toluidine, Me⟨ ⟩$\overset{+}{N}Me_3\}\overset{-}{X}$. Two isomeric products are possible, but 65 per cent. aqueous ethanol saturated with the synthetic substance of structure Me⟨ ⟩$\overset{+}{N}Me_3$ (SO_3^-) extracted nothing from the reaction product, which was thus shown not to contain as much as 0·5 per cent. of any other component.

The third method to be discussed here depends on the progressive dissolution of the mixture in a solvent, the amount passing into solution being plotted against the amount added. In the course of this operation the mixture is in effect successively extracted with solvent saturated with 1, 2, . . . n − 1 components.

This device was first put forward as a criterion of chemical homogeneity and as a method of analysis by Northrop and Kunitz in 1930,[8] and it has been of great value in controlling the isolation of pure proteins (which have no sharp melting points). A general account of the method has been given by Herriott.[9]

This method has recently been applied to other mixtures by Thorp.[10]

The principle may be illustrated by reference to Fig. 2, which represents the examination of an ideal mixture of three components A, B and C. At first the whole of the mixture dissolves and the amount found in solution is equal to the amount added, but at A the solvent becomes saturated with A. Thereafter only B and C dissolve and the slope of AB, which is 2 in 3, must represent the fraction of the whole dissolving, *i.e.*, $\frac{2}{3}$. At B the solution becomes saturated with B as well as A and along BC only C is dissolving. The fraction of C in the mixture is given by the slope of BC, *i.e.*, $\frac{1}{2}$. Thus the proportion of A is $1 - \frac{2}{3}$ or 33 per cent.; that of B is $\frac{2}{3} - \frac{1}{2} = 17$ per cent., and that of C is 50 per cent. The composition can be arrived at graphically by drawing the triangle PQT and making the lines PQ, PR and PS respectively parallel to OA, AB, and BC. Then if PT is unity the lengths QR, RS and ST will give the fractions of the mixture consisting of A, B and C respectively.

At the same time, if BA, CB, and DC are produced backwards to cut the axis in A′, B′ and C′, it can be seen that OA′, A′B′ and B′C′ give the solubilities of A, B and C respectively. However, these will not be the true solubilities in the solvent, but solubilities as modified by the mutual solubility effects.

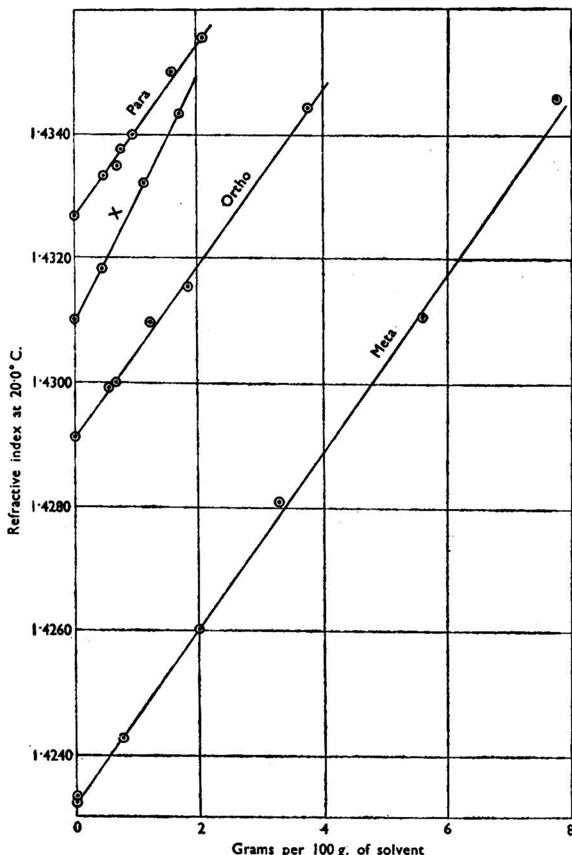


Fig. 1. Variation of refractive index of amyl valerate, saturated with three of the components, on adding increasing weights of the fourth.

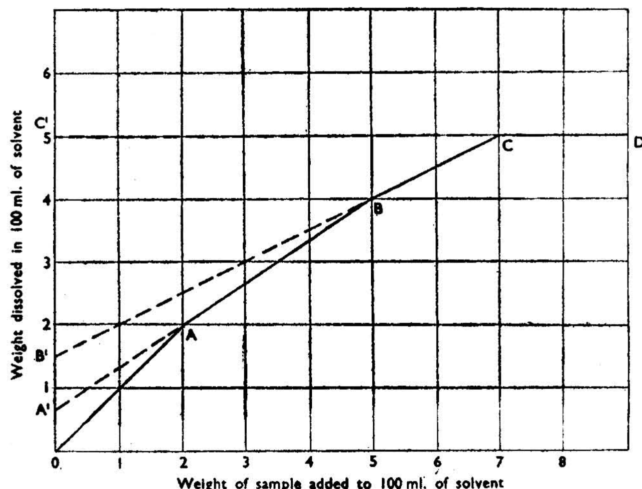
Apart from the possible complications of mixed crystal formation, which may cause AB to curve to the right, the solid phase here being variable, a difficulty may arise where two components A and B are present in the ratio of their solubilities. The solution will then become saturated with the two simultaneously and A will coincide with B. This will not, however, generally be so if another solvent is used, and for this reason more than one solvent should be tried out with any new mixture. Such an occurrence is by no means unlikely to be encountered, since when a material is extracted by a solvent the latter may well become saturated simultaneously with two components and so yield a mixture fulfilling the condition mentioned.

As a test of homogeneity this method has been applied repeatedly to protein problems. Northrop and his colleagues,[11] for instance, in 1940 showed that a specimen of pepsin was a mixture. They isolated a single individual and showed it to be homogeneous by the solubility test, using various aqueous solvents of different *p*H and containing sodium or magnesium sulphate. The method was also examined by Butler.[12] More recently a further development of this device has been made by Falconer and Taylor,[13] using a variable medium.

The dissolution method is a powerful one for detecting new components. Thorp showed that commercial DDT gave a solution curve of the type of Fig. 2 in which component A was DDT and B the *o,p′*-isomeride. C was thus detected as a third constituent, at that time unidentified but afterwards shown to be DDD (dichlorodiphenyldichloroethane) by Forrest, Stephenson and Waters.[14]

The quantitative use of this method is shown by Thorp's examination of an 80 per cent. : 20 per cent. mixture of DDT and its *o,p′*-isomeride. From his curve the slope of AB is 6·1/30

= 20·3 per cent.; whence DDT = 79·7 per cent. A commercial sample in the crude form gave slope of AB 8·6/25 = 34·4 per cent., corresponding to DDT = 65·6 per cent.; when crystallised it gave slope of AB 4·15/20 = 20·7 per cent., corresponding to DDT = 79·3 per cent.



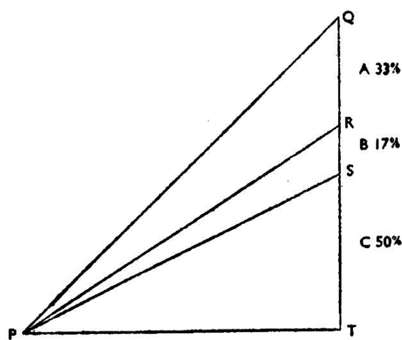


Fig. 2. Progressive dissolution of 3-component mixture in a solvent.

Here again the result must carry a small error arising from mutual solubility effects. As more sample is added beyond the first saturation point A, not only B and C will dissolve but also a little A, owing to the slight increase of its solubility. The experimental line AB may be expected to be linear but slightly too great in slope, leading to a slight defect in the calculated percentage of A. A closer investigation of this point is desirable.

For the examination of a series of similar samples this method could be made more rapid, and the mutual solubility error allowed for, by using measurement of refractive index or other convenient property to determine concentration in solution, the measurement being standardised in the first instance by direct weighing of evaporated solutions. The preliminary standardisation would then permit of the exact assessment and elimination of the mutual solubility error.

Of the three methods discussed here the second has given the most accurate results in actual use, but it requires exact knowledge of the components present. The third method is the most powerful for yielding both qualitative and quantitative information about an unknown mixture, and could be made equally convenient and accurate for routine analyses.

References

1. Cristol, S. J., Hayes, R. A., and Haller, H. L. *Ind. Eng. Chem. Anal. Ed.* 1945, **17**, 470.
2. Holleman A. F., "*Die direkte Einführung von Substituenten in den Benzolkern,*" 1910, p. 17.
3. Huisinga, J., *Rec. Trav. Chim.*, 1908, **27**, 260.
4. Holleman, A. F., and de Bruyn, B. R., *Ibid.*, 1900, **19**, 79.
5. Bennett, G. M., and Youle, P. V., *J. Chem. Soc.*, 1938, 887.
6. ——, ——, *Ibid.*, 1938, 1816.
7. Brand, J. C. D., private communication.
8. Northrop, J. H., and Kunitz, M., *J. gen. Physiol.*, 1930, **13**, 781.
9. Herriott, R. M., *Chem. Reviews*, 1942, **30**, 413.
10. Thorp, D., *J. Soc. Chem. Ind.*, 1946, **65**, 414.
11. Herriott, R. M., Desreux, V., and Northrop, J. H., *J. gen. Physiol.*, 1940, **24**, 213.
12. Butler, J. A. V., *Ibid.*, 189.
13. Falconer, J. S., and Taylor, D. B., *Biochem. J.*, 1946, **40**, 835.
14. Forrest, J., Stephenson, O., and Waters, W. A., *J. Chem. Soc.*, 1946, 333.

Methods of Penicillin Assay: their Purpose, Scope and Validity

A Symposium held at a Joint Meeting of the Physical Methods Group and the Biological Methods Group on Thursday, January 29th, 1948.

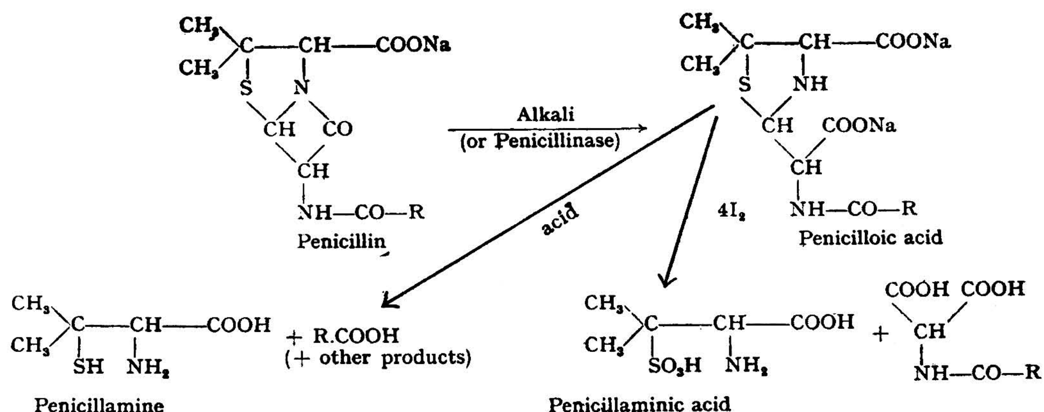
The following papers were read at the afternoon session.*

CHEMICAL AND PHYSICAL METHODS FOR PENICILLIN ASSAY

Introductory Survey

By E. LESTER SMITH

THIS historical survey of the many chemical and physical methods that have been described for the estimation of penicillin covers mainly those that for one reason or another have fallen into disuse, because most of the more interesting and valuable methods are the subjects of other papers in this symposium. I shall also mention, however, a few techniques that have been published too recently to permit critical appraisal.



It is first necessary to review briefly some aspects of the chemistry of penicillin.[1] The molecule contains a thiazolidine ring, carrying a carboxyl and two methyl groups, fused to a β-lactam ring carrying a long side-chain. The various penicillins differ only in the nature of the group R in the formula, as shown in Table I. Evidence has been presented for the occurrence of at least eleven different penicillins, of which five commonly occur in appreciable proportions in commercial samples. The nomenclature is confused, owing to the use of numbers in this country and letters in America. There is now a move to replace both, where possible, by an abbreviated chemical name composed of the name of the group R followed by "penicillin," *e.g.*, benzyl penicillin instead of penicillin G or II. The nomenclature of the salts is clumsy because penicillin was not originally named as an acid; thus we must say sodium benzyl penicillin, or benzyl penicillin sodium salt. A recent American paper introduced the new style "sodium benzylpenicillinate." This is slightly confusing, because "penicillinic acid" has been employed already for a degradation product, although it fell into disuse with the discovery that the substance so named was a mixture. It should be observed that the alternatives "penillate" or "penicillate" are not admissible because "penillic acid" has been used for an acid degradation product of penicillin, and "penicillic acid" for another mould metabolite unrelated to penicillin.

* The papers on Biological Methods of Penicillin Assay, read at the evening session, will be published in the May issue.

Most of the reactions that are utilised analytically are shown in the formulae. Alkali or penicillinase opens the lactam ring: the resulting dibasic penicilloic acid is split by hot acid into penicillamine and a penaldic acid, which breaks down further to yield eventually the side-chain acid R.COOH. Iodine oxidises penicilloic acid vigorously, to yield presumably the products shown.

TABLE I

THE PENICILLINS

(In order of separation on partition chromatograms)

Nomenclature			I.U./mg.		$\frac{B. subtilis}{S. aureus}$
U.S.A.	Great Britain	R Groups	*S. aureus*	*B. subtilis*	ratio
S_1			?	?	
S_2			?	?	
X	III	$-CH_2.C_6H_4OH(p)$	900	1450	1·61
S_3	VI		?	?	
G	II	$-CH_2.C_6H_5$	*1670	*1670	*1·00
F	I	$-CH_2.CH : CH.CH_2.CH_3$	1640	1100	0·67
Flavicidin		$-CH_2.CH_2.CH : CH.CH_3$	1400	1000	0·72
Dihydro-F	(?)IV	$-CH_2.CH_2.CH_2.CH_2.CH_3$	1610	1010	0·63
"K-type"	(?)VII		?	?	
K		$-(CH_2)_6.CH_3$	2400	750	0·31
"K-type"			?	?	

* By definition.

One must distinguish sharply between methods for the assay of total penicillin and those for the assay of individual penicillins. The unit of penicillin is defined in biological terms; the Oxford unit was an arbitrary one and has now been replaced by the international unit, which has the same magnitude but is defined as the anti-bacterial activity against a certain organism of 0·6 μg. of the international standard preparation of benzyl penicillin sodium salt. A chemical or physical method can give a precise result for total penicillin expressed on a weight or on a unitage basis when the sample contains this variety of penicillin only. Many commercial samples, however, contain in addition other varieties of penicillin.

Besides differing slightly in molecular weight, these penicillins have different anti-bacterial activities expressed in units per mg. Chemical and physical methods therefore are not capable of giving precise results for mixtures of penicillins in unknown proportions; it is only possible to quote the results in terms of the *chemically* equivalent weight or unitage of penicillin G. To put it differently, chemical and physical methods determine the molarity of a penicillin solution. It happens that the factors for conversion to international units do not differ very greatly from one penicillin to another; this, combined with the very fortunate circumstance that most penicillin samples now available commercially contain a high proportion of benzyl penicillin means that the non-biological methods can give results, despite the above limitation, that often agree with the biological results within the usually accepted limits of experimental error.

ESTIMATION OF TOTAL PENICILLINS

POLAROGRAPHIC METHOD—

The polarographic method[2] is properly taken first because it was the first non-biological method for penicillin estimation to be described. It was developed by my colleague Dr. J. E. Page, and was described in confidential reports in 1944; the method has not yet appeared in other publications. The penicillin is first hydrolysed with dilute alkali to penicilloic acid and then with dilute acid to break this down further to penicillamine. This compound is dimethyl cysteine and has a free SH group which is utilised for the polarographic estimation. The sensitivity, compared with that of the more usual polarographic estimations, can be much increased by carrying out the measurements in presence of ammonia and a cobalt salt. Under these conditions the polarographic SH step is enormously magnified by the catalytic effect of the cobalt, making it possible to estimate penicillin solutions as dilute as 1 unit per ml. One disadvantage of this method is its indirectness and lack of specificity; there is the risk that it might respond to breakdown products of penicillin and other sulphur-containing impurities in crude penicillin; obviously, it cannot be used for fermentation liquors. The other disadvantage is that it is more laborious than methods developed subsequently, and

indeed rather more so than routine biological methods. Statistical examination of adequately replicated determinations on a number of samples showed that the accuracy is of the same order as that of the plate method of biological assay.

COLORIMETRIC AND FLUORIMETRIC METHODS—

The colorimetric method developed by Scudi[3] came next. This depends on the fact that amines react under suitable conditions with penicillin, the β-lactam ring opening to yield a substituted amide of penicilloic acid. Scudi utilised this phenomenon in a very ingenious fashion by using a coloured amine, namely, N-(1-naphthyl-4-azobenzene)-ethylenediamine. The coupling is effected in a chloroform solution of penicillin (free acid). The penicillin is extracted into chloroform from acidified aqueous solution (about 10 i.u. per ml.), and the extract is left standing for 3 hours with a benzene solution of the reagent, containing a trace of acetic acid, which catalyses the reaction. The condensation product, being acidic by virtue of the carboxyl group of the penicillin, is separated from excess of the basic reagent by extraction with 0·05*N* sodium hydroxide. After acidification, the red product is extracted into butanol - benzene mixture to separate it from any remaining traces of reagent. The extract is finally diluted with alcoholic hydrochloric acid for colorimetric measurement. This method is very sensitive and is almost specific for penicillin. It can be used for fermentation liquors, provided a blank determination is included on the same sample after inactivation of the penicillin with penicillinase. It suffers from the disadvantage that the numerous manipulations involved are time-consuming and can give rise to serious errors unless great care and skill are exercised.

The fluorimetric method[4] depends on exactly the same principle, the only difference being that the coupling agent, 2-methoxy-6-chloro-9-(β-aminoethyl)-aminoacridine, is fluorescent. Thus the manipulations are practically the same, except that the condensation requires 1 hour only, and the final estimation is done fluorimetrically. The sensitivity is thereby increased, so that blood and urine samples containing 0·1 i.u. or even less per ml. may be assayed, but the other disadvantages remain.

A colorimetric method,[5] based on the reaction between ninhydrin and the penicillamine liberated on acid hydrolysis, does not appear to have been much used.

A very rapid colorimetric method has recently been published[6]; it depends on the reaction between penicillin and hydroxylamine to form a hydroxamic acid which gives a purple colour with ferric salts. The reactions are complete in 10 minutes, a period that must be timed accurately because the colour fades quickly. The method is not specific for penicillin, but the effect of interfering substances can be eliminated by a blank determination after inactivation of the penicillin with penicillinase. The method then becomes applicable directly to fermentation liquors.

HYDROLYTIC METHODS—

The β-lactam ring of penicillin can be opened by dilute aqueous alkali or the enzyme penicillinase, liberating the potential second carboxyl group. This permits estimation of penicillin by what is virtually a saponification value determination. The reaction is complete in a few hours at room temperature, and the excess of alkali merely has to be titrated back. This method is of the utmost simplicity, is specific in absence of esters and is accurate. Its main disadvantage is that it requires more penicillin than the previous methods. Its capabilities are described more fully in another paper (p. 207). Alternatively, the hydrolysis can be accomplished with penicillinase, provided adequately pure specimens of the enzyme are available; the resulting increase in acidity can be titrated directly,[7] or assessed manometrically[8] by performing the reaction in bicarbonate solution. It has been proposed to estimate penicillin by oxidation with hydrogen peroxide.[9]

IODIMETRIC METHOD—

This method[10,11] depends on the observation that penicilloic acid, but not penicillin itself, will react with iodine. The precise course of the reaction is somewhat obscure and appears to depend on reaction conditions. Complete reaction presumably involves oxidation of the sulphur atom to the sulphonic acid state and oxidation of the potential aldehyde group of the thiazolidine ring to the carboxylic acid state, involving in all a total of 8 atoms of iodine per molecule of penicillin. On this account, and because iodine can be estimated accurately in dilute solution, the method is much more sensitive than the direct hydrolytic method,

is more rapid and is applicable to strongly buffered solutions, which is not true of the direct hydrolytic methods.

It is also more selective, because the blank determination on a portion of the solution that has not been treated with alkali eliminates interference by most other oxidisable bodies. The method has been adopted officially in the United States and is regarded with increasing favour in this country. The Ministry of Supply laboratories have introduced a useful modification in which the iodine solution is buffered, thus reducing greatly the temperature coefficient of the reaction and obviating the need for a thermostat.

To 5 ml. of the penicillin solution, containing not more than 5000 units, is added 1 ml. of *N* sodium hydroxide. The solution is neutralised after standing for 30 minutes and treated with 10 ml. of 0·01 *N* iodine buffered at *p*H 6·24. After another 30 minutes the solution is titrated with 0·01 *N* thiosulphate. A blank is done, omitting the alkali treatment and allowing only 5 minutes standing with iodine.

POLARIMETRIC METHOD—

Commercial penicillins, of 800 i.u. or more per mg., appear to contain no significant amounts of optically active impurities. The penicillins, on the other hand, are strongly dextro-rotatory and to the same degree. Accordingly, it has recently been suggested[12] that the penicillin in such samples could be estimated polarimetrically.

SPECTROSCOPIC METHOD—

A spectroscopic method[13] for total penicillins, based on the absorption at 322 mμ. induced by heating at *p*H 4·6, is described in another paper (p. 211).

ESTIMATION OF INDIVIDUAL PENICILLINS

We turn now to an entirely different aspect of the subject, namely, the determination of particular individual penicillins present in mixtures, as distinct from the estimation of total penicillin irrespective of variety. The methods available for this purpose can be divided roughly into chemical, physical and bio-physical methods.

CHEMICAL METHODS—

Since the penicillins differ only in the nature of their side-chains, it is clear that only these side-chains are available for differential chemical attack. The benzene ring of benzyl penicillin can be nitrated and the determination made by adapting methods[14,15,16] based on the estimation of phenylalanine in this way. In the method of Page and Robinson the penicillin is nitrated, reduced with zinc dust, diazotised and finally coupled with N-(1-naphthyl) ethylenediamine to yield an azo dye suitable for colorimetric measurement. Unfortunately the method is by no means specific and will respond, for instance, to phenylacetic acid, an impurity liable to be present in yellow penicillin.

It should be possible to determine *p*-hydroxybenzyl penicillin (X) by taking advantage in one way or another of its phenolic hydroxyl group; for example, the determination of acetyl value might be used, or advantage be taken of the fact that this type of penicillin will take up iodine. In practice, penicillin X is more easily determined by partition methods.

The differing unsaturation in the side-chains of the various penicillins has been suggested as a basis for differentiation. It would be advantageous if some mild hydrogenation technique could be found that would reduce only the aliphatic double bond in penicillin F (or flavicidin); but such methods, or indeed others that might be devised, depending on the peculiar reactivity of aliphatic double bonds, do not appear to have been described. Mention has been made in confidential reports[17] of a vigorous hydrogenation technique that reduces, in addition, the aromatic double bonds of penicillins G and X. This method would obviously only discriminate between two groups of penicillins, namely, the fully saturated heptyl and amyl penicillins and all the others.

A method has been published that purports under favourable conditions to separate benzyl penicillin from all other penicillins by precipitation as the ethylpiperidine salt.[18] As this method is described in another paper (p. 205), I will only remark that it would be very useful if only it were as specific as its authors claim.

PHYSICAL METHODS—

Methods involving ultra-violet and infra-red spectroscopy have been published[19,20] and will be described in another paper (p. 211).

The possibilities of X-ray crystallography for analysing mixtures of pure crystalline penicillins have been considered in more than one laboratory, but no results have yet been published.

A great deal of work has been done on the separation of penicillins by taking advantage of the differences in their partition behaviour between buffer solutions and organic solvents. For the most part these differences are small, but roughly quantitative methods for *p*-hydroxybenzyl and heptyl penicillins (X and K) have been devised depending on "bulk partition" as distinct from counter-current techniques. Thus penicillin X is virtually not extracted by chloroform from acidified aqueous solutions under conditions that remove other penicillins nearly quantitatively. Penicillin K is largely extracted by chloroform from a buffer at *p*H 5·5, while other penicillins are extracted only to a small degree. This partition separation can be made more nearly perfect by using multiple counter-current partitions. This can be done with the Craig apparatus,[21] a machine designed to apply, without too much mechanical effort, a total of 20 or 25 "theoretical plates." Vastly more "theoretical plates" can be applied by using a partition chromatogram in which the stationary phase is silica gel impregnated with a strong buffer solution, a technique adapted by I.C.I. chemists from that originally developed by Martin and Synge for the separation of acetyl amino acids. The penicillin fractions arising from the Craig machine or from chromatograms[22,23] can obviously be estimated either biologically or chemically. There is, however, an ingenious modification of the chromatographic technique, also elaborated by I.C.I. workers,[24,25,26] in which buffer-impregnated filter paper strips form the stationary phase. This micro separation is of necessity followed up by biological estimation of the separated penicillins, as described in further detail in a later paper (p. 203).

Some attempts have been made to separate the penicillins by adsorption chromatography on alumina, charcoal and other adsorbents.

Vigorous hydrolysis will split the amide linkage in the side-chains of the penicillins, liberating the corresponding acids R.COOH. These have been used for identification of individual penicillins, and we have been experimenting lately (as doubtless have other laboratories) on methods of fractionating these acids by the Craig apparatus or by partition chromatography as a means of indirect analysis of mixed penicillins.

Brodersen[27,28] has recently described a technique based on measurements of the rate of inactivation by acid. On plotting the logarithm of penicillin concentration against time, changes of slope in the curve are observed, due to the presence of penicillins of differing lability. A rather complicated geometrical analysis permits calculation of the percentage of penicillin G present, and of the more labile penicillins, X, F and K, which cannot be differentiated. A number of samples were found to contain traces (under 1 per cent.) of a less labile penicillin, Y.

Each method has its advantages and its disadvantages, and probably no single method provides the perfect solution to this difficult problem of the accurate assay of penicillin mixtures. To make matters worse it has been claimed that the clinical efficacy of penicillin is enhanced by impurities of unknown constitution that are present in some samples of crude penicillin.

References

1. Committee on Medical Research (Office of Scientific Research and Development, Washington) and the Medical Research Council (London), *Nature*, 1945, **156**, 766.
2. Hems, B. A., Page, J. E., and Robinson, F. A., C.P.S. 225, 290 and 384.
3. Scudi, J. V., *J. Biol. Chem.*, 1946, **164**, 183.
4. Scudi, J. V., and Jelinek, V. C., *Ibid.*, 1946, **164**, 195.
5. Allinson, M. J. C., *Proc. Soc. Exp. Biol. Med.*, 1945, **60**, 293.
6. Ford, J. H., *Anal. Chem.*, 1947, **19**, 1004.
7. Murtaugh, J. J., and Levy, G. B., *J. Amer. Chem. Soc.*, 1945, **67**, 1042.
8. Henry, R. J., and Housewright, R. D., *J. Biol. Chem.*, 1947, **167**, 559.
9. Colon, A. A., and Frediani, H. A., *Bol. Col. Quim. Puerto Rico*, 1947, **4**, 20 (*cf. Chem. Abs.*, 1947, **41**, 7662).
10. Alicino, J. F., *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 619.
11. Mundel, M., Fischbach, H., and Eble, T. E., *J. Amer. Pharm. Assoc.*, 1946, **35**, 373.
12. Grenfell, T. C., Means, J. A., and Brown, E. V., *J. Biol. Chem.*, 1947, **170**, 527.
13. Herriott, R. M., *Ibid.*, 1946, **164**, 725.
14. Page, J. E., and Robinson, F. A., *Nature*, 1946, **158**, 910.
15. Del Vecchio, G., and Argenziano, R., *Boll. Soc. Ital. Biol. sperim.*, 1946, **22**, 1190.
16. Canback, T., *Farm. Rev.*, 1947, **46**, 97 (*Chem. Abs.*, **41**, 3258).

17. Eli Lilly Inc., C.P.S. 472.*
18. Sheehan, J. C., Mader, W. J., and Cram, D. J., *J. Amer. Chem. Soc.*, 1946, **68**, 2407.
19. Philpotts, A. R., Thain, W., and Twigg, G. H., *Nature*, 1947, **159**, 839.
20. Barnes, R. B., Gore, R. C., Williams, E. F., Linsley, S. G., and Petersen, E. M., *Anal. Chem.*, 1947, **19**, 620.
21. Craig, L. C., Hogeboom, G. H., Carpenter, F. H., and Du Vigneaud, V., *J. Biol. Chem.*, 1947, **168**, 665.
22. Fischbach, H., Mundell, M., and Eble, T. E., *Science*, 1946, **104**, 84.
23. Fischbach, H., Eble, T. E., and Mundell, M., *J. Amer. Pharm. Assoc., Sci. Ed.*, 1947, **36**, 220.
24. Goodall, R. R., and Levi, A. A., *Nature*, 1946, **158**, 675.
25. ——, —— ANALYST, 1947, **72**, 277.
26. Winsten, W. A., and Spark, A. H., *Science*, 1947, **106**, 192.
27. Brodersen, R., *Acta Pharmacol.*, 1946, **2**, 1.
28. —— *Ibid.*, 1947, **3**, 124.

