# THE ANALYST

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




*Published for the Society by*
W. HEFFER & SONS LTD., Cambridge, England

Volume 74 Price 3s. 6d. March, 1949
No. 876, Pages 153—222

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# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, February 2nd, in the Hall of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1, with the President, Mr. Lewis Eynon, in the chair. The following papers were presented and discussed:—"A Rapid and Accurate Volumetric Method for the Determination of Silica," by H. N. Wilson, F.R.I.C.; "The Analysis of Nylon and Related Polymers," by M. Clasper, A.R.I.C., and J. Haslam, M.Sc., F.R.I.C.; "The Quantitative Separation of Beryllium and Aluminum," by W. C. Coppins, M.Sc., A.R.I.C.

### NEW MEMBERS, ELECTED FEBRUARY 2ND, 1949

Alma Aranshew Christie, A.R.I.C.; Sam Collett, Ph.C., M.P.S.; Nathan Goldenberg, M.Sc. (Lond.), F.R.I.C.; Miss Audrey Mabel Jones; Oswald Hilton Keys, M.Sc. (N.Z.), A.R.I.C.; Norman Kirby, B.Sc. (Lond.), A.R.I.C.; Roy Albert Knight, B.Sc. (Lond.); James Ravantós, M.D.; Edward John Rolfe, B.Sc. (Lond.), A.R.I.C.; Edwin Weedon Smith, B.Sc. (Lond.), A.R.I.C.; George Alexander Stewart, B.Sc. (Lond.); Ronald Wellwood, B.Sc. (Q.U.B.).

### ANNUAL GENERAL MEETING

THE Annual General Meeting of the Society was held at 3.30 p.m. on Wednesday, March 9th, 1949, 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 1948 was presented by the Hon. Treasurer and approved, and the Auditors for 1949 were appointed. The Report of the Council for the year ending March, 1949 (see pp. 157–162) was presented by the Hon. Secretary and adopted. The following were elected Officers and Council for the coming year.

*President*—George Taylor, O.B.E., F.R.I.C.

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

*Vice-Presidents*—C. A. Adams, H. E. Cox, J. R. Nicholls and, *ex officio*, J. G. Sherratt (Chairman, North of England Section), and J. Sword (Chairman, Scottish Section).

*Hon. Treasurer*—J. H. Hamence.

*Hon. Secretary*—K. A. Williams.

*Other Members of Council*—N. L. Allport, R. C. Chirnside, J. F. Clark, D. C. Garratt, J. G. A. Griffiths, E. T. Illing, J. King, J. E. Page, C. J. Regan, F. A. Robinson, N. Strafford, A. M. Ward 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 the retiring President, Mr. Lewis Eynon, B.Sc., F.R.I.C., delivered his Presidential Address (see pp. 163–167).

### NEW MEMBERS, ELECTED MARCH 9TH, 1949

David George Allen, B.Sc. (Lond.), A.R.I.C.; Charles Gerald Barlow, M.A. (Cantab.), A.R.I.C.; John Brinley Bowen, B.Sc. (Wales), A.R.I.C., Dip. Ed. (Wales); Eugene Henry William James Burden, B.Sc. (Lond.), A.R.I.C.; Brian Crossley Christian, B.Sc., Ph.D. (Liv.), F.R.I.C.; Walter Charles Coppins, M.Sc. (Lond.), A.R.I.C.; Ernest Walter Courts, B.Sc. (Birm.), A.R.I.C.; Stuart Herbert Henry Davison, B.Sc. (Lond.), A.R.I.C.; George Drewery, B.Sc. (Lond.); Edward Alfred Elbury, F.R.I.C.; Robert Alexander Fraser, B.Sc. (Lond.),

A.R.I.C.; Jack Arthur Gilby, B.Sc. (Lond.), A.R.I.C.; Maurice Speakman Green, B.Sc. (Lond.), A.R.I.C.; Donald Hanson, B.Sc. (Lond.), F.R.I.C.; Laurence John Arthur Haywood; Leslie Frank Hewitt, B.Sc., Ph.D. (Lond.), F.R.I.C.; Leslie Charles Horace Holyday, B.Sc. (Lond.), A.R.I.C.; Robert Knox, A.R.I.C.; Charles Michael Lavender, B.A. (Oxon.); David Evan Lightfoot, B.Sc. (Birm.), A.R.I.C.; James Kidd McLellan, M.A., B.Sc. (Glas.), A.R.I.C.; Maurice Pereira Mendoza, B.Sc. (Lond.), A.R.C.S.; Francis Albert Oliver, B.Sc. (Lond.), A.R.I.C., A.C.T.C. (Birm.); Victor Harold Parks, F.RI.C.; Ernest Pedley, M.Sc. (Manc.), Ph.C., A.R.I.C.; William Patrick Pepper, M.Sc. (Liv.), F.R.I.C.; Giulio Richard Primavesi, B.A. (Cantab.); Norman Frank Rapps, B.Sc. (Lond.), A.R.I.C.; Edward Rogers, A.R.I.C.; Jack Palmer Savage, B.Sc. (Liv.), A.R.I.C.; Edward John Skerrett, A.R.I.C.; William Cuthbert John Smith, A.R.I.C.; William Arthur Stephens, A.R.I.C.; Alfred McMullon Taylor, B.Sc., Ph.D. (Lond.), F.R.I.C.; Edmund Alan Taylor, B.Sc. (Lond.); Ivan Waide, A.R.I.C.; William Ward; Richard John Whiffin, Ph.C.; Arthur William Williams.

## DEATHS

WE regret to record the deaths of

Charles Thomas Bennett.
John Henry Coste.
William Rhys Davies.
Thomas Wentworth Glass.
James Hendrick.
Rowland Williams.

# Anniversary Dinner

IN the evening of the day of the Annual General Meeting the Society held a Dinner at the Trocadero Restaurant, Piccadilly, London, W.1, to celebrate its seventy-fifth anniversary.

The members and guests, numbering 140, were received by the President, Mr. Lewis Eynon, and Mrs. Eynon. The President afterwards took the chair at the Dinner.

The guests of the Society and of the President included: Sir Robert Robinson, M.A., Hon.D.Sc., Hon.LL.D., Hon.D.Pharm., F.R.I.C., President of the Royal Society, and Lady Robinson; Professor E. K. Rideal, M.B.E., M.A., Ph.D., D.Sc., F.R.S., Chairman of the Chemical Council; Dr. G. Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.R.I.C., President of the Royal Institute of Chemistry, and Miss Roche Lynch; Dr. G. M. Bennett, C.B., B.A., Ph.D., M.A., Sc.D., F.R.I.C., Government Chemist; H. W. Cremer, Esq., C.B.E., M.Sc., M.I.Chem.E., M.Inst.F., F.R.I.C., President of the Institution of Chemical Engineers; Dr. R. P. Linstead, C.B.E., M.A., Ph.D., D.Sc., F.R.I.C., Vice-President of the Chemical Society; H. N. Linstead, Esq., M.P., O.B.E., Ph.C., Joint Secretary of the Pharmaceutical Society.

After the loyal toasts had been honoured, Professor E. K. Rideal proposed the toast of the Society. He said he was not sure why he had been chosen to do so when others more worthy were present, but perhaps it was because his father had at one time been president of the Society. He congratulated the Society on its age and growth. As Chairman of the Chemical Council he was particularly interested in this Society and the Faraday Society—the two most recent member bodies of the Chemical Council—and wondered which of them would reach the 2000 membership mark first.

It was not only the most important Society dealing with analytical chemistry, but was also a publishing body, whose journal, *The Analyst*, was unique in the chemical world. The activities of the Society covered and recorded a very wide range of specialist subjects, and for that reason, in days in which the specialist was becoming more than ever important, it was doing work of real value.

He had been astonished by the relatively small place that instruction in analysis occupied in the curricula of our leading chemical teaching institutions, and hoped that this condition of affairs would be speedily remedied.

The President, replying to the toast, thanked Professor Rideal—a distinguished son of a distinguished Past President of the Society—for his kind remarks on the work of the Society. The work was of an unobtrusive character, but none the less very important. Its complexity and many-sidedness came as a surprise to many people. He recalled a

conversation he had had with a friend to whom he mentioned that he had to attend a meeting of the Society. The friend remarked: "But I didn't know you were a Public Analyst." He replied that he was not; he was one of the Other Analytical Chemists. His friend asked: "But is analytical chemistry the only thing you have to talk about?" to which he replied that "the more we talk about analytical chemistry, the more there is to say." It was indeed true, for new problems were always arising, new methods were always being discovered and known methods improved. The progress and development of analytical chemistry had kept pace with the demands made upon it. Thanks to Public Analysts, gross adulteration of food had almost disappeared. But Public Analysts were now presented with much more difficult and subtle problems than formerly; for example, the determination of certain substances present only in traces had become of great importance. Here the work of the Other Analytical Chemists had helped very much. It was the business of many of the Other Analytical Chemists to see that accidental contamination of food did not occur. There was now hardly any industry that did not rely, for its proper control, directly or indirectly, on analytical chemists.

For many years past the Society had had Local Sections in the North of England and in Scotland. These had been a valuable means of fostering mutual help amongst members in those areas. Within the past few years, Subject Groups had also been formed, to promote the development of microchemical, physical and biological methods of analysis, respectively. Those Groups had more than justified their formation, familiarising the Society with new and sometimes rather astonishing techniques, and incidentally with some strange terminology. It might almost be said that one of the differences between the Sections and the Groups was that the Sections spoke the same language as the parent Society, but the Groups seemed to be developing their own languages. The Society was being faced with the necessity of developing a language that would be intelligible to all.

Sir Harry Jephcott, who proposed the toast of the Guests, coupled with the names of Mr. H. W. Cremer, C.B.E., President of the Institution of Chemical Engineers, and Mr. H. N. Linstead, M.P., Joint Secretary of the Pharmaceutical Society, said that the guests included many eminent persons, and the Society did most heartily welcome them. There were the President of the Royal Society, the President of the Royal Institute of Chemistry, the Chairman of the Chemical Council, and a Vice-President of the Chemical Society. It might seem presumptuous to comment on them, but he would like to mention one observation made to him when on a recent visit to the United States. He had remarked, on the subject of research, that this country had not at its disposal the large number of chemists available in the States, to which the reply was made: "No, but you have some very distinguished ones, in fact, the most distinguished organic chemist in the world, Sir Robert Robinson." As an industrial chemist himself, he was particularly pleased to welcome the President of the Institution of Chemical Engineers. He could not too highly praise the contribution that had been made by that body and its President in helping to raise the productive output of this country. In welcoming Mr. Hugh Linstead, Secretary of the Pharmaceutical Society, he experienced a personal gratification, not unmixed with hopes for the future, at the recollection of most excellent lunches that for several years past he had been able to attend as an Honorary Auditor of that Society. He greatly admired the skill, tact, and diplomacy with which Mr. Linstead was able to conduct his Council into what he considered the right path of life. On a recent occasion the same tact appeared to have induced a very large number of people to enter the polling booth and put a cross in the right place. He would like to refer also to the Government Chemist, who represented the scientific officers of his own and several other Departments. His position was unique and represented a magnificent example of co-operation between Government Departments, industry and other organisations. It was significant that many of his scientific officers were well-known and very highly respected members of the Society. Sir Harry ended by extending a particular welcome to the ladies, who had added so greatly to the pleasure of the evening.