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The Determination of Individual Penicillins: A Critical Review of Some Proposed Methods

BY W. R. BOON

THE problem of determining individual penicillins varies with the nature of the particular sample to be examined and also with the sort of information required. Thus one may wish to know, at one extreme, the relative amounts of the various penicillin species in a sample that may contain, say, six different penicillins as well as a quantity of inert material, and at the other extreme the amount of a particular penicillin present in a substantially pure sample. In this paper the different methods that have been proposed from time to time to deal with this problem will be examined briefly, and an attempt will be made to indicate what are the particular advantages and disadvantages of each. Spectroscopic methods will not be discussed, as they are dealt with by Dr. Twigg (p. 211).

One of the earliest methods that found any extended application was the so-called differential assay. In this method the biological activity of the sample under investigation was determined by means of two different test organisms, usually *Staphylococcus aureus* and *Bacillus subtilis*, and a single standard substance. Obviously, in this simple form, it is only possible to determine the composition of a two component mixture; with more complex mixtures the figures can only be interpreted in terms of a large number of possible combinations. It has been suggested from time to time that the method can be extended by the addition of a test using a further organism for each additional penicillin present. Whilst this is theoretically possible, there is the practical difficulty that one seldom knows how many penicillins are present in a mixture. Nevertheless the method still has some value in the control of manufacturing operations where the previous history of any sample under examination is known.

Since all the penicillins have very similar chemical properties, methods for their estimation in complex mixtures containing much inactive material must necessarily involve separation, partial or complete, of the different individuals by physico-chemical methods, followed by measurements of the penicillin content of the various fractions by biological assay, by a chemical method, *e.g.*, the iodimetric titration method of Alicino,[1] or by a physical means such as light absorption or optical rotation.[2]

At the present time the most useful method of separating the penicillins is undoubtedly by distribution between two liquid phases. Distribution between a liquid and a solid phase, as in adsorption chromatography, is not nearly so satisfactory, as no suitable adsorbent has yet been found for this purpose. Owing to the small differences in partition coefficient and dissociation constant of the different penicillins, simple solvent extraction is of little use in effecting a separation and resort must be had to counter-current methods. There is one exception, however, to this general statement, since *p*-hydroxybenzyl penicillin is virtually insoluble in chloroform and may be estimated by extracting the other penicillins with this solvent from an acidified aqueous solution.

Of the counter-current extraction procedures the oldest is the silica - buffer - solvent chromatogram introduced in our laboratories in the middle of 1942 and since then used

* Progress reports of the Committee for Penicillin Synthesis are available for inspection at the places listed in *Nature*, 1947, **159**, 565.

extensively elsewhere. In the method, which is an adaptation of the partition chromatogram of Martin and Synge,[6] one of the liquid phases, a phosphate buffer solution, is held stationary absorbed on silica gel, while the other, mobile, phase moves past it. It was by this method that in 1943 we were able to demonstrate conclusively the existence of more than one penicillin by separating what are now known as benzyl penicillin and Δ^2-pentenyl penicillin from one another.[3] We also used it somewhat later to estimate the composition of the crude penicillin produced by various combinations of mould strain and medium.[4] The method, although very effective for preparative purposes, is rather cumbersome as an analytical tool. Unless the amount of penicillin put on to a column is very low in proportion to the amount of silica employed, there is often considerable overlapping of the bands and in order to obtain a complete separation in the less favourable cases it is necessary to analyse fractions from the first column on one or more further columns. Furthermore, it is necessary to determine the total penicillin activity in a large number of samples, either of eluate or of portions of the column material, in order to obtain a measure of the distribution of activity. For rapid approximate determinations the polarimeter is of considerable help, for the penicillins are strongly dextro rotatory. Care should be exercised, however, in the interpretation of results obtained by this method since some batches of crude penicillin contain optically active but biologically inert material, both dextro and laevo rotatory.

Recently, Fischbach, Eble and Mundell[5] have written a paper on the use of this method for the analysis of mixed penicillins. Their technique differs little from that developed by us some five years previously, except that they estimate the penicillin content of their fractions by means of the iodimetric method of Alicino. From the results given in this paper, which may be taken as typical, it would appear that *n*-heptyl penicillin can be estimated fairly readily, for it moves so much faster through the column than any other of the penicillins that there is no overlapping. The picture given by Fischbach *et al.* of the distribution of activity is in accord with this supposition, for the shape of the band is characteristic of a pure substance, having a sharp front edge, a zone of constant concentration and then a gradual fall-off at the tail. There is a possibility that this happy state of affairs in which only one penicillin migrates faster than the others will not always hold, for we have evidence that in some batches of crude penicillin there may be more than one active substance present in the zone normally occupied by *n*-heptyl penicillin. Fischbach *et al.* claim that by this method they are able to estimate *n*-heptyl penicillin with a reproducibility of ± 2 per cent. They make no claim for the determination of other penicillins, although they state that they were able to isolate some sodium Δ^2-pentenyl penicillin. Judging by the shape of the curves given in their paper it appears that there is considerable overlapping between the different zones of activity, which would render the method of little value for the estimation of penicillin other than *n*-heptyl penicillin.

Perhaps the most generally useful method for determining the composition of a complex mixture of penicillins is the micro-chromatographic method of Goodall and Levi.[7] This method is similar, in general principle, to the partition chromatogram discussed above, the main difference being that the inert support for the phosphate buffer solution is a strip of filter paper instead of silica gel. In addition, the distribution of activity between the various fractions is determined in one operation by placing the paper strip, containing the developed chromatogram, on a sheet of nutrient agar that has been inoculated with a suitable penicillin-sensitive micro-organism. After incubation the nature of the penicillins and the amount of each present may be estimated from the position and size of the inhibition zones.

The main advantages of this method are as follows—

(i) It is fairly readily adapted to routine use, because the manipulations involved are simple and easily learned by the average laboratory assistant, though strict attention to detail is necessary.
(ii) It employs only a very small amount of material, the actual amount applied to a strip being a few micrograms.
(iii) Replication is easy, a series of six replicates on one sample being easily run on one plate.
(iv) All the penicillins are estimated at one time.
(v) The efficiency of separation of one penicillin from another is extremely high. Thus, it is possible to separate benzyl penicillin (II) from Δ^2-pentenyl penicillin (I) the ratio of whose partition coefficients is only 1·5. More difficult is the separation

of Δ^2-pentenyl penicillin from what, in our laboratories, we call penicillin IV, but even in this case, where the ratio of the partition coefficients is less favourable, a complete separation can be effected. This complete separation of penicillins I and IV is not shown in the illustrations in the paper of Goodall and Levi,[7] but recent slight changes in technique have rendered it possible, though it is usually necessary to develop the K group of penicillins off the strip to achieve it

(vi) The amount of inert material in the sample to be analysed is unimportant provided the sample is not so impure that it is impossible to obtain a solution of the required concentration, 10,000 units per millilitre.

(vii) The method indicates the presence of any novel penicillins that may be present in the sample, subject to the proviso that sufficient resolution has been obtained during development. Although it is not possible to say categorically that the method will permit of the separation of all penicillins, it can be said with confidence that the resolution obtained is much greater than with any other method available at the present time.

Against the many advantages indicated above some defects of the method must be set. The chief one is that results can only be expressed as a proportion of the total penicillin activity measured in terms of the particular test organism and standard that are used. Translation of these into either percentage of total weight of penicillin or of sample is theoretically possible, but is only likely to be accurate if the activity of the individual penicillins in the pure state is known. Unfortunately, this is not always so at the present time. Calculation of the relative proportions in terms of total activity is based on an empirical equation whose constants vary somewhat from plate to plate and probably also from one penicillin to another and with the total amount applied to the strip. These weaknesses, the effect of which it is impossible to assess accurately, do not detract from the general utility of the method, since it enables consistent and reasonably reliable results to be obtained on complex mixtures that cannot be analysed by any other technique available at the present time. It has also proved useful for the examination of nearly pure, and reputedly pure, samples of individual penicillins, for small quantities of other penicillins are readily detected.

Results obtained by this method for the benzyl penicillin content of commercial crystalline sodium penicillin are usually in good agreement with those obtained by the N-ethylpiperidine salt method or by infra-red spectroscopy.

There is one other method depending on distribution that remains to be considered. That is the method introduced by Craig at the Rockefeller Institute using an ingenious piece of apparatus which is, in effect, a series of tubes or separating funnels so arranged that the top layer in one funnel may be transferred to the next one while at the same time fresh top layer is brought in from the one on the other side.[8] By this means it is possible to submit a solute to a number of counter-current distributions. If the solute is a pure substance and if its partition coefficient is constant over the range of conditions met with in the experiment, the total amount of solute present in each tube is given by the successive terms in the expansion of

$$\left[\frac{1}{K+1} + \frac{K}{K+1}\right]^n \quad \ldots \quad (1)$$

where K is the partition coefficient and n is the number of "plates" in the distribution. If the solute contains two substances that do not interact and whose partition coefficients are K and K′, constant throughout the conditions of the experiment, and the fractions of each present initially are p and $1-p$ respectively, then the amount in any given tube will be the corresponding term in the expansion of

$$p\left[\frac{1}{K+1} + \frac{K}{K+1}\right]^n + (1-p)\left[\frac{1}{K'+1} + \frac{K'}{K'+1}\right]^n.$$

Provided the difference between K and K′ is sufficiently great there will be two maxima in the distribution and it is possible to estimate accurately the relative amounts of the two solutes present from the shape of the curve and the positions of the maxima.

The method has been applied to penicillin by Craig, Hogeboom, Carpenter and du Vigneaud,[9] using ether and a $2N$ phosphate buffer solution as the two immiscible solvents. These authors' results show that it is fairly easy to separate n-heptyl penicillin and

p-hydroxybenzyl penicillin from benzyl penicillin by this method, but very little separation was achieved with a mixture of benzyl penicillin and Δ^2-pentenyl penicillin. The penicillin in each tube was determined by transferring all the material to the ether layer, evaporating to dryness and weighing, or by a spectrophotometric procedure.

This method, although very ingenious, appears to be far too cumbersome and insensitive to be useful as a routine analytical method. There is also a weakness in it which does not seem to have been appreciated by Craig, who assumes that the partition coefficient is constant over a wide range of conditions. This is not true, in general, for organic acids, and penicillin is no exception. The effective partition coefficient of penicillin is in fact a function of several variables, the most important of which have been found by my colleagues Mr. Greenhalgh and Dr. Pryce to be as follows.

(i) The nature of the organic solvent.
(ii) The hydrogen ion concentration in the aqueous phase.
(iii) The salt strength of the aqueous phase.
(iv) The concentration of penicillin, particularly in the solvent phase.

Under the conditions of Craig's method, (i) and (iii) can be kept constant and (ii) is approximately constant, for in order to obtain a partition coefficient of unity it is necessary to work at a *p*H value very near the limits of the buffering range of the phosphate system employed. The most important factor is undoubtedly the last, since owing to association in the solvent phase the effective partition coefficient is higher at higher concentrations, *e.g.*, at the beginning of the distribution or at the peak, than it is at low concentrations, *e.g.*, at the tails of the distribution. The effect of association is probably not of much importance when ether is used as the organic solvent, as then the association constant of benzyl penicillin is less than 10; with other solvents it is much higher, being about 20 for amyl acetate and at least 600 for chloroform. It will be realised that the use of solvents such as these, which might be considered on grounds of greater convenience, is to be avoided since with them the mathematical expressions used by Craig for interpreting the results are no longer valid.

If we assume all factors other than the concentration in the solvent layer to be kept constant, the effective partition coefficient is given, to a first approximation, by the expression

$$K = \frac{K_0}{2}\left[1 + \sqrt{8\alpha C_s + 1}\right] \quad \ldots \quad (2)$$

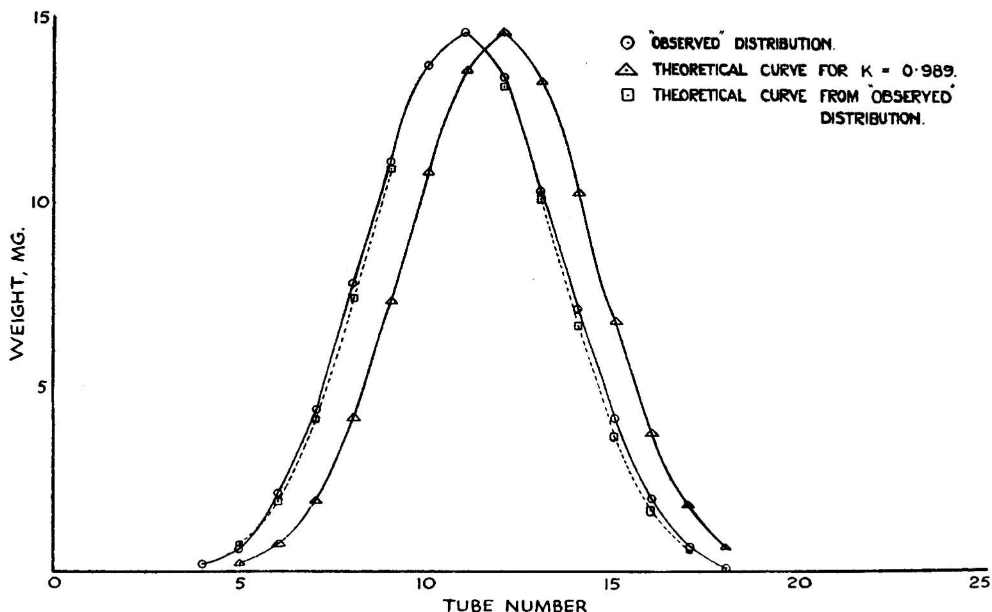
where K_0 is the true partition coefficient at zero concentration, α is the association constant (association into double molecules) and C_s is the concentration in the solvent phase in gram molecules per litre.

Then assuming an association constant of 10, an effective distribution coefficient of 0·989 at a concentration of 0·0175 g.-mol./litre in the solvent in the first tube, a calculation, term by term, for a twenty-four plate distribution shows that the maximum will occur one tube sooner than would be expected from Craig's expression. If, on the other hand, the "observed" partition coefficient at the maximum is used, a "theoretical" curve in much better agreement with the "observed" one is obtained; the agreement is still better if one fits the maximum of the "theoretical" curve to the maximum of the "observed" one, using the method of Williamson and Craig.[10] These effects are illustrated in the figure.

To sum up, Craig's method is too cumbersome and insufficiently sensitive for the routine examination of complex mixtures. It is probably of use for detecting heterogeneity in "pure" samples of individual penicillins though it is unlikely to be of much use in detecting, say, a trace of Δ^2-pentenyl penicillin in benzyl penicillin. In addition, Craig's theoretical treatment is too simple to be applied indiscriminately to all systems, though it is probably adequate under the conditions actually employed by Craig *et al.* in their examination of penicillin. In a later paper by Sato, Barry and Craig[13] on the distribution of fatty acids, the effect of association on the theoretical treatment is realised and an attempt is made to find a system in which the partition coefficient does not change with concentration.

Of the purely chemical methods for the estimation of individual penicillins only that of Sheehan, Mader and Cram,[11] involving the precipitation of the N-ethylpiperidine salt of benzyl penicillin, has been used to any extent. This method is official in the United States.[12] In the original paper it was claimed that it could be used to determine the benzyl penicillin in a mixture provided this penicillin represented at least 50 per cent. of the total penicillin and that the potency was over 800 units/mg. This claim was based on the melting point, specific rotation, ultra-violet absorption and elementary analysis of the precipitated salt.

Unfortunately these measurements are all such as would be altered only slightly by contamination with several per cent. of another penicillin and are therefore useless as criteria of homogeneity. Some time ago my colleague Dr. Dobson examined this point critically by analysing the starting material and the precipitate by the micro-chromatographic method and by re-analysing the precipitated salt of supposedly pure benzyl penicillin. Thus a sample of calcium penicillin, of potency 1320 u./mg. and containing 65 per cent. of benzyl penicillin, 10 per cent. of Δ^2-pentenyl penicillin, 1 per cent. of *p*-hydroxybenzyl penicillin and 15 per cent. of penicillin IV, gave a precipitate equivalent to 57 per cent. of benzyl penicillin in the sample. This precipitate on micro-chromatographic analysis was found to contain only 83 per cent. of benzyl penicillin, together with 7 per cent. of Δ^2-pentenyl penicillin, 1 per cent. of *p*-hydroxybenzyl penicillin and 9 per cent. of penicillin IV. This N-ethylpiperidine



salt was then analysed and gave a 90 per cent. recovery of a salt containing 90 per cent. of benzyl penicillin by micro-chromatographic analysis and only small amounts of other penicillins. It is now generally accepted in this country that the method should only be applied to samples substantially free from inert material and containing at least 90 per cent. of their penicillins as benzyl penicillin. Even with this limitation the method is still rather tricky and the analysts of the main producers, together with a representative of the Ministry of Supply, are endeavouring to standardise details of procedure so that results obtained from different laboratories will be in as good agreement as those that can be obtained by any one of them. One of the biggest sources of variation has been found to be the N-ethylpiperidine used. This is probably due to the fact that piperidine as usually available contains several other substances, notably partially reduced pyridines and higher homologues. If, however, the reagent is carefully purified in a precision still, more concordant results can be obtained.

Page and Robinson[14] have suggested a colorimetric procedure for benzyl penicillin in "purified" penicillin, involving nitration, reduction with zinc, diazotisation and coupling with N-(1-naphthyl)-ethylenediamine. This method does not appear to have found any very extensive use.

Finally, a few words about methods of expressing results. There is, of course, the broad division into percentage on total weight and percentage on total penicillin. The first presents little difficulty other than the fact that penicillin is sold not by weight but in terms of a biological assay. If the result is expressed in terms of total penicillin then the figures will vary with the method of assay employed. This is perhaps best illustrated by an example. Suppose we have a sample of sodium penicillin of which 90 per cent. by weight of the total

penicillin is benzyl penicillin and 10 per cent. by weight is *n*-heptyl penicillin; if the penicillin activities are determined by the usual assay procedure using *S. aureus* the benzyl penicillin will appear as only 85 per cent. of the total penicillin, but if the total penicillin is determined by the iodimetric method of Alicino or by optical rotation, then the correct result will be obtained, because the molecular weights of these two penicillins are almost identical.

A fair comment on the methods discussed above would be that there are no methods available that are entirely adequate for the determination of individual penicillins, but that the two most generally useful are the micro-chromatographic analysis of Goodall and Levi because of its range of application and simplicity, and, for the determination of benzyl penicillin in nearly pure material, the ethyl piperidine salt method of Sheehan *et al.*

REFERENCES

1. Alicino, J. F., *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 619.
2. Grenfell, T. C., Means, J. A., and Brown, E. V., *J. Biol. Chem.*, 1947, **170**, 527.
3. Boon, W. R., Calam, C. T., Gudgeon, H., and Levi, A. A., *Biochem. J.* In the press.
4. C.P.S. Report No. 49, 12.4.44.
5. Fischbach, H., Eble, T. E., and Mundell, M., *J. Amer. Pharm. Assoc.*, 1947, **36**, 220; Preliminary note in *Science*, 1946, **104**, 84.
6. Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, 1941, **35**, 1358.
7. Goodall, R. R., and Levi, A. A., ANALYST, 1947, **72**, 277.
8. Craig, L. C., *J. Biol. Chem.*, 1944, **155**, 519.
9. Craig, L. C., Hogeboom, G. H., Carpenter, F. H., and du Vigneaud, V., *Ibid.*, 1947, **168**, 665.
10. Williamson, B., and Craig, L. C., *Ibid.*, 1947, **168**, 687.
11. Sheehan, J. L., Mader, W. H., and Cram, D. J., *J. Amer. Chem. Soc.*, 1946, **68**, 2407.
12. *United States Federal Register*, 1946, **11**, 13905.
13. Sato, Y., Barry, G. T., and Craig, L. C., *J. Biol. Chem.*, 1947, **170**, 501.
14. Page, J. E., and Robinson, F. A., *Nature*, 1946, **158**, 910.

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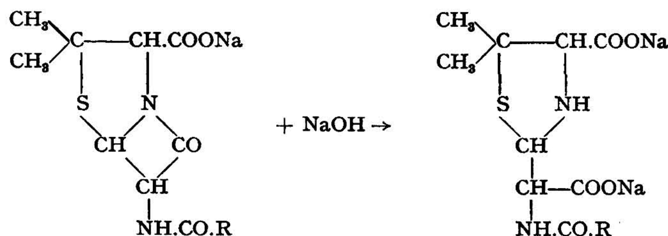
The Determination of Penicillin by Alkaline Hydrolysis

BY STELLA J. PATTERSON AND W. B. EMERY

ABRAHAM and Chain[1] reported that penicillin was inactivated by alkali and record that electrometric titration of an aqueous solution of the barium salt with alkali gave evidence of a reaction occuring between *p*H 11 and 12.

It was later stated by Lester Smith (private communication) that alkaline hydrolysis took place quantitatively, in a manner analogous to the saponification of oils; he demonstrated that theoretical results could be obtained on pure penicillin, but that for the low potency material then generally available the results were high, because impurities present also consumed alkali.

The reaction is represented thus—



The product formed is sodium penicilloate; the radical R differs in the different forms of penicillin.

A report[2] received later from the research laboratories of Chas. Pfizer and Co., New York, gave details of a quantitative method for penicillin estimation, based on alkaline hydrolysis. Their procedure was as follows.

Pipette 10 ml. of a penicillin solution containing from 60,000 to 150,000 units into a 50-ml. beaker equipped with the usual glass and calomel electrodes, connected to a *p*H meter,

and a motor-driven stirrer. Add dropwise sufficient 0·1 *N* sodium hydroxide to bring the *p*H to 8·0. Add exactly 10·0 ml. of the standard alkali, which will bring the *p*H to approximately 12. Stir the solution at room temperature for 10 minutes and then titrate back to *p*H 8·0 with standard 0·1 *N* hydrochloric acid and note the volume of acid consumed.

Calculation—Units of penicillin = (10 − ml. of *N* HCl) × 594,000.

We have found a modification of this method very useful for routine assays on solid penicillin of high potency. In order to avoid the use of a *p*H meter we tried several indicators, singly and in combination, and found cresol red to be satisfactory. It gives at the required *p*H a colour change that is sharp even in presence of the yellow pigment of commercial penicillin. The technique we finally adopted is as follows.

REAGENTS—*Cresol red solution*—0·1 per cent. solution of cresol red in 70 per cent. alcohol, neutralised with 0·1 *N* sodium hydroxide.

Carbon dioxide free water—Boil distilled water for ¼ hour, stopper the flask with a rubber bung fitted with a soda-lime tube and allow to cool.

PROCEDURE—Weigh between 0·1 and 0·2 g. to the nearest mg. into a small dry conical flask, taking all precautions necessary to minimise absorption of moisture. Add 50 ml. of cold carbon dioxide free water and 1·0 ml. of cresol red solution followed by 0·1 *N* sodium hydroxide slowly from a micro-burette until a red coloration is obtained (a 5-ml. micro-burette delivering drops of from 0·02 to 0·03 ml. is suitable). Add from a pipette 10 ml. of 0·1 *N* sodium hydroxide; stopper the flask with a rubber bung and allow to stand at room temperature for 3 hours.

After this period, add 10 ml. of 0·1 *N* hydrochloric acid and immediately titrate the excess of acid with 0·1 *N* alkali to the red colour obtained on initial neutralisation of the penicillin solution (*a* ml.). Carry out a blank (*b* ml.) on 50 ml. of the carbon dioxide free water with 1 ml. of indicator solution treated in the same way (usually this requires about 0·13 ml. of 0·1 *N* alkali).

Penicillin, i.u./mg. = $(a - b) \times 59{,}340$/weight of sample taken, in mg.

An alternative procedure used by one of us (W. B. E.) on production material is as follows.

Weigh out the sample as before and dissolve in 50 ml. of cold carbon dioxide free water. Add 10 drops of a 0·1 per cent. solution of α-naphtholphthalein in 50 per cent. alcohol and add 0·01 *N* sodium hydroxide until a green (with yellow penicillin) or blue colour (with white penicillin) is obtained. Add 10 ml. of 0·1 *N* sodium hydroxide, stopper the flask with a rubber bung and allow to stand at room temperature for 3 hours. Then titrate back with 0·05 *N* hydrochloric acid to a pale greenish-yellow colour (with yellow penicillin) or to almost complete absence of colour (with white penicillin).

OBSERVATIONS ON THE METHODS—

1. The use of water free from carbon dioxide was found to be essential, but we found it unnecessary to prepare carbon dioxide free standard acid and alkali.

2. Some workers find no great advantage in using standard acid and alkali more dilute than 0·1 *N*, as the end-point then becomes less distinct. Using 0·1 *N* solutions with cresol red, a sharp end-point is obtained with 1 drop from a micro-burette unless the penicillin solution is strongly buffered; this represents a possible titration error of approximately ±1 per cent. when the above quantities are taken for assay.

3. We normally find it convenient, when carrying out several assays at the same time, to dissolve the penicillin in 50 ml. of water and allow the solution to stand for 3 hours to complete the inactivation after addition of the sodium hydroxide. Lester Smith (private communication), however, has pointed out that the reaction can be completed in a shorter time by reducing the total volume of the solution: with 20 ml. (including the 10 ml. of 0·1 *N* sodium hydroxide) 1 hour's standing is sufficient; with a total volume of 30 ml., 1½ hour's standing is sufficient.

4. The temperature of the solution during inactivation is not critical. Inactivation is completed in the stated time at temperatures above 4° C.

5. The statement of the equivalence of 1 ml. of 0·1 *N* sodium hydroxide is derived from the molecular weight of sodium penicillin G (356) and the fact that 1 mg. has a potency of 1667 i.u. Thus 1 ml. of 0·1 *N* sodium hydroxide $\equiv 35{\cdot}6 \times 1667 = 59{,}340$ i.u. Clearly also 1 ml. of 0·1 *N* sodium hydroxide ≡ 59,340 i.u. of penicillin G present as the calcium or any other salt.

For penicillin K, with a molecular weight of 364 and a potency of 2300 i.u./mg., 1 ml. of 0·1 *N* sodium hydroxide is equivalent to 83,720 i.u. Thus, in the analysis of commercial samples of penicillin G containing some penicillin K, the use of the 59,340 factor will give results approximately 3 per cent. lower than the true biological potency for every 10 per cent. of penicillin K present. Appreciable quantities of the other penicillins, if present, will also affect the accuracy of the results.

6. Although the chemical assay cannot in all circumstances have the same value as a biological determination of potency, it is nevertheless very useful where assays are required, both in production control and on various penicillin products. The bio-assay is the more consuming of time, and the number of these assays that can be carried out is often restricted; there is therefore a great advantage in having available a quick chemical method with a known degree of reliability.

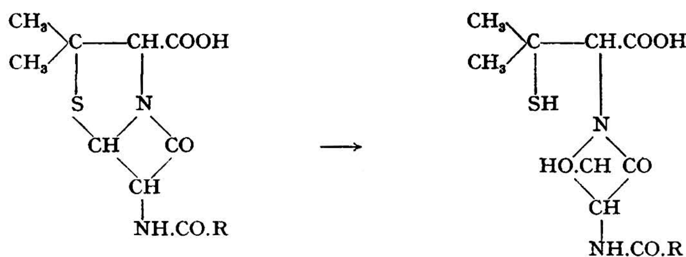
The method just described has been of great use in investigations into the stability of penicillin and some of its preparations; thus, a considerable bulk of information has become available from the results of many hundreds of keeping tests which could not have been carried out by biological assay alone.

LIMITATIONS OF THE METHOD—

1. Since 1 ml. of 0·1 *N* sodium hydroxide is equivalent to 59,340 i.u. of penicillin G, it is clear that the method as described is not of use in the examination of products of low levels of potency, such as the official ointment (500 i.u./g.) and lozenge (500 i.u.). It is useful, however, in the routine examination of bulk penicillin powder having a potency greater than 900 i.u./mg., vials containing from 0·1 to 1×10^6 units, penicillin in oil and beeswax suspension, high-potency lozenges and ointments and certain other special pharmaceutical preparations. The penicillin must normally be first extracted before the hydrolysis can be carried out; we give later in this paper the method we have adopted for the suspension of penicillin in oil and beeswax.

2. While carrying out an investigation into the effect of boiling on penicillin solutions, some wide discrepancies between the results of chemical assay and those of the biological method were observed.[3] On boiling, the biological potency fell more quickly than the apparent potency found by alkaline hydrolysis. After about 10 minutes boiling, in some instances an unbuffered solution initially of 50,000 i.u./ml. showed no biological activity, but the chemical assay still indicated a considerable potency.