Mr. H. W. Cremer, President of the Institution of Civil Engineers, said he greatly appreciated the honour done to his Institution and to himself, in being selected, with Mr. Hugh Linstead, to reply on behalf of the guests. He did so with some embarrassment in the presence of some much more distinguished chemists, but he was relieved to feel that he was not regarded as a complete outcast from the chemical profession. He was, in fact, brought up in the orthodox chemical faith and passed the examinations of the Institute of Chemistry, which had not then its Royal status. He hoped he could say that since then

his stay in a far country had not been spent in riotous living—war time abroad could hardly be so described. All the guests had greatly enjoyed the feast that had been prepared for them and the charming company, and they drank to the prosperity of the Society and its influence in the time to come.

Mr. H. N. Linstead, M.P., Joint Secretary of the Pharmaceutical Society, said he was pleased to have the honour of replying on behalf of the guests and the opportunity of thanking Sir Harry Jephcott and the President. He understood that the membership of the Society had now reached 1500, and wondered if as the cunning of mankind increased there became a greater need of Public Analysts. Many of the members of the Society were Public Analysts and many others were more or less associated with Government Departments, but he hoped there would always be independent analysts to whom the ordinary citizen could feel that he could turn for independent advice. He would like to congratulate Dr. Nicholls, the Chairman of the Publication Committee, and the Editors on the high standard maintained by *The Analyst*. It was one of the journals that came to his desk every month; he could not say that he read it, but he took his hat off to it. He would like also to pay a tribute to the late Dr. C. A. Mitchell, who made *The Analyst* part of his life's work. On the subject of published papers he wondered if it was possible to change the very common practice in which the authors at the end of a paper thanked their employers A and B for permission to publish. It seemed to him that the direction of the thanks would more appropriately be reversed. He thought that in one respect analysts were in a much more enviable position than many other practitioners; they had definite problems to solve and had the satisfaction of completing them and knowing they were finished. How different from the work of those in Parliament who, however carefully they analysed their problems, never seemed to get a final solution!

The President then proposed the toast of the President Elect, Mr. George Taylor. He had known him many years, having been a fellow student with him at college. It certainly did not then occur to either that they would be successive Presidents of the same Society. Mr. Taylor had the advantage of working for very many years with the late Dr. Bernard Dyer, and it would hardly be possible to imagine a better school for the training of a President. With that recommendation and the knowledge of Mr. Taylor's personal qualities, they could look forward with confidence to the maintenance of the prestige and welfare of the Society.

Mr. Taylor expressed his thanks to the President for his kind remarks and to the Society for the cordial way in which the toast had been received.

At the request of the President, Sir Robert Robinson made some concluding remarks. He expressed his pleasure at being made an Honorary Member of the Society. Analytical chemistry was a subject near to his heart. Referring to the projected International Conference on Analytical Chemistry, which it was proposed to hold in London in 1952, he sincerely hoped that it would be arranged and receive the necessary support from the powers that be. He was very interested in the subject of tuition in analytical chemistry at our universities, and was glad to know that it was one of the main topics dealt with in the President's address that afternoon. From the educative point of view he thought the fundamental principles of analysis—the use of the balance and the ordinary processes of analytical chemistry—were essential. The small amount of analytical work usually done by the student was to be deplored, and more analysis should be insisted on in the first years of a student's course. But he did not attach much educative value to the more specialised and complex apparatus, *e.g.*, apparatus that would do almost everything except write a paper.

# Annual Report of Council: March, 1949

THE roll of the Society numbers 1496, an increase over the membership of a year ago of 107.

HONOURS—The Council is glad to record that during the year the C.B. has been awarded to Dr. G. M. Bennett, the C.B.E. to Professor Alexander Findlay, Mr. B. C. Aston and Mr. C. A. Adams, the O.B.E. to Mr. George Taylor and the M.B.E. to Mr. G. W. Baker, Mr. R. C. Frederick, Mr. W. A. Godby and Dr. G. V. James. Mr. S. E. Melling has been awarded the honorary degree of M.Sc. by the University of Manchester. The Council offers its congratulations to the recipients of these honours.

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

| | | |
|---|---|---|
| J. E. Byles | T. W. Glass | A. H. Mitchell |
| U. A. Coates | E. Halliwell | G. Rudd Thompson |
| J. H. Coste | C. A. Hill | S. R. Trotman |
| J. Davies | W. G. Leach | Rowland Williams |

Byles, who died in his 59th year, was educated at Banham Grammar School and the University of Manchester. He entered the Government Laboratory, working from 1911 to 1926 mainly on the analysis of foods, fertilisers and feeding stuffs. For the next six years he was stationed at the Custom House, Liverpool, and for a further six years at the Custom House, London. He then came back to the Government Laboratory, where he was a Senior Principal Scientific Officer at the time of his death.

Coates joined the Society in 1920 and was for many years Joint Honorary Auditor of the North of England Section.

Coste was trained at Finsbury Technical College in 1888–91 and was a member of the staff of Augustus Voelcker from 1891 to 1894. He then joined the Chemical and Gas Testing Department of the London County Council, and when the main part of the work was transferred in 1912 to the Public Health Department, he became what is now described as the Chemist-in-Chief of the Chemical Branch. He retired in 1936. He became a Fellow of the Institute of Chemistry in 1896 and was a founder Fellow of the Institute of Physics. He served on the Councils of the Institute of Chemistry and the Society, and was for some years Honorary Secretary of the London Section of the Society of Chemical Industry. He was one of the first members of the Atmospheric Pollution Research Committee, now a constituent Committee of the Fuel Research Board, D.S.I.R. He joined the Society in 1907.

Davies was the last of the original members associated with A. Norman Tate who conducted a large analytical practice and a School of Chemistry in Liverpool. On the death of his colleagues he took over the firm of A. Norman Tate & Co. Unassuming and simple in his tastes, he had a profound knowledge of the chemistry and technology of oils and fats. In his youth he was an ardent cricketer, and all his life he was devoted to photography. He joined the Society in 1934.

Glass joined the Society in 1887 and served on the Council in 1917–18. He was for many years associated in practice with Mr. Edward Hinks.

Halliwell died in his 75th year. He was trained at the Yorkshire College, Leeds, becoming an Associate of the Royal Institute of Chemistry in 1894. He was then appointed an assistant to Thomas Fairley, Public Analyst for Leeds and the North Riding of Yorkshire. In 1897 he took charge of the laboratory of the West Riding Rivers Board. He became Chief Inspector and Chemical Adviser to the Ribble Joint Committee in 1902, retiring from that position in 1938. He joined the Society in 1897.

Hill joined the Society in 1906 and died in his 75th year. He was educated at Winchester, at the Pharmaceutical Society, at St. Thomas's Hospital and at King's College, London. From 1895 to 1896 he was analyst to A. S. Hill & Son and then directed the laboratories of Davy Hill & Son, Yate & Hicks, in Southwark. He was the prime mover in forming, in 1909, the British Drug Houses Ltd., becoming managing director and later chairman. He was Master of the Salters' Company and played an important part in the formation of the Salters' Institute of Industrial Chemistry. He served twice on the Council of the Royal Institute of Chemistry; he was a Vice-President of the Society in 1917–18.

Leach joined the Society in 1946. He was educated at John Ruskin School and Selhurst Grammar School and at Birkbeck College. After a period with British Drug Houses Ltd. he spent some years with the Printing and Allied Trades Research Association. From 1940 he

was research chemist and analyst to British Waxed Wrappings Ltd. until 1944, when he moved to a similar position with T. Hubbuck & Son Ltd.

Mitchell died in his 90th year. He was educated at Longwood Grammar School, Huddersfield, and St. John's College, York, and received scientific training at Birkbeck College and the Royal College of Science, London. After holding the position of Science Master at Camberwell Grammar School he went to Tiverton Technical School as head of the Chemical Department. In 1895 he became Borough Analyst for Tiverton. He joined the Society in 1901.

Rudd Thompson became a Fellow of the Royal Institute of Chemistry in 1908 and had been a member of the Society since 1893. He died in his 80th year. He was President of the Society in 1924–5 during the Jubilee year, and he was the last President whose term of office was described in detail by Dr. Bernard Dyer in "*Fifty Years of the Society of Public Analysts.*"

Trotman was 79 at the time of his death. He was Exhibitioner and Prizeman of St. John's College, Cambridge, and graduated as M.A. of the University of Cambridge in 1892. He became Science Master at Nottingham and in 1896 Public Analyst for the City of Nottingham, retiring from this post in 1937. He was Lecturer in Applied Chemistry at University College, Nottingham, from 1909 to 1938. He joined the Society in 1901.

Rowland Williams died in his 88th year. He assisted A. H. Allen in the preparation of Volume II of *Commercial Organic Analysis*. In 1885 he started a consulting practice in Manchester. He later became chief chemist of James Williamson & Son Ltd., of Lancaster, manufacturers of linoleum, leather cloth, etc. He became a member of the Society in 1887 and was elected a Fellow of the Institute of Chemistry in the same year. He was a Gas Identification Officer for Lunesdale during the recent war, in spite of his advanced age.

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

"The Freezing Point of Bulk Milk." By F. J. Macdonald.

"The Determination of Cerium in Cast Iron." By W. Westwood, B.Sc., and A. Mayer.

"The Determination of Linoleic Acid in Edible Fats." By W. J. Stainsby, Ph.D., F.R.I.C.

"The Determination of *p,p'*—DDT in Commercial Samples." By A. E. Martin, B.Sc., F.R.I.C., and R. L. Wain, M.Sc., Ph.D., F.R.I.C.

"The Composition of Concentrated Tomato Puree and the Estimation of the Tomato Content of Tomato Ketchup." By J. C. Morpeth, B.Sc., A.R.I.C.

"Ether Peroxide as a Possible Source of Error in the Röse-Gottlieb Butter-fat Test." By M. M. Muers, Ph.D., F.R.I.C., and Miss M. A. House, B.Sc.

"The Standardisation of Hortvet Thermometers." By R. Aschaffenburg, Ph.D., and J. A. Hall, A.R.C.S., B.Sc., D.I.C.

"A Micro Method for the Determination of Unsaturation." By Miss W. M. Phillips and W. C. Wake, M.Sc., A.R.I.C.

"The Determination of Small Amounts of Hydroquinone in Styrene." By S. M. A. Whettem, B.Sc., F.R.I.C.

"A Method for Determining the Tin Content of Tungsten High Speed Tool Steel." By B. Bagshawe, A.Met., F.I.M., and E. Dyke, A.Met., A.I.M.

JOINT MEETING—The December Meeting was, as usual, a Joint Meeting with the Food Group of the Society of Chemical Industry. The subject was "Food Standards and Labelling," with the following special contributions:—

"Introduction." By C. A. Adams, C.B.E., B.Sc., F.R.I.C.

"The Viewpoint of the Manufacturer." By L. H. Lampitt, D.Sc., F.R.I.C., M.I.Chem.E.

"The Viewpoint of the Public Analyst." By H. E. Monk, B.Sc., F.R.I.C.

Mr. Monk, in making his contribution to the meeting, deputised at short notice for Mr. Stanley Dixon who was unable through indisposition to take part in the meeting.

NORTH OF ENGLAND SECTION—There have been five meetings during the year including a Joint Meeting with the Physical Methods Group and, for the first time since 1939, a Summer Meeting was held.

The following papers have been read and discussed:—

"The Government White Paper on the Post-War Loaf." By D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.

"The Examination and Analysis of Shellac." By A. Wright.
"Methods of Water Analysis." By W. Gordon Carey, F.R.I.C.
"Chemistry in the Kitchen." By E. B. Hughes, D.Sc., F.R.I.C.
"Analysis of Rare Earth Oxides by means of Emission Spectra." By D. M. Smith, B.Sc., D.I.C., F.Inst.P., and G. M. Wiggins.
"Determination of the Rare Earths, using the intermittent Arc." By J. A. C. McClelland, B.Sc., Ph.D., A.R.I.C.
"The Chromatographic Estimation of Vitamin A in Whale Liver Oil." By N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson and J. P. Savage, B.Sc., A.R.I.C.
"The Determination of the Meat Content of Sausages." By R. W. Sutton, B.Sc., F.R.I.C., and J. Markland, B.Sc., F.R.I.C.

SCOTTISH SECTION—In addition to the Annual General Meeting, two ordinary meetings were held during the year.

One meeting in Edinburgh took the form of an Exhibition of Films. The subjects of the films included the Cathode Ray Oscillograph, Crystals, Colour, Atomic Fission, Medicinal Gases and Colloidal Chemistry. The show of films was followed by a Dinner at which the members of the Section joined with the members of the Association of Public Analysts of Scotland.

The second meeting was held in Glasgow and the following paper was presented and discussed:—

"The Determination of Vitamin $B_1$, Riboflavine and Nicotinic Acid by Microbiological and Chemical Methods." By James Cassidy.

The number of members of the Section is now 74, an increase of eight since last year.

MICROCHEMISTRY GROUP—Three meetings have been held during 1948, in London, Aberdeen and Leeds respectively. The Aberdeen meeting was held jointly with the Local Sections of other Chemical Societies. The Leeds meeting was held with the Leeds Area Section of the Royal Institute of Chemistry and Leeds University Chemical Society.

The following papers have been read:—

"The Microchemical Aspects of Electrical Conductivity." By J. T. Stock, M.Sc., F.R.I.C.
"A New Micro Blowpipe for the Manipulation of Capillaries." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"A Diaphragm Pump for Air and Other Gases." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"A Melting Point Indicating Device." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"A Transmitting Manometer for Micro Oxygen Uptake Experiments." By J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill, A.R.I.C.
"Ultra Micro Methods." By Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.
"Simultaneous Concentration of Trace Elements with Organic Precipitants." By R. L. Mitchell, B.Sc., Ph.D., A.R.I.C.
"Trace Determinations by Means of the Polarograph." By G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P.
"Micro Diffusion Analysis." By T. G. Brady.
"The Microscope as a Chemical Tool." By Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C.
"General Account of Microchemical Methods in Forensic Investigations." By J. B. Firth, M.Sc., D.Sc., M.I.Chem.E., F.R.I.C.
"Microchemical Methods in Forensic Toxicology." By G. E. Turfitt.
"Rapid Colorimetric Methods for the Detection and Estimation of Alkaloids and Related Compounds." By E. Pedley.

The number of Group members is now 258, an increase of 48 since the last report.
The Committee has met three times during the year.

The University Grants Committee, having intimated that it was considering strengthening the teaching of microchemistry, invited the views of the Committee. A small Sub-Committee consisting of the Chairman, Professor Briscoe and Mr. Belcher prepared a memorandum on the subject which has been submitted to the U.G.C.

Steps were taken to form a Micro-analytical Methods Panel, to initiate investigations into microchemical methods, but the Committee found themselves unable to have a free hand in the organisation of such a panel and the matter was abandoned.

The report on "The Progress of Microchemistry in Germany" has now been published as "B.I.O.S. Final Report No. 1606" and is available to the general public.

Owing to pressure of work and also for health reasons the Hon. Secretary, Mr. Ronald Belcher, had to relinquish the office about July, and Mr. D. F. Phillips was appointed as Acting Hon. Secretary until the next Annual General Meeting.

PHYSICAL METHODS GROUP—During the past year the Physical Methods Group has held two meetings in London and one each in Birmingham, Leeds and Liverpool. One London meeting and the Liverpool meeting were held jointly with the Biological Methods Group and the North of England Section respectively. The Leeds meeting had been organised by the Polarographic Discussion Panel. The meetings had an average attendance of over 80 members and visitors. The following papers were read at meetings of the Group:—

Electron Microscope Meeting in London on November 25th, 1947.

"Electron Microscopy." By B. S. Cooper, B.Sc., F.Inst.P.

Penicillin Assay Meeting in London on January 29th, 1948.

"Introductory Survey of Physical and Chemical Methods." By E. Lester Smith, D.Sc., F.R.I.C.

"A Critical Review of some Proposed Methods for the Determination of Individual Penicillins." By W. R. Boon, B.Sc., Ph.D., F.R.I.C.

"The Determination of Penicillin by Alkaline Hydrolysis." By Stella J. Patterson, B.Sc., A.R.I.C., and W. B. Emery, B.Sc., A.R.I.C.

"The Spectroscopic Estimation of Penicillin." By G. H. Twigg, B.Sc., Ph.D.

"Introductory Survey of Biological Methods." By N. G. Heatley, M.A., Ph.D.

"Serial dilution Method" and "Differential Assay by Charcoal Adsorption." By C. G. Pope.

"The Microbiological Assay of Penicillin by the Turbidimetric Method using *Staphylococcus aureus*." By C. R. Bond, M.Sc.Tech., F.R.I.C., and O. L. Davies, M.Sc., Ph.D.

Tracer Isotope Meeting at Birmingham on April 2nd, 1948.

"Measurement of $\beta$-activity." By A. G. Maddock.

"Measurement of Radioactive Isotopes." By F. E. Whitmore.

"The Mass Spectrometer." By E. R. S. Winter, B.Sc., Ph.D., A.R.C.S., D.I.C., A.R.I.C.

"Determination of Abundance Ratios of Non-radioactive Isotopes." By E. R. Roberts.

"Tracers in Biochemical Investigations." By W. J. Arrol.

Polaragraphic Meeting at Leeds on April 9th, 1948.

"Polarography in Germany." By G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P.

"The Polarographic analysis of Light Alloys and Metals." By W. Stross, M.D., F.R.I.C.

"The Polarography of Anions." By W. Furness, B.Sc., F.R.I.C.

Spectroscopy Meeting at Liverpool on October 2nd, 1948.

"Analysis of Rare Earth Oxides by means of Emission Spectra." By D. M. Smith, B.Sc., A.R.C.S., D.I.C., F.Inst.P., and G. M. Wiggins.

"Determination of the Rare Earths using the Intermittent Arc." By J. A. C. McClelland, B.Sc., Ph.D., F.R.I.C.

"The Chromatographic Estimation of Vitamin A in Whale Liver Oil." By N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson and J. P. Savage, B.Sc., A.R.I.C.

The Polarographic Discussion Panel held, in addition to the Leeds meeting, which was organised as a Group meeting, ordinary meetings at Norwood Technical College on December 12th, 1947, and at Imperial College on October 29th, 1948. The discussion at these meetings was opened by Mr. F. L. Steghart, Dr. F. L. Warren, Dr. S. G. Tudor Jones and Dr. J. E. Page. Mr. J. T. Stock, who has resigned from the office of Hon. Secretary of the Panel, must be thanked for the way in which he has organised the Panel during the last two years. Dr. W. Cule Davies is the Chairman of the Panel which now has 55 members.

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 250, an increase of 67 since the last Annual Report.

BIOLOGICAL METHODS GROUP—The Group has held four meetings during the year. After the Annual General Meeting on December 16th, 1947, an ordinary meeting was held at which the following papers were read:—

"A Modified Method for the Microbiological Assay of Tryptophan, Methionine, Cystine and Tyrosine." By E. C. Barton-Wright, D.Sc., F.R.I.C., and N. S. Curtis.

"The Use of *Neurospora Crassa*, mutant 9185, for the Assay of Aneurine." By J. S. Harrison, B.Sc., M.Sc., and E. J. Miller.

"A Note on the Cup Method of Microbiological Assay and its Limitations." By W. F. J. Cuthbertson, B.Sc., Ph.D., F.R.I.C.

A Joint Meeting was held with the Physical Methods Group on January 29th, 1948, the subject being—

"Methods of Penicillin Assay—their Purpose, Scope and Validity."

Biological methods of assay were dealt with in papers by N. G. Heatley, M.A., Ph.D., C. G. Pope, and C. R. Bond, M.Sc.Tech., F.R.I.C., and O. L. Davies. Dr. A. A. Miles summed up at the end of a most vigorous and stimulating discussion. The meeting attracted many visitors, and undoubtedly served a most useful purpose in enabling the merits and applicability of physical and chemical methods to be assessed in relation to biological methods. The proceedings at the meeting appeared in full in *The Analyst* and have since been published separately in booklet form.

Dr. A. A. Miles, who is Head of the Department of Biological Standards at the National Institute for Medical Research, read a paper on Biological Standards at a meeting held on May 11th. A symposium was held on October 21st on—

"The Assay of Curare and Curarimimetic Substances."

Papers were read by C. A. Mogey, M.B., B.Ch., B.A.O., and J. W. Trevan, M.B., B.S., B.Sc., F.R.C.P.; G. B. West, Ph.D., B.Pharm.; J. Raventós, M.D.; H. O. J. Collier, B.A., Ph.D.; F. C. McIntosh, M.A., Ph.D.

The membership of the Group is now 135, 37 new members having been added during the year.

Dr. E. C. Wood, who has been Hon. Secretary of the Group since its inception, has resigned from this post, and Mr. S. A. Price has succeeded him.

PUBLIC ANALYSTS AND OFFICIAL AGRICULTURAL ANALYSTS COMMITTEE—The Committee met on three occasions during 1948. Amongst subjects discussed were how the Society could assist with mutual advantage the Consulting Pathological Group of the British Medical Association on the chemical side of pathological practice; examination of commercial petrol; and matters dealing with the welfare of Public Analysts in general.

ANALYTICAL METHODS COMMITTEE—The past year has shown the results of the considerable activities and progress of work of the Committee and its Sub-Committees. Five Reports from the Committee have been published during the year:—

The Evaluation of Powdered Tragacanth (*Analyst*, 1948, p. 368).
The Assay of Yohimba (*Analyst*, 1948, p. 309).
The Assay of Jaborandi (*Analyst*, 1948, p. 311).
The Assay of Ephedra and of Ephedrine in Nasal Sprays (*Analyst*, 1948, p. 312).
Determination of Traces of Zinc in Foodstuffs (*Analyst*, 1948, p. 304).

Sub-Committees have been active and progress reports indicate that a further report from the Tragacanth Sub-Committee is imminent and that the Standard Methods Sub-Committee has compiled a considerable bibliography. A Liaison Committee has been appointed to consider all cases in which the Society is invited to co-operate in the formulation of Standards and Standard Methods of Analysis. The Freezing Point of Milk Sub-Committee has been reconstituted and new Sub-Committees have been formed to investigate Standard Methods for Meat Extracts and for Soapless Detergents.

HON. TREASURER'S REPORT—The financial position of the Society continues to receive the closest attention. The Council set up, during the year, a small Sub-Committee to make a special study of the finances of the Society in view of the great increase in the cost of publication of *The Analyst* and of administrative expenses, and the preliminary recommendations of the Sub-Committee are now before the Council.

THE ANALYST—Some recovery from the effects of the fuel crisis of 1947 on the paper supplies for *The Analyst* is reflected in the increased size of the journal for 1948, which totals 704 pages, compared with 588 in 1947 and 600 in 1946. The numbers of original papers and notes published in 1948 were 78 and 33 respectively, compared with 61 and 19 in 1947, and the abstracts 470 compared with 304. In spite of this increased size the paper restriction to which the journal was subject led this year to a serious accumulation of papers and abstracts awaiting publication, and near the end of the year application was made for increased paper allocation. This has been granted and the immediate problem of how best to cope with the enlargement of the journal is receiving the attention of the Council.