It was clear that the penicillin itself had been completely destroyed, but some alkali-consuming substance remained. Lester Smith and Page (private communication) postulated that some of the loss of activity was caused by a breakdown of the penicillin molecule owing to cleavage of the sulphur-containing ring, as follows:—



That this is so has been partially confirmed by the results of polarographic experiments. Since this (or something similar) occurs or may occur when penicillin decomposes, it follows that the use of the chemical method for the assay of solutions or preparations that have partially lost potency must necessitate occasional checks against bio-assay to establish the reliability of the chemical results in the particular circumstances.

Samples of freeze-dried (yellow) penicillin were kept at 100° F. for about 12 to 14 months and were examined to see whether decomposition had occurred in the solid substance over this long period. The samples (21) were assayed chemically and biologically; on the average the chemical were 10 per cent. higher than the biological results. The average loss of biological potency over the period was 25 per cent. It must be pointed out that the samples were of loose powder in stoppered vials, *i.e.*, not in the normal commercial freeze-dried vial pack;

it is not claimed that the experimental conditions were carefully controlled, particularly in respect of moisture content. It is likely, however, in view of the higher chemical results, that the type of decomposition that occurs on boiling the solution had been slowly taking place.

In the interpretation, therefore, of chemical assay results on old samples of solid penicillin, it will be necessary to bear this phenomenon in mind.

ASSAY OF PENICILLIN OIL AND BEESWAX SUSPENSIONS—

The B.P. oily injection is a suspension of calcium penicillin in a mixture of arachis oil with 4·5 per cent. of beeswax; it contains 125,000 i.u./g. The penicillin may be extracted for assay by dissolving the mixture in chloroform and shaking out with water; good recoveries have been obtained by this method, but emulsions are readily formed. These may be avoided by using a large excess of chloroform, but several extractions with water are then necessary; the layers take several minutes to separate completely and the procedure is tedious.

TABLE I

Batch	Input i.u./g.	Found i.u./g.
1	272,000	276,000
2	136,000	135,000
3	113,000	112,000
4	120,000	118,000
5	130,000	130,000

It was originally hoped to apply the chemical method of assay to these suspensions without previously removing the oil and beeswax; a number of solvents were used, but in our experience some degree of saponification of the oil always took place, results being consistently high. We realised that it would be necessary to separate the penicillin from the oil and beeswax and the following method, depending on the negligible solubility of calcium penicillin in anhydrous ether, was finally adopted.

PROCEDURE—Weigh between 1 and 2 g. of the sample to the nearest mg. into a dry 50-ml. centrifuge tube; add 50 ml. of dry ether to dissolve the oil and beeswax, leaving the calcium penicillin undissolved, and centrifuge for 3 minutes. Decant the ether layer carefully and as completely as possible; centrifuge with a further 50 ml. of dry ether and to the residue in the tube add 50 ml. of carbon dioxide free water, proceeding with the chemical assay in the manner described above. Alternatively the penicillin recovered in this way can be dissolved in buffer solution and assayed biologically if necessary.

TABLE II

Batch	Original potency i.u./mg.		Age months	Re-assay i.u./mg.		Storage temperature
	Biological	Chemical		Biological	Chemical	
8	149,000		11	169,000	140,000	Room
9	136,000		11	144,000	117,000	,,
12	142,000		11	152,000	127,000	,,
14	137,000		11	122,000	131,000	,,
16	145,000		11	—	144,000	,,
17	134,000		11	138,000	127,000	,,
20	156,000		11	110,000	115,000	100° F.
26	146,000		11	107,000	114,000	,,
34	141,000		10	112,000	106,000	,,
37	126,000		10	116,000	105,000	,,
52		144,000	7		146,000	,,
54		136,000	7		123,000	,,
3		256,000	10	222,000	231,000	100° F.
7		264,000	10	249,000	235,000	,,
9		276,000	10	282,000	292,000	,,
11		284,000	9	270,000	281,000	,,
12		276,000	9	—	266,000	,,
15		278,000	9	223,000	249,000	,,

The dry ether used in the above extraction is prepared by shaking anaesthetic ether with powdered calcium chloride for 1 hour, followed by filtration and distillation.

Table I gives the results on five experimental batches; they show that the extraction process is for all practical purposes satisfactory.

Similarly, the method has been found adequate for the control of large-scale production batches both of the B.P. product and of oil and beeswax suspensions of higher potencies.

STABILITY OF OIL AND BEESWAX SUSPENSIONS—

The figures in Table II were obtained on routine samples taken from stock, some stored at 100° F. and others at room temperature.

These figures indicate that the oil and beeswax suspensions are remarkably stable products, considering the readiness with which penicillin itself can be destroyed.

SUMMARY

1. The alkaline hydrolysis method for quantitative determination of penicillin, originally reported by the Pfizer research laboratory, has been adapted to the routine examination of large numbers of samples.

2. Reference is made to certain limitations of the method.

3. Some applications of the method are described; they include a particular reference to a rapid method for the extraction of penicillin from suspensions in oil and beeswax.

The authors are indebted to Miss H. B. Johnson and Mr. G. Norris for most of the chemical and biological assays respectively, and to Mr. A. F. Lerrigo for direction and advice.

REFERENCES

1. Abraham, E. P., Chain, E., and Holiday, E. R., *Brit. J. Exp. Path.*, 1942, **23**, 103.
2. Committee for Penicillin Synthesis Report No. 602.
3. Johnson, H. B., and Lerrigo, A. F., *Quart. J. Pharm.*, 1947, **20**, 3, 183.

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The Spectroscopic Estimation of Penicillin

BY G. H. TWIGG

THE complexity and uncertainty of the biochemical methods used in the estimation of penicillin, and especially in the estimation of the different penicillins, has led to the development of chemical and physical methods, one of which is the use of ultra-violet and infra-red spectroscopy. With this technique, the aim is to discover in the absorption spectrum a band which is characteristic of each penicillin molecule as a whole. In practice, this is an ideal unlikely to be realised and use has to be made of absorptions arising from the separate parts of the molecule. While such a procedure may, with little ambiguity, be made to provide an estimate of total penicillin, it leads to a fundamental difficulty in assaying individual penicillins. Impurities and deactivation products containing similar molecular groupings may have bands almost identical with those of the penicillins. It seems probable that the spectroscopic estimation of individual penicillins is applicable only to pure materials, but more work is necessary to find out how far this is true.

1. ULTRA-VIOLET ANALYSIS OF TOTAL PENICILLIN—

One of the first applications of spectroscopic methods was made by Herriott[1] for the determination of total penicillin. The method depends on the fact that an absorption band develops at 3220 A. on treatment of aqueous penicillin solution with acid. The band is due not to the end product of acid deactivation but to an intermediate. The absorption band, therefore, disappears in time, and the analysis thus depends on an empirical technique designed to catch this band at its maximum intensity. In practice, the sample, which may contain as little as 35 units, is heated in a 0·4 *M* acetate buffer solution at *p*H 4·6 for 15 minutes and cooled rapidly. Using a Beckman ultra-violet spectrophotometer, measurement is made of the increase in absorption at 3220 A. compared with a blank in the same buffer but kept at room temperature. The penicillin concentration is then obtained from a calibration chart.

This method can be used with impure material, as it depends on an increase in absorption. An accuracy to within 5 per cent. is claimed. Since the development of this technique,

other methods have been found for the estimation of total penicillin and it is doubtful whether this method can compete with any of the chemical methods of assay for speed and accuracy.

2. ULTRA-VIOLET ANALYSIS OF PENICILLIN G—

It is in the estimation of the individual penicillins that spectroscopic methods offer greater promise. Two methods have been developed; they depend on the absorption bands in the ultra-violet due to the phenyl group in penicillin G. In the first of these (Philpotts, Thain and Twigg[2]), the ultra-violet spectrum of a solution of the sodium penicillin sample in water - ethanol (1 : 30) is photographed several times on a plate, using a series of path-lengths which are varied by means of a Hilger micrometer cell. Alongside each exposure is recorded the spectrum of a standard penicillin G solution, using the same path-length each time. The path-length at which the spectrum of the unknown solution matches that of the standard is estimated by eye, with interpolation between spectra when necessary. The relative concentrations of the two solutions are then given by the inverse ratio of these path-lengths. As an alternative to penicillin G, an alcoholic solution of phenyl acetamide can be used as a sub-standard. Its spectrum is not identical with that of penicillin G since the unspecific absorption of the penicillin molecule overlies some of the phenyl absorption bands, but matching is possible at the longer wavelength bands at 2685 A. and 2645 A.

This method appears to be accurate to ± 2 per cent. for pure samples of penicillin. Certain impurities produce a continuous unspecific absorption, so that direct matching of the intensities of the bands will give an incorrect result. However, if this absorption is not too strong, matching can be effected by the pattern and width of the bands. With more impure samples, an oxidation with permanganate was carried out and the benzoic acid produced estimated by the same method of matching spectra.

The chief limitation of this method is that, since it is based on the absorption produced by the phenyl group, it will include all phenyl-containing substances, whether active penicillin or not. This is particularly true of the estimation *via* benzoic acid. The result, however, may be a useful guide in setting a maximum value to the penicillin G content of a sample. A further limitation is the possible presence of penicillin X, which has a broad and strong absorption band; its presence to the extent of more than 1 per cent. will invalidate the analysis. This analytical method appears to hold for any salt of penicillin, a correction for molecular weight only being necessary. One advantage of photographing the whole spectrum is that it is easily seen whether the sample has suffered any acid degradation by the appearance of a band at 3220 A.

A method similar in principle to the above has been devised by Grenfell, Means and Brown[3] for the estimation of penicillin G. Using a Beckman ultra-violet spectrophotometer, the optical density of an aqueous solution is measured at 2630 A., where there is a phenyl absorption band. Pure penicillin G has almost no absorption at 2800 A.; decomposition products and other impurities, however, have. To correct for these, the optical density at 2800 A. is subtracted from that at 2630 A. Both penicillin K and F show some absorption at 2630 A. and to take account of this all measurements of optical density are made with solutions of a constant total penicillin content (1·8 mg./ml.). A graph of the optical density difference ($E_{2630} - E_{2800}$) plotted against percentage of penicillin G in the sample is then found to give a straight line; analyses are evaluated from this calibration curve.

The authors state that this method becomes inaccurate when the optical density at 2800 A. is greater than 0·1. This is similar to what was found by Philpotts, Thain and Twigg.[2] Grenfell, Means and Brown overcome this difficulty by a purification of the penicillin. To the aqueous penicillin solution is added 30 per cent. of ammonium sulphate, the mixture is cooled to $-5°$ C. and the precipitate separated and analysed as before. It is stated that for fairly pure samples there is no change in the ratio of the penicillins in this process and that the recovery is over 90 per cent. With very impure samples, this purification may not be adequate; it is then repeated and, if a change in penicillin G content is found, it is corrected for by a linear extrapolation to 100 per cent. purity.

There appears to be nothing to choose between either of these two methods of using ultra-violet absorption for the estimation of penicillin G. Both suffer from similar defects, particularly in respect of phenyl-containing inactive material, although the purification technique of Grenfell, Means and Brown probably circumvents this source of trouble. It should be noted that in both methods the presence of pencillin X in quantities greater than 1 per cent. will invalidate the results. Grenfell, Means and Brown detail a method of estimating the amount of penicillin X present.

3. Infra-red analysis of individual penicillins—

Theoretically, infra-red analysis should be the ideal method, as the infra-red spectrum of any substance is unique. In practice there are serious difficulties. One of these may arise through the penicillin containing impurities or inactive materials that have absorption bands in the same position as the bands used for analysis. Another arises through penicillin being chiefly in the form of a salt (usually the sodium salt) which is insoluble in all the solvents that are of use in infra-red measurements; conversion of the salt to the free acid, and transfer to a suitable dry solvent is not easy and may result in degradation.

Barnes, Gore, Williams, Linsley and Petersen[4] claim to have overcome most of these and other difficulties in a method which uses the solid sodium salt. The salt is ground with a small quantity of Nujol and pressed to a suitable thickness between two rock-salt plates; the spectrum is recorded and these authors use a Perkin - Elmer spectrometer. A qualitative examination of the whole spectrum is always made and may reveal the presence of impurities. It should show a band at 1770 $cm.^{-1}$, which is common to all the penicillins.

From an examination of pure samples of the five known penicillins, Barnes *et al.* show that each has a characteristic band, which is usually free from interference by the bands of the other penicillins. This characteristic band is used for quantitative analysis. One difficulty in the analysis of solids is to know the thickness of the absorbing layer of the substance under examination. This has been overcome by the use of an internal standard in known concentration, and for this purpose the authors recommend *dl*-alanine. Weighed amounts of penicillin and *dl*-alanine are ground together, a drop of Nujol is added and the mixture is squeezed between rock-salt plates. Most of the work described was on the analysis of penicillin G, which has a characteristic band at 703 $cm.^{-1}$ For comparison the alanine band at 851 $cm.^{-1}$ was used, and the ratio $R = \log (I_0/I)_{703}/\log (I_0/I)_{851}$ was determined. (I_0 and I are respectively the intensities of the incident and transmitted radiation.) A calibration curve of R against percentage of penicillin G was made up by dilution of pure penicillin G with magnesium oxide; it was claimed that by means of this curve analysis of unknown mixtures could be made accurate to ± 2 per cent.

A similar method was used for the analysis of the other penicillins; characteristic bands used were penicillin F_1 971 $cm.^{-1}$, F_2 (amyl) 1166 $cm.^{-1}$, K 1330 $cm.^{-1}$ and X 831 $cm.^{-1}$.

There are many technical difficulties in this method of analysis. The effect of crystal structure is not known and a separate calibration may be necessary with amorphous material. It is not easy accurately to measure I_0, the intensity of the incident radiation; in the present work it was measured by interpolation of the background intensity, a procedure which may be inaccurate, especially if impurities are present having absorption bands close to the band being measured.

One of the most serious difficulties, and one that may limit the technique to crystalline penicillin of high purity, is the possible presence of substances such as deactivation products that have absorption bands at the same wavelength as the characteristic bands of the penicillins. For example, the band used in the analysis of penicillin G is one associated with the presence of phenyl groups; deactivation products as well as substances such as phenylacetic acid will thus interfere with the analysis. It is true, as Barnes *et al.* point out, that such impurities may be detected by the additional bands they produce at other wavelengths, *e.g.*, those of phenylacetic acid at 682 $cm.^{-1}$ and 728 $cm.^{-1}$ It is possible, though unlikely, that a unique band characteristic of the active penicillin may be found. In their work on the quantitative analysis, Barnes *et al.* have used only pure specimens of the penicillins, and it is obvious that further investigation is required before the validity of this method of analysis is completely established.

An interesting effect due to crystal orientation was found by Barnes *et al.* One sample of penicillin G was found to give an anomalous result on analysis. Microscopical examination revealed that the crystals were in the form of flat plates that had become oriented on squeezing the Nujol mull between the rock-salt plates; the intensities of absorption along the different crystal axes are not equal. The practical difficulty caused by this could be overcome by a more complete grinding of the sample and the production of a thoroughly random distribution of the crystals. The effect, if present, can be detected by observing whether the apparent penicillin concentration (as measured against the alanine) is altered when the sample is rotated in the beam of radiation.

From the foregoing it may be concluded that the application of spectroscopic methods to the analysis of penicillin has been moderately successful, the limitations being imposed mainly by impurity of the material. In a sample consisting very largely of active penicillin, the G content can be determined accurately by means of ultra-violet absorption, and the technique is fairly quick and simple. It appears possible to extend the analysis to less pure samples by making a purification under standard conditions. Infra-red measurements can be applied to the determination of the other penicillins, but here the technique is more complicated, and further exploratory work appears to be necessary to put the method on a sound basis.

SUMMARY

The applications of ultra-violet and infra-red spectroscopy to the estimation of total and individual penicillins are reviewed and the limitations of the methods are discussed.

1. *Ultra-violet analysis of total penicillin*—Total penicillin can be measured by the absorption band at 3220 A., which develops when an aqueous penicillin solution is treated with acid under standard conditions.

2. *Ultra-violet analysis of penicillin G*—The analysis depends on the absorption bands due to the phenyl group. In one method using a photographic technique the spectrum of the unknown is compared with that of a standard. In another method using a spectrophotometer, the difference in optical densities at 2630 A. and 2800 A. gives a measure of the penicillin G concentration. Penicillin X, in more than 1 per cent. concentration, invalidates these methods. The effect of impurities and de-activation products is discussed.

3. *Infra-red analysis of individual penicillins*—Spectra are recorded of the penicillin in the form of its solid sodium salt. The individual penicillins each have characteristic absorption bands which are substantially free from interference by each other and can be used for quantitative analysis. For estimations, a suitable solid internal standard is mixed with the penicillin. Some of the difficulties of the method are discussed.

The author thanks the Directors of the Distillers Company Limited for permission to publish this paper.

REFERENCES

1. Herriott, R. M., *J. Biol. Chem.*, 1946, **164**, 725.
2. Philpotts, A. R., Thain, W., and Twigg, G. H., *Nature*, 1947, **159**, 839.
3. Grenfell, T. C., Means, J. A., and Brown, E. V., *J. Biol. Chem.*, 1947, **170**, 527.
4. Barnes, R. B., Gore, R. C., Williams, E. F., Linsley, S. G., and Petersen, E. M., *Anal. Chem.*, 1947, **19**, 620.

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RESEARCH AND DEVELOPMENT DEPARTMENT
GREAT BURGH, EPSOM, SURREY

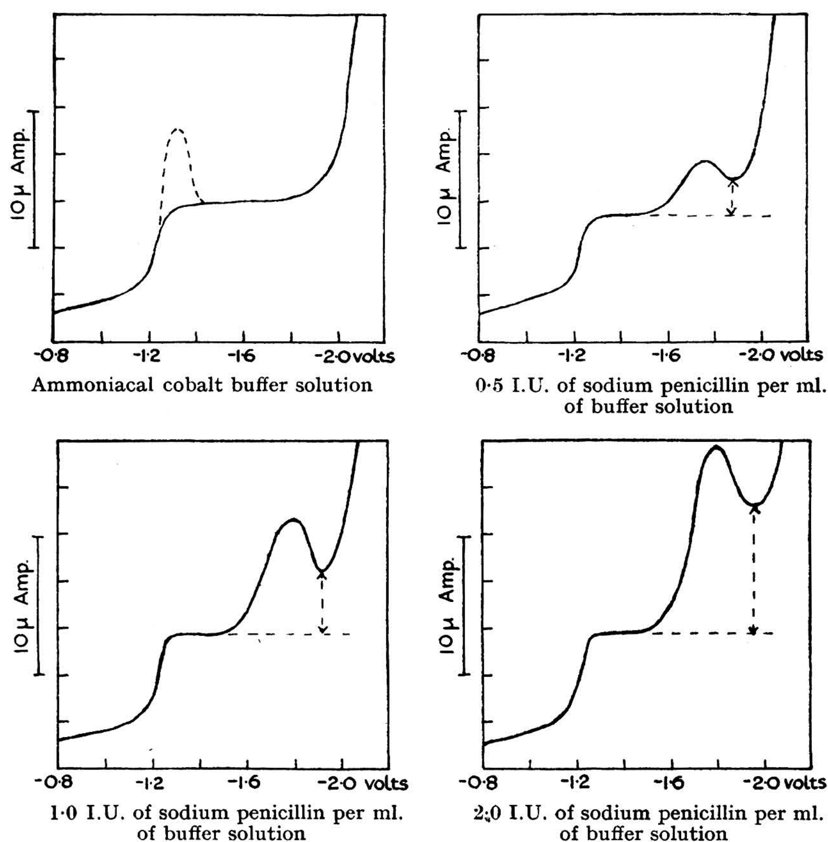
DISCUSSION ON THE PRECEDING FOUR PAPERS ON PHYSICAL AND CHEMICAL METHODS OF PENICILLIN ASSAY

DR. J. E. PAGE gave some particulars about the polarographic determination of penicillin. The procedure depends on the observation that penicillamine in an ammoniacal cobalt buffer solution forms a catalytic step similar to that given by cysteine (*cf.* Brdička, *Coll. Czech. Chem. Comm.*, 1933, **5**, 148). A fresh solution of pure penicillin does not give a catalytic step, but if the buffer solution is allowed to stand in the polarograph cell in the presence of the dropping mercury electrode, hydrolysis takes place and the catalytic step slowly develops. For analytical work, the penicillin is inactivated with 0·1 *N* sodium hydroxide, hydrolysed by warming with 1·0 *N* hydrochloric acid, and dissolved in Brdička's ammoniacal cobalt buffer solution. The hydrolysis of the penicillin and preparation of the buffer solution must be carried out under carefully controlled conditions; the final solution must be polarographed immediately. Small changes in *p*H value have an appreciable effect on the height of the catalytic step.

Some typical polarograms for hydrolysed penicillin are reproduced in Figure 1. If a trace of gelatin is added to the solution, the maximum on top of the cobalt step is suppressed but the height of the catalytic maximum is not affected. The vertical distance from the top of the cobalt step to the characteristic catalytic minimum provides a measure of the penicillin originally present.

The results for purified penicillin solutions listed in the Table show that the accuracy of the polarographic method is of about the same order as that of the biological procedure. In these experiments, 2 ml. of each penicillin solution were inactivated, hydrolysed and diluted to 50 ml. for polarographic examination.

The method is not suitable for metabolism solutions since any substance containing a free sulphydryl group will interfere with the assay, and certain amino acids such as tryptophan, histidine and arginine exert a suppressive action on the height of the catalytic step.



Ammoniacal cobalt buffer solution

0·5 I.U. of sodium penicillin per ml. of buffer solution

1·0 I.U. of sodium penicillin per ml. of buffer solution

2·0 I.U. of sodium penicillin per ml. of buffer solution

COMPARISON OF RESULTS OF POLAROGRAPHIC AND BIOLOGICAL ASSAYS

No. of replicates	Biological assay in i.u./ml.	Mean polarographic assay in i.u./ml.	Percentage coefficient of variation of polarographic values
11	20	20	25
12	40	42·5	8
10	50	57·5	9
9	100	95	8
11	150	155	7
9	200	210	4
8	300	300	5
8	500	510	4

In reply to Dr. G. E. Foster, Dr. PAGE said that the polarographic method was not suitable for the determination of penicillin in samples contaminated with hydrolytic decomposition products but that it could be used to study the nature of such products.

Mr. L. J. BELLAMY asked Dr. Lester Smith for information on the hydrolysis of penicillin with alkali. He suggested that as temperature seemed to influence the rate and possibly the degree of hydrolysis, the simple opening of the β-lactam ring might not be the only reaction involved.

Dr. LESTER SMITH said he had no further information on the alkaline hydrolysis of penicillin. From the practical point of view, sufficient evidence had been presented by Patterson and Emery to show that the hydrolysis was quantitative enough to give results in reasonable agreement with the biological method, bearing in mind the uncertainty of the conversion factor when dealing with mixed penicillins. From personal experience with the chromatography of penicillins he agreed with nearly all Dr. Boon's comments. With silica gel columns 80 per cent. recovery of penicillin seemed as much as one could expect, and the claims of some American workers to get nearly quantitative recovery (except with amyl penicillin) seemed rather remarkable. Some other American workers described a modification of the paper strip method said to be applicable directly to fermentation liquors. The strips are buffered at *p*H 5·0 and developed

for a few hours only at room temperature, using amyl acetate. This method revealed some new penicillins called S_1 and S_2 near the top of the strip, but did not give good separation of penicillins F and G. The I.C.I. method, however, was capable, under favourable conditions, of completely separating penicillins II, I and IV (in the I.C.I. nomenclature).

The Craig apparatus had proved rather cumbersome and laborious in use and appeared incapable of doing anything more than separating penicillin K from all the others. Moreover, some destruction of penicillin was observed during the protracted manipulations.

Dr. Lester Smith said that his colleague Mr. Hickman had measured the partition coefficient for pure penicillin G between various solvents and buffer solutions and found hardly any changes in the values over the concentration range of 50 to 5000 i.u. per ml. On the other hand, the partition coefficient *was* influenced by other acids such as those present in crude penicillin.

As to the ethyl piperidine method, he had also demonstrated by the paper strip method the presence of penicillins other than G in the precipitate and, in addition, the presence of a substantial part of the original penicillin G in the filtrate.

The method for purification of crude penicillin by precipitation with ammonium sulphate, referred to by Dr. Twigg, did not appear satisfactory with some penicillin samples. Some batches, rich in penicillin K and assaying 500 to 800 i.u. per mg., had given no precipitate at all with saturated ammonium sulphate solution.

Dr. W. R. Boon said that from the results obtained by Mr. Greenhalgh and Dr. Pryce it was clear that in some solvents penicillin was markedly associated. This effect was most important with chloroform. It also appeared that there was cross association between different species of penicillin and impurities such as phenylacetic acid. This might explain the difficulty of separating individual penicillins on the micro-chromatogram when solvents other than ether were employed. He also agreed with Dr. Lester Smith that the ammonium sulphate precipitation method did not always work within the limits set by American workers. Many samples that should have been satisfactory gave either no precipitate or a sticky brown mess.

Mr. C. W. Munday had found that the infra-red method could be used for the analysis of penicillin provided that the total penicillin content was greater than 90 per cent. Two-component mixtures of sodium penicillin G and sodium penicillin F had been examined and the infra-red results were in fair agreement with those obtained by Goodall and Levi's micro-chromatographic method. The difficulty of mixing the sample and internal standard could be overcome by suitable experimental technique. Infra-red spectroscopy was of considerable value for following the changes that take place in penicillin during storage.

Mr. K. A. Brownlee said that it was perhaps not generally realised that the iodimetric method could give seriously erratic results compared with biological assay. He had found that the difference between iodimetric and biological assay for freeze-dried penicillin varied appreciably according as the penicillin solution was freeze-dried in 0·1 or 0·2 mega unit vials.

Dr. Page asked Dr. Twigg if he had any information about the X-ray method for penicillin (*cf. Ind. Eng. Chem.*, 1947, November, p. 14A).

Dr. Twigg said that he did not know about the method, but he thought that such a technique could only be applied to crystalline penicillin

Ministry of Food

STATUTORY INSTRUMENTS*

1948—No. 131. The Manufactured and Pre-packed Foods (Control) Order, 1942 (Amendment No. 5.) Order, 1948. Price 1d.

This Order, as from February 1st, 1948, *excludes from the scope of the main Order* (*No.* 1863 *of* 1942) *as amended* (*No.* 2073 *of* 1942, *No.* 167 *of* 1944, *No.* 250 *of* 1945 *and No.* 1486 *of* 1946), *soft drinks in solid, semi-solid or powder form, and brings within the scope of the Order toffee apples and any fresh fruit covered with chocolate, treacle, syrup or sugar confectionery.*

— No. 397. The Canned Fruit and Vegetables (No. 2) Order, 1946 (Amendment No. 4) Order, 1948. Price 1d.

The main Order (*No.* 1724 *of* 1946) *as amended* (*Nos.* 333, 1317 *and* 1979 *of* 1947) *is hereby amended as from February 29th,* 1948. *The minimum weight of fruit per can prescribed for canned apples in syrup is reduced. The maximum syrup strengths prescribed for canned fruit* (*except for canned apples and fruit canned in light syrup*) *are abolished. The minimum syrup strength prescribed for canned cherries is reduced to 32° Brix. Bottled cocktail cherries are freed from price control.*

British Standards Institution

A few copies of the following draft specification, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7–8, Idol Lane, London, E.C.3.

Technical Committee LBC/11—Microchemical Apparatus.

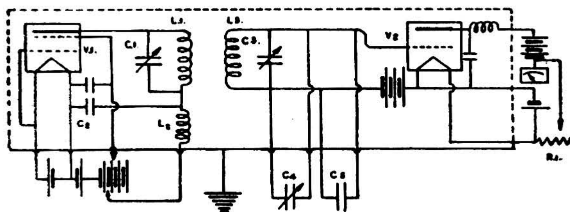
CJ(LBC)5953—Fourth Draft British Standard for Micro-Nitrometers.

* Italics signify changed wording.

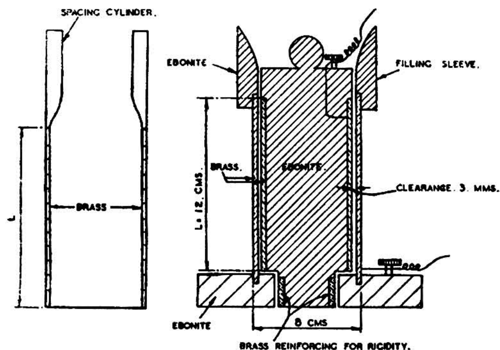
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of the Moisture Content of Cereals by Measurement of Specific Inductive Capacity. L. G. Groves and J. King (*J. Soc. Chem. Ind.*, 1946, **65**, 320–324)—The apparatus uses well-established principles and is easily constructed in the laboratory. The circuit is similar to that of Groves and Sugden (*J. Chem. Soc.*, 1934, 1094) and consists of a high-frequency oscillator loosely coupled to a valve voltmeter



receiver. The tuned circuit of the latter contains a measuring condenser cell into which the sample is introduced. A calibrated variable condenser, C_3, connected across the cell allows the electrical capacity change thus produced to be compensated and the receiver to be kept in resonance with the oscillator. Resonance is indicated by a maximum reading of the galvanometer in the anode circuit of the receiver. The apparent specific inductive capacity is given by the ratio of the capacities of the cell when filled with the sample and when empty. When moisture is the only variable, there is a straight-line relationship between moisture content and specific inductive capacity. However, each cereal requires its own calibration curve.