Following the precedent of the cloth-bound reprints of Symposia on Polarography, Chromatography and Spectroscopic Analysis issued in 1946 (Annual Report, April, 1947), a similar reprint of the papers on "Methods of Penicillin Assay: their Purpose, Scope and Validity," held at a Joint Meeting of the Physical Methods Group and the Biological Methods Group, in January, 1948, has been published.

SPECIAL COMMITTEE ON TRAINING IN ANALYSIS—The Special Committee has drawn up a Memorandum during the year, which has been submitted to the Royal Society. It is hoped that the Memorandum will prove useful in drawing attention to the immediate need for much greater facilities for the training of students at Universities in Analytical Chemistry and will pave the way for prompt improvements.

SPECIAL COMMITTEE ON THE NAME OF THE SOCIETY, THE MEMORANDUM AND ARTICLES OF ASSOCIATION, AND RELEVANT MATTERS—The Special Committee appointed by the Council to investigate these matters has obtained the opinion of the members on the subject of the title of the Society. The information thus gained has been carefully studied by the Council, and, as a result of their study, the Council expects to make recommendations to an Extraordinary General Meeting of the Society in the near future.

BIENNIAL LECTURE OF THE SOCIETY—Council has agreed unanimously that the Biennial Lecture given after alternate Annual General Meetings of the Society should be named: "The Bernard Dyer Memorial Lecture."

CHEMICAL COUNCIL—A grant of £500 has been made to the Society by the Chemical Council from funds accumulated during the war to assist in bringing the publication of original papers up to date.

INTERNATIONAL CONGRESS ON ANALYTICAL CHEMISTRY AT UTRECHT—The Society was well represented at this Congress, held last June.

PROPOSED INTERNATIONAL CONFERENCE ON ANALYTICAL CHEMISTRY—The success that attended the International Congress held at Utrecht has led to exploratory talks being held in London to investigate the possibility of holding a Conference in England in 1952. A General Committee, under the Chairmanship of Sir Robert Robinson, and an Executive Committee, with Mr. R. C. Chirnside as Honorary Secretary, were set up at a representative meeting held in the Rooms of the Royal Society to make preliminary arrangements for the Congress or Conference. The Society has made a grant for the preliminary expenses.

PLACE OF ORDINARY MEETINGS OF THE SOCIETY—For many years the Society has held its Ordinary Meetings in the Rooms of the Chemical Society in Burlington House, Piccadilly. The expansion of the Chemical Society has rendered it necessary for them to terminate this association at least temporarily. Ordinary Meetings of the Society have been held during the year at Gas Industry House, Hyde Park Corner, and at Manson House, Portland Place, London.

SEWAGE AND SEWAGE EFFLUENTS—The Society has appointed Dr. Hamence as representative on a technical committee of the Ministry of Health set up to consider necessary revisions in methods of chemical analysis in this field.

BRITISH STANDARDS INSTITUTION—Dr. G. W. Monier-Williams has been nominated as the Society's representative on the Chemical Divisional Council of the B.S.I. in place of Dr. E. B. Hughes, whose term of office has been completed.

BRITISH NATIONAL COMMITTEE ON CHEMISTRY OF THE ROYAL SOCIETY—Dr. A. M. Ward has been appointed the representative of the Society in place of Mr. F. W. F. Arnaud, whose term of office has been completed.

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

# Address of the Retiring President

LEWIS EYNON, B.Sc., F.R.I.C.

*(Delivered after the Annual General Meeting, March 9th, 1949)*

BEFORE dealing with the main subject of my address, I propose to make a short survey of the recent progress and activities of the Society.

The best criterion of the prosperity of a society is, I think, the rate of growth of membership. Taking the membership of our Society, in round numbers, at ten-year intervals for the last forty years: 350 in 1909, 450 in 1919, 600 in 1929, 850 in 1939 and 1500 in the present year, we are justified in feeling satisfied with the present and hopeful for the future. The membership has increased more than fourfold since 1909 and the increase has been greater in the past ten years than in the preceding thirty.

The strength of a society, however, depends not only on its numbers but on the spirit and activity of its individual members, and here again we have good reason for satisfaction. As compared with their London brethren, the country members of a society with its headquarters in London are at a considerable disadvantage, since for them attendance at meetings involves much expense and sacrifice of time. In 1925 members of the Society resident in the North of England met that difficulty by forming the North of England Section, with a Committee of Management, and in 1936 the Scottish members followed suit with the Scottish Section. The two Sections have flourished greatly and have added and do add to the strength and influence of the parent Society. I have been privileged to attend four Summer Meetings of the North of England Section and these gatherings remain as some of my pleasantest memories. A still more recent manifestation of the spirit and activity of our members has been the formation of three Groups for special subjects, called into existence by the rapid development of certain branches of analysis and covering this development very completely. I shall have occasion to refer to the Groups later.

Despite the upheavals of two World Wars and the consequent great increase in the cost of living, there is one commodity, and I can think of no other, the price of which remains the same as it was in 1914, and that is membership of the Society; we are legitimately proud of such a record. How has it been achieved?

Firstly, we owe a debt of gratitude to a succession of Honorary Treasurers whose careful husbandry has done so much to maintain our financial stability. Of our present Honorary Treasurer, Mr. G. Taylor, and of his immediate predecessor, Dr. E. B. Hughes, I can speak from personal experience, since I have served with them for years on the Finance Committee and on the Council, and know how jealously they have guarded the finances of the Society.

Secondly, we are indebted to a succession of Editors of *The Analyst*, under whose control our journal has not only acquired a very high prestige, but has become an asset of great value to the Society. Here again I can speak from personal experience of the work of our present Editor, Mr. J. H. Lane, and his immediate predecessor, the late Dr. C. A. Mitchell, since I have served with them for years on the Publication Committee. The editorial work has increased very much during the past few years, and this has necessitated the appointment of an Associate Editor, Mr. L. S. Theobald, and an Assistant Editor, Mr. F. L. Okell, and our thanks are due to them for the able assistance which they render to Mr. Lane.

I have one further observation to make with reference to our membership subscription. Although it is very gratifying that the subscription should have remained the same through the vicissitudes of the past thirty-five years, the welfare of the Society is our primary object and if, to ensure that, it became necessary to raise the subscription, I am sure that the Council would not hesitate to ask you to sanction that step.

I am glad to take this opportunity, the last I shall have, of thanking the Honorary Secretary, Dr. K. A. Williams, for his help to me during my term as President. Dr. Williams came new to his office two years ago and has carried out his duties as "to the manner born."

For some years past it has been our custom, in alternate years, to invite a distinguished chemist to give a lecture following the Annual General Meeting. The Council has decided that in future this biennial lecture shall be known as "The Bernard Dyer Memorial Lecture" in honour of the late Dr. Bernard Dyer who, through so many years, served the Society so

well. It will be a most appropriate form of posthumous recognition, and will keep the memory of Dr. Dyer green long after those who knew him have passed away.

I now come to the main theme of my address—the fundamental importance of analysis to the progress of the science of chemistry, and the necessity of giving a prominent place to analysis in the training of the chemical student.

The same subject has been dealt with by two former Presidents of the Society—A. C. Chapman in 1915 and 1916[1] and E. R. Bolton in 1928[2]—and both deplored the inadequacy of training in analytical chemistry. The subsequent years, however, have shown little advance in the status of analysis as a subject of instruction in our universities and technical colleges; indeed, the position has, if anything, worsened owing to the increasing and admittedly irresistible claims of other branches of the science on the student's time. No apology, therefore, is needed for again pleading the claim of analytical chemistry to be regarded as the basis of the science and of prime importance in chemical education.

That this claim is fully justified is abundantly proved by the history of chemistry from the time of Lavoisier who, by quantitative experimental work on combustion, overthrew the Phlogiston Theory and transformed chemistry from an art into a science. The etymologist might reasonably object that Lavoisier's work on the conversion of elements into oxides is not analysis but synthesis; to the chemist, of course, the word "analysis" has so changed its meaning as to include its opposite.

A secondary effect of Lavoisier's work—hardly less important than the overthrow of the Phlogiston Theory—was the conviction that it gave to the then rising generation of chemists of the importance of exact quantitative work in furthering the progress of the science and the following period of sixty or seventy years saw an enormous development in the scope and accuracy of chemical analysis, a development which was of inestimable value both to theory and practice. It was during this period that methods of analysis were devised and perfected—and many of them by leaders of chemical thought—which are used to-day with little or no modification. It was during this period that the atomic weights of most of the elements were determined to a high degree of accuracy, the laborious work of many chemists on which the brilliant generalisation of the Periodic Law was built. It was during this period that Stas determined the atomic weights of a few of the elements, to a degree of accuracy that has not been surpassed, for the purpose of testing the validity of Prout's hypothesis that all elements are built up from hydrogen. The work of Stas led to the rejection of this hypothesis, and although more recent work on the nature of atomic structure has shown that the hypothesis has a basis of truth, it is, I think, quite certain that if the hypothesis had been accepted in its original, crude form, the development of atomic chemistry would have been hindered rather than helped. How many theories have been strangled at birth by the results of quantitative work? Most of us, I suppose, have found quantitative experiment to be a very salutary corrective of theoretical speculations.

Indeed, the first half of the nineteenth century might be called the Golden Age of analytical chemistry. As pointed out by Chapman it was followed by a period of comparative neglect which he justly ascribed to the enormous development of organic chemistry, a development to which the activity of research was chiefly directed. Chapman went on to prophesy that "just as modern organic chemistry was responsible for the neglect of the study of analytical chemistry, so the still more modern physical chemistry is likely to be responsible for its vigorous revival . . . and the same may be said of the many biological methods which are now being pressed into the service of analytical chemistry."

Chapman's prevision has been abundantly confirmed as is proved by the formation of the Physical Methods Group and the Biological Methods Group, and a new weapon has been added to the armoury of the analyst in the application of complex organic compounds as specific precipitants for various metals. Indeed, the rate of progress of analytical chemistry during the past thirty or forty years recalls the "Golden Age" of a century ago. The estimation of "trace" substances in various commodities has acquired an importance undreamt of previously. The metallurgist and engineer require accurate estimations of traces of foreign metals in the metals and alloys used in industry. The agricultural chemist requires accurate estimations of those traces of elements which have such remarkable effects on the fertility of soil, the quality of crops and the well-being of cattle. The food manufacturer requires accurate estimations of those "trace" substances—the vitamins—which are so necessary to health. The increasing stringency of Food Regulations requires accurate estimations of traces of substances injurious to health.

These requirements have been met by the development of physical and biological methods of analysis in recent years and, in addition, a new field of chemical analysis—microchemistry—of especial value when only very small samples of material are available, has been opened up. Traces of elements so small that they cannot be estimated by purely chemical methods or only by long and laborious processes, can be estimated by spectrographic and polarographic methods so rapidly that the results are available for the running control of factory processes. The possibilities of spectrographic methods have, of course, long been known, but their practical application in industry is of comparatively recent growth. It is only a few years since it was realised that traces of cobalt and boron are very important constituents of soil. It is safe to assume that in the future, other and perhaps more exacting demands will be made on the analyst, and that traces of substances not yet known to be of significance will prove to be so. More than that, substances now known to be poisonous when present even in traces may prove to be, not merely harmless, but actually beneficial when present in still smaller traces; indeed, fluorine is such a substance. There is another important branch of chemical analysis in which much more may be demanded from the chemist in the future, and perhaps in the near future, than in the past, *viz.*, the analysis of fertilisers and feeding stuffs. For many years it has been customary to make "omnibus" determinations of nitrogen and digestible carbohydrates without regard to the availability of these constituents. G. Taylor[3] has recently pointed out the inadequacy of such determinations for some types of fertilisers and feeding stuffs, and has foreshadowed the possibility of a new Fertilisers and Feeding Stuffs Act requiring the determination of "available" constituents.

In order that these increasing demands on the analyst shall be met it is necessary that the chemical student of to-day should be thoroughly trained in analysis.

Our Society was founded during the period of neglect of analytical chemistry and it has contributed greatly to the present or "Second Golden Age" by its meetings for the presentation and discussion of papers on analytical chemistry, by the work of its Analytical Methods Committee in devising new methods of analysis or modifying existing ones, by the publication of *The Analyst* and, within the last few years, by the formation of Groups for special subjects—the Microchemistry Group, the Physical Methods Group and the Biological Methods Group. The formation of these Groups is evidence, not only of rapid progress in but also of widened scope of analysis; it is evidence, too, that the individual chemist cannot hope to be a master in all branches of analysis.

It is fitting, therefore, that the Society should be concerned with the question of training the student of chemistry in analysis, since it is on this training that future progress in this branch of the science depends. One of the objects for which the Society was formed, as laid down in the Memorandum of Association, is "To encourage, assist and extend the knowledge and study of analytical chemistry."

None would question the great utilitarian value of training in analysis; the uses of analysis are too obvious and manifold. It serves as guide and control in manufacturing operations of the most diverse kinds, it is an indispensable aid to the medical man in the diagnosis and cure of disease and to the water engineer in ensuring supplies of wholesome drinking water, it is a protection to the public against the fraudulent trader and it is a Court of Appeal for the research worker in other branches of the science.

There does appear, however, to be an assumption by many, expressed or tacit, that training in analysis, though useful, is not of great educational value and that it should be subordinate to the study of the fundamental principles of chemistry. This assumption has no foundation in fact. Most of the fundamental principles of chemistry are derived from and based upon accurate quantitative work and the student learns in the most effective way possible, *i.e.*, from his own practice in quantitative analysis, the truth of those principles. As he becomes practised, beginning with the simplest determinations and progressing to the more difficult ones, he finds that after a time, depending on his aptitude, he can count on obtaining results within the commonly accepted limits of experimental error and that these limits can be narrowed but only by taking extraordinary precautions, and that there are limits which cannot be further narrowed. Thus, in learning to become a competent analyst the student not only acquires manipulative skill and dexterity, and familiarity with the use of various instruments of measurement; he learns for himself that chemistry is an exact science based on experimental work, and that the exact laws of chemistry, such as Avogadro's Law and the Law of Multiple Proportions, are reasonable deductions from experimental data, subject to experimental error. Such training must have the most salutary effect on

the student's scientific mental outlook and critical faculty. He has been taught verbally the fundamental principles of chemistry; some of them at least he examines and verifies in the laboratory. His developed critical faculty leads him to investigate the causes of unexplained differences in quantitative results; it was the slight difference between the densities of "chemical" and "atmospheric" nitrogen that led Ramsay and Rayleigh to the discovery of argon, and the former to the discovery of the rarer gases of the atmosphere.

No one, I think, would suggest that the student should spend time on the study of special analytical methods devised for the special needs of industry; he could not hope to acquire the speed of those to whom such work is part of routine. If he has been thoroughly trained in the principles of analysis he will quickly pick up such specialised skill as and when required, and he will be competent to suggest improvements in special methods and to devise new ones.

What is now the status of analytical chemistry from the educational aspect in our teaching institutions? All the available evidence goes to show that it leaves much to be desired. A few years ago, under the auspices of the Committee of the Microchemistry Group, a questionnaire was addressed by Dr. C. L. Wilson to universities and technical institutions, and on the basis of the replies Dr. Wilson prepared a most admirable report entitled "The Teaching of Analytical Chemistry with Special Reference to Microchemistry,"[4] from which one conclusion may be quoted: "There is undoubtedly a general recognition among teachers of the importance of analytical chemistry in general, and microchemical methods have obviously recommended themselves strongly to teachers as a useful instructional topic. Recognition of this by the professional and other examining bodies is desirable, particularly in view of the probable post-war development. Every effort ought to be made to secure this recognition, and to make possible further development of the regard for analytical chemistry in teaching institutions."

Of the "professional and other examining bodies" referred to by Dr. Wilson, the Royal Institute of Chemistry fully recognises the importance of training in analysis. In 1940 the Institute added to its list of Branches of the Fellowship examination, Branch H, General Analytical Chemistry. The subject is an extremely wide one and, unlike some of the more specialised Branches, might be expected to attract a large proportion of candidates. Examination of the figures published in the *Proceedings* of the Institute, however, shows that the reverse is the case. During the years 1944–48, out of 188 candidates for the Fellowship examination (nine Branches) only 9 presented themselves for examination in Branch H. It seems that the only possible inference to be drawn from these figures is that candidates have not been sufficiently trained in analysis to satisfy the high standard set by the Institute. Anyone who regards analytical chemistry as a subject of subordinate importance in chemical education would do well to study the past examination papers of the Institute in Branch H; he should be effectively cured of that illusion.

In 1945 the then President of the Society, Dr. Monier-Williams, addressed a questionnaire to chemists occupying positions which would enable them to judge the competence in chemical analysis of recently qualified chemists. The majority of the replies were to the effect that the study of analysis did not receive sufficient time and attention during the years of studentship.

I think that the sum of the evidence from Dr. Wilson's report, from the figures of the Fellowship examination of the Royal Institute of Chemistry and from the replies to the questionnaire of Dr. Monier-Williams is amply sufficient to show that training in analysis does not occupy in the curricula of our teaching institutions the status that its importance warrants. To this it might be objected that progress in chemical analysis shows no sign of lessening, but the answer to that objection is that the effect of neglect of training does not of course become manifest for perhaps ten or twenty years. Once it does become manifest the cure is correspondingly slow, and the cure should obviously be begun as soon as possible.

What are the practicable steps in effecting the cure? Chapman[1] recommended the establishment of Chairs of Analytical Chemistry in our universities and colleges, a recommendation subsequently endorsed by Bolton[2] and again by Sir John Fox, then Government Chemist, at the 1937 Summer Meeting of the North of England Section. The adoption of this recommendation would undoubtedly give to analytical chemistry a status in our teaching institutions attainable in no other way, and some other countries, less industrialised than our own, have set the example. Further, Chairs of Analytical Chemistry would serve as foci of research in analysis which at present is too dependent on the unco-ordinated efforts of individual chemists generally made to meet an immediate need. More than the establish-

ment of Chairs of Analysis is required, however; as pointed out by Chapman and others a longer period of college training is necessary if chemical analysis is to be adequately taught, and this would certainly be welcomed by the teacher as well as by the student.

The chemical student of to-day has very much more to learn than was the case with his predecessor of fifty years ago. All branches of chemistry have developed greatly during this period, and new branches, which must be studied, have come into being. Fifty years ago the student was taught to assume that the atom was a homogeneous, indestructible particle, and that it was, so to speak, a "dead end" of chemistry. The development of atomic chemistry since then, and world events of the past four years have taught us that the atom is anything but a "dead end" and to-day the study of the structure of the atom and of the nature of valency is of the utmost importance in the student's training; no one would suggest that less time should be given to it. The enlargement of our chemical vocabulary too has kept pace with the development of the science, and this entails a further tax on memory.

These increased demands on the student can only be met by a longer period of training, either by lengthening the ordinary course or by the provision of post-graduate courses. Unless one of these alternatives is adopted and if the student continues to be offered more mental food than he can assimilate in the time now at his disposal, he will either suffer from mental indigestion or, what perhaps is more probable, he will, consciously or unconsciously, reject the excess. In either case the progress of chemistry as a whole, and of chemical analysis in particular, will suffer.

There is no body in this country so closely concerned with the question of training in analysis as our Society. Two years ago the Council appointed a Special Committee to consider the question and make recommendations with a view to improving the present state of affairs. The Committee prepared a Report, subsequently approved by the Council, and this Report has been submitted to the appropriate authorities.

If the present unsatisfactory conditions of training in chemical analysis are allowed to continue there is serious danger that within, say ten or twenty years, the analyst himself may be "weighed in the balance and found wanting."


REFERENCES

1. *Analyst*, 1915, **40**, 77; 1916, **41**, 31.
2. *Ibid.*, 1928, **53**, 190.
3. *Thirtieth Streatfeild Memorial Lecture*, 1947 (Royal Institute of Chemistry publication).
4. *Nature*, 1945, **156**, 588.


# The Micro-Determination of Potassium as Cobaltinitrite in Biological and Agricultural Materials

## Part II. A New Turbidimetric Method

BY J. TINSLEY

THE development of turbidimetric methods of analysis for a wide variety of purposes was described up to 1929 by Yoe.[1] In agricultural science such methods have been applied chiefly to studies on soil fertility and plant nutrition. Much attention has been paid to potassium and the various procedures used for the turbidimetric determination of this element in soil extracts have been reviewed by Tinsley.[2] For the routine examination of extracts obtained with a solution of sodium acetate and acetic acid prepared according to Morgan,[3] the method devised by Tinsley and Pizer[4] for use with the Spekker absorptiometer, has proved very useful. In this method a stock solution of sodium cobaltinitrite was prepared according to Bennett[5] and freshly diluted 1 in 5 with Morgan's solution before use. For each series of tests 0·5 ml. of diluted sodium cobaltinitrite reagent was mixed with 1 ml. of soil extract in a small glass tube and the potassium cobaltinitrite cloud precipitated with 1 ml. of a cold mixture of methyl and *iso*propyl alcohols. This latter mixture was added as a layer at 0° C. and then mixed in a standard manner by means of a mechanical reciprocating shaker. Interference by ammonium ions was effectively prevented by adding 1 drop of a 1 per cent. solution of sodium hypochlorite to the soil extract before the addition of the sodium cobaltinitrite. Turbidity was measured directly in the cylindrical test tube mounted

in a brass holder on the "Spekker." The only serious defect of the method was the interference caused by appreciable amounts of sulphate present in some extracts.

It was considered that if this interference by sulphate could be overcome and the procedure for cloud formation simplified, with improved stability of the cloud, the turbidimetric determination might be applied generally to other solutions containing potassium. Bechold and Hebler[6] prepared stable dispersions of barium sulphate in glycerol and Baver and Bruner[7] advocated the use of glycerol to stabilise the oxalate precipitate obtained in their turbidimetric test for calcium. The author has investigated the possibility, using glycols either in place of or combined with the usual alcohols, to form the potassium cobaltinitrite cloud.

## Experimental

### Preliminary work—

It was found that Bennett's sodium cobaltinitrite solution could be mixed with alcohol - glycol mixtures to give reagents that produced a cobaltinitrite precipitate suitable for turbidimetric measurement when added to various solutions containing small quantities of potassium. Cloud formation was investigated with various mixtures of methyl, ethyl (95 per cent.) or *iso*propyl alcohol with glycerol, ethylene glycol, or propylene glycol (1 : 2 dihydroxypropane).

*Reagents*—(i) Morgan's acetate solution containing 10 g. of AnalaR hydrated sodium acetate and 3 ml. of AnalaR glacial acetic acid per 100 ml.