The cell consists of two concentric brass cylinders forming an annular space 3 mm. wide. The outer cylinder is connected to the earthed side of the system, is let into a stout ebonite base plate and has at the upper end a detachable ebonite filling sleeve. The inner cylinder is connected to the grid of the receiver triode and is driven on to an ebonite plug. The latter is reinforced with brass at the lower end and fits into a hole in the base plate. An appropriate weight of sample is fed into the filling sleeve; after each small addition, a brass tamping cylinder is inserted, rotated, and withdrawn helically. To empty the cell, the plug is withdrawn, when the cereal either falls or is brushed through the hole in the base plate.

The nature of the sample must allow packing of uniform density from a standard weight. Sieving is usually necessary and biscuit, tapioca, and the like, require grinding before sieving. The method is not applicable to cereals that contain angular starch grains (*e.g.*, maize) and form tight aggregates. Uniform packing of fine powders such as flour requires considerable practice. The prepared sample must be kept in a stoppered bottle until equilibrium is attained. Its conductivity must be low and its specific inductive capacity high, so that the tuning curve is sharp.

The apparatus is suitable for the rapid examination of numerous samples. It should be possible to estimate the moisture content to within 0·25 to 1 per cent. Measured over the range 0° to 30° C., the temperature coefficient of the apparatus is much smaller than the experimental error.

J. T. Stock

Determining the Presence of Olive Oil extracted from Oil-cake, or of Arachis Oil, in Olive Oil obtained by Pressing. G. Loew (*Ind. y Quim.*, 1947, **9**, 9–12)—The method of detecting the presence of oil-cake oil depends on the observation of Dorta (*Atti X° Congr. intern. Chim.*, 1939, **4**, 517) that the oil extracted from oil-cake contains a greater proportion of unsaponifiable matter, and also contains waxy substances and their polymerisation and oxidation products. These substances are stated to be unaffected during refining, and therefore appear in the final product, together with the unsaponifiable matter normally present in olive oil; the presence of these substances causes a rise in the m.p. of the unsaponifiable matter, and a determination of the m.p. permits an estimate of the proportion of oil-cake oil present, even in presence of arachis oil.

Procedure—The unsaponifiable matter is separated by a modification of the method of Spitz and Hoenig. Distil off the ether used for extraction in several stages, using a small flask; when the volume has been reduced to 5 ml., transfer the solution to a test tube of 12-mm. diameter and remove the remaining solvent by carefully immersing the tube in boiling water for 5 min. Immediately transfer the test tube to a 250-ml. flask containing water at 50° C., fixing the tube in place by means of a cork, and arranging in the tube a thermometer graduated in 0·1° up to 50° C. Note the temperature at which the entire contents of the tube become turbid (separation of crystals at temperatures below 25° C. is not characteristic of the non-saponifiable matter from oil-cake olive oil). This temperature is the "turbidity point", *Pe*. The determination of the turbidity point must be carried out

immediately after the elimination of the ether, as partial polymerisation of the unsaponifiable matter will otherwise lead to a rise in the solidification point. It is essential that the temperature should drop slowly during the determination of *Pe*, and a surrounding temperature of 20° C. is recommended; if necessary, the flask should be immersed in a water-bath at 20° C.

After determining *Pe*, allow the temperature to fall further, tilt the flask frequently, and record the temperature at which the contents of the test tube become gelatinous and cease to drain down the walls of the tube; this temperature is the solidification point, *Ps*.

Unrefined oil obtained by pressing is said to contain not more than 0·9 to 1·2 per cent. of unsaponifiable material, which remains clear and drains well at 20° C.; after long standing, small crystals separate. Refined olive oil contains about 1 per cent. of unsaponifiable matter, which, in general, behaves in the same way as that from the unrefined oil, though it may become opalescent at 26° to 25° C., but without becoming turbid; it drains down to 22° to 20° C. If, before refining, the oil is of very poor quality, and contains much acid, such as the "washed" or "infierno" oils, the unsaponifiable matter may become turbid at 30° C. and solidify at 25° C. A refined oil-cake oil has *Pe* between 45° and 40° C. and *Ps* between 44° and 38° C., and the content of unsaponifiable matter is about 2 per cent. Table I shows the variation of *Pe* and *Ps* with different proportions of oil-cake oil and pressed oil.

TABLE I

Oil obtained by pressing, per cent.	Oil-cake oil, per cent.	*Pe*	*Ps*
100	—	clear at 22°–20° C.	clear at 22°–20° C.
—	100	turbid at 45°–40° C.	gelatinous at 44°–38° C.
50	50	„ 43°–42° C.	„ 42°–41° C.
60	40	„ 40·5°–40° C.	„ 38°–37° C.
70	30	„ 39°–38·5° C.	„ 36·8°–36·5° C.
80	20	„ 38°–37° C.	„ 35°–33·8° C.
90	10	„ 36°–33·8° C.	„ 31°–30° C.

The method recommended for detecting arachis oil in olive oil obtained by pressing is that of Jaffe (*Annali di Chimica Applicata*, 1928, **18**, 368), and depends on the very slight solubility of the lithium salt of arachidic acid, a characteristic component of arachis oil. The test is not invalidated by the presence of oil-cake olive oil. E. M. POPE

Riboflavine in Malt Extract. C. Klaztkin, F. W. Norris, and F. Wokes (*Quart. J. Pharm.*, 1946, **19**, 376–387)—Fluorimetric assays have been conducted on 25 samples of malt extract and malt preparations made by 10 different manufacturers. The results indicate wide variations, ranging from 0·09 to 0·3 mg. per fl. oz., in riboflavine content. With one exception, which is being further investigated, the figures obtained are in good agreement with those derived from microbiological assays, being, on the average, 103 per cent. of the latter. A study of the effect of malting and brewing on the riboflavine content of malt extract showed that this content may increase greatly, especially during brewing. Differences in brewing conditions may thus account for most of the variations found in the riboflavine content of malt extract. The fluorimetric assay applied to barley and malted barley gave figures for the riboflavine content ranging from 72 to 89 per cent. of those obtained microbiologically; and sometimes the fluorimetric result was even lower. These differences do not affect the above conclusions. J. ALLEN

Colorimetric Determination of Morphine. D. C. M. Adamson and F. P. Handisyde (*Quart. J. Pharm.*, 1946, **19**, 350–359)—The method based on the colour produced by treating a solution of morphine with nitrous acid and making the mixture alkaline has been examined, and details of an improved photometric technique employed are given.

Procedure—Transfer a volume of a solution of the alkaloid in 0·1 *N* hydrochloric acid such as might be expected to contain not more than 1 mg. of morphine to a 50-ml. Nessler cylinder, and dilute to 20 ml. with 0·1 *N* hydrochloric acid. Add 8 ml. of a 1 per cent. aqueous solution of sodium nitrite, mix well with a plunger, and allow to stand for exactly 15 min. Then add 12 ml. of 10 per cent. aqueous ammonia solution, immediately dilute to 50 ml. with distilled water, and measure the extinction by means of a Spekker absorptiometer, using 4-cm. cells, and Ilford 601 (spectrum violet) and H.503 heat filters. Read off the quantity of morphine present in the volume of solution used by referring to a curve correlating known quantities of the alkaloid with the extinction produced by the same procedure. The colour is adequately stable, a diminution of about 1 per cent. during 1 hr. being observed when the final solution is exposed to artificial light. The stability is much less in daylight. The morphine and nitrite standard described in the 7th Supplement to the B.P.C., 1934, is shown to be unsatisfactory in that no time of standing before alkalising is specified and no age limit for the reagent is given.

The absorption curve of the ammoniacal nitrosomorphine has three maxima, at 235, 342, and 442 mμ., the corresponding extinctions, based on the morphine content, being 360, 169, and 124, respectively. The presence of a comparatively high peak in the ultra-violet region may prove of value in detecting small amounts of morphine. Several samples of papaveratum have been examined by the official procedure and by the proposed method; the results are quoted. The compensating "blank"

technique of Nicholls ("*Aids to the Analysis of Food and Drugs*," London: Baillière, Tindall & Cox, 1942, p. 371) was used and the agreement obtained was about ±2 per cent. The proposed method is not specific for morphine (*see* Garratt, *Quart. J. Pharm.*, 1937, **10**, 467), since it is a general reaction for most phenols. In injection of papaveratum, B.P.C. 2nd Supplement, there is 0·2 per cent. of chlorocresol for 1 per cent. of morphine. In such a preparation, the morphine content as determined by the proposed method would be subject to a positive error of not more than 1·5 per cent. When subjected to the proposed method, both phenol and chlorocresol give a colour, but this is not directly proportional to the concentration over the range studied. J. ALLEN

Simple Colour Reaction for Piperazine. R. D. Barnard (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1947, **36**, 224)—To about 5 ml. of test solution add, dropwise, sufficient of a 5 per cent. aqueous solution of potassium ferricyanide to produce a permanent pale yellow tint, add a droplet of mercury, and shake well. In presence of piperazine, a lilac colour that fades after 10 min. is developed, but if an excess of solid sodium bicarbonate be first added to the test solution, a permanent red colour is produced on completing the test. Solutions containing proteins become turbid when shaken with the mercury, although the colours can still be detected, whilst if polyphenols are present, they must first be removed by extracting the acidified sample with ether.
A. H. A. ABBOTT

Determination of Barbituric Acid Derivatives. H. A. Mangouri and L. Milad (*Quart. J. Pharm.*, 1947, **20**, 109–113)—Critical investigation of the available methods for the determination of barbituric acid derivatives revealed in each sources of error not easy to control. The following technique, which trials showed to be the most suitable set of conditions for utilising the property of these derivatives of forming silver salts, was evolved as an alternative.

Procedure—Suspend about 0·17 to 0·25 g. of the material in 20 ml. of water, add 10 ml. of 10 per cent. sodium acetate solution, warm gently, and add dilute aqueous ammonia dropwise until the suspension is dissolved. Boil off the excess of ammonia, add a known volume of 0·1 *N* silver nitrate and 0·1 g. of pure calcium carbonate, boil the solution for 2 to 3 min., cool, filter, and wash the precipitate with 5-ml. quantities of freshly boiled, cooled water until free from silver. Acidify the combined filtrate and washings with dilute nitric acid and titrate the excess of silver nitrate with 0·1 *N* ammonium thiocyanate, using ferric ammonium sulphate as indicator.

Calculation—From the volume of 0·1 *N* silver nitrate consumed, calculate the quantity of the barbituric acid derivative from the following data: 1 g.-molecule of the barbituric acid derivative reacts with 2 g.-equivalents of silver if it is one of the acid derivatives or their sodium salts, with 4 g.-equivalents if it is one of the thiobarbituric acids or their sodium salts, and with 1 g.-equivalent if it is one of the N-methylated derivatives or their sodium salts.

For thiobarbituric acid derivatives the boiling period should be increased to 5 to 6 min., but for sodium N-methylated derivatives warming to 60° to 70° C. is sufficient to coagulate the silver compound. The addition of ammonia is not necessary when the two imido hydrogen atoms in the nucleus of the derivative are replaced by a metal and a radical, respectively. A. H. A. ABBOTT

Biochemical

Colorimetric Determination of Streptomycin in Clinical Preparations, Urine, and Broth. G. E. Boxer, V. C. Jelinek, and P. M. Leghorn (*J. Biol. Chem.*, 1947, **169**, 153–165)—The most selective chemical reaction for characterising streptomycin is the formation of maltol, 2-methyl-3-hydroxy-γ-pyrone, on heating with dilute alkali. Maltol, in acid and alkaline solutions, shows characteristic absorption spectra in the ultra-violet and reacts with ferric ions to give a purple-red colour stable in acid solution, and with the phenol reagent of Folin and Ciocalteu to give a blue colour. These reactions are used as the basis of a colorimetric assay of streptomycin.

DETERMINATION OF STREPTOMYCIN IN CLINICAL PREPARATIONS—*Maltol formation*—To 5 ml. of streptomycin solution in a test tube, add 1 ml. of 2 *N* sodium hydroxide. Immerse the tube in boiling water for 3 min. and cool for 3 min. in cold water.

Colour reactions. (*a*) *Ferric ammonium sulphate reagent*—To the cooled alkaline solution, add 4 ml. of a 1 per cent. solution of ferric ammonium sulphate in 0·75 *N* sulphuric acid. Shake vigorously and after 10 min. determine the intensity of the purple colour in an Evelyn photo-electric colorimeter with filter 540. Determine the reagent blank, which is small.

(*b*) *Phenol reagent*—To the cooled alkaline solution, add dropwise 1 ml. of Folin and Ciocalteu phenol reagent (*Ibid.*, 1927, **73**, 627). Mix well, set aside for 1 to 2 min., add 3 ml. of 20 per cent. sodium carbonate solution, and shake vigorously. After 10 min., take the reading in the photo-electric colorimeter with filter 660. Prepare a blank simultaneously. Carry out an additional blank with 5 to 10 times the quantity of sample. Proceed as above, but do not heat with alkali. Add the phenol immediately after the addition of the alkali, as maltol is slowly formed at room temperature. This blank is for the impurities that react directly with the phenol reagent.

Methanolic solutions of streptomycin—To 4 ml. of the methanol solution in a measuring cylinder, add 2 ml. of *N* sodium hydroxide, and immerse in a water-bath at 65° ± 1° C. for 20 min. Cool and make up to 10 ml. with the ferric ammonium sulphate reagent. Set aside for 10 min. and then measure the colour intensity.

Calibration—For hygroscopic standards, weigh out two samples at the same time. Determine the moisture in one sample by drying for 3 hr.

at 100° C. over phosphorus pentoxide *in vacuo*. Use the other for calibration, applying a correction for water content.

Dihydrostreptomycin, *N*-methyl-*l*-glucosamine, streptidine, and streptobiosamine do not form maltol in the above procedure. The phenol reagent is relatively unselective, reacting with carbohydrate degradation products and phenols. However, its sensitivity (20 to 250 μg.) is greater than that of the ferric ammonium sulphate reagent (500 to 2500 μg.). The latter is selective, although acid phenols, *e.g.*, salicylic acid, interfere. The reproducibility with both reagents was ±3 per cent. and comparison with microbiological assay on samples of potency above 450 μg. per mg. showed agreement within ±5 per cent. in 67 per cent. of the assays, and within ±10 per cent. in 95 per cent. of the assays. In 20 to 30 per cent. of samples of potency below 450 μg. per mg. the chemical values were 10 to 50 per cent. greater than those by the microbiological assay. This may be due to the low accuracy of the bio-assay or to interference with the chemical assay.

With broth and urine samples, the low concentration of streptomycin requires the sensitive phenol reagent. The maltol must be separated from interfering impurities by a chloroform extraction from acid solution. The impurities that come through here are corrected for by performing a blank estimation after removing the streptomycin. With broth samples this is accomplished by boiling with *N* hydrochloric acid; and with urine, where acid treatment releases further interfering impurities, by adsorption by Lloyd's reagent.

DETERMINATION IN URINE—*Special reagents*—*Phosphate buffer*, *p*H 7, saturated with sodium chloride. To 2 litres of 0·2 *M* disodium phosphate, add 28 ml. of 0·1 *M* citric acid and 690 g. of sodium chloride. *Chloroform*—Wash daily thus: once with an equal volume of approximately 0·5 *N* sodium hydroxide and 3 times with an equal volume of distilled water. Dry with anhydrous sodium sulphate.

Procedure—Adjust the urine sample to *p*H 5·0 to 5·2 with a few drops of glacial acetic acid, shake with an equal volume of chloroform, and centrifuge. This initial extraction diminishes emulsions in the subsequent procedure and removes any free phenols.

To prepare the blank, mix in a centrifuge tube 5 ml. of the urine, containing not more than 100 μg. per ml., with 500 mg. of Lloyd's reagent measured from a calibrated spoon. Shake vigorously, allow to stand for 15 min., shaking occasionally, centrifuge and use the clear supernatant urine in the blank.

Take 1 to 3 ml. of the urine (containing a total of 50 to 500 μg. of streptomycin), and separately an equal volume of blank urine, and dilute each to 5 ml. in a test tube. Add 1 ml. of 3 *N* sodium hydroxide, immerse in boiling water for 3 min., and then cool for 3 min. Transfer to a 60-ml. separating funnel, using two rinses with 2 ml. of 1·5 *N* sulphuric acid. Extract with 10 ml. of washed chloroform for 15 sec., shaking vigorously. Repeat this extraction in a second funnel with another 10 ml. of chloroform. Combine the chloroform extracts and shake for 15 sec. with 20 ml. of the phosphate buffer. Transfer the chloroform layer to a third separating funnel. Wash the buffer solution with 5 ml. of chloroform and add this to the third funnel. Add 5 ml. of water and 1 ml. of 2 *N* sodium hydroxide to the combined chloroform extracts and shake for 15 sec. Transfer the aqueous phase to a 15-ml. centrifuge tube with a conical bottom and centrifuge for 2 to 3 min. Pipette 5 ml. of the aqueous layer into a test tube and add dropwise 2 ml. of the phenol reagent, diluted 1 : 1. Mix well, allow to stand for 1 to 2 min., add 3 ml. of 20 per cent. sodium carbonate solution, and shake vigorously. After 10 min., read the colour intensity in a photo-electric colorimeter at 660 mμ., setting the instrument at 100 per cent. light transmittance with the solution obtained from the blank urine. Complete the above procedure in 20 to 30 min., as protracted contact gives low results.

DETERMINATION IN BROTH — *Procedure* — To remove anti-foam agents, shake 10 to 20 ml. of broth for 1 min. in a 40-ml. centrifuge bottle with about an equal volume of washed chloroform, and centrifuge for 10 min. Transfer 1 to 3 ml. of the clear supernatant layer, containing a total of 50 to 500 μg. of streptomycin, into each of two test tubes. Dilute to 4 ml. In one tube destroy the streptomycin by adding 1 ml. of 5 *N* hydrochloric acid and immersing in boiling water for 5 min. Cool for 3 min. and add 1 ml. of 7 *N* sodium hydroxide. To the other tube add 2 ml. of 1 *N* sodium hydroxide and immerse both tubes in boiling water for 3 min. Cool for 3 min. and transfer the contents of each tube to 60-ml. separating funnels, rinsing twice with 2 ml. of 0·75 *N* hydrochloric acid. Proceed now as for the urine determination, omitting the washing of the chloroform with the phosphate buffer if only 1 ml. of broth is used.

Calibration—The calibration curve is the same for urine and broth. Treat solutions of the standard containing 50 to 500 μg. of the free base to form maltol and extract as above, omitting the inactivation of streptomycin by acid or adsorption by Lloyd's reagent. Beer's law is strictly obeyed.

The calibration curve is reproducible to within ±3 per cent. The values on actual samples of urine or broth can be reproduced to within ±10 μg. If 3 ml. of sample are used, 10 μg. per ml. of streptomycin can be detected. The percentage errors are correspondingly large at very low concentration.

W. S. WISE

Estimation of Progesterone in Ethyl Oleate Solution. W. F. Elvidge (*Quart. J. Pharm.*, 1946, **19**, 260–269)—Photometric methods suitable for the determination of progesterone in ethyl oleate solution have been investigated. The Zimmermann method (*Z. physiol. Chem.*, 1935, **233**, 257; 1936, **245**, 47), based on the formation of a red colour by various 17-ketosteroids with *m*-dinitrobenzene in alkaline solution, and various modifications of the Liebermann - Burchard colour reaction have been found to be unsatisfactory either because of unsuitable or varying colours produced with progesterone, or because of the high chromogenic

reaction with ethyl oleate. By adapting the reaction with salicylic aldehyde and alkali, a method that overcomes these difficulties has been evolved. The procedure, based on the method of Behre and Benedict (*J. Biol. Chem.*, 1926, **70**, 487) for the determination of acetone bodies in urine, has been examined and the following technique evolved.

Method—Calibration curve—Into four, graduated 25-ml. flasks introduce 0·1, 0·2, 0·4, and 0·8 ml., respectively, of a solution of progesterone in absolute alcohol containing 500 μg. per ml. and add to each sufficient absolute alcohol to produce a volume of 2 ml. Into a fifth flask, introduce 2 ml. of absolute alcohol. Continue as described below.

Procedure—To a 25-ml. graduated flask add an amount of the ethyl oleate solution of progesterone that might be expected to contain between 80 and 500 μg. of the hormone and dilute to 2 ml. with absolute alcohol. Into a similar flask introduce the same volume of ethyl oleate as that of the solution used and dilute to 2 ml. with absolute alcohol. To each of the seven flasks, add 1 ml. of a 10 per cent. solution of salicylic aldehyde in absolute alcohol followed by 10 ml. of 7·5 N sodium hydroxide. Immerse all the flasks at the same time in a water-bath maintained at 80° ± 2° C. Leave for 10 min., then cool immediately, add absolute alcohol to increase the volume to about 22 ml., and dilute to 25 ml. with distilled water. Take readings in a Spekker absorptiometer, using 4-cm. cells and Ilford spectrum 605 and heat-resisting filters. Prepare a calibration curve from the standard progesterone solutions and read off the result of the test after deducting the value obtained for the ethyl oleate "blank." Three experiments are cited in which amounts of progesterone and ethyl oleate equivalent to solutions containing 2·0, 5·0, and 10·0 mg. per ml. were subjected to the above procedure. The results obtained were 2·11, 5·0, and 11·2 mg. per ml., respectively. The blank correction due to ethyl oleate is small: if a sample of ethyl oleate had not been available for these three experiments, the figures obtained would have been increased by 0·1, 0·3, and 0·01 mg., respectively.

J. Allen

Modified Kober Method for the Determination of α- and β-Oestradiol. J. Carol and J. C. Molitor (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1947, **36**, 208–210)—This method depends on the fact that the β-form reacts with Kober's reagent at room temperature but α-oestradiol does not; the modified procedure determines α-oestradiol as the difference between the total oestradiols and β-oestradiol.

Kober's reagent—Add slowly, with continual stirring, 5·6 g. of sulphuric acid to 3·6 g. of freshly distilled phenol, taking precautions to keep the mixture cool.

Procedure—Prepare an alcoholic solution of the sample and transfer two aliquots containing 25 to 100 μg. of total oestradiol to test tubes. Transfer 1 ml. of a standard solution of α-oestradiol (100 μg. per ml. in 95 per cent. alcohol) to a third tube, and 1 ml. of a standard solution of β-oestradiol (50 μg. per ml. in 95 per cent. alcohol) to a fourth tube. Evaporate all the solutions on a steam-bath and dry over sulphuric acid for 1 hr. *Total oestradiol*—To one of the sample tubes, the α-oestradiol standard tube, and a blank tube, add exactly 3 ml. of Kober's reagent, close the tubes with corks wrapped in tin-foil and heat in boiling water for 20 min., mixing the contents of each tube by vigorous shaking twice during the first 10 min. Transfer the tubes to an ice-bath, cool for at least 5 min. and, while they are still in the ice-bath, add exactly 3 ml. of water to each. Mix and re-heat the tubes in the boiling water for 3 min., transfer them to the ice-bath again, cool for at least 5 min., and dilute each solution to 15 ml. with 25 per cent. v/v sulphuric acid. Mix well, and determine the extinction, at 420 and 526 mμ., of the sample and standard against the blank in a spectrophotometer or photometer. Total oestradiols in the aliquot

$$= \frac{E526_{sample} - \frac{1}{2}E420_{sample}}{E526_{standard} - \frac{1}{2}E420_{standard}} \times 100\ \mu g.$$

β-Oestradiol—To the other sample tube, the β-oestradiol standard tube, and a blank tube, add exactly 3 ml. of Kober's reagent, mix, and allow to stand at room temperature for 20 min. Transfer the tubes to an ice-bath, proceed as previously described, and finally determine the extinction of the sample and standard against the blank at 420 and 528 mμ. β-Oestradiol in the aliquot

$$= \frac{E528_{sample} - \frac{1}{2}E420_{sample}}{E528_{standard} - \frac{1}{2}E420_{standard}} \times 50\ \mu g.$$

α-Oestradiol = total oestradiols − β-oestradiol.

Good results are obtained with mixtures containing as little as 5 μg. of one isomeride in presence of 100 μg. of the other.

A. H. A. Abbott

Design and Statistical Analysis of Microbiological Assays. E. C. Wood and D. J. Finney (*Quart. J. Pharm.*, 1946, **19**, 112–126)—The common-zero 3-point design (Wood, Analyst, 1936, **71**, 1) is the most precise way of assaying a test preparation against a standard preparation if the measured response is linearly related to the dose and if this relation holds to the zero point; in many types of microbiological assay the latter condition can always be ensured by a simple modification of the basal medium. This design is not, however, suitable in practice since it provides no data for the detection of departures from linearity, the occurrence of which would indicate that either the form of statistical analysis used in truly linear assays was in this case inappropriate or that, because of faults in the experimental technique, the assay was invalid. If the validity of the assay and the statistical analysis is in question, intermediate dose levels must be included in spite of the consequent reduction in accuracy with which the potency is determined for a fixed number of observations. A design that gives a reasonably sensitive test of validity without too great a sacrifice of efficiency, and is yet convenient in practice, is one employing equal numbers of tubes at five levels, *viz.*, those used in the 3-point assay together with half-doses of each preparation. Whatever design is used, the distribution of tubes

between the doses of the test preparation should be the same as for the standard, but there are other 5-point designs, which may sometimes be useful, conforming to this condition. Formulae are given, applicable to the common-zero 5-point design, for estimating the potency and its standard error, for testing the validity of the assay, and for assessing the efficiency of these estimations and tests for the best distribution that could be obtained with a specified total number of tubes. General formulae are also given for deriving the estimate of potency from designs involving the use of a greater number of dose levels, such as may be required by research workers, and, in addition, the simpler forms of these expressions appropriate to a symmetrical common-zero $(2k + 1)$-point design are quoted. The method of setting out the calculations in the form of an analysis of variance is described. J. ALLEN

Determination of Glycine in Protein Hydrolysates with *Leuconostoc Mesenteroides* P-60 S. Shankman, M. N. Camien, and M. S. Dunn (*J. Biol. Chem.*, 1947, **168**, 51–59)—*Method*—The assay procedure was essentially that described previously (*Idem, ibid.*, 1945, **161**, 643; ANALYST, 1946, **71**, 241) and the composition of the medium was the same as that given for medium D, Table I, by Dunn *et al.* (*J. Biol. Chem.*, 1944, **156**, 703; ANALYST, 1945, **70**, 182), except that glycine was omitted and double the concentration of total amino acids was used. Glycine was added to the medium at a level of 3 µg. per ml. of final solution to overcome an induction period. The inoculum was diluted with a volume of sterile saline three to nine times that of the medium from which it was centrifuged. Standards were run at fifteen levels (0 to 28 µg.) and the test-mixtures and hydrolysates at five levels, with five to six replicate tubes at each level. The tubes were incubated for 3 days at 35° C.

Results—Recoveries of glycine from amino acid test-mixtures and protein hydrolysates were close to 100 per cent., and results of assays agreed well. The glycine content of casein was 1·9 ± 0·1 per cent. and of silk fibroin, 43·6 ± 1·0 per cent.

J. S. HARRISON

Micro-Colorimetric Estimation of Plasma Proteins. A. A. Albanese, B. Saur, and V. Irby (*J. Lab. Clin. Med.*, 1947, **32**, 296–299)—The authors recently described (*J. Biol. Chem.*, 1946, **166**, 231; ANALYST, 1947, **72**, 208) a colorimetric method utilising the Sakaguchi reaction for estimating total proteins and protein fractions in human plasma, nephrotic urine, and abdominal fluid in terms of arginine. Modifications now introduced, including the elimination of the 3-hr. incubation period, render the method rapid and easy of operation and it is recommended for routine clinical work.

Special reagents—(*a*) 0·1 per cent. α-naphthol solution in 95 per cent. ethanol, stored in a brown bottle and kept in a refrigerator, (*b*) 22 per cent. aqueous sodium sulphate (anhydrous) solution stored in an incubator at 37° C., and (*c*) standard arginine solution, containing 100 µg. of free arginine per ml., prepared by dissolving arginine hydrochloride in a saturated aqueous solution of benzoic acid.

Procedure—Total plasma proteins—Dilute 0·1 ml. of plasma (derived from blood collected over lithium oxalate) to 2 ml. with 10 per cent. sodium hydroxide solution, and mix thoroughly. Transfer 1 ml. to a colorimeter tube and add rapidly 5 ml. of water and 1 ml. of the alcoholic naphthol solution (*a*). Allow to stand for 5 min., add 1 ml. of 0·06 *N* sodium hypochlorite and, after exactly 1 min., 2 ml. of 20 per cent. w/v urea solution. Mix, allow to stand for 5 min., and evaluate the colour intensity in a colorimeter. Conduct parallel determinations on 1 ml. of the standard and on a reagent blank.

Plasma albumin and globulin—Introduce 0·2 ml. of plasma into a centrifuge tube, add 7 ml. of the sodium sulphate solution (*b*) and 3 ml. of ether (reagent grade). Shake vigorously for 2 min., centrifuge for 10 min., and separate by careful decantation the globulin that collects at the ether - water interface. Dilute the aqueous albumin-containing fraction to 10 ml. with water, transfer 5 ml. to another tube, and add 1 ml. of sodium hydroxide solution. Determine the arginine content as described for total proteins beginning at the words "and 1 ml. of the alcoholic naphthol solution (*a*)." Dissolve the disc of precipitated globulin in 2 ml. of sodium hydroxide solution, add 2 ml. of water, and warm gently to remove the ether and dissolve the protein. Transfer 2 ml. to a colorimeter tube, add 4 ml. of water, and determine the arginine as described for total proteins, beginning at the words "and 1 ml. of the alcoholic naphthol solution (*a*)."

It is essential that in all deviations from these sample sizes the adjusted sample be contained in a volume of 6 ml. (which shall include the equivalent of 1 ml. of 10 per cent. sodium hydroxide solution) prior to the addition of the other reagents.

Calculation of results—The arginine is estimated in the protein samples by comparison with the standard arginine solution (*c*). To obtain the protein equivalent, multiply the arginine values by the dilution factors and 19·2, a factor derived from the mean arginine content of human plasma albumin and globulin. Although it is recommended that blood be collected over lithium oxalate, other oxalates or other anti-coagulants can be used in this method. A. H. A. ABBOTT

Whole Blood or Other Clotting Systems in Assaying Heparin. O. Jalling, J. E. Jorpes, and G. Linden (*Quart. J. Pharm.*, 1946, **19**, 96–105)—Various published methods for the assay of heparin are criticised and the advantages of those based on the use of fresh whole blood are discussed. Since heparin exerts a multiple effect on the blood, acting both on the thrombokinase and on the prothrombin, and particularly strongly on the thrombin, the whole-blood method offers a better opportunity for heparin to develop its full effect than other methods based on the use of a single component. The unfavourable influence of citrate ions is avoided, as is also the effect of a non-physiological concentration of calcium ions.

Procedure—Determine the coagulation time in non-paraffined test tubes of Pyrex glass, each of

2·5 ml. capacity (70 × 8 mm.) without admission of air. Ten tubes are held in an oak rack (30 × 4 × 2 cm.) fitted with an oak cover of the same size. The top of the rack and the under face of the cover are faced with rubber sheeting, and provision is made for the two components of the stand to be tightly clamped together to exclude air from the tubes. Into each tube place a glass bead somewhat smaller in diameter than the inside of the tube, and 0·2 ml. of a diluted heparin solution. Five of the tubes contain the standard substance and five the unknown. The diluted solutions of standard heparin contain, in the first tube, a concentration of 10 mg. of the water-free substance in 32 ml. of physiological saline solution, in the second 5 mg., and so on; the same concentrations of the unknown are employed. In the summer, solutions of twice this strength should be employed, whilst, in the winter, the concentrations may be halved. Quickly fill each tube with blood when the animals are slaughtered at the abattoir, tighten the cover without allowing any air to remain in the tubes and invert the rack several times, when the glass beads give sufficient mixing. It is important that the contents of the tubes be thoroughly mixed before the stands are left. For the best results, examine the tubes after 4, 8, 16, and 24 (26) hr. The time for the initial coagulation of a tube should be noted as well as for complete clotting, the former being indicated by the slowing down of the speed of the glass bead during the inversion of the rack.

Calculation—The evaluation of the readings is a simple empirical one according to the following scheme:—

A figure is noted according to the difference between the number of tubes containing the standard preparation, of which the contents have coagulated, and the number containing the unknown, in this condition, as follows:—

The same number of coagulated tubes in both standard and unknown	0
One more coagulated on the side of the standard	+1
Two more tubes on the side of the standard	+2
One more on the side of the unknown ..	−1
Two more on the side of the unknown ..	−2

Incipient coagulation is counted as half of full clotting. Thus, if two of the tubes containing the unknown have clotted, whilst, of those containing the standard, two have completely clotted and one partially coagulated, the figure assigned to the rack for this particular examination would be +0·5. For each examination, after 4, 8, 16, and 24 (26) hr., respectively, a figure is noted, making four for each rack. All the figures obtained are added together and the algebraic sum is divided by the number of figures. This mean figure is transposed into percentage of the standard according to the following scheme:—

−1	=	50 per cent.
−0·5	=	75 per cent.
0	=	100 per cent.
+0·5	=	150 per cent.
+1	=	200 per cent.

The method has been checked by conducting seven series of assays on samples of heparin of known concentration. The results cited indicate that the accuracy is about 5 per cent. It is, however, emphasised that the precision of the method is entirely dependent upon the skill of the operator in filling the tubes with fresh blood. [The author used ox-blood.—ABSTRACTOR.] J. ALLEN

Extraction Procedure for the Microbiological Determination of Vitamin B_6. J. C. Rabinowitz and E. E. Snell (*Anal. Chem.*, 1947, **19**, 277–280)—Investigations by various workers to compare the effectiveness of different procedures for liberating vitamin B_6 from natural products have shown that hydrolysis for 1 hr. at 20 lbs. per sq. in. with 0·055 *N* sulphuric or hydrochloric acid is often more effective than a similar treatment with 1 *N* or 2 *N* acid. Moreover, treatment with clarase can liberate vitamin that is not available after hydrolysis with 2 *N* acid. Some of this unusual behaviour may result from the presence of pyridoxal phosphate and, to test this, various hydrolytic procedures on the synthetic co-enzyme have been studied.

Method—The method used for most of this work was the *Saccharomyces carlsbergensis* 4228 assay of Atkin *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 141) with the following modifications, which gave a lower blank and increased response to added vitamin B_6. The casein was hydrolysed by the method of Snell and Rannefeld (*J. Biol. Chem.*, 1945, **157**, 475). Assays were carried out on one half the original scale. Samples, with water to make the volume 2·5 ml., were steamed for 10 min. in 25 × 200-mm. Pyrex tubes. The basal medium was steamed in a separate container, cooled, and inoculated with 0·01 mg. of moist yeast per 2·5 ml., and 2·5 ml. of the inoculated basal medium were added aseptically to each tube. The samples were protected from light because of its destructive effect on vitamin B_6. The tubes were shaken mechanically for 18 hr. at 30° C. and then steamed for 5 min. to stop growth, and 2 ml. of water were added before readings were taken in an Evelyn colorimeter. Standard levels of pyridoxine hydrochloride ranged from 0 to 25 mμg.

Results—A sample of the synthetic co-enzyme, pyridoxal barium phosphate, which had been shown to contain 32 to 36 per cent. of pyridoxal by chemical methods, was assayed by the yeast method and also by means of *Lactobacillus helveticus*, *Streptococcus faecalis* and *Leuconostoc mesenteroides*, after different hydrolytic procedures. The activity of the untreated co-enzyme for all the organisms was only 3 to 10 per cent. as great as after hydrolysis. Clarase digestion and hydrolysis with 0·1 *N* hydrochloric acid released the expected amount of the vitamin for yeast, and slightly smaller amounts for the other test organisms. The rate of release of pyridoxal from the co-enzyme by treatment of 50 μg. with 10 ml. of 0·055 *N* hydrochloric acid was much more rapid than when 2 *N* acid was used; 1 hr. at 15 lbs. per sq. in. gave 100 per cent. liberation with 0·055 *N* acid, compared with 75 per cent. in 5 hr. with 2 *N* acid. The time taken to

liberate pyridoxal from the co-enzyme was much less than was required for liberation of vitamin B_6 from natural products, 5 hr. being required to reach 100 per cent. with 0·055 *N* acid at 20 lbs. per sq. in. for dried yeast. This led to the conclusion that, in the latter process, more was involved than hydrolysis. Moreover, the optimal conditions for treatment of various natural products were different; rice bran concentrate required 2 *N* hydrochloric acid, and this treatment was also satisfactory for many other plant products, whilst yeast and animal products required 0·055 *N* acid. There was no evidence of destruction of the vitamin by the more concentrated acid, as further treatment with clarase released the remaining vitamin.

For liberation of vitamin B_6 in a form available to micro-organisms the recommended single general method is treatment with 0·055 *N* hydrochloric acid at 20 lbs. per sq. in. for 5 hr. Results of vitamin B_6 assays on various natural materials are given.

[ABSTRACTOR'S NOTE—Two recent papers present further evidence that the optimal conditions for liberation of vitamin B_6 in an available form require careful control.

S. H. Rubin, J. Scheiner, and E. Hirschberg (*Ibid.*, **1947**, **167**, 599) showed that the efficiency of extraction from yeast of vitamin B_6 activity for *S. carlsbergensis* during an extraction time of 1 hr., was greatest within the *p*H range 1·5 to 2·0, and diminished progressively on either side of these limits. The lower response at *p*H levels outside the optimal range could be raised to maximal, or near-maximal, by further hydrolysis at *p*H 1·7 (0·055 *N*) for 1 hr., or by 2 *N* sulphuric acid for 8 hr., or by enzymic treatment. Similar effects were demonstrated with liver.

R. H. Hopkins and R. J. Pennington (*Biochem. J.*, 1947, **41**, 110) found that hydrolysis with 0·1 *N* sulphuric acid at 120° C. for 5 hr. released a maximal amount of vitamin B_6 from yeast as assayed with *S. carlsbergensis*, and that the lower value obtained with 2 *N* acid could be increased to near-maximal by digestion with papain and takadiastase.]

J. S. HARRISON

Estimation of Types of Penicillin in Broths and Finished Products. A Microbiological Method. K. Higuchi and W. H. Peterson (*Anal. Chem.*, 1947, **19**, 68–71)—This assay of penicillins G, X, F, and K (*n*-heptyl) is based on the response of *Staphylococcus aureus*, *B. brevis*, and "Organism E" to the different penicillins.

Cultures—Carry the organisms, *S. aureus* 209-P, *B. brevis*, and "Organism E," an unidentified spore-forming lactic acid bacillus, as streaks on 0·3 per cent. yeast extract, 0·2 per cent. glucose agar slant. Use the following media for both the inoculum and the assay. Grow the *S. aureus* and *B. brevis* inocula for about 15 hr. at 37° C.

Calibration—Make up stock solutions of pure, crystalline penicillins G, X, F, and K. For each assay, dilute to 10 and 2·5 units per ml. with 0·3 per cent. sterile yeast-extract water. Pipette 10 ml. of the appropriate medium into uniform tubes (18 × 150 mm.), plug, and autoclave for 10 min. at 15 lb. pressure.

S. aureus assay—From the solution containing 2·5 ml. of penicillin G, add to the prepared tubes 0·005, 0·0075, 0·010, 0·015, 0·020, and 0·025 unit of penicillin per ml. of medium. Make up a similar set of tubes for the other penicillins and the unknowns at levels of 0·010, 0·015, and 0·020 unit per ml. Add 1 drop of inoculum and incubate for about 15 hr. at 37° C. Measure the bacterial growth by turbidity readings in a photometer with a 660-mμ. filter. Plot galvanometer deflections against concentrations of penicillin.

The curves for other penicillins are not always identical with the G curve, but there are no consistent differences, and the average concentration is calculated over the three levels 0·01, 0·015, and 0·02 unit per ml.

B. brevis assay—Here there is a different sigmoid curve for each penicillin, and the concentration must be arranged so that the calibration comes on the steep section of the curve. For penicillin G, the range is between 0·015 and 0·030 unit per ml.; for X, between 0·02 and 0·06; for F, between 0·04 and 0·09; for K, between 0·05 and 0·13. Proceed as above, using the 10 units per ml. solutions, except for penicillin G, and take about 10 tubes at 5 levels in duplicate. For unknowns, the concentration range should cover 0·015 to 0·13 unit per ml.

"Organism E" assay—The concentration ranges here are: penicillin G, 0·015 to 0·03 unit per ml.; X, 0·0075 to 0·020; F, 0·3 to 0·06; and K, 0·04 to 0·10. Incubate at 45° C. for 15 hr.

Calculation—Obtain from the calibration curves the ratio of the potency of the two other penicillins to that of penicillin G for the particular organism. Thus, for "Organism E" the ratios are 1·8, 0·53, and 0·31 for X, F, and K, respectively. If the letters *G*, *X*, etc., represent the amounts of penicillin present we have:—

$$G + 1{\cdot}8X + 0{\cdot}53F = \text{Response of "Organism E" expressed as units of pure G}$$

For the *S. aureus* assay all the ratios are unity.

In this way three simultaneous equations can be set up and solved for three unknowns. If more than three penicillins are present, a preliminary separation must be made.

COMPOSITION OF ASSAY BROTH MEDIA

Organism	Difco yeast extract g./litre	Bacto peptone g./litre	Glucose g./litre	Armour beef extract g./litre	KH_2PO_4 g./litre	K_2HPO_4 g./litre	*p*H
S. aureus	3	6	2	1·5	—	—	6·3
"Organism E" ..	3	6	2	—	5·0	0·5	6·0
B. brevis	3	6	—	—	—	—	6·6

W. S. WISE

Determination of Thiamine [Aneurine] and Riboflavine in Presence of Reduced Iron. E. D. Ritter and S. H. Rubin (*Anal. Chem.*, 1947, **19**, 243–248)—In the analysis of flour pre-mixes containing reduced iron (*ferrum redactum*) for subsequent enrichment of larger bulks of flour, low and erratic results for aneurine and riboflavine were obtained when the extraction was made in the usual way with 0·1 *N* sulphuric acid at 100° C. To ascertain the role of the acid, comparative trials were made with hot acid extraction and with water extraction at room temperature. Aneurine was determined in direct extracts (without enzyme or Decalso treatment) by both the thiochrome method and the diazotised aminoacetophenone (red dye) reaction, and riboflavine by the fluorescence method and by micro-biological assay. With pre-mixes containing reduced iron, both thiochrome and red dye values for aneurine were 20 to 26 per cent. lower after acid extraction than after water extraction. With samples containing sodium ferric pyrophosphate as the enriching agent, both extraction procedures gave the same results, and purification with Decalso showed that the difference between the thiochrome and the red dye values is due to inhibition of the red dye reaction. A greater loss of riboflavine (32 to 40 per cent.) occurred during hot acid extraction of samples containing reduced iron and again there was no loss with samples containing sodium ferric pyrophosphate. The low results from the fluorimetric and the microbiological assays suggest that the riboflavine is actually destroyed and not merely reduced to leucoflavine, because aeration of the extract causes no increase in the fluorescence. When these pre-mixes were extracted by shaking with acid at room temperature, the loss of aneurine was even greater (50 per cent.) than at 100° C. Assays by the fermentation method of Schultz *et al.* (*Ind. Eng. Chem. Anal. Ed.*, 1942, **14**, 35) also gave low values, thereby providing further evidence of the destruction of aneurine. Riboflavine losses at room temperature were also greater than those found with hot extraction. The microbiological results were somewhat lower than the fluorimetric results, apparently owing to the presence of derivatives of riboflavine, which, though fluorescent, are less active than riboflavine towards *L. casei*. Again, there was no loss from the pre-mix containing sodium ferric pyrophosphate.

The effects of *p*H, time of shaking, and the iron - vitamin ratio were also investigated. McIlvaine's series of phosphate - citrate buffer solutions were used to cover the wide *p*H range from 2 to 8 with the same buffer ions and, to rule out the effect of specific buffer ions, similar experiments were made with acetate buffer solutions over the range 3 to 6·5. With phosphate - citrate buffer, low recoveries of both vitamins were found up to *p*H 6·5, but from 6·6 to 7, recoveries were practically complete, but dropped again above *p*H 7, probably owing to the instability of aneurine in alkali. With acetate buffers, the recoveries at the lower *p*H values were considerably higher, nearly complete recoveries being obtained above *p*H 5·5. At *p*H 5 only 0·13 per cent. of the reduced iron had dissolved in the acetate buffer, whereas in the phosphate - citrate buffer 75 per cent. had dissolved. The greater losses of vitamins are thus associated with the solubility of the reduced iron, but not entirely, since the losses after 30-min. shaking in 0·1 *N* sulphuric acid (in which the reduced iron is completely soluble) are considerably less than in the phosphate - citrate buffers at *p*H 5 to 6. Determinations of the effect of the time of shaking showed that, in presence of reduced iron, extraction at low *p*H even for only 2 min. is not safe. The effect of changes in the iron/vitamin ratio on the recovery in 0·1 *N* sulphuric acid is that the losses of both vitamins are approximately the same and increase progressively as the ratio is increased.

The foregoing evidence indicates that the low values found for both vitamins after acid extraction in presence of reduced iron are not due to interference in the terminal part of the assay methods, and the effect is probably due to partial reduction of the two vitamins. Experiments were made to ascertain the effect of adding, before extraction, some easily reducible compound which, by being selectively reduced, would protect the vitamins from the reducing action of the iron. Cystine proved satisfactory for this purpose in the hot extraction but, in the cold extraction, it did not provide complete protection. Stoicheiometrically, 1 g.-mol. of cystine should be required per g.-mol. of iron; good recovery is in fact obtained at a cystine/iron ratio of only 0·3. Cystine itself does not interfere with either fluorimetric assay, neither does it affect the microbiological assay of riboflavine. The reduction product, cysteine, however, inhibits the red dye reaction and must be removed by treatment with Decalso.

Procedures recommended for extraction—Finely divided samples—For products such as flour pre-mixes, which do not require hot extraction or enzyme treatment, shake the sample mechanically for 30 min. at room temperature in an acetate buffer solution of *p*H 6 prepared by mixing 10 parts of 0·1 *N* sulphuric acid and 25 parts of 0·75 *M* sodium acetate solution. Take 1 part of this solution and 5 parts of water for the extracting solution, varying the amount of buffer if necessary to bring the *p*H of the extract to between 5 and 6·5.

Samples requiring hot extraction—Dissolve enough cystine in 20 ml. of 5 *N* sulphuric acid so that after dilution to 1 litre the aliquot of the resulting solution taken for extraction contains about 13 mg. of cystine per mg. of reduced iron in the sample to be assayed. Extract at 100° C. for 45 min. with occasional shaking.

After extraction, dilute the liquid as required and determine aneurine by the thiochrome method and riboflavine by either the fluorimetric method or by microbiological assay. The red dye method may be used for aneurine provided that, after extraction with the acid solution of cystine, an aliquot of the extract is passed over Decalso according to the procedure of Hochberg *et al.* (*Cereal Chem.*, 1945, **22**, 83; ANALYST, 1946, **71**, 146) in order to remove cysteine. A. O. JONES

Micro-method of Phosphate Determination. B. Soyenkoff (*J. Biol. Chem.*, 1947, **168**, 447–457)—The method, based on a colour change of quinaldine red, is at least twice as sensitive as the Bodansky stannous chloride method (*Ibid.*, 1932–33, **99**, 197), and about fifteen times more sensitive than the Fiske - Subbarow method (*Ibid.*, 1925, **66**, 382); the precision is about 1 per cent. The change of colour intensity with time is much smaller than in the Fiske - Subbarow method (*loc. cit.*), but the temperature coefficient is slightly larger and *p*H adjustments must be more accurate. Good agreement with the amidol - molybdenum blue method (Mueller, *Z. Physiol. Chem.*, 1935, **237**, 36; and Allen, *Biochem. J.*, 1938, **34**, 858) was obtained.

Reagents and apparatus—Glassware, except the colorimeter tubes, should be made of Pyrex glass. Purify the water used by a single distillation through a tin condenser, and store in glass-stoppered, Pyrex bottles. Rinse the reaction vessels and colorimeter tubes with concentrated sulphuric acid after use. Rinse the photometer cells (which have fused-on windows) quickly with 50 to 75 per cent. sulphuric acid, then immediately with water, and leave them filled with water for 0·5 hr. in order to soak the acid out of the joints. This procedure is necessary to avoid changes in *p*H during a determination. *Quinaldine red - gum arabic solution*—To 50 mg. of quinaldine red (2-*p*-dimethylaminostyrylquinoline ethiodide) and 25 mg. of gum arabic add 500 ml. of water. Heat on a steam-bath for 1 hr., stirring at 10-min. intervals. The dye should dissolve in 40 min. Cool under the tap and decant into a glass-stoppered, Pyrex bottle. Store out of direct sunlight. The gum arabic should be the best commercial grade; lumps were found to be better than powder, which gave a high blank. The lumps were powdered before use. The reagent is stable for 3 to 5 weeks, but is best used from the seventh to the twenty-first day after preparation, during which period change is slight. The dye solution forms a coating on burettes, and this often interferes with proper drainage; it is readily removed by detergents. *Molybdate - sulphate solution*—Dissolve 8·85 g. of ammonium molybdate (81·4 per cent. MoO_3) in 250 ml. of 10 *N* sulphuric acid, and dilute to 1 litre with water. *Stock phosphate solution*—Dissolve 0·4390 g. of potassium dihydrogen phosphate in 10 ml. of 10 *N* sulphuric acid and dilute to 1 litre with water; this solution contains 100 mg. of phosphorus per litre.

Standards for calcified tissue—From the stock solution of phosphate prepare solutions containing 0·3 and 0·5 mg. of phosphorus per litre with addition of 7 ml. of 10 *N* sulphuric acid per litre.

Standards for blood sera—Prepare solutions containing 0·2 and 0·4 mg. of phosphorus per litre from stock phosphate solution with addition of 90 ml. of 0·55 *N* trichloroacetic acid plus 0·5 g. of anhydrous sodium carbonate per litre.

Preparation of samples for analysis—(1) Dissolve 3 mg. of enamel or ashed tissue or 4 mg. of dentine or bone in 0·8 ml. of 1 *N* hydrochloric acid, add 6·75 ml. of 10 *N* sulphuric acid, and dilute to 1 litre with water. Allow any undissolved residue to settle and withdraw samples (containing 0·4 to 0·5 mg. per litre of phosphorus) from the top. (2) De-proteinise blood sera with 9 volumes of 0·55 *N* trichloroacetic acid and dilute the filtrate ten-fold with water.

Procedure—Mix 2 volumes of sample (or standard) solution with 2 volumes of quinaldine red - gum arabic solution. Add 1 volume of molybdate - sulphate solution from a pipette held against the wall of the vessel, and stir continuously during the addition. As the rate of addition and mixing influences colour development, the pipette must be adjusted to have a delivery time of at least 40 sec. Allow the mixture to stand for 10 min. and then read the colour in a suitable instrument, in this work a Lumetron colorimeter No. 402-E. For samples containing down to 0·04 mg. of phosphorus per litre, 14·5-mm. tubes were used, 5-ml. volumes being mixed in the tubes themselves; more dilute samples were mixed in beakers and read in Pyrex cells 2 to 5 cm. deep.

Many experiments on the effects of temperature, small variations in the amounts of dye solution and molybdate solution, rate of molybdate addition, ageing of the dye solution, *p*H, and interfering substances, are described. Variation of *p*H had the greatest effect on the final colour and, of the interfering substances likely to be present in sera, only citric acid had any effect. The tolerance level of citric acid was increased to 0·00001 *M* by making the molybdate - sulphate solution 0·4 *M* in boric acid.

Improvements that might be made in the method to increase its sensitivity and the stability of the dye solution include the use of molybdate - perchlorate instead of molybdate - sulphate and the use of Tween 20 (The Atlas Powder Co.) instead of gum arabic as a stabiliser.

The large effects of *p*H and of some interfering substances limit the use of the present method to serum filtrates and solutions in which phosphate is one of the main dissolved constituents.

Five tables of results and the effects of variations of procedure and of interfering substances are given.

G. R. Primavesi

Colorimetric Micro-Determination of Antimony [in Biological Materials] with Rhodamine B. T. H. Maren (*Anal. Chem.*, 1947, **19**, 487–491)—In conventional wet-ash methods for the preparation of organic material for the determination of antimony, recovery of antimony was found to be highly erratic, ranging from an apparent complete loss to complete recovery. Since small amounts of organic matter can be digested without loss of antimony if sulphuric acid alone is used, the losses encountered are not due to volatilisation, and under the proper conditions of acidity the tervalent metal may be completely oxidised by ceric sulphate to the quinquevalent state. With the sulphuric acid and nitric acid mixture, only about 20 per cent. is thus oxidised and, although there was wide variation in this percentage, complete oxidation was rare. When ceric sulphate was added to the antimony residue, the recovery reached only 60 per cent. In absence of an active oxidising agent such as nitric acid, the antimony remains in the tervalent state and then

there is no loss when it is oxidised to the quinquevalent state with ceric sulphate. This procedure cannot, however, be applied practically, because sulphuric acid alone digests only centigram amounts of tissue.

Oxidation of antimony with nitric acid yields a mixture of the trioxide, the tetroxide, and the pentoxide, and apparently the tetroxide resists oxidation to the quinquevalent state. If the antimony is first reduced to the tervalent state by sodium sulphite and subsequently heated to the fuming point of sulphuric acid before treatment with any of the usual oxidising agents, it is completely recovered, the unreactive tetroxide being thus reduced and rendered available for quantitative oxidation to the quinquevalent state. In digesting 10- to 15-g. amounts of whole blood and tissue, it became convenient to hasten the later stages with perchloric acid. Antimony was thus oxidised to the quinquevalent state and, since rhodamine B gives the required reaction with quinquevalent antimony, the use of perchloric acid eliminated both the sulphite reduction and the subsequent oxidation with ceric sulphate.

Procedure—To not more than 15 g. of blood or tissue, or 50 ml. of urine or plasma in a flask, add 5 ml. of concentrated nitric acid, 5 ml. of concentrated sulphuric acid, a few glass beads or alundum chips, and 1 drop of capryl alcohol, and allow the digestion to start spontaneously. Place the flask on a hot-plate at low heat and when digestion is proceeding rapidly (1 to 2 hr.) increase the heat moderately. If charring occurs, add 1 or 2 ml. of nitric acid to the cooled digest and continue to heat, repeating this operation as often as may be necessary. When no further charring is evident, increase the heat. If iron is present the solution will be yellow when hot but colourless, with frequently a granular precipitate, when cold. When the cooled solution is colourless or only slightly yellow and no further charring occurs, add 2 drops of perchloric acid and heat strongly until fumes of sulphur trioxide are evolved. If the liquid chars or becomes yellow, add more nitric acid and repeat the treatment with perchloric acid. Finally, add 3 ml. of water and heat again to fuming point. The time of digestion varies with the type and amount of material. Whole blood (15 ml.) will take about 20 hr. and 15 ml. of plasma about 4 hr. Immerse the flask in a cold water-bath and add 5 ml. of 6 *N* hydrochloric acid. If a deep yellow colour forms it is due to iron in excess of 1 mg., and if the amount of antimony expected is very small the *iso*propyl ether method B (*infra*) should be used. Otherwise, at this stage, method A is recommended.

(A) *Benzene method*—Add 8 ml. of 3 *N* phosphoric acid and 5 ml. of 0·02 per cent. rhodamine B solution, shake the flask, and cool if necessary. Do not interrupt the procedure at any point after addition of the phosphoric acid. Rinse the liquid into a separating funnel with 10 ml. of benzene and shake 150 times. Separate the aqueous layer and collect the benzene layer, which is coloured red if antimony is present, in a test tube, and set it aside for several minutes for water to separate. Place 6 to 8 ml. in a suitable cell and read the colour intensity at 565 mμ. or with a green filter.

(B) Iso*propyl ether method*—After the addition of hydrochloric acid (*supra*), add 13 ml. of water and rinse the solution into a separating funnel with 15 ml. of *iso*propyl ether. Shake 100 times, discard the lower aqueous layer, add 5 ml. of rhodamine B solution, and shake 150 times. Discard the aqueous layer and collect the ether layer in a test tube. Read the colour intensity immediately at 545 mμ. or with a green filter.

To construct a standard reference curve add incremental values from 0 to 40 μg. of antimony to 5 ml. of sulphuric acid, treat this with nitric acid and perchloric acid, and continue in the manner already described. To prepare the standard antimony solution, dissolve 0·1 g. of antimony in 25 ml. of heated sulphuric acid, cool, and dilute to 1 litre. This solution contains 100 μg. of tervalent antimony per ml. and is stable for at least two years. Dilute the solution as required to prepare working standards of 10 and 1 μg. per ml.