(ii) Standard solutions of potassium chloride dissolved in Morgan's solution, containing from 5 to 50 p.p.m. of potassium.

(iii) Similar standard solutions containing in addition to potassium known amounts of purified gum arabic, calcium acetate, magnesium acetate, sodium phosphate and sodium sulphate, respectively.

(iv) Standard potassium solutions in water alone, and in solutions containing respectively 22 per cent. of sodium perchlorate, 0·5 *N* acetic acid, 0·5 *N* calcium acetate, 1 per cent. of citric acid, an equivalent amount of calcium citrate, and 4 per cent. of trichloracetic acid.

(v) Sodium cobaltinitrite solution. Initially Bennett's solution was used containing 5 g. of hydrated cobalt nitrate, 30 g. of sodium nitrite and 2·5 ml. of acetic acid per 100 ml. This was replaced after trial by a solution containing 20 g. of AnalaR sodium cobaltinitrite and 20 g. of AnalaR sodium nitrite per 100 ml. which proved more satisfactory for cloud formation, easier to prepare and more stable when stored.

(vi) Alcohol - glycol mixtures. Each alcohol was re-distilled and mixed in different proportions with each glycol.

(vii) Reagent mixtures of the sodium cobaltinitrite solution (v) with the alcohol - glycol mixtures were freshly prepared before use at the same temperature as was used for cloud formation, because of the slow decomposition of the cobaltinitrite. The solubility of the sodium cobaltinitrite in the reagent mixture decreased as the proportion of alcohol was increased. The order of solubility in the presence of alcohols was in methyl > in ethyl > in *iso*propyl; as to glycols, the solubility in presence of glycerol was about the same as in presence of ethylene glycol and much greater than in presence of propylene glycol. With glycerol especially there was a strong tendency to supersaturation, but only those reagent mixtures that were true stable solutions proved satisfactory for cloud formation.

*Procedures for cloud formation and measurement*—The potassium solution and reagent mixture were mixed in five different proportions by volume, which are distinguished as procedures (*a*), (*b*), (*c*), (*d*) and (*e*). Usually a final volume of 3 ml. of test liquid was obtained, and for this the following volumes were required.

| Procedure | Reagent mixture ml. | Potassium solution ml. |
|---|---|---|
| (*a*) | 1·00 | 2·00 |
| (*b*) | 1·20 | 1·80 |
| (*c*) | 1·50 | 1·50 |
| (*d*) | 1·80 | 1·20 |
| (*e*) | 2·00 | 1·00 |

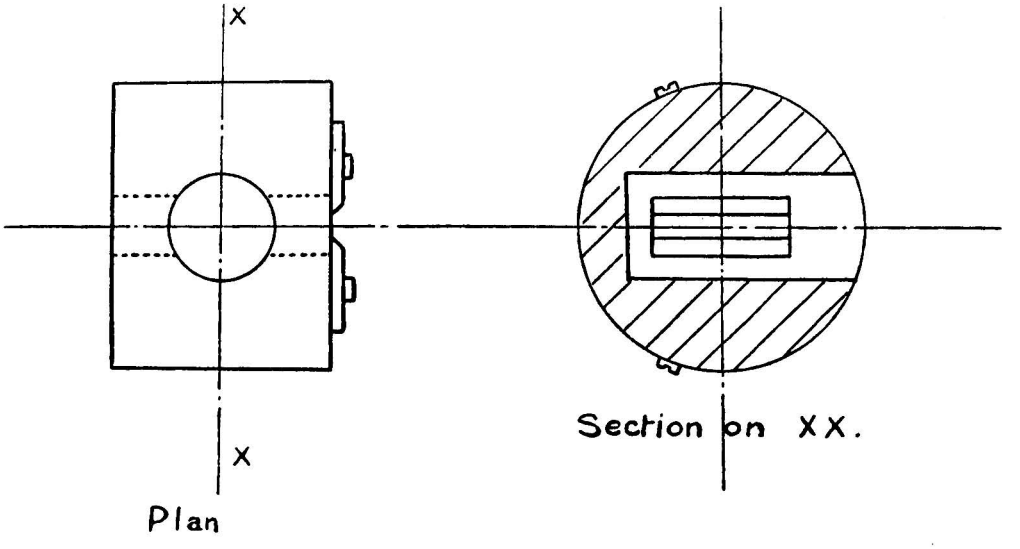
(i) *Micro procedure* using the test tubes directly for turbidity measurements on 3 ml. of final mixture.

Small round-bottomed glass tubes* 70 mm. long by 14 mm. outside diameter were used,

* Supplied by Wood, Bros. Glass Co., Barnsley.

as previously employed by Tinsley and Pizer. The reagent mixture was added in two ways, either by running it carefully down the side of the tube to form a layer above or below the potassium solution depending on its density, or by rapid delivery from a pipette with a large orifice to achieve mixing as quickly as possible. In each case mixing was completed immediately with a glass plunger made from a piece of rod by forming a flattened disc at one end about 10 mm. in diameter so that it could be moved up and down in the tube. The tubes were held in a metal tray which could be immersed in an ice-bath or water-bath to secure a controlled temperature. Turbidity measurements were made on the "Spekker" with the aid of a brass tube holder and with the No. 1 red glass filters and a drum setting for the blank test of 1·50 or 1·00 depending on the range required.

Fig. 1—Design of Brass Test Tube Holder




The design of the tube holder is shown in Fig. 1. It was made from a piece of brass cylinder of diameter 38 mm. (1·5 inch). A hole just slightly exceeding 14 mm. in diameter was bored 30 mm. deep in a central position between the two plane parallel faces of the cylinder which had an over-all length of 30 mm. when finished. A rectangular window was cut from each face to the central hole; these two windows were centrally placed, parallel, and measured 8 mm. by 18 mm. The glass test tube when in position in the hole and containing the test liquid, formed a cylindrical lens of focal length approximately 14 mm. Two semi-circular plates were fixed to one face of the holder by means of threaded screws to form a slit of variable width behind the window. The slit was finally adjusted to an optimum width of 3 mm. which, when facing the photo-cell, cut off most of the dispersed light, yet allowed sufficient to pass for adequate sensitivity on the ordinary Spekker model. A small screw on either side of the holder ensured that when it was placed on the sliding cell carrier of the instrument the test tube was always in a vertical position.

(ii) *Macro procedure* using standard 1-cm. rectangular cells for measurement.

By using hard glass test tubes 18 mm. in diameter the same procedure for cloud formation could be followed but with four times the volumes, giving a final volume of test liquid of 12 ml. This amount was quite adequate to fill the 1-cm. cells usually employed with the "Spekker," and thus enabled slightly greater accuracy of measurement to be attained.

*Results*—These have been recorded in detail by Tinsley[8] and only the more important points arising from the preliminary experiments are mentioned here. The five procedures were compared at 0° and 15° C., with two levels of Bennett's cobaltinitrite solution, namely, 0·15 ml. and 0·30 ml. per 3 ml. of the final mixture. With all the reagent mixtures examined procedures (*c*) and (*d*) gave the best turbidity. The differences between turbidities at the two temperatures and the two levels of cobaltinitrite were irregular but not very pronounced. Turbidity increased with the concentration of alcohol in the reagent mixture up to the limit of solubility of the sodium cobaltinitrite, this effect being most evident with the lower potassium

standards and reagent mixtures containing glycerol and ethylene glycol. Propylene glycol gave good turbidity without any alcohol. Methyl alcohol gave the most satisfactory turbidity with each glycol and the best results were secured with mixtures of methyl alcohol and propylene glycol.

Two variations of the original method of Tinsley and Pizer were tried. Firstly, with each glycol incorporated in the diluted sodium cobaltinitrite solution and mixed with the potassium solutions before addition of the alcohols separately. Secondly, with the sodium cobaltinitrite solution added to the potassium solutions before the addition of the alcohol - glycol mixtures separately. Neither procedure proved as successful as the use of the combined reagents containing sodium cobaltinitrite, methyl alcohol and propylene glycol.

CLOUD FORMATION WITH REAGENT MIXTURES CONTAINING METHYL ALCOHOL, PROPYLENE GLYCOL AND GLYCEROL—

Good turbidity was secured with mixtures containing methyl alcohol and propylene glycol with a smaller proportion of glycerol or ethylene glycol. The presence of either of the last two enabled a higher proportion of alcohol to be present in the reagent mixture than with propylene glycol alone. Turbidity tests favoured glycerol rather than ethylene glycol, and in this series of experiments different proportions of these three organic reagents were used in combination with 10 volumes per cent. of sodium cobaltinitrite solution (20 per cent. + 20 per cent. of sodium nitrite). Procedure (*c*) was used at 15° C., with standards in Morgan's solution and in water, the reagent mixture being added as a layer before mixing except in tests on the duplicated standards, and for these the cloud was formed in one tube by the "layering" procedure, and in the other tube by rapid mixing. Readings were taken 20 and 40 minutes after mixing but only the former are given here; they are recorded as drum readings × 100 in Table I.

TABLE I

CLOUD TURBIDITY WITH REAGENT MIXTURES CONTAINING METHYL ALCOHOL, PROPYLENE GLYCOL AND GLYCEROL

| Mixture | | P.p.m. potassium in acetate solution | | | | | | | | P.p.m. K in water | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | M.P.G. | 5 B | 5 | 10 | 20 | 30 | 40 | 50 | 50 B | 50 | 50 B |
| 1 | 7.1.1 | 130 | 129 | 110 | 87 | 67 | 65 | 45 | 56 | 26 | 54 |
| 2 | 7.0.2 | 135 | 134 | 114 | 76 | 53 | 51 | 16 | 31 | 4 | 20 |
| 3 | 6.2.1 | 133 | 132 | 116 | 96 | 73 | 66 | 47 | 60 | 27 | 10 |
| 4 | 6.1.2 | 133 | 133 | 113 | 74 | 42 | 22 | 1 | 0 | 4 | 0 |
| 5 | 6.0.3 | 139 | 140 | 116 | 80 | 46 | 24 | 4 | 9 | 11 | 5 |
| 6 | 5.3.1 | 135 | 135 | 114 | 77 | 45 | 23 | 4 | 4 | 16 | 4 |
| 7 | 5.2.2 | 135 | 136 | 115 | 79 | 45 | 26 | 11 | 11 | 34 | 8 |
| 8 | 5.1.3 | 145 | 145 | 117 | 81 | 52 | 27 | 17 | 31 | 9 | ↓ 16 |
| 9 ↓ | 5.0.4 | 147 | 147 | 121 | 82 | 49 | 32 | 7 | 23 | 11 | 18 |
| 10 | 4.4.1 | 136 | 136 | 114 | 77 | 45 | 23 | 1 | 2 | 10 | ↓ 8 |
| 11 | 4.3.2 | 138 | 136 | 114 | 79 | 46 | 29 | 6 | 24 | 9 | ↓ 10 |
| 12 ↓ | 4.2.3 | 146 | 146 | 124 | 88 | 51 | 26 | 8 | 10 | 10 | 10 |
| 13 ↓ | 4.1.4 | 150 | 150 | 124 | 86 | 51 | 27 | 13 | 13 | 12 | 16 |
| 14 ↓ | 4.0.5 | 150 | 150 | 128 | 88 | 52 | 30 | 13 | 13 | 13 | 13 |
| 15 | 3.6.0 | 134 | 134 | 114 | 79 | 47 | 26 | 5 | 5 | 10 | ↓ 9 |
| 16 | 3.5.1 | 137 | 138 | 118 | 78 | 48 | 27 | 5 | 4 | 22 | ↓ 8 |
| 17 ↓ | 3.4.2 | 143 | 142 | 119 | 86 | 54 | 25 | 4 | 7 | 14 | 13 |
| 18 ↓ | 3.3.3 | 144 | 146 | 120 | 84 | 45 | 27 | 5 | 5 | 12 | 8 |
| 19 | 2.7.0 | 138 | 137 | 119 | 78 | 47 | 24 | 3 | 5 | 33 | ↓ 7 |
| 20 ↓ | 2.6.1 | 140 | 138 | 124 | 80 | 48 | 26 | 3 | 4 | 22 | 5 |
| 21 ↓ | 2.5.2 | 144 | 145 | 118 | 80 | 47 | 28 | 6 | 14 | 14 | 9 |
| 22 ↓ | 1.8.0 | 137 | 137 | 118 | 80 | 49 | 30 | 25 | 7 | 16 | 16 |
| 23 ↓ | 1.7.1 | 142 | 141 | 120 | 81 | 48 | 34 | 11 | 12 | 11 | 19 |
| 24 ↓ | 0.9.0 | 137 | 140 | 118 | 80 | 44 | 26 | 4 | 8 | 10 | 11 |

M = Methyl alcohol, volumes per 10 volumes of reagent mixture.
P = Propylene glycol, volumes per 10 volumes of reagent mixture.
G = Glycerol, volumes per 10 volumes of reagent mixture.
B = Reagent blown into potassium solution.
↓ = Reagent sunk *below* potassium solution.