Bismuth, zinc, lead, mercury, molybdenum, cobalt, or copper, in amounts up to 10,000 μg., do not interfere with the determination of 2 μg. of antimony. Interference by arsenic, tin, and iron begins at 1000 μg. A. O. JONES

Rhodamine-B Method for Micro-Determination of Antimony. L. D. Freedman (*Anal. Chem.*, 1947, **19**, 502)—Although the method described by Maren (*cf.* preceding abstract) is sensitive and rapid, certain modifications increase both the accuracy and the selectivity.

In order to avoid complications due to the effect of varying amounts of sulphuric acid on the colour intensity, a constant amount of 10 ml. of 18 *N* sulphuric acid should be used in the digestion, with precautions against loss of acid. As shown by Maren, the use of perchloric acid (0·5 ml. of a 60 per cent. solution) renders unnecessary both the sulphite reduction and the ceric sulphate oxidation. As reported by Maren, the presence of more than 1 mg. of iron seriously interferes with the method, owing to formation of a similar colour with rhodamine B. This interference can be eliminated by addition of solid sodium pyrophosphate, after the addition of the 6 *N* hydrochloric acid and the 3 *N* phosphoric acid, until the yellow colour disappears. The 0·02 per cent. rhodamine B solution is then added and the subsequent procedure is exactly as described by Maren.

The colour intensity was read in a Beckman quartz spectrophotometer at 562 mμ., the point of maximum extinction. Gellhorn, Krahl, and Fertig (*J. Pharmacol.*, 1946, **87**, 159) have used the same instrument at 565 mμ.

With these modifications, excellent recoveries of antimony from biological material have been obtained. A. O. JONES

Agricultural

Rapid Turbidimetric Determination of Inorganic Nitrogen in Soil and Plant Extracts. B. Wolf (*Anal. Chem.*, 1947, **19**, 334–335)—

Aluminium foil and Devarda's alloy proved unsuitable for the rapid reduction of nitrate and nitrite to ammonia in cold solutions, but titanous chloride effected the reduction of nitrates quantitatively in alkaline solution at room temperature in a few minutes. It was less satisfactory for the reduction of nitrites but, since these are present in soil and plant extracts in only very small amounts, it was still possible to use titanous chloride as a means of determining inorganic nitrogen fractions in such extracts.

Procedure—To prepare the soil extract shake 12·5 g. of the sample with half a teaspoonful of activated carbon (Darco grade 0-97) and 25 ml. of Morgan's universal extracting solution (*N* sodium acetate buffered at *p*H 4·8 with acetic acid) in a small flask for 1 min., filter the mixture through Whatman No. 1 filter paper, and collect the filtrate. To prepare the plant extract agitate 5 g. of fresh, minced tissue or 1 g. of dry ground tissue with half a teaspoonful of activated carbon and 200 ml. of extracting solution (*supra*) in a Waring Blendor for 5 min., filter through Whatman No. 1 paper, and collect the filtrate. Pipette 5 ml. of soil extract or 10 ml. of plant extract into a 50-ml. Erlenmeyer flask and, with soil extract, add 5 ml. of extracting solution. Add 2·25 ml. of water and 2·5 ml. of 15 per cent. sodium hydroxide solution, mix well, and add 0·25 ml. of 20 per cent. titanous chloride solution. After 10 min., filter through a Whatman No. 1 paper. Pipette 10 ml. of modified Graves's reagent (40 g. of sodium chloride and 3·5 g. of mercuric chloride dissolved in 750 ml. of water and diluted to 2000 ml.) into a photometer cell, add 0·5 g. of gum arabic solution (0·25 per cent. by weight), and mix thoroughly by means of a flat-ended glass rod. Pipette 10 ml. of the filtered extract into the cell, mix by means of the glass rod, and allow the mixture to stand for 15 min. Read the turbidity in a photometer using a 425-mμ., blue filter and adjusting the instrument to give 100 per cent. transmission with a blank cell prepared by carrying 10 ml. of extracting solution through the entire procedure. Compare the readings obtained with those of a series of standards (0 to 20 p.p.m. of nitrogen) treated as described and prepared from a solution of ammonium sulphate in extracting solution containing 50 p.p.m. of nitrogen.

The reduction by titanous chloride is almost instantaneous, but a 10-min. waiting period allows the handling of a number of samples. The rapid reduction is apparently limited to very simple forms of nitrogen, such as nitrates and nitrites; alloxantin, glycine, benzidine, urea, brucine, and diphenylamine failed to show any reduction. The gum arabic limits the size of the particles of precipitate formed and prevents precipitation of mercuric oxide. The precipitate tends to darken in strong light, especially with plant extracts, but this photo-chemical reduction may be prevented by placing the photometer cells in suitable holes in a wooden block until the readings are made. The amount of alkali added in the reduction is important, insufficient alkali giving dark or turbid extracts and excess of alkali causing precipitation of mercury oxides. Substances such as alcohol and formaldehyde interfere with the reduction and must not be used for preserving plant extracts.

The method can be applied to the determination of from 8 to 80 p.p.m. of inorganic nitrogen in soil, 1 part of soil being extracted with 2 parts of extracting solution. With greenhouse soils and soils recently fertilised, it is necessary to use a smaller aliquot. Extraction of 1 part of plant tissue with 40 parts of extracting solution permits the determination of 80 to 800 p.p.m. of nitrogen, and again higher concentrations can be handled by using smaller aliquots. Analyses can be repeated with a precision of ± 10 per cent. When the concentration of nitrites is high, as in alkali soils, the nitrites must be determined separately for accurate results.

The titanous chloride should be stored under an atmosphere of hydrogen. A. O. Jones

Determination of 2 : 4-Dichlorophenoxyacetic Acid and its Compounds in Commercial Herbicides. H. A. Rooney (*Anal. Chem.*, 1947, **19**, 475–476)—Since 2 : 4-dichlorophenoxyacetic acid (2 : 4-D), is only slightly soluble in water, it is most frequently sold in powdered form mixed with sodium carbonate or bicarbonate and, less frequently, as the free acid dissolved in polyethylene glycol. Other salts, *e.g.*, those of ammonium, diethanolamine, morpholine, and triethanolamine, have been used and also the butyl, ethyl and *iso*propyl esters dissolved in dispersing agents such as alcohols and glycols with or without soaps and neutral oils.

The methods for its determination now to be described depend upon either the titration of the acid group or, with salts and esters, separation of the free acid followed by its titration in alcoholic solution.

Procedure—1. *Salts*—Dissolve an amount of sample equivalent to about 1 g. of the pure material in 100 ml. of water in a separating funnel, acidify with diluted sulphuric acid (1 + 1) and extract the aqueous phase with two 75-ml. portions of ether. Wash the combined extracts with 10-ml. portions of water until they are free from mineral acid, add a few carborundum chips and, with the aid of a stream of air, reduce the volume to about 25 ml. on the steam-bath. Complete the removal of solvent in a stream of air at room temperature to prevent loss of 2 : 4-D by volatilisation. Dissolve the residue in 75 ml. of alcohol and titrate with 0·1 *N* sodium hydroxide, using thymolphthalein as indicator. Calculate the percentage of 2 : 4-D salt from its equivalent (*e.g.*, 1 ml. of 0·1 *N* sodium hydroxide ≡ 0·0238 g. of ammonium 2 : 4-dichlorophenoxyacetate).

2. *Esters and amides*—Heat a sample weight equivalent to about 0·8 g. of the ester or amide with about 1 g. of potassium hydroxide and 90 ml. of 95 per cent. alcohol for 1 hr. under reflux. Transfer the product to a beaker, add 50 ml. of water, and reduce the volume to about 50 ml. on the steam-bath. Cool, transfer the liquid to a separating funnel, acidify, and proceed as described for the salts.

3. *Esters in presence of soap, acids, alcohols, and*

oils—Saponify a sample weight equivalent to 0·7 g. of the ester by the method already described for esters and amides and, after removal of the alcohol by evaporation, transfer the liquid to a separating funnel and extract with 75 ml. of light petroleum to remove unsaponifiable oils. Draw off the aqueous phase into a 200-ml. graduated flask, add a few drops of phenolphthalein indicator, then diluted hydrochloric acid (1 + 1) until the pink colour disappears and, finally, diluted aqueous ammonia (1 + 1) until the liquid is slightly alkaline. Dilute with water to 150 ml., add, slowly, enough barium chloride solution to precipitate the fatty acids, dilute to 200 ml., and filter. The solution must be alkaline after the addition of barium chloride, otherwise the 2 : 4-D will be precipitated. Acidify an aliquot in a separating funnel with hydrochloric acid, and proceed as in (1) with the ether extraction, evaporation, and titration.

4. *2 : 4-D in presence of oil, soap, and alcohol*—Dilute a sample weight equivalent to 0·5 g. of 2 : 4-D to 75 ml. with light petroleum, transfer to a separating funnel, and extract with six, 10-ml. portions of a 50 per cent. alcohol and water mixture to each portion of which has been added a few drops of 40 per cent. potassium hydroxide solution and a few drops of phenolphthalein indicator. Draw off the combined alcohol - water extracts into a 250-ml. beaker, and remove the alcohol on the steam-bath. Transfer the aqueous residue to a 200-ml. volumetric flask and cool to slightly below room temperature. Proceed as in (3) beginning with the direction to add diluted hydrochloric acid (1 + 1) until the pink colour disappears.

5. *2 : 4-D in alcohol or glycol carriers*—Dilute a sample weight equivalent to about 0·8 to 1·0 g. of 2 : 4-D to 75 ml. with 95 per cent. alcohol, and titrate directly with 0·1 *N* sodium hydroxide.

If other organic acids are present, titration methods will yield high results. The total chlorine should then be determined either by combustion in a Parr bomb with sodium peroxide, sugar, and potassium nitrate, or by the sodium - alcohol method (Stepanow, *Ber.*, 1906, **39**, 4056; Umhoefer, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 383). The latter method, however, is tedious and requires a sample free from water. A. O. Jones

Gas Analysis

Rapid Determination of Small Amounts of Carbon Monoxide. Preliminary Report on the NBS Colorimetric Indicating Gel. M. Shepherd (*Anal. Chem.*, 1947, **19**, 77–81)—Silica gel impregnated with ammonium molybdate and a solution of palladium oxide in sulphuric acid is highly sensitive to small amounts of carbon monoxide, the colour of the gel changing from yellow to blue-green. This report describes the preparation of the gel, and the technique used during the war for carrying out tests for carbon monoxide in air, in the laboratory, and in the field.

Preparation of the indicating gel—Silica gel—Place about 1·45 kg. of a suitable silica gel in a 4-litre Pyrex serum bottle and digest with nitric acid (sp.gr. 1·42) for several days. Remove the acid by suction, using an immersion filter, and wash the gel with distilled water. Dry the gel at 100° C. and then for 3 days at 320° C. Close the bottle with a stopper carrying a guard tube containing purified gel and allow to cool. *Palladium sulphate solution*—Heat about 14 g. of finely divided palladium metal at 600° C. for about 12 hr. Transfer the oxide and remaining metal to a 500-ml. Pyrex flask having a thermometer well and a ground joint, add 175 ml. of concentrated sulphuric acid, connect the flask to a reflux condenser, and boil until the metal and oxide have been converted into sulphate. Add portions of water not larger than 1 ml. through the top of the condenser, heating after each addition until no more sulphur dioxide is evolved, and continue the addition until the crystalline precipitate has dissolved and the boiling temperature is about 200° C. Cool, filter through a filter crucible, and dilute the filtrate to 500 ml. in a graduated flask. Dilute a 5-ml. portion of this solution to 250 ml. and determine the palladium content by precipitation with dimethylglyoxime, and the sulphuric acid content by titrating the filtrate and washings from the palladium precipitation with standard alkali. Adjust the concentration of the palladium solution to 0·013 g. of palladium and 0·33 g. of sulphuric acid per ml. *Molybdenum solution*—Dissolve 50 g. of pure ammonium molybdate in 1 litre of water. *Impregnation of the gel*—Mix 750 ml. of distilled water, 450 ml. of the molybdenum solution, and 100 ml. of the palladium solution in a Pyrex 4-litre serum bottle. Add slowly 2 litres of the silica gel, rotating the bottle so that the gel is always submerged. Allow to stand overnight. Heat the bottle to 60° C., and reduce the pressure in the bottle to 4 cm. to evaporate the excess of water. Rotate the bottle vigorously every few minutes. Transfer equal portions of the partially dried gel to fourteen, 500-ml., round-bottomed flasks fitted with ground joints, attach to a pumping system and evacuate until, after about 20 hr., the pressure has dropped to about 0·028 mm. Admit, slowly, air dried by passage through silica gel and Hopcalite. Determine the moisture content of the gel by heating weighed portions for 16 hr. at 105° C. in a vacuum. The optimum water content is 8 to 12 mg. per g. Gels of lower water content may be blended with gels of higher water content and the blend stored for 1 month to secure equilibrium.

Indicator tubes for laboratory use—Use 15-cm. lengths of 7-mm. diameter, cleaned Pyrex tubing. Close one end of each tube with a cork, insert a small pad of adsorbent cotton and fill the tube in order with 4 cm. of guard gel (purified silica gel), 15 mm. of indicating gel, and 3 cm. of guard gel. Insert a cotton pad and close the tube with a cork. Use within 8 hr. To make tubes for field use, use fibre-glass instead of cotton and seal the tube at each end by means of a blow-pipe.

Laboratory estimation of carbon monoxide in air—Pass the air to be tested through the indicating tube at a rate of 90 ml. per min. for a definite time. Compare the colour of the tube with the colours of indicating tubes exposed to known amounts of carbon monoxide.

The gel will detect 1 part of carbon monoxide in 5×10^8 parts of air in 20 min., and 0·001 per cent. by volume in less than 1 min. The optimum concentration for the laboratory estimation is 0·01 per cent. by volume, at which concentration the values for carbon monoxide concentration are within ±1 part in 10 of the correct values. Reducing and oxidising gases interfere if they pass the guard gel. B. Atkinson

Micro-determination of Organic Sulphur in Gases. J. Hambersin (*Bull. Soc. Chim. Belg.*, **1947, 56,** 134–141)—Two methods are described for the determination of traces of organic sulphur in the synthesis gases used in the manufacture of ammonia. One method, used primarily for continuous determinations, is based on hydrogenation to hydrogen sulphide, and thence to lead sulphide, whilst the other is based on combustion, the sulphur dioxide being trapped in hydrogen peroxide and titrated.

Procedure for hydrogenation—Pass the synthesis gas at 3 litres per hr. through three wash-bottles containing 2 per cent. "plumbite of soda" solution, sulphuric acid, and 10 per cent. sodium hydroxide solution, respectively, and then over a red-hot platinum spiral. Trap the resulting hydrogen sulphide in a sintered-glass wash-bottle containing the "sodium plumbite" solution with gelatin added to prevent precipitation of the lead sulphide. Determine the latter colorimetrically.

The method is said to determine 0·15 mg. of sulphur in 1 cubic metre of gas in a 24-hr. run.

Procedure for combustion—Pass the gas through a flow-gauge and through four wash-bottles containing lead or zinc acetate solution, diluted sulphuric acid, caustic soda solution, and water, respectively, into a quartz-packed tube heated to 900° to 1000° C. Pass oxygen through a flow-meter into the same tube. Trap the sulphur dioxide in a sintered-glass wash-bottle containing "4-volume" hydrogen peroxide, removing any water left in the end of the tube by heating with a bunsen burner. Titrate with 0·01 *N* sodium hydroxide.

With 100 litres of gas containing 0·01 mg. of sulphur, the method is said to be accurate to ±0·002 mg. of sulphur. With very small concentrations of sulphur, the errors due primarily to the formation of nitric acid assume greater significance, and the authors discuss some points concerning these errors and others that occur if the solutions are evaporated to dryness.
W. J. Gooderham

Determination of Cadmium Oxide Suspended in Air. H. A. Bewick, A. J. Cruikshank, and F. E. Beamish (*Anal. Chem.*, 1947, **19,** 269–270)—*Method*—The air is filtered through dry, 5·5-cm., No. 50 Whatman papers, which are destroyed by nitric and sulphuric acids. Interfering elements are removed from the solution by means of iron wire, and the cadmium is precipitated by β-naphthoquinoline in presence of iodide. The precipitate is decomposed and the liberated iodine titrated with potassium iodate solution.

Reagents—Hydrazine hydrate solution: 10 ml. of 50 per cent. w/w solution diluted to 500 ml. *Rochelle salt - potassium iodide solution*: 25 g. of potassium iodide and 125 g. of potassium sodium tartrate tetrahydrate dissolved in 425 ml. of water. *β-Naphthoquinoline solution*: 12·5 g. dissolved in 7 ml. of concentrated sulphuric acid and 15 ml. of water, diluted to 50 ml., filtered, and diluted to 500 ml. *Dilute potassium iodide solution*: 32 g. per litre. *Wash liquid*: 40 ml. of the dilute potassium iodide solution, 40 ml. of the β-naphthoquinoline solution, 1 ml. of saturated sulphurous acid, and 320 ml. of water. *Potassium hydroxide solution*: 420 g. in 340 ml. of water. *Potassium cyanide solution*: 50 g. in 450 ml. of water. *Potassium iodate solution*, 0·0156 *M*; 1 ml. ≡ 1 mg. of cadmium oxide.

Procedure—Add 3 ml. of concentrated sulphuric acid to the paper containing the cadmium deposit and heat for 1·5 min. Add slowly 1 ml. of red, fuming nitric acid, and two 0·5-ml. portions at 1-min. intervals. Heat the flask over a burner until fumes of sulphur trioxide displace the oxides of nitrogen. Cool, and add 30 ml. of hydrazine hydrate solution. Add 3 ft. of 36-gauge iron wire, and heat just below the boiling point for 15 min., filter through a fritted-glass crucible, and wash twice with water. To the filtrate, in a 250-ml. beaker, add 0·25 ml. of saturated sulphurous acid, and 20 ml. of the Rochelle salt - iodide solution, and stir. Stir in β-naphthoquinoline solution, 9 ml. for 25 mg. of cadmium oxide, 12 ml. for 50 mg., and 15 ml. for 75 mg. Leave for 15 min. and filter on an asbestos pad in a Gooch crucible. Grind the asbestos before use to facilitate dissolution of the precipitate. Wash the beaker twice with 15 ml. of wash liquid, and wash the crucible with 5 ml. Add a little dry asbestos to the crucible, moisten, rub round with a rod, and rinse into the beaker, using a small amount of water. Break up the pad with a rod. Add 5 ml. of potassium hydroxide solution and heat the beaker over a flame, taking precautions against bumping. Stir vigorously for 2 min., add 16 ml. of concentrated hydrochloric acid down the sides of the beaker, and stir for 15 sec. Cool to below 30° C., add 5 ml. of potassium cyanide solution, and titrate with 0·0156 *M* potassium iodate solution until the colour becomes light brown. Add 2 ml. of 1 per cent. starch solution, and continue the titration until the liquid is colourless. After adding the cyanide, the work must be done in a fume cupboard. No purple particles of undecomposed cadmium complex should be visible after the titration. L. A. Dauncey

Organic

Determination of the Saponification Value of Natural Waxes. B. H. Knight (*Anal. Chem.*, 1947, **19,** 359)—The procedure is the result of a search for a method that would give consistent values without special apparatus and manipulation. A solution of potassium hydroxide in Carbitol (diethyleneglycolmonoethylether), which offered promise as a high-boiling (200° C.) saponifying agent, had a tendency to develop a colour that interfered with the end-point of the subsequent

titration, but excellent results were obtained when an equal volume of Carbitol was added to alcoholic potassium hydroxide solution.

Procedure—Grind small lumps representing the entire consignment of wax first in a loosely set laboratory plate mill and screen and then to pass through a 20-mesh screen. Roll the screened sample on paper to ensure uniformity, and to 0·5 g. in a 200-ml. Erlenmeyer flask add 20 ml. of 0·5 *N* alcoholic potassium hydroxide from a pipette and 20 ml. of Carbitol from another pipette. Make a blank determination with the same volumes of the reagents. Heat over a low flame under refluxing conditions so that the liquid boils gently for 1 hr. Titrate the blank mixture with aqueous 0·5 *N* hydrochloric acid to phenolphthalein indicator. Heat the sample mixture to the boiling point so that the precipitate disappears, and titrate with 0·5 *N* hydrochloric acid, swirling the mixture vigorously as the precipitate reappears. After the first disappearance of the pink colour, heat the mixture to the boiling point and continue the titration. Finally, re-heat the mixture and continue the titration if necessary. Usually the colour is all discharged in the second titration. Subtract the final titre from the titre of the blank mixture and calculate the saponification value.

Consistent results were obtained with crude and refined carnauba, candelilla, and ouricury waxes, and twenty determinations with the same sample of candelilla wax had an over-all variation of only 0·05 ml. of 0·5 *N* alkali.

Occasionally, specimens of Carbitol develop colour when heated with alcoholic potassium hydroxide; each batch must be tested, and any that develops colour rejected. An advantage of the use of Carbitol is that the residue left on glassware after saponification is easily removed. [See also ANALYST, 1946, **71**, 687.] A. O. JONES

African Drying Oils. I. Seed Oil of *Tetracarpidium Conophorum*. F. D. Gunstone, T. P. Hilditch, and J. P. Riley (*J. Soc. Chem. Ind.*, 1947, **66**, 293–296)—The seed oil of *Tetracarpidium conophorum* is qualitatively similar to linseed oil in composition, but its component acids contain a higher proportion of linolenic acid. Its drying properties should therefore be superior to those of linseed oil. Shell nuts of the 1943 and 1944 Nigerian crops (*a*) and (*b*), and kernels of the latter crop (*c*), which were examined at Liverpool, gave oils containing 76·8, 75·2, and 73·5 per cent. of free fatty acid (as oleic), respectively. Kernels of the 1946 crop, which were heated immediately after harvesting at 100° C. for some hours, yielded oil containing little free fatty acid; samples examined in Nigeria (*d*) and Liverpool (*e*) contained 2·7 and 1·2 per cent., respectively. The percentages of oil in kernels were: (*a*) 48, (*b*) 48 to 49, (*c*) 44, (*d*) 44, and (*e*) 49 to 52. Other characteristics were: iodine value, per cent.: (*a*) 206·4, (*b*) 205·4, (*c*) 198·6, (*d*) 199·4, and (*e*) 198·2; saponification equivalent: (*a*) 283·7, (*b*) 286·0, (*c*) 281·3, (*d*) 287·8, and (*e*) 287·0; unsaponifiable, per cent.: (*a*) 0·3, (*b*) 0·5, (*c*) 0·4, (*d*) 0·5, and (*e*) 0·6; n_D^{25} : (*a*) 1·4758, (*b*) 1·4751, (*c*) 1·4747, (*d*) 1·4833, and (*e*) 1·4830. Component acids of the oil are: linolenic 64 to 68, linoleic 10 to 12, oleic 10 to 11, and saturated acids 10 to 12 per cent. (wt.). The oil forms a clear, hard film on exposure to air. The rapid formation of free fatty acid in the air-dried kernels is due to lipolytic enzymes, which must be inactivated, immediately the fruit has been harvested, by heating the kernels at 100° C. The shrub, which grows fairly rapidly, is already cultivated for native edible purposes in S. Nigeria. E. B. DAW

Fatty Oil from the Seeds of *Argyria Speciosa*, Sweet (N.O. *Convolvulaceae*). G. M. Kelkar, N. L. Phalnikar, and B. V. Bhide (*J. Indian Chem. Soc.*, 1947, **24**, 83–86)—*Argyria speciosa*, Sweet (N.O. *Convolvulaceae*), known as elephant creeper, is abundant in India and is cultivated in Java. Its seeds give a fatty oil containing the glycerides of the following acids: palmitic 6·73, stearic 29·12, behenic 6·64, linolenic 6·09, linoleic 18·17, and oleic 33·23 per cent. The constants of the oil are: sp.gr. at 30° C. 0·9251, iodine value (Hanus) 76·0 per cent., Reichert-Meissl value 0·53, acetyl value 6·0, n_D^{30} 1·4554, saponification value 197·5, acid value 1·8, and unsaponifiable matter 0·3 per cent. Full experimental details of the examination are given. E. B. DAW

Fatty Oil of the Seeds of *Ipomoea Muricata*, Jacq. (N.O. *Convolvulaceae*). G. M. Kelkar, N. L. Phalnikar, and B. V. Bhide (*J. Indian Chem. Soc.*, 1947, **24**, 87–90)—*Ipomoea muricata*, Jacq, is an annual Indian creeper, known as Bhonvari, which grows in the rainy season. Its seeds, black when mature, have three sides, two plane and one convex. Fruit and seeds are similar to those of *Argyria speciosa*, Sweet. The seeds yield 8·7 per cent. of fatty oil. The oil constants are: sp.gr. at 30° C., 0·9164, n_D^{30} 1·45288, iodine value (Hanus) 68·6 per cent., saponification value 200·0, and acid value 1·7. The percentage composition of the component acids of the oil is: palmitic 13·66, stearic 22·55, behenic 3·78, linolenic 3·92, linoleic 15·15, and oleic 40·97. E. B. DAW

Fat from the Seeds of *Vanguera Spinosa* (N.O. *Rubiaceae*). M. D. Nadkarni, J. W. Airan, and S. V. Shah (*J. Indian Chem. Soc.*, 1947, **24**, 25–30)—Fat from the seeds of *Vanguera spinosa* contains palmitic and stearic acids (together) 27·85 per cent. (palmitic/stearic = 67·5/32·5), oleic acid 32·46 per cent., and linoleic acid 39·69 per cent. The characteristics and constants of the fat are: sp.gr. at 24°/26° C. 0·9515, n_D^{26} 1·4780, acid number 3·9, saponification value 190·7, iodine value 88·6, Reichert-Meissl value 1·56, Polenske number 0·48, acetyl value 5·8, and unsaponifiable matter 0·95 per cent. E. B. DAW

Chemical Composition of the Seed Oil of *Chorisia Insignis* "Palo Borracho." P. Cattaneo, G. Karman, and L. Uberti (*Anales Assoc. Quim. Argentina*, 1946, **34**, 5–14)—The seeds of *Chorisia insignis*, a member of the Bombacaceae, contain approximately 24 per cent. of

oil, the chief physical and chemical properties of which are tabulated in this paper. The acids present are the following, the figures in brackets giving the percentages of the total acids, and the percentages in the oil, respectively: palmitic (17·93; 16·73), oleic (31·45; 29·35), linoleic (42·04; 39·23), myristic (2·12; 1·98), stearic (0·78; 0·73), arachidic (0·08; 0·07), behenic (0·92; 0·86), eicosenic (4·68; 4·37); all unsaturated acids present having more than 18 carbon atoms in the molecule are included in the value for eicosenic acid. Linolenic acid is not found in the oil. E. M. POPE

Analytical Methods for Carotenes of *Lycopersicon* Species. F. P. Zscheile and J. W. Porter (*Anal. Chem.*, 1947, **19**, 47–51)—The spectrophotometric method can be used for the simultaneous routine estimation of β-carotene and lycopene in *Lycopersicon* species. If other carotenes are present, however, a chromatographic estimation is necessary.

Procedure—Homogenise fresh, whole, unpeeled fruits, previously cored, if large, in a Waring Blendor until blended into a thick liquid. Pour a 20-g. sample into a Blendor cup, add 75 ml. of acetone and 60 ml. of hexane (b.p. 65° to 67° C.), blend for 2 min., and filter on a Buchner funnel after the two phases have separated. Wash the residue with acetone and then with hexane. If the residue, other than skin flakes, retains pigment, shred it and the paper, blend with 50 ml. of acetone, and re-filter. Transfer the combined filtrates to a separating funnel, and drain off the lower phase; if this is coloured, add water carefully before draining. Wash the upper phase three times with water, avoiding emulsion formation. In order to remove carotenols and chlorophyll, wash once with 20 ml. of 90 per cent. aqueous methanol for 30 sec. Use mechanical agitation throughout. Remove the lower phase after it has cleared (15 to 30 min.), and wash with 20 ml. of a 20 per cent. solution of potassium hydroxide in methanol (1 min.). When the lower phase is clear (maximum 30 min.), discard it, and wash again with another 20 ml. for 30 sec. Remove the lower phase and wash vigorously three times with much water. Dry the funnel stem, and run the hexane solution into a 100-ml. volumetric flask; if the solution is not clear and bright, dry over sodium sulphate. Dilute to 100 ml. The solution can be stored in a refrigerator up to a few days before spectroscopic analysis.

In the above extraction, skin pigments are not completely extracted by the acetone and hexane, but this can be ignored for work on provitamin A. Lycopene is not extracted from the hexane solution by alkaline methanol or aqueous acetone. Control experiments showed that losses of β-carotene were negligible.

Spectroscopic analysis—A photo-electric spectrophotometer employing a large, Muller - Hilger, universal double monochromator with crystal quartz optics was used. Absorption curves of various carotenes are given. A wavelength of 487·5 mμ. was used to determine total carotene (β-carotene and lycopene) using an average specific absorption coefficient of 181. At 502 mμ., the specific absorption coefficient of lycopene is 279, and that of β-carotene, 42. It is assumed that β-carotene is isomerised to the extent of 18 per cent. to neo-β-carotene B, and that lycopene is 20 per cent. isomerised to neo-lycopene A.

If the ratio of log.(I_0/I) at 487·5 mμ. to log.(I_0/I) at 437·5 mμ. does not lie between 0·94 (lycopene) and 0·88 (β-carotene), then other carotenes are present and the chromatographic method must be used to determine β-carotene. Traces of polar solvent (less than 0·5 per cent.) do not affect the spectrographic analysis.

Chromatographic analysis—Take a chromatogram of a 20-ml. sample of the carotene solution on a column, 4 cm. × 1·6 cm. (internal diameter), of a 1 : 1 mixture of magnesia and Super Cel. After adsorption, wash the column with a small amount of hexane and then with 10 per cent. acetone in hexane until the β-carotene has been eluted. Wash the eluate twice with water, dilute it to 25 ml., and analyse spectrographically at 436 and 478 mμ. (*cf.* Beadle and Zscheile, *J. Biol. Chem.*, 1942, **144**, 21). W. S. WISE

Colorimetric Determination of Less than 0·001 per cent. of Sulphur as Carbon Disulphide in Benzoles. Colorimetric Test for Small Quantities of Carbon Disulphide in Pure Benzene. T. A. Dick (*J. Soc. Chem. Ind.*, 1947, **66**, 256–257)—The test is applicable to the determination of sulphur present as carbon disulphide in concentrations ranging from 0·0002 to about 0·01 per cent. by weight in pure benzene, and may also be applied to samples containing unsaturated compounds if, under the conditions of the test, the colour of the sample does not interfere. The sample is mixed with diethylamine and copper acetate and is then suitably diluted with sulphur-free benzene. The yellow colour developed is compared with that produced by a sample of sulphur-free benzene containing a known amount of carbon disulphide, treated in the same manner.

Reagents—To prepare benzene free from carbon disulphide, heat pure benzene (Benzole, N.B.A. Specification No. 2, 1938) under refluxing conditions for 1 hr. with a third of its volume of alcoholic potassium hydroxide. After washing the product three times with equal volumes of water, dry it over calcium chloride, and re-fractionate it through a 12-pear column, collecting the fraction distilling at 80° to 80·5° C. When 25 ml. of this purified benzene are mixed with 1 ml. of diethylamine and 1 ml. of a 0·03 per cent. alcoholic solution of copper acetate, the colour as seen in Nessler glasses should be not darker than that of an equal amount of the benzene itself.

To prepare the standard solution weigh about half the desired amount of the purified benzene into a stoppered flask, add the appropriate amount of carbon disulphide, re-weigh, and finally dilute to the graduation mark with the purified benzene. By dilution of this solution with carbon-disulphide-free benzene prepare the standard solution containing 0·001 g. of carbon disulphide per 100 ml. From this standard solution prepare daily a series

of colour standards covering the desired range in increments of 0·00001 g. of carbon disulphide by mixing the appropriate volume with the reagent and making up to 25 ml. (*infra*).

Procedure—Pipette an appropriate volume of the sample containing 0·00004 to 0·00012 g. of carbon disulphide, determined by a preliminary test, into a 25-ml., glass-stoppered cylinder and add 1 ml. of pure, freshly distilled diethylamine and 1 ml. of a 0·03 per cent. solution of copper acetate in absolute alcohol. Dilute the mixture to 25 ml. with carbon-disulphide-free benzene and shake. After 8 min., transfer the mixture to a Nessler glass and match the colour against standards in Nessler glasses similar in every respect, including colour. The glasses should be viewed while they are held vertically 3 in. above the surface of an opaque glass sheet reflecting diffused daylight, and the final matching should be made 10 min. after the mixing of the reagents. The percentage by weight of sulphur, present as carbon disulphide, is given by $100C/V\rho$, where C is the sulphur (g.) present as carbon disulphide in the colour standard most nearly matching the sample, V is the volume of sample used, and ρ is the specific gravity of the sample at the temperature of the test. The test should be made at 20° ± 5° C.

The error in estimating the carbon disulphide will depend upon the amount of sample used, but the quantity found in this volume should be within 0·000005 g. of the true value. A. O. JONES

Ethanolamine Molybdate as a Reagent for o-Dihydric Phenols. A. André Thomas (*Chimie Analyt.*, 1947, **29**, 15–16)—The reagent yields with *o*-dihydric phenols a red colour suitable for colorimetric determination of catechol, pyrogallol, and gallic acid (in wood tar oils). The sensitivity is 0·3 mg. per ml.

Procedure—Warm 2·88 g. of molybdic oxide and 1·30 g. of monoethanolamine with 9 ml. of water until dissolution is complete, add 35 ml. of alcohol, 5 g. of monoethanolamine, and water to a volume of 50 ml. Take a suitable quantity (0·5 to 1 ml.) of the liquid for test, dilute to 9 ml. with 90 per cent. alcohol, add 0·5 ml. of the reagent, and adjust the volume to 10 ml. Compare with standards made with catechol. Of 54 other substances tested, only benzoquinone yielded a red colour, which persisted on acidification; salicylaldehyde and 8-hydroxyquinoline yielded yellow colours.

W. C. JOHNSON

Mixed Perchloric and Sulphuric Acids in the Analysis of Chrome Tanned Leather. Destruction of Organic Matter and Determination of Chromium. G. F. Smith and J. S. Fritz (*J. Amer. Leather Chemists Assoc.*, 1947, **42**, 195–201)—The properties of a mixture of perchloric and sulphuric acids as an oxidising agent for the destruction of organic matter and the oxidation of chromium have previously been described (Smith, *Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 229). New features of the procedure now given for the determination of chromium in leather are the use of an equimolecular mixture of the acids instead of the more costly 2 : 1 mixture, and the addition of potassium permanganate to destroy the hydrogen peroxide produced in side reactions of the perchloric acid.

Procedure—Place 1 g. of the finely divided leather sample in a 500-ml. resistant-glass (Vycor) Erlenmeyer flask and add 10 ml. of concentrated nitric acid and 20 ml. of an equimolecular mixture of perchloric and sulphuric acids. Attach a refluxing still-head to the flask and digest the contents of the flask at 195° C. until a clear green solution is obtained. Add 5 ml. of concentrated nitric acid to ensure complete removal of organic matter. Heat at 210° C. until the red-orange colour of chromic acid is apparent and for 1 min. longer. Allow the flask to cool to 175° C., add 20 mg. of potassium permanganate down the still-head and immediately plunge the flask into a mixture of ice and brine. Swirl the flask for half a minute, remove from the cooling-bath, raise the still-head, and add 60 to 70 ml. of distilled water to the flask. Add 10 ml. of diluted hydrochloric acid (1 + 4) and boil the solution for 3 to 5 min. to destroy the permanganate. Cool, add 1 drop of 0·025 *M* ferrous-*o*-phenanthroline sulphate solution, and titrate with standard ferrous sulphate solution.

For samples of leather containing the equivalent of about 0·03 g. of chromium sesquioxide, results obtained by this procedure are accurate to within ±0·5 mg. B. ATKINSON

Inorganic

Qualitative Test for Sodium. P. Galet (*Chimie Analyt.*, 1947, **29**, 17)—*o*-Dinitro*cyclo*hexylphenate (a commercial insecticide) yields a characteristic precipitate of golden leaflets with sodium at concentrations down to 0·02 g. of sodium ion per litre. All other cations give precipitates varying in colour from cream to deep red and must be removed before testing for sodium.

Procedure—To remove all but magnesium and the alkali metals, add aqueous ammonia and ammonium carbonate, warm, filter, evaporate the filtrate to dryness, and ignite to volatilise the ammonium salts. Remove magnesium by the Gooch and Eddy method (Treadwell and Hall, "*Analytical Chemistry*," Vol. II) and again volatilise ammonium salts. Remove potassium by precipitation as the acid tartrate in presence of alcohol, evaporate the filtrate, ignite the residue, dissolve it in hot water, and filter. To the neutral solution add 1 ml. of 10 per cent. reagent solution and rub the wall of the test tube with a rod to obtain the sodium compound. If a preliminary test shows magnesium and potassium to be present in no more than traces, their removal is not necessary.

W. C. JOHNSON

Use of Potassium Ethyl Xanthogenate for Determination of Zinc and Cadmium. M. T. Berkovich (*J. Anal. Chem. Russ.*, 1947, **2**, 215–218)—Zinc and cadmium may be determined by precipitating them as the ethyl xanthogenates, $M(C_2H_5OCS_2)_2$, treating the insoluble salts with silver nitrate solution to give soluble zinc and

cadmium nitrates and insoluble silver ethyl xanthogenate, and determining the excess of silver nitrate, without filtration, by titration with potassium iodide potentiometrically. Direct potentiometric titration of the insoluble zinc and cadmium salts with silver nitrate solution was found to be impossible. A new method of separating zinc and cadmium is described.

Determination of zinc or cadmium—Procedure—To 150 ml. of the solution containing zinc or cadmium, add aqueous ammonia solution dropwise until a slight permanent precipitate forms, heat to 50° to 60° C., add 0·5 g. of potassium ethyl xanthogenate, mix carefully, allow the solution to cool to room temperature, filter, wash the precipitate with diluted potassium ethyl xanthogenate solution, transfer it together with the filter to a beaker, add a known amount of standard silver nitrate solution, mix, and titrate potentiometrically, using a silver electrode, and completing the cell with a calomel electrode and saturated potassium nitrate solution.

Separation of zinc from cadmium—In sodium carbonate solution, cadmium is precipitated as the ethyl xanthogenate, but zinc gives the basic carbonate only. *Procedure*—To the solution containing zinc and cadmium salts, at 50° to 60° C., add 5 to 10 g. of sodium carbonate, and 0·5 g. of potassium ethyl xanthogenate, cool, filter, wash first with hot water, and then with 0·1 *N* sulphuric acid. The cadmium salt remains on the filter, and may be determined as described above.

G. S. Smith

2 : 2-Dipyridine Ferrous Complex Ion as Indicator in the Determination of Iron. F. W. Cagle, jun., and G. F. Smith (*Anal. Chem.*, 1947, **19**, 384–385)—The complex dipyridine ferrous cation, $(C_{10}H_8N_2)_3Fe^{\cdot\cdot}$, which has an intense red colour, is oxidised at a formal oxidation potential of 0·97 v. to the corresponding ferric cation, which is faint blue and slightly less stable in mineral acid solutions. 2 : 2-Dipyridine, mol. wt. 156·18, m.p. 69·5° C., and molecular extinction coefficient 8650 at 522 mμ., is cheaper than other indicators containing the reactive =N—C—C—N= grouping.

Preparation of reagents — Indicators — The ferrous perchlorate complex of 2 : 2′-dipyridine, $(C_{10}H_8N_2)_3Fe(ClO_4)_2$, may now be obtained commercially. To prepare it, add 11·7135 g. of the dye base, with stirring, to a freshly prepared solution of 6·9505 g. of ferrous sulphate heptahydrate in water. The red, water-soluble ferrous sulphate complex, $(C_{10}H_8N_2)_3FeSO_4$, forms at once, and sulphuric acid, which retards its formation, is not necessary to prevent hydrolysis, so long as the ferrous sulphate solution is used at once. Dilute the aqueous solution to 300 ml. and add enough diluted perchloric acid (72 per cent. acid diluted ten-fold) to precipitate the dye base as the red perchlorate complex. Filter on a sintered-glass crucible, wash thoroughly with distilled water, and dry at 90° to 100° C.

As indicator, use 1 ml. of 0·165 per cent. aqueous solution of the perchlorate complex, or 1 drop of 0·025 *M* solution of the dipyridine sulphate complex, prepared as above and diluted directly to 1 litre (instead of 300 ml.), without conversion to the perchlorate.

Sulphato-cerate solution—Suspend 20·8 g. of ceric hydroxide in 120 ml. of water and dissolve by slow addition of 38 to 39 ml. of concentrated sulphuric acid (density, 1·84), with continual stirring. On dilution to 1 litre, an approximately 0·1 *N* solution of oxidant that is 0·5 *M* with respect to sulphuric acid is obtained.

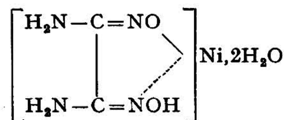
Dipyridine ferrous perchlorate as redox indicator—25-Ml. portions of approximately 0·1 *N* ferrous sulphate in 1 *M* sulphuric acid were diluted to 150 ml. with 1 *M* sulphuric acid and 1 ml. of the perchlorate indicator was added from a pipette. A deep pink coloration formed. Titration with 0·116 *N* sulphato-ceric acid in 1 *M* sulphuric acid gave a sharp colour change from orange to practically colourless at the equivalence point. Using a pipetted volume of 1 ml. of the indicator, a blank correction of 0·02 ml. of 0·1 *N* oxidant must be applied.

The titration values obtained are concordant. The indicator is reversible, and stable for more than 5 min. in presence of 1 ml. excess of the oxidant, and for approximately 2 hr. with 1 drop of oxidant in excess.

Indicator characteristics in presence of hydrochloric acid—The conditions described are suitable for use with the Jones amalgamated zinc reductor. The Walden silver reductor requires hydrochloric acid, and 0·1 *N* solutions of ferric chloride in 1 *N* hydrochloric acid have been as accurately determined with the same oxidising agent, but the colour change is then from orange to yellow because of the colour of the complex ferric chlorides formed. Phosphoric acid must not be used to decolorise the ferric ions because of the formation of insoluble cerium phosphates.

M. E. Dalziel

"Niccolox," a new Reagent for Nickel. M. Kuraš (*Coll. Czech. Chem. Comm.*, 1947, **12**, 198–203)—Oxalenediamidoxime, for which the name "niccolox" is proposed, yields an orange-yellow complex of the composition



with a solution of nickel chloride containing ammonium acetate or ammonia. The sensitivity of the reaction is almost equal to that with dimethylglyoxime, but since the yellow colour is less visible than the red of the nickel dimethylglyoxime complex, the latter is preferable for micro-qualitative tests. For quantitative work, the new reagent has the advantages of being easily prepared and of being soluble in water, so that even a large excess does not precipitate with the nickel complex, and a saving in alcohol is effected. The complex does not adhere to the side of the vessel, and does not clog the filter.

Procedure—To a neutral or slightly alkaline solution of 0·1 to 0·2 g. of nickel chloride, sulphate,

or nitrate in 50 to 100 ml. of water add a slight excess (0·3 g.) of the reagent dissolved in hot water and cooled. Neutralise the liberated acid by adding aqueous ammonia. Alternatively, employ an ammoniacal solution of the reagent or of the nickel salt. After mixing, filter in a crucible, wash with a little water and dry for 0·5 hr. at 110° C. to obtain the anhydrous complex, $Ni(C_4H_{10}N_8O_4)$, containing 20·05 per cent. of nickel.

Metals of Groups I and II must first be eliminated. Alkaline earth metals do not interfere with the reaction itself, but may yield insoluble carbonates with the carbonate usually present in aqueous ammonia. Ammonium salts in moderate amount and alkali metals do not interfere. High results are obtained in presence of iron or cobalt, and the method is not applicable.

Separation of nickel from manganese, chromium, and aluminium—Add sufficient citric acid to form complex salts, boil, cool, and precipitate the nickel with niccolox and aqueous ammonia.

Separation of nickel from zinc—Render ammoniacal in presence of sufficient ammonium chloride to prevent precipitation, then acidify slightly with hydrochloric acid, and precipitate with an ammoniacal solution of niccolox. If all four of the above metals are present, add ammonium chloride and citric acid, boil, cool, and precipitate with ammoniacal niccolox solution. Micro-analytical procedure is similar, a solution containing 2 to 4 mg. of nickel in 10 to 20 ml. of water being used.

W. C. JOHNSON

Colorimetric Determination of Cobalt in Stainless Steel. H. M. Putsché and W. F. Malooly (*Anal. Chem.*, 1947, **19**, 236–238)—Use is made of the blue colour produced by adding sodium thiocyanate to a cobaltous solution and then adding acetone.

Procedure—Dissolve 0·25 g. of the steel by warming with 5 ml. of hydrochloric acid, 5 ml. of nitric acid and 2 drops of hydrofluoric acid in a 250-ml. conical flask. Boil for several minutes, cool somewhat, add 10 ml. of perchloric acid, and heat until all the chromium is oxidised and fumes of perchloric acid are evolved freely. Cool, add 25 ml. of water and diluted aqueous ammonia (1 + 1) until the solution is almost neutral. Cool, transfer the solution to a 250-ml. graduated flask, and add a suspension of zinc oxide in water until precipitation is complete. Dilute to the mark and allow to settle. Take 25·0 ml., filter if necessary, add 5 ml. of sulphurous acid, and boil to expel the excess of sulphur dioxide. Cool the solution and add it to 15 ml. of 40 per cent. sodium thiocyanate solution in a 50-ml. graduated flask. Dilute to the mark and mix. Measure the optical absorption of a convenient volume of this solution diluted with an equal volume of water. Discard the solution and place an equal volume of the solution in the cell, and this time dilute with an equal volume of acetone. Allow the blue colour to develop for 5 to 10 min. and measure. Correct the figures by the results of blank experiments, using sodium thiocyanate solution and distilled water, and refer to data obtained with standard cobalt solutions to convert the results into amounts of cobalt. (The paper gives in greater detail the operations when a Klett - Summerson photo-electric colorimeter, with a Corning red filter No. 2424, 3·5 mm. thick, and a Corning blue filter No. 4308, 3 mm. thick are used.) To obtain the best results, the maximum amount of cobalt, when using 1·3-cm. cell depth, should be 16 μg. per ml. The method is applicable to many alloys and few, if any, elements interfere; it has been employed on stainless steels containing from 0·008 to 60 per cent. of cobalt, with appropriate modifications of the sample weight or the size of aliquot portions.

L. A. DAUNCEY

Hexamethylenetetramine in Separations of Titanium and Niobium. K. W. Traub (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 122–124)—In a reduced solution, hexamethylenetetramine precipitates titanium and leaves iron in solution, and the separation has many advantages over the hydrogen sulphide method for removing iron.

Gravimetric determination of titanium in iron-titanium alloys—Procedure A—Heat 0·5000 g. of the 80-mesh alloy with 100 ml. of 5 per cent. sulphuric acid solution until reaction ceases, then add nitric acid dropwise until the iron is oxidised. Separate the vanadium by means of sodium hydroxide, and return the washed precipitate and paper to the original beaker. Destroy the organic matter with 15 ml. of sulphuric acid, 25 ml. of nitric acid, 10 ml. of perchloric acid (70 to 72 per cent.), and 5 to 8 g. of sodium sulphate; evaporate the solution until the sulphuric acid fumes strongly, and ensure the complete removal of perchloric acid. Cool, dilute to 150 ml., and heat to dissolve the salts. Reduce the solution with an amalgamated zinc spiral until the violet colour of reduced titanium appears. Remove the spiral, add 3 or 4 drops of 10 per cent. aerosol, wetting-agent solution to eliminate aerial oxidation of the ferrous iron by lowering the surface tension of the solution, and aqueous ammonia solution to just dissolve the first-formed black precipitate. Add 10 per cent. hexamethylenetetramine solution until the solution is alkaline to methyl red and some macerated filter-paper pulp, and then boil for 1 min. Filter the solution through paper, using a gentle suction, and wash the precipitate 5 or 6 times with hot, 0·5 per cent. hexamethylenetetramine solution. Return the paper and precipitate to the beaker and destroy the organic matter as before. Cool, dilute to 100 ml., heat to boiling, and filter. Discard the residue after washing it thoroughly with hot water. Reduce the filtrate and washings, and repeat the separation with ammonia and hexamethylenetetramine.

If copper is present, dilute the solution to 200 ml., boil with a 5 per cent. excess of ammonia, filter, and wash the paper with hot, 5 per cent. aqueous ammonia solution. Digest the paper and precipitate in the same beaker with 30 ml. of sulphuric acid, dilute hot to 100 ml., and when dissolution is complete cool to 10° C., and make a cupferron separation. Ignite the precipitate in platinum and correct the result for traces of silica and ferric

oxide. Any zirconium present will accompany the titanium. The maximum deviation in the results given is 1 part in 500 for samples containing 25 per cent. of titanium.

The caustic soda separation may be avoided by reducing the vanadium to the bivalent condition with the zinc spiral, but it may not be easy to recognise complete reduction.

Procedure B—Heat 0·5000 g. of the sample with 100 ml. of 10 per cent. sulphuric acid solution, and introduce the zinc spiral for 15 to 30 min. Add 3 or 4 drops of aerosol solution and proceed with two hexamethylenetetramine and ammonia separations as in *A*. Precipitate the titanium with cupferron and ignite to titanium dioxide. The maximum deviation of the results given is 1 in 418.

Determination of titanium and niobium in high chromium-nickel steels—The usual method involving hydrolysis is inefficient in presence of much titanium, when quantitative hydrolysis takes too long. When tungsten, vanadium, or molybdenum is also present, the determination of niobium and titanium is further complicated. *Procedure*—Heat 5·000 g. of the sample with 100 ml. of diluted hydrochloric acid (1 + 2) until the reaction ceases. Cool to 10° C., and precipitate niobium and titanium by the usual cupferron method for titanium in steels. Precipitate also sufficient iron to avoid loss of niobium and titanium in the subsequent sodium hydroxide separation (25 ml. of 6 per cent. cupferron solution should be enough). Return the filtered precipitate to the beaker and treat it with 10 ml. of sulphuric acid, 25 ml. of nitric acid, 10 ml. of perchloric acid, and 3 g. of sodium sulphate, and then concentrate to fuming. Cool and dilute the solution. Separate the tungsten and vanadium with sodium hydroxide and wash the precipitate with hot, 2 per cent. sodium hydroxide solution to prevent re-dissolution of the niobium. The separation is unnecessary if tungsten and vanadium are absent, as molybdenum is not precipitated, and tantalum remains with niobium and titanium throughout.

Return the paper and precipitate to the beaker, and destroy the paper as above, removing all perchloric acid by fuming. Cool, dilute to 150 ml., add 5 ml. of hydrochloric acid, and heat. Reduce the iron with amalgamated zinc, add 3 or 4 drops of aerosol, and aqueous ammonia solution until the solution is nearly neutral to methyl red; disregard any white precipitate due to titanium or niobium. Add 10 per cent. hexamethylenetetramine solution until the solution is alkaline to methyl red, and some paper pulp, and boil for 1 min. Filter, wash the precipitate 5 times with a hot, 0·5 per cent. solution of the reagent, and destroy the paper and precipitate in the original beaker as above; repeat the separation. Transfer the precipitate to a beaker, treat it with 30 ml. of diluted sulphuric acid (1 + 1), dilute with hot water to 100 ml., and cool to 10° C. Precipitate with cupferron and determine the mixed oxides by ignition, making a correction for silica present. Fuse the residual oxides with sodium bisulphate, dissolve the cooled melt, and determine the titanium present colorimetrically with hydrogen peroxide in 5 per cent. sulphuric acid. Determine niobium by difference. Results are reproducible to within 1 in 45 on a titanium content of 0·4 per cent., and 1 in 25 on a niobium content of 0·78 per cent.

The precipitates are not contaminated with mercury or zinc from the reducing spirals.

M. E. Dalziel

Volumetric Titrations in Strongly Coloured Solutions. M. Brut (*Chimie, Analyt.*, 1947, **29**, 85)—The principle consists of adding to the coloured solution another solution of complementary colour to produce a grey tint that does not obscure the colour change to be observed. The material added must not affect the chemical reaction. Thus, cobalt sulphate solution (10 per cent. w/v) may be employed to neutralise the green colours present in the following titrations: (1) the determination of chromate by reduction with ferrous sulphate and titration of the excess of ferrous sulphate with permanganate, (2) the titration of chromic acid solutions containing a considerable quantity of nickel, and (3) the determination of manganese by oxidation to permanganate, reduction with ferrous sulphate and titration of the excess of ferrous sulphate, in presence of a high nickel content. In (3), the cobalt sulphate is added before the oxidation. Conversely, solutions coloured by cobalt salts may be treated with nickel sulphate solution to obtain a neutral grey colour.

W. C. Johnson

Rapid and Accurate Method for Determining Available Chlorine in Hypochlorite. V. Sinn (*Chimie, Analyt.*, 1947, **29**, 58)—The method is not affected by chlorates and is more accurate than the method of Pénot (Treadwell and Hall, "*Analytical Chemistry*," Vol. II), which can give values 1 per cent., or more, in error.

Procedure—Dissolve 19·782 g. of arsenious oxide in 120 ml. of 2·5 *N* sodium hydroxide, dilute to 1·5 litres, add 56 g. of sodium bromide dihydrate, neutralise to litmus with acetic acid, add a further one-tenth of the quantity of acid required, and dilute to 2 litres. Dilute *Eau de Javel* by 1 to 50, or 1 to 100, or dissolve 7 g. of bleaching powder in 1 litre of water. Add the diluted hypochlorite solution, or the [turbid?] solution of bleaching powder, from a burette to 10 ml. of the 0·2 *N* arsenious solution using as indicator 2 to 3 drops of a 0·2 per cent. solution of quinoline yellow (sulphonated). The end-point is marked by a sharp change from yellow-green to colourless.

W. C. Johnson

Alkalimetric Determination of Cadmium and Zinc. E. Carrière (*Chimie, Analyt.*, 1947, **29**, 83–84)—In the titration of a cadmium sulphate solution with sodium hydroxide there are three sharp *p*H changes, 3·3 to 7 corresponding to the neutralisation of free acid, 9 to 10·6 corresponding to the production of 4 CdO,SO_3, and 11·2 to 12·6 corresponding to the liberation of cadmium hydroxide.

Procedure—Titrate the acid cadmium sulphate solution with 0·2 *N* sodium hydroxide to the yellow colour of methyl red, and then to the blue colour of thymolphthalein. The difference between

the two titrations is the volume of 0·2 *N* sodium hydroxide required to precipitate all the cadmium as $4CdO,SO_3$.

Solutions of zinc chloride or nitrate show two sharp changes, one at *p*H 3 to 6·5, corresponding to the neutralisation of free acid, and the other at 8·5 to 11·8, corresponding to the formation of zinc hydroxide.

Procedure—To the solution of zinc chloride or nitrate add 2 drops of methyl orange and 5 drops of phenolphthalein, and run in the sodium hydroxide, noting the two changes. The solution is agitated and boiled to determine the final end-point.

Zinc sulphate solutions exhibit three changes—*p*H 2·8 to 6·4 (neutralisation of free acid), 7·6 to 9·2 (precipitation of $5ZnO,SO_3$), and 9·4 to 11·5 (formation of zinc hydroxide). In practice, using methyl orange and phenolphthalein, the second change occurs at a point corresponding to the formation of $4ZnO,SO_3$. All the titrations may be repeated after re-acidification. W. C. JOHNSON

Determination of Triphosphoric and Pyrophosphoric Acids in Presence of Ortho- and Metaphosphoric Acids. R. N. Bell (*Anal. Chem.*, 1947, **19**, 97–100)—The amount of sulphuric acid liberated by adding zinc sulphate solution to a sample solution at *p*H 3·8 is related to the sum of the pyrophosphate and triphosphate. Zinc pyrophosphate is precipitated and used to determine pyrophosphate gravimetrically.

Owing to the complex nature of the phosphates and some confusion in the nomenclature a table of compositions and some reactions is included.

Apparatus—A glass-electrode *p*H-meter with the electrodes in a titration vessel with a mechanical stirrer.

Special reagent—Zinc sulphate solution containing 12·5 per cent. of the heptahydrate, adjusted to *p*H 3·8.

Method—Weigh 0·5 g. of the sample and dissolve in 50 ml. of water containing a slight excess of sodium hydroxide. Add sufficient 0·04 per cent. bromophenol blue indicator solution to give a distinct blue colour and add 0·2 *N* hydrochloric acid until a yellow colour is produced. Dilute to 100 ml. and, using the glass electrode, adjust the *p*H to 3·8. Add 70 ml. of the zinc sulphate solution, agitate for 1 to 2 min., and titrate to *p*H 3·8 with 0·1 *N* sodium hydroxide. Any pyrophosphate is precipitated as the zinc compound in this titration. Wash in the electrodes and allow the precipitate to settle for 30 to 60 min. Filter, and wash the precipitate three times with cold water. Rinse the precipitate into a 400-ml. beaker, dissolving any that remains in the funnel with 10 ml. of 0·2 *N* hydrochloric acid. Dilute to 250 ml. and add enough 0·2 *N* hydrochloric acid to dissolve all the precipitate. Without delay, add 25 ml. of the zinc sulphate solution and adjust the *p*H to 3·8. Allow the precipitate to settle, filter, and wash well with cold water. Ignite at 500° to 600° C. until the residue is grey, and then at 900° C. until it is light grey or white. Weigh as $Zn_2P_2O_7$, calculate the titration equivalent of the pyrophosphate and subtract it from the titration figure. The balance is due to triphosphate.