With reagent mixtures 1 to 3, containing 60 to 70 per cent. of methyl alcohol, layering was not good because diffusion occurred at the interface, and turbidity was poor with the higher standards both in acetate and water solution. For the rest it was noticed that as the glycerol concentration increased, visible cloud formation was delayed. With reagent mixtures 12 to 14, containing 30 to 50 per cent. of glycerol, this delay amounted to about 5 seconds with the higher standards, but with the lowest standard cloud formation was very slow and the turbidity increased for 4 hours after mixing. Thus glycerol retards cloud formation, possibly by restricting molecular movement within the solution. Because mixing could be completed before the cloud formed appreciably, reproducibility of the turbidity was good and also the stability of cloud was good, but the slow rate of formation with the lower standards would be a serious disadvantage when testing solutions covering a wide range of potassium content.

Rapid mixing was generally as good as the layering procedure with the acetate solutions; and with the water solutions rapid mixing gave greater turbidity with the 50 p.p.m. standard. This was encouraging, because if the reagents proved satisfactory in other respects mixing could be done in a simple standard manner. Reagent mixtures Nos. 6, 10 and 15 appeared to give the best gradation of turbidity with adequate sensitivity for the 5 p.p.m. solution. These gave closely similar results when tested under a wide variety of conditions, but No. 6 appeared to be slightly superior over-all, and in the following account only results for this reagent are given.

CLOUD FORMATION WITH REAGENT MIXTURES PREPARED FROM 50 VOLUMES OF METHYL ALCOHOL, 30 VOLUMES OF PROPYLENE GLYCOL AND 10 VOLUMES OF GLYCEROL PER 100 VOLUMES—

(*i*) *Effect of sodium cobaltinitrite concentration*—Reagent mixtures containing 7·5, 10 and 12·5 volumes per cent. of sodium cobaltinitrite solution were freshly prepared and used at 15° C. by procedure (*c*). The results recorded in Table II show that the turbidity of the lower standards decreased as the concentration of sodium cobaltinitrite fell below 10 per cent., but was not significantly increased by raising the concentration above this level. The reagent mixture containing 12·5 per cent. proved to be supersaturated and a precipitate of sodium cobaltinitrite was deposited after use.

TABLE II

EFFECT OF SODIUM COBALTINITRITE CONCENTRATION ON TURBIDITY

| Cobaltinitrite solution % of reagent mixture | Time, min. | P.p.m. K in acetate solution | | | | | | P.p.m. K in water |
|---|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 20 | 30 | 40 | 50 | 50 |
| 7·5 | 20 | 140 | 119 | 84 | 54 | 25 | 6 | 13 |
| | 40 | 138 | 120 | 86 | 56 | 29 | 10 | 16 |
| 10 | 20 | 132 | 113 | 82 | 49 | 27 | 14 | 16 |
| | 40 | 132 | 112 | 84 | 52 | 30 | 17 | 20 |
| 12·5 | 20 | 132 | 111 | 76 | 45 | 23 | 1 | 8 |
| | 40 | 129 | 109 | 75 | 47 | 24 | 6 | 10 |

(*ii*) *Effect of alcohol - glycol concentration*—Reagent mixtures containing 12·5, 10 and 8·3 volumes per cent. of sodium cobaltinitrite solution were prepared and used at 15° C. by procedures (*b*), (*c*) and (*d*) respectively, thus giving 35, 45 and 55 volumes per cent. of alcohol - glycol mixture in the final state after cloud formation with 1 per cent. each of sodium cobaltinitrite and sodium nitrite throughout. The results recorded in Table III show that

TABLE III

EFFECT OF ALCOHOL - GLYCOL CONCENTRATION ON TURBIDITY

| Procedure used | Time, min. | P.p.m. K in acetate solution | | | | | | P.p.m. K in water |
|---|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 20 | 30 | 40 | 50 | 50 |
| (*b*) | 20 | 140 | 117 | 74 | 35 | 7 | 0 | 6 |
| | 40 | 140 | 114 | 73 | 36 | 12 | 0 | 8 |
| (*c*) | 20 | 136 | 115 | 79 | 47 | 23 | 5 | 7 |
| | 40 | 135 | 113 | 80 | 49 | 25 | 9 | 12 |
| (*d*) | 20 | 137 | 124 | 94 | 65 | 40 | 22 | 47 |
| | 40 | 137 | 123 | 94 | 65 | 42 | 25 | 49 |

the turbidity, as measured, was least for the lowest standards and greatest for the highest standards by procedure (*b*). However, when the readings were plotted against actual concentrations of potassium in the final mixture it was evident that procedure (*b*) gave a lower turbidity throughout. Procedures (*c*) and (*d*) gave almost identical turbidities, showing that the maximum effect of the alcohol - glycol mixture was obtained in each case.

(*iii*) *Effect of temperature*—It was decided to compare the turbidity produced at 10°, 15° and 20° C. by procedures (*c*) and (*d*), using the same reagent mixture throughout. The results recorded in Table IV show that with both procedures the turbidity decreased with rise of temperature, the difference between 10° and 15° C. being smaller than between 15° and 20° C. The decrease in turbidity was relatively most marked with the lowest potassium concentration as would be expected. 15° C. seemed to be well justified as the most convenient temperature for ease of laboratory control combined with good turbidity.

TABLE IV

EFFECT OF TEMPERATURE ON TURBIDITY

| | | P.p.m. potassium in acetate solution | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Procedure (*c*) | | | | | | Procedure (*d*) | | | | | |
| Temp. | Time, min. | 5 | 10 | 20 | 30 | 40 | 50 | 5 | 10 | 20 | 30 | 40 | 50 |
| 10° C. | 20 | 134 | 116 | 78 | 45 | 21 | 0 | 131 | 116 | 89 | 61 | 38 | 20 |
| | 40 | 133 | 115 | 78 | 47 | 24 | 5 | 131 | 116 | 88 | 61 | 38 | 20 |
| 15° C. | 20 | 136 | 115 | 80 | 46 | 23 | 4 | 134 | 119 | 92 | 64 | 39 | 21 |
| | 40 | 135 | 115 | 79 | 49 | 26 | 8 | 134 | 119 | 92 | 66 | 41 | 24 |
| 20° C. | 20 | 141 | 118 | 83 | 53 | 28 | 11 | 137 | 122 | 94 | 68 | 46 | 25 |
| | 40 | 139 | 116 | 84 | 55 | 32 | 16 | 137 | 122 | 94 | 68 | 45 | 27 |

(*iv*) *Effect of calcium, magnesium, phosphate and sulphate*—Using the same standard solutions as in the preliminary experiments and reagent mixture of the same composition as in the previous experiment, clouds were formed at 15° C. by procedures (*c*) and (*d*). The turbidity readings were taken 30 minutes after mixing. The results recorded in Table V show that none of these elements present in the amounts stated, either separately or all together, has any serious effect on the turbidity.

TABLE V

EFFECT OF CALCIUM, MAGNESIUM, PHOSPHATE AND SULPHATE AND ALSO AMMONIUM CHLORIDE AND SODIUM HYPOCHLORITE ON TURBIDITY

| | Procedure (*c*) | | | | | | Procedure (*d*) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P.p.m. K in | 5 | 10 | 20 | 30 | 40 | 50 | 5 | 10 | 20 | 30 | 40 | 50 |
| Acetate solution .. .. | 135 | 118 | 80 | 50 | 25 | 5 | 132 | 118 | 89 | 62 | 44 | 24 |
| + 5000 p.p.m. Ca .. | 135 | 118 | 80 | 51 | 25 | 1 | 132 | 119 | 86 | 64 | 40 | 26 |
| + 1000 p.p.m. Mg .. | 137 | 120 | 86 | 54 | 26 | 6 | 132 | 118 | 87 | 64 | 45 | 27 |
| + 100 p.p.m. P .. .. | 137 | 116 | 86 | 55 | 27 | 6 | 133 | 118 | 88 | 64 | 45 | 26 |
| + 1000 p.p.m. $SO_4$ .. | 135 | 117 | 85 | 51 | 25 | 6 | 132 | 119 | 88 | 64 | 43 | 27 |
| + Ca + Mg + P + $SO_4$ .. | 136 | 117 | 83 | 54 | 29 | 6 | 132 | 118 | 86 | 62 | 45 | 26 |
| + 1 drop 10% NaOCl solution .. .. .. .. .. .. | | | | | | | 132 | 117 | 90 | 63 | 44 | 25 |
| + 250 p.p.m. $NH_3$ + 1 drop NaOCl solution .. .. .. .. | | | | | | | 130 | 116 | 86 | 62 | 44 | 23 |

(*v*) *Effect of ammonium chloride and sodium hypochlorite*—In these experiments 1 drop of B.D.H. 10 per cent. sodium hypochlorite solution was added to each tube containing 1·2 ml. of standard potassium solution in two sets, one containing the usual range of potassium standards in Morgan's acetate solution, and the other containing also 250 p.p.m. of ammonia ($NH_3$) added as ammonium chloride. Turbidity readings taken 30 minutes after mixing and recorded in Table V show that the ammonium ion was completely immobilised by the sodium hypochlorite solution, which had no effect by itself.

(*vi*) *Method of mixing for cloud formation and accuracy of the results*—Duplicate tests were made with procedures (*c*) and (*d*), at 15° C., in one case the reagent mixture being layered and in the other blown in rapidly from the pipette before completing the mixing with a glass plunger. Each set of tests was replicated ten times on different occasions and the readings

were made 30 minutes after mixing. The results are summarised in Table VI showing the mean "Spekker" reading for each standard solution, 150 minus the mean, the standard deviation and the coefficient of variation. Similar information is included for the previous method of Tinsley and Pizer.