Grams of $Zn_2P_2O_7$ × 0·572 ≡ g. of P_2O_7
× 0·584 ≡ g. of $H_4P_2O_7$
× 0·872 ≡ g. of $Na_4P_2O_7$
× 1·463 ≡ g. of $Na_4P_2O_7.10H_2O$
× 65·5 ≡ ml. of 0·1 *N* sodium hydroxide

Ml. of 0·1 *N* sodium hydroxide not due to pyrophosphate
× 0·0177 ≡ g. of P_3O_{10}
× 0·0181 ≡ g. of $H_5P_3O_{10}$
× 0·0258 ≡ g. of $Na_5P_3O_{10}$
× 0·0333 ≡ g. of $Na_5P_3O_{10}.6H_2O$

	Trisodium orthophosphate $Na_3PO_4.12H_2O$ (See note *a*)		Tetrasodium pyrophosphate $Na_4P_2O_7.10H_2O$		Sodium triphosphate $Na_5P_3O_{10}.6H_2O$		Sodium trimeta-phosphate $(NaPO_3)_3.6H_2O$		Sodium hexameta-phosphate $(NaPO_3)_6$	
	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
P_2O_5, per cent.	18·7	18·6	31·8	32·3	44·7	44·9	51·4	53·4	69·6	69·6
Loss on ignition, per cent.	56·8	55·8	40·3	40·5	22·7	23·1	26·1	24·7	None	None
Na_2O, per cent.	24·5	25·1			32·6	32·75				
Orthophosphate as P_2O_5, per cent.		18·4		Trace		Trace		None		Trace
Precipitates with $BaCl_2$ at *p*H 8		Yes		Yes		Yes		No		Yes
" at *p*H 2·2 ..		No		No		No		No		Yes
Precipitates with $ZnSO_4$ at *p*H 3·8		No		Yes		No (See note *b*)		No		No
Precipitates with $AgNO_3$		Yes		Yes		Yes		No		Yes

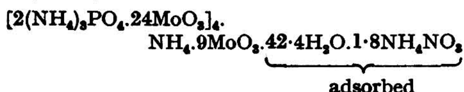
Note *a*. Possibly more complex, approximately $9(Na_3PO_4.12H_2O).2NaOH$.
Note *b*. Crystals of $NaZn_2P_3O_{10}.8H_2O$ form if the solution is seeded or heated.

The triphosphate factors are empirical ones for the method given. Any change affecting the zinc sulphate concentration will change them.

Sodium zinc triphosphate does not crystallise readily unless the solution is seeded. To avoid seeding, it is advisable to rinse the apparatus with approximately *N* hydrochloric acid before a

determination. Orthophosphates, silicates, and carbonates as used in detergent mixtures do not interfere. L. A. DAUNCEY

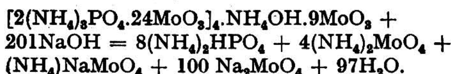
Volumetric Determination of Phosphoric Acid by a Modification of the N. v. Lorenz Method. L. Gisiger (*Z. anal. Chem.*, 1938, **115**, 15–29)—According to the investigations of Lorenz (*Ibid.*, 1907, **46**, 193; 1935, **102**, 233) the phosphomolybdate precipitate obtained by using a molybdenum-rich reagent containing sulphate has a ratio $MoO_3 : P_2O_5$ of 24 : 1. The composition of the precipitate, washed with dilute ammonium nitrate solution and acetone and dried over diluted sulphuric acid (1 + 1), is now found to be



$$[2(NH_4)_3PO_4.24MoO_3]_4.NH_4.9MoO_3.\underbrace{42\cdot4H_2O.1\cdot8NH_4NO_3}_{\text{adsorbed}}$$

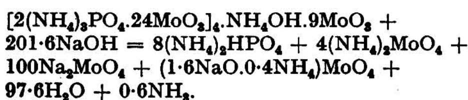
giving a mean ratio of 26·25 ± 0·084MoO_3 : 1P_2O_5. Some ammonium nitrate is removed by prolonged washing with water, but this does not affect volumetric determinations when no formalin is added before titration. In gravimetric determinations, it causes a variation of 1 part in 180. Washing the precipitates obtained by the Lorenz method with water, or 2 per cent. ammonium nitrate solution, or 1 per cent. sodium sulphate solution does not affect the phosphorus pentoxide content of the precipitate, but washing with the ammonium nitrate solution increases the ammonia content of the precipitate by approximately 50 per cent. Further, owing to base exchange with ammonium ions, washing with 1 per cent. potassium sulphate or nitrate solution leads to lower contents of phosphorus pentoxide in the titration method. For this method, the Lorenz precipitate is best washed with water.

The theoretical equation for the titration, based on this $MoO_3 : P_2O_5$ ratio, is



$$[2(NH_4)_3PO_4.24MoO_3]_4.NH_4OH.9MoO_3 + 201NaOH = 8(NH_4)_2HPO_4 + 4(NH_4)_2MoO_4 + (NH_4)NaMoO_4 + 100\ Na_2MoO_4 + 97H_2O.$$

Thus P_2O_5 is equivalent to 50·25 Na, but in practice this value is 50·4 owing to a slight loss of ammonia. The following relation then holds, for a precipitate washed either with water or ammonium nitrate solution.



$$[2(NH_4)_3PO_4.24MoO_3]_4.NH_4OH.9MoO_3 + 201\cdot6NaOH = 8(NH_4)_2HPO_4 + 4(NH_4)_2MoO_4 + 100Na_2MoO_4 + (1\cdot6NaO.0\cdot4NH_4)MoO_4 + 97\cdot6H_2O + 0\cdot6NH_3.$$

METHOD—*Molybdate reagent*—Dissolve 1500 g. of ammonium molybdate in 4 litres of boiling water and cool to room temperature. Pour this solution in a thin stream into 4500 ml. of concentrated nitric acid containing 500 g. of ammonium sulphate. Mix well, filter, and store in a brown glass flask in a dark place for at least two days before use.

Procedure—Treat about 0·16 g. of sample in a 400-ml. beaker with 40 ml. of diluted nitric acid (1 in 5), warm, and swirl for 5 to 10 min. and then heat to boiling. Run in 50 ml. of the molybdate-sulphate reagent from a burette, and shake well until most of the reagent is added. When all has been added, leave in a dust-free place for at least 2 hr., and, after filtering and washing as described below, titrate with standard alkali.

Filter by means of a filter-stick with a fritted disc about 2 cm. in diameter, attached to a 3-way tap so that it may be directly connected to a pump or to a flask. Withdraw the supernatant liquid and wash the precipitate at least six times with distilled water. When the precipitate is washed and dry, detach the filter stick and allow water to flow in from a syphon, thus rinsing the precipitate from the disc into the beaker. Add 25 ml. of 0·584 *N* sodium hydroxide and several drops of phenolphthalein solution, swirl to dissolve the precipitate, and titrate the excess of alkali with standard acid.

The titrated liquid can be acidified with nitric acid to precipitate the ammonium phosphomolybdate almost quantitatively after several hours standing. After removal of the supernatant liquid, the precipitate can, if desired, again be titrated.

The method is suitable for the determination of phosphorus pentoxide in fertilisers.

M. E. DALZIEL

Rapid Determination of Silicic Acid in Ignited and Un-ignited Silicates. S. J. H. Spronck (*Chem. Weekblad*, 1947, **43**, 259–264)—The use of gelatin in the determination of silica offers several advantages over the usual method. The amount of silica remaining in the filtrate is only about 0·5 per cent., and is nearly constant, so that a correction may be applied. The silica obtained is very pure, and it is not necessary to check its purity by treatment with hydrofluoric acid. The addition of gelatin is also of advantage in other analyses in which an acid solution containing silica is produced, as it greatly increases the rate of filtration.

Procedure—Fuse 1 g. of the silicate with 8 g. of sodium hydroxide, extract the melt with water, and transfer the solution to a porcelain basin of 150 mm. diameter. Cover with a clock-glass and add 50 ml. of hydrochloric acid (sp.gr. 1·19). After heating for several minutes on the water-bath, rinse the clock-glass, add a small amount of bromine water, and evaporate the liquid to dryness. It is essential that the residual salts should form a fine-grained mass. This is attained by pressing the nearly dry mass with a flattened glass rod. While the basin is still on the water-bath, add 20 ml. of hydrochloric acid (sp.gr. 1·19) and 10 ml. of alcohol, and stir and press the mass. After 10 min., repeat the operation with a further 10 ml. of acid and of alcohol, and add 200 mg. of solid gelatin. Stir well from time to time to distribute the gelatin uniformly, keeping the basin covered in the intervals, and continue the heating for 10 min. Add 85 ml. of warm water and, after the salts have dissolved, warm, and filter off the residue. Wash with warm water containing a trace of gelatin, and ignite the residue to constant weight at 1150° C. Apply a total correction of +0·4 per cent. for dissolved silica (+0·5 per cent.) and non-siliceous

residue in the ash (−0·1 per cent.). This correction should be subtracted from the weight of the R_2O_3 precipitate. G. MIDDLETON

Rapid Gravimetric Determination of Silicon in Aluminium Alloys. P. Lisan and H. L. Katz (*Anal. Chem.*, 1947, **19**, 252–253)—Existing colorimetric methods for the rapid determination of silicon are criticised because they are successful only under very precisely controlled conditions. Gravimetric procedures based on alkali attack of the alloy are slow, and acid attack often results in loss of silicon as hydride or incomplete oxidation to silica. If the acid reagent is chosen to overcome these difficulties precise control of later operations must be introduced, and this control is difficult. A method employing acid attack, which is comparatively simple to use, is now described.

METHOD—*Reagent*—An acid mixture of 150 ml. of phosphoric acid (*d.* 1·71), 200 ml. of concentrated nitric acid, and 50 ml. of concentrated sulphuric acid.

Procedure—Dissolve 2 g. of sample containing 1·5 to 4·5 per cent. of silicon, or 1 g. containing 4·5 to 10 per cent., in 80 ml. of the acid mixture in an 800-ml. beaker. For samples containing over 10 per cent. of silicon use 0·5 g. and 60 ml. of acid. Warm to assist solution. Evaporate until the solution clears, and immediately add a pinch of ammonium nitrate to oxidise any sulphur, swirl, and remove the beaker from the hot-plate. Cool slightly, add 60 ml. of 70 per cent. perchloric acid and evaporate to fuming. Cover the beaker, and boil the solution gently for 8 or 9 min. Cool somewhat, cautiously add 300 ml. of hot water and 40 ml. of 1 per cent. gelatin solution, and stir well. Filter immediately on a No. 41 Whatman paper containing some pulp. Transfer all the precipitate on to the paper and wash twelve times with hot, diluted sulphuric acid (1 + 99) and a few times with hot water. Burn off the paper in a platinum crucible, ignite the silica to 1100° C., and weigh. Treat with hydrofluoric acid and sulphuric acid, ignite, and re-weigh as usual.

Statistical examination of the results indicates that the method is not significantly different in accuracy from that of the Aluminium Company of America ("*Chemical Analysis of Aluminium,*" 2nd Ed., pp. 28–30, 1941), which is considered to be an umpire method and is considerably longer.

L. A. DAUNCEY

Microchemical

Micro-Kjeldahl Determination of Nitrogen. Use of Potassium Bi-iodate in the Iodimetric Titration of Ammonia. R. Ballentine and J. R. Gregg (*Anal. Chem.*, 1947, **19**, 281–283)—*Procedure*—Weigh or pipette the sample, containing 0·1 to 1·4 mg. of nitrogen, into a 10-ml. Kjeldahl flask, add 1 ml. of a mixture of 3 volumes of concentrated sulphuric acid and 1 volume of concentrated phosphoric acid, and a glass bead. Remove the excess of water over the low flame of a micro-burner or in a drying-oven at 125° C. To the cooled contents of the flask, add about 100 mg. of potassium persulphate and 5 to 8 drops of water and heat the flask over a micro-burner until white fumes are evolved. Cool the flask and, if the liquid is not clear, repeat the treatment with potassium persulphate. Finally, add about 50 mg. of potassium persulphate with 5 to 8 drops of water and heat the flask on a sand-bath at about 375° C. for at least 5 hr.

Pipette 10 ml. of 0·01 *M* potassium bi-iodate into a 50-ml. Erlenmeyer flask placed under the delivery tip of the still (*infra*) so that the tip is well beneath the surface of the liquid. Wash the cooled digest into the still with five, 1-ml. portions of water containing a little phenolphthalein, and make the liquid strongly alkaline with 20 per cent. sodium hydroxide solution. Distil for 6 min. (about 15 ml. are collected in this time) then lift the delivery tip about 2 cm. above the surface of the liquid and rinse it with water. Continue the distillation for 2 min. more before removing the receiver. To the distillate add about 25 mg. of potassium iodide and titrate the liberated iodine with 0·11 *M* sodium thiosulphate (containing 3 drops of chloroform per litre), adding a drop of starch indicator near the end-point. Correct the result by means of a blank determination. To prepare the starch indicator make a paste of 0·5 g. of soluble starch with a small amount of water and pour on it with constant stirring 50 ml. of boiling, 20 per cent. sodium chloride solution. The nitrogen (mg.) in the sample is given by the expression $(A - B) \times 10\,N \times 14{\cdot}008/A$, where A is the amount (ml.) of sodium thiosulphate solution required for the blank titration, B is that required for the sample titration and N is the normality with respect to hydrogen ions of the standard potassium bi-iodate solution.

A statistical examination of the results of a number of blank determinations and of applications of the method to substances of known composition showed that the reproducibility is satisfactory with amounts of nitrogen ranging from 0·125 mg. to 1·2 mg., especially when each step in the procedure is standardised. The first distillation of a series may yield low values. This may be prevented by "seasoning" the still by distilling ammonia from one or two samples of ammonium sulphate before each series of determinations.

The micro-distillation unit (Scientific Glass Apparatus Co., Bloomfield, N.J., Catalogue No. M-3074) may be connected with an adapter to the centre outlet of a 500-ml., three-arm, round-bottomed flask containing a heating coil of nichrome wire, connected through the side-arms to a 110-volt source and an adjustable resistance to control the rate of heating. A. O. JONES

Determination of Micro-quantities of Cyanide (and Thiocyanate). J. Epstein (*Anal. Chem.*, 1947, **19**, 272–274)—*Method*—The cyanide is converted to cyanogen chloride by means of chloramine-T; and the addition of a reagent containing pyridine, 1-phenyl-3-methyl-5-pyrazolone, and *bis*-pyrazolone produces a blue colour suitable for absorptiometry.

Reagents—*bis*-(1-Phenyl-3-methyl-5-pyrazolone) is prepared by Knorr's method (*Ber.*, 1884, **17**, 2044).

Dissolve 17·4 g. of phenylmethylpyrazolone, twice recrystallised from alcohol, in 100 ml. of 95 per cent. alcohol, add 25 g. of freshly distilled phenylhydrazine, and heat under refluxing conditions for 4 hr. Filter while hot and wash the insoluble *bis*-pyrazolone (m.p. >320° C.) with hot alcohol. *Pyridine - pyrazolone reagent*—Dissolve 0·1 g. of the *bis*-pyrazolone in 100 ml. of pyridine and mix with 500 ml. of a saturated solution of phenylmethylpyrazolone in water. Store in a dark bottle. The solution becomes pink on standing, but remains useful, although a fresh supply should be prepared every 3 days.

Procedure—To 1 ml. of sample solution containing up to 1·2 µg. of cyanide ion, in a test tube, add 0·2 ml. of 1 per cent. aqueous chloramine-T solution. Stopper the tube, shake, after 1 min. add 6 ml. of reagent, mix, stopper, and leave for 20 min. Measure the absorption at 630 mµ., setting the instrument with a reagent blank. Prepare a calibration curve from known cyanide solutions. The *p*H of the cyanide solution may be between 2·8 and 9·0. Recovery of cyanide is between 98 and 100 per cent. Phosphate, carbonate, chloride, borate, cyanate, oxalate, ferricyanide, ferrocyanide, and sodium and ammonium ions do not interfere. Iron salts catalyse the reaction with chloramine-T, but have no adverse effect. Any substance that reacts to give cyanogen chloride interferes. Thiocyanate reacts slowly with chloramine-T and the method, modified by addition of a trace of iron to catalyse the reaction, is also used to determine thiocyanate.

Procedure—To 1 ml. of solution containing up to 2·5 µg. of thiocyanate ion add 0·2 ml. of 0·1 per cent. ferric chloride solution and 0·2 ml. of 1 per cent. chloramine-T solution. After 3 min., add 6 ml. of pyridine - pyrazolone reagent, and proceed as for cyanide.

L. A. DAUNCEY

Reviews

A TEXTBOOK OF PRACTICAL ORGANIC CHEMISTRY, INCLUDING QUALITATIVE ANALYSIS. By A. I. VOGEL, D.Sc., D.I.C., F.R.I.C. Pp. xxiii + 1012. London: Longmans, Green & Co., Ltd. 1948. Price 42s.

It is not so long ago that a German chemist gratefully acknowledged a gift of a few ounces of that "rare" substance *cyclo*hexanol. The technical advances which have made this a "carload" chemical and thereby opened the way to various other products of commercial importance can be traced back to the laboratory bench. Some chemists have a natural aptitude for dealing with organic compounds, whether in preparative or qualitative work, but all need a practical foundation on which to build. No better basis could be given than that provided by this book, which opens with a brief theoretical review and then proceeds to give at some length (155 pp.) all the varied aspects of experimental technique. Distillation in particular is dealt with in some detail and no points are overlooked. It is good to see the warning that the method of mixed melting point is not infallible; a recent journal mentioned yet another pair of substances which show no depression of melting point on mixing. Then comes the main subject of the book, divided for convenience into groups, *e.g.*, aliphatic, aromatic and heterocyclic. These are subdivided as necessary and the scheme adopted is to open the sections with a brief general description and to follow this with the appropriate practical details. In order to allow variety, most of the preparations are accompanied by one or more closely related items. The sections conclude with notes on the reactions and methods of characterising the compounds of the group; these notes serve as an introduction to the qualitative side of the subject. Short chapters on more specialised topics follow. The preparations of organic reagents are not only useful exercises but also afford a considerable economy; it is indicative to see here 3 : 4 : 5-tri-iodobenzoyl chloride, only recently introduced to give solid derivatives of the β-alkoxyethyl alcohols. Several dyes and indicators are described, and among the physiologically active compounds may be found DDT and some of the barbiturates and of the "sulpha" drugs. A few experiments serve as a brief introduction to the subject of synthetic polymers. In all, over 600 preparations are described.

The chapter on qualitative analysis (67 pp.) is intended to be read in conjunction with the notes referred to above. The tables of compounds and their derivatives are quite extensive but, as the author comments, they are far from complete. There are many substances of relatively simple structure manufactured in substantial quantities and not used merely as intermediates in the works where they are made. Among those which come to mind in this connection are penta-erythritol, diphenylolpropane, propylene oxide, 4-nitro-*o*-anisidine, various chlorotoluidines, β-naphthoxyacetic acid and possibly in the future 5-nitro-2-*n*-propoxyaniline. The student of to-day may not meet them in the laboratory but he may later, as the chemist of to-morrow. It has long seemed that a practical extension of tabulations on these lines is very desirable, though admittedly it is not easy to decide just where to draw the line. A useful feature is the greater emphasis laid on the simpler physical properties of liquids generally. The large majority of the figures are sound, though one or two errors from the literature have been carried on; thus, the density and refractive index of *cyclo*hexylamine are 0·867 and 1·459 respectively (not the 0·819 and 1·437, apparently of Konovaloff, 1895) and the melting point of the benzoyl derivative of *m*-bromoaniline is 135° (not the 120° of Kottenhahn, 1891). As a small suggestion, it would be an advantage if the dash printed in absence of information were replaced by a dot; this would facilitate the manual insertion of figures either when they are retrieved from their present obscurity (*e.g.*, the *p*-toluenesulphonyl derivative of *cyclo*hexylamine, m.p. 87° C.) or when they are encountered in the future.

There are a few misprints, generally self-evident, but the direction to crystallise *as*-dimethylsuccinic acid from "cyanohydrochloric acid" looks odd. An appendix gives an introduction to the literature of organic chemistry and includes a description of the Beilstein system of classification and of the Dyson notation. Finally, safety precautions and first-aid measures are not neglected.

The narrative on the dust-cover refers to the subject becoming "alive"; there is, in fact, a strong feeling of vitality which pervades the book, springing no doubt from the author's own experience. For the combination of quantity with quality, the volume is not expensive, and subject to limitations imposed by paper quota it should prove a best-seller.

B. A. Ellis

Poisons: Their Isolation and Identification. By Frank Bamford, B.Sc. Second Edition revised by C. P. Stewart, M.Sc., Ph.D. Pp. 304. London: J. and A. Churchill, Ltd. 1947. Price 21s.

When this book first appeared, in 1940, it was welcomed as a practical handbook embodying a lifelong experience by the late Director of the Medico-Legal Laboratory at Cairo, Mr. F. Bamford. It bore on every page the stamp of personal and painstaking experience—and Cairo is a rare place for experience in chemical toxicology! Now a second edition has been prepared, by Dr. Stewart of the University of Edinburgh, which maintains the outstanding personal characteristics of Bamford's work, only adding notes and details of further tests, qualitative and quantitative, which have been found reliable. There is also a foreword by Professor Sydney Smith, who testifies to the practical and trustworthy character of the work.

Biochemists, toxicologists, public analysts and all who have occasion to undertake toxicological analyses know how often the standard textbooks on poisons or toxicology, while describing effects, symptoms, etc., are sketchy and lacking in precision on all-important practical details of analytical procedure. Bamford is pre-eminently a laboratory handbook: it gives just those practical points, hints and instructions that make all the difference in the clear, competent and convincing execution of a difficult and delicate branch of our science. No one who has to undertake this kind of work should be without it. Having said this perhaps one should indicate briefly what are the contents of the book. First there is a useful chapter on organisation, equipment and the preliminary dealing with specimens; this is important and should not be overlooked, especially by those who have not evolved a special system for themselves. Then follow chapters on classification of poisons from the chemical—not the pathological or clinical—aspect and on the detection of each of different classes of volatile poisons, metallic and non-metallic poisons, acids, alkalis, alkaloids, non-basic organic poisons and their isolation. Particularly to be noted is the author's systematic method for the identification of more than sixty alkaloids and for non-basic poisons such as barbiturates, sulphonal, polyhydric phenols, saponins, digitoxin, glycosides and purgative drugs. The careful following of the systematic scheme will be of particular value in those cases in which clinical or post-mortem indications are not available or do not afford a good clue to the identity of the poison. There is also a chapter on toxalbumins, poisonous plants, sulphonamides, fur dyes, gaseous poisons and drugs of addiction. Each chapter has been carefully corrected and brought up to date in detail by Dr. Stewart. The whole is a most valuable contribution, not merely to the bookshelf, but to the laboratory bench of the practical analyst.

H. E. Cox

Water Supply and Sewage. By Ernest W. Steel. Second Edition. Pp. xv + 666. New York and London: McGraw-Hill Company, Inc. 1947. Price 30s.

This book, by an American civil engineer, is written primarily for engineers to provide, in a limited space, the essentials of water supply and sewage practice. Since the book deals mainly with engineering it follows that it is not of paramount interest to the analyst, and, as it covers so wide a field, detailed information is not possible. Nevertheless, it is of great interest and value to chemists concerned with water supply and sewage treatment.

The first half of the book deals with water and there are sections on rainfall and run-off, underground water, self-purification of rivers, pumping machinery, the conveyance of water, sedimentation and coagulation, filtration and the quality of water.

Impurities normally found in water are mentioned: lead poisoning, iodide enrichment as a protection against goitre, and fluorides, all have paragraphs, while the coliform group and the coliform index are discussed. Chlorination has about 11 pages devoted to it and break-point chlorination is well described. Coagulation and filtration are dealt with in some 50 pages and among coagulants is mentioned the mixture of ferric sulphate and chloride known as "chlorinated copperas."

The section on sewage deals with the characteristics, treatment and disposal of sewage and storm sewage, and with sewer design, construction and maintenance. Of particular interest to the chemist is the chapter devoted to the characteristics of sewage, in which sampling, chemical tests and methods of treatment are discussed. Septic tanks, contact beds and trickling filters are described and the activated sludge process has a chapter to itself.

The book is excellently produced and well indexed and some chapters have mathematical problems (without solutions) appended to them. There are some 260 illustrations and numerous references, but as in many American books there is an almost complete disregard of English work. We are glad to know, however, that at "Manchester, England, the first spiral flow tank was constructed," and also to see that Queen Elizabeth (presumably of England) is mentioned, if only in a footnote.

W. G. Carey

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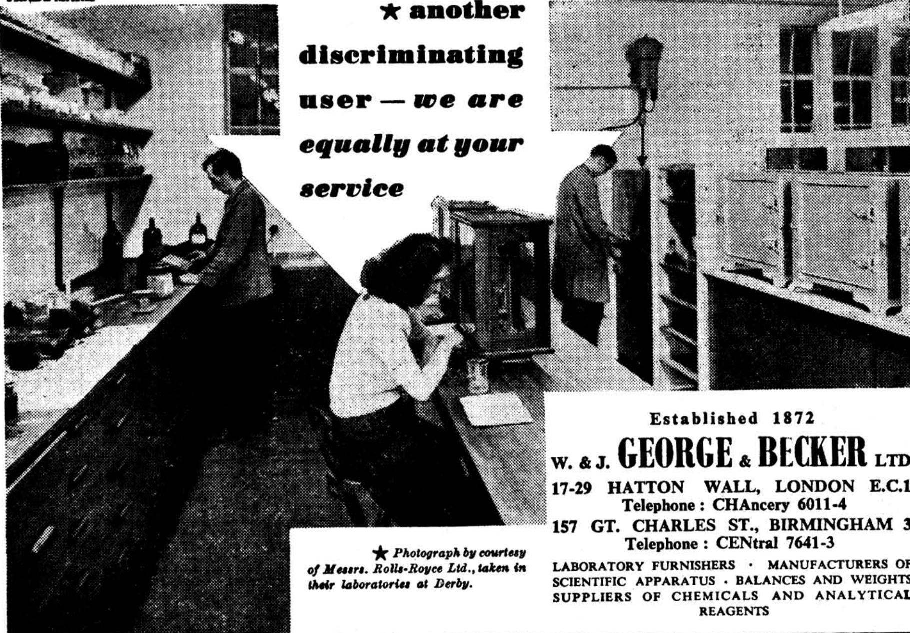

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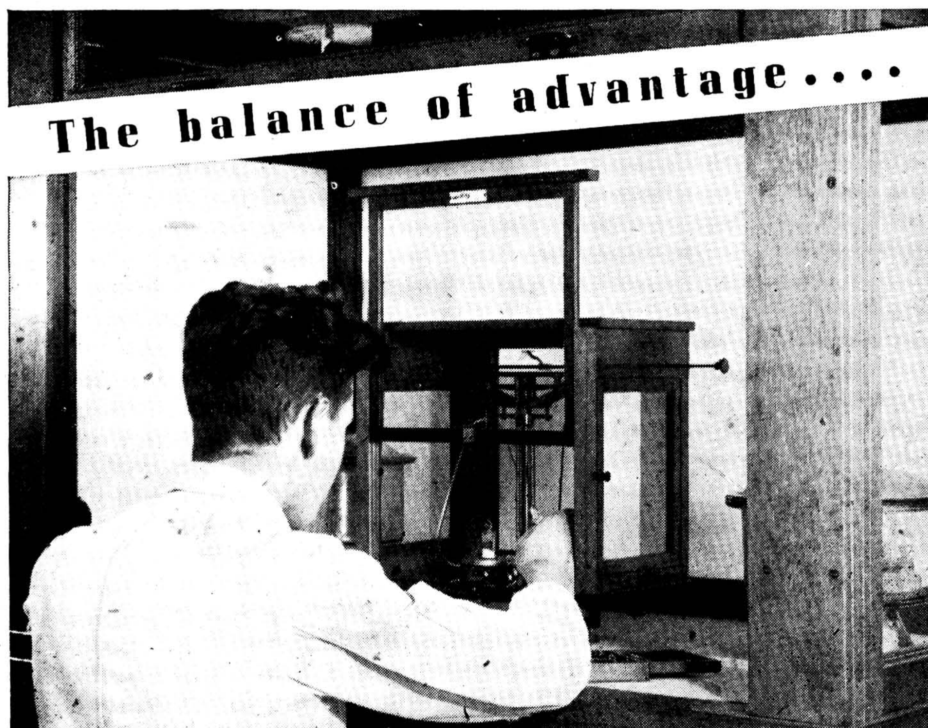

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