TABLE VI

COMPARISON OF METHODS OF CLOUD FORMATION

| | P.p.m. K in acetate solution | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 20 | 30 | 40 | 50 |
| Procedure (*c*) layered: | | | | | | |
| Mean reading × 100 | 134·9 | 115·1 | 80·4 | 50·8 | 26·2 | 7·4 |
| 150 — Mean | 15·1 | 34·9 | 69·6 | 99·2 | 123·8 | 142·6 |
| Standard deviation | 1·20 | 1·60 | 1·62 | 2·55 | 2·17 | 2·72 |
| Coefficient of variation | 7·9 | 4·6 | 2·3 | 2·6 | 1·7 | 1·9 |
| Rapid mixing: | | | | | | |
| Mean reading × 100 | 135·9 | 115·8 | 81·1 | 49·8 | 25·9 | 6·4 |
| 150 — Mean | 14·1 | 34·2 | 68·9 | 100·2 | 124·1 | 143·6 |
| Standard deviation | 1·00 | 1·84 | 2·24 | 1·81 | 1·45 | 2·35 |
| Coefficient of variation | 7·0 | 5·4 | 3·2 | 1·8 | 1·2 | 1·7 |
| Procedure (*d*) layered: | | | | | | |
| Mean reading × 100 | 134·9 | 119·8 | 91·5 | 65·5 | 43·6 | 23·7 |
| 150 — Mean | 15·1 | 30·2 | 58·5 | 84·5 | 106·4 | 126·3 |
| Standard deviation | 1·52 | 2·20 | 3·22 | 2·20 | 1·74 | 2·67 |
| Coefficient of variation | 10·1 | 7·3 | 5·5 | 2·6 | 1·7 | 2·1 |
| Rapid mixing: | | | | | | |
| Mean reading × 100 | 134·7 | 119·5 | 90·4 | 64·4 | 42·0 | 22·2 |
| 150 — Mean | 15·3 | 30·5 | 59·6 | 85·6 | 108·0 | 127·0 |
| Standard deviation | 1·64 | 2·17 | 2·35 | 1·62 | 1·33 | 1·51 |
| Coefficient of variation | 10·7 | 7·2 | 3·9 | 1·9 | 1·2 | 1·2 |
| Method of Tinsley and Pizer: | | | | | | |
| Mean reading × 100 | 138·5 | 121·4 | 93·1 | 70·8 | 51·8 | 35·8 |
| 150 — Mean | 11·5 | 28·6 | 56·9 | 79·2 | 98·2 | 114·2 |
| Standard deviation | 1·96 | 1·31 | 1·66 | 0·92 | 1·84 | 1·40 |
| Coefficient of variation | 17·0 | 4·6 | 2·8 | 1·2 | 1·5 | 1·2 |

Comparison of the coefficients of variation indicates that procedure (*c*) gave slightly better reproducibility than procedure (*d*) and there was little difference between layering and rapid mixing with either procedure. Rapid mixing gave slightly greater turbidity with the higher standards by procedure (*d*). The new reagent mixture gives better turbidity than the old method using methyl and *iso*propyl alcohols with the 5 p.p.m. standard, but otherwise there is little difference, apart from the facts that the new reagent mixture does not show any effect of sulphate present in solution, and the "life" of the cloud is longer.

CLOUD FORMATION WITH SOLUTIONS OF POTASSIUM CHLORIDE IN WATER, 0·5 *N* ACETIC ACID, 4 PER CENT. TRICHLOROACETIC ACID AND 22 PER CENT. SODIUM PERCHLORATE SOLUTION—

Sodium perchlorate solution was included because of its extensive use by Bray[9] for extracting the exchangeable potassium from soils, but a sample of the salt obtained from a

TABLE VII

TURBIDITY WITH SOLUTIONS OF POTASSIUM CHLORIDE IN WATER, 0·5 *N* ACETIC ACID, 4 PER CENT. TRICHLOROACETIC ACID AND 22 PER CENT. SODIUM PERCHLORATE SOLUTION

| Solution in | Time, min. | P.p.m. potassium | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Procedure (*c*) | | | | | | Procedure (*d*) | | | | | |
| | | 5 | 10 | 20 | 30 | 40 | 50 | 5 | 10 | 20 | 30 | 40 | 50 |
| Water | 20 | 143 | 120 | 85 | 54 | 30 | 19 | 137 | 123 | 94 | 71 | 48 | 21 |
| | 40 | 141 | 120 | 84 | 55 | 32 | 22 | 135 | 123 | 94 | 71 | 50 | 26 |
| 0·5 *N* acetic acid | 20 | 145 | 120 | 84 | 54 | 29 | 8 | 137 | 122 | 94 | 66 | 44 | 22 |
| | 40 | 143 | 120 | 84 | 55 | 32 | 12 | 135 | 122 | 94 | 66 | 47 | 25 |
| 4% $CCl_3COOH$ | 20 | 150 | 150 | 100 | 66 | 32 | 13 | 137 | 123 | 91 | 64 | 42 | 21 |
| | 40 | 150 | 150 | 96 | 65 | 35 | 17 | 135 | 123 | 91 | 64 | 45 | 24 |
| 22% $NaClO_4$ solution | 20 | 142 | 118 | 80 | 52 | 30 | 8 | 137 | 120 | 89 | 64 | 41 | 21 |
| | 40 | 140 | 114 | 80 | 54 | 32 | 10 | 135 | 120 | 90 | 64 | 45 | 25 |

chemical supplier contained an appreciable quantity of potassium and it was necessary to prepare a solution free from potassium; this was prepared from A.R. perchloric acid and sodium bicarbonate. Clouds were formed by procedures (*c*) and (*d*) at 15° C. and readings were taken 20 and 40 minutes after mixing. The results recorded in Table VII show that the turbidity obtained with water, acetic acid and sodium perchlorate solutions was closely similar to that with Morgan's acetate solution, particularly with the latter two solutions. With trichloroacetic acid the readings for the lower standards showed very low turbidity by procedure (*c*) but were almost normal by procedure (*d*). This was thought to be due to the greater buffering capacity of the larger quantity of reagent employed in the latter and this point was examined more fully later. Procedure (*d*) gave slightly improved turbidity with the lowest standard for each solution and the calibration curves when plotted were more nearly linear.

## Method recommended for general use

Procedure (*d*) appeared from the experimental work to be most suitable for the turbidimetric determination of potassium in various solutions containing from 5 to 50 p.p.m. The following method was adopted for routine use.

### Reagents—

(i) *Potassium standards* prepared in the appropriate solution for calibration of the "Spekker" and routine checking of the method, especially when a new batch of reagents is prepared. It is convenient to maintain a stock solution containing 1·907 g. of AnalaR potassium chloride per litre, corresponding to 1000 p.p.m. of K, from which the working standards of 5, 10, 20, 30, 40 and 50 p.p.m. are prepared by dilution as required.

(ii) *Sodium hypochlorite solution.* A commercial 10 per cent. stock solution should be stored in a refrigerator if possible and freshly diluted to 1 per cent. with water each day as required.

(iii) *Alcohol - glycol mixture.* This can be prepared and stored in bulk by mixing 5 volumes of methyl alcohol with 3 volumes of propylene glycol and 1 volume of glycerol. Re-distillation of these reagents is not usually necessary provided they are quite free from suspended material.

(iv) *Sodium cobaltinitrite solution* containing 20 g. of sodium cobaltinitrite and 20 g. of sodium nitrite per 100 ml. The special precautions necessary for its preparation and storage are discussed below.

(v) *Sodium cobaltinitrite - alcohol - glycol reagent mixture.* This is freshly prepared before use by mixing 1 volume of (iv) with 9 volumes of (iii). The special precautions necessary are also discussed below.

### Apparatus—

Small test tubes for micro-tests, 70 mm. long by 14 mm. in diameter, selected for uniformity. Pyrex test tubes for macro-tests, 150 mm. long by 18 mm. in diameter. Metal racks of convenient size for holding tubes when immersed in the water-bath. Thermostat bath with a stirrer and well lagged, to maintain 15° C. Glass rods of two sizes approximately 80 mm. and 170 mm. long with flattened discs at one end approximately 10 mm. and 14 mm. in diameter respectively; one rod is used for each test tube. Pipettes 1 ml., 2 ml., 5 ml. and 10 ml. straight form, Grade B, those used for the reagent mixture having a large orifice and graduated to the tip.

Spekker photo-electric absorptiometer or other suitable instrument. Brass test tube holder as described previously (p. 169) for use with the "Spekker."

### Procedure—

As many as 20 to 30 tests may be carried out in one batch. When interference by ammonium ions must be eliminated it is convenient to add the necessary drops of 1 per cent. sodium hypochlorite solution to the test tubes prior to the potassium solution so that mixing is automatically secured. The tubes containing the test solution are held in the appropriate rack and immersed to a convenient depth in the water-bath adjusted to 15° C. by adding ice or warm water when necessary. The reagent mixture, prepared as described below, is also immersed in the bath for 5 to 10 minutes before use. Then the required volume is delivered as a jet of liquid into the potassium solution at a rate of approximately 1 ml. per

second from a suitably calibrated pipette having a large orifice and held in a vertical position in the tube. If the outside of the pipette becomes contaminated by splashing with the test liquid or in other ways it must be wiped clean since it is most important not to introduce crystal nuclei into the bulk of the reagent mixture.

| Volumes for | Micro-tests | Macro-tests |
|---|---|---|
| 1 per cent. sodium hypochlorite .. .. | 1 drop | 4 drops |
| Potassium solution .. .. .. .. | 1 ml. or 1·2 ml. | 4 ml. |
| Reagent mixture .. .. .. .. | 1·5 ml. or 1·8 ml. | 6 ml. |
| Total volume .. .. .. .. | 2·5 ml. or 3·0 ml. | 10 ml. |

A blank test on the reagents without potassium is included in each batch in order to set the "Spekker" with a drum reading of 1·50 or 1·00, using the red light filters. It is also advisable to include a standard potassium solution with each batch as a check on the calibration curve.

The tubes are removed from the water-bath 15 minutes (or 30 if desired) after mixing was started and the turbidity measurements are made in the same order so that each tube is always read at about the same time after mixing. For the micro-tests each tube is carefully wiped clean and dry on the outside and then inserted in the brass tube holder mounted on the cell carrier close to the slider. A final volume of 2·5 ml. in the tube may not be sufficient to cover the full depth of the window of the holder, in which event the larger volumes must be used to give 3 ml. of test liquid. For macro-tests the contents of the tube are poured into the 1-cm. glass cell in the usual manner.

PREPARATION AND PROPERTIES OF STOCK SODIUM COBALTINITRITE SOLUTION AND REAGENT MIXTURES—

Experience has shown that careful attention to detail is necessary in the preparation of the reagent mixture if reliable results are to be secured from day to day. During some measurements on aqueous solutions it was observed that the turbidity was frequently considerably below normal, especially when the freshly prepared reagent was first used; after some time it generally improved. This was thought to be due to the formation of crystal nuclei of sodium cobaltinitrite in the reagent mixture. These may arise either in the stock sodium cobaltinitrite solution or be formed when it is added to the alcohol - glycol mixture. The following recommendations are made for the preparation of sodium cobaltinitrite solution and reagent mixtures.

Dissolve the required amount of sodium cobaltinitrite in water. Add the same amount of sodium nitrite crystals, stir until dissolved and dilute to the required volume to give 20 per cent. w/v of each constituent. Store for three or four days, preferably in a refrigerator and then filter. This may be done with a No. 44 Whatman filter paper, or by means of a sintered glass filter funnel of the finest porosity, but neither method appears to remove crystal nuclei completely. The best results are secured by using a sintered glass funnel of small Buchner type, having a disc of coarse porosity impregnated with an acetic acid - collodion membrane. This may be prepared quite simply by pouring on the 2 per cent. solution of collodion in glacial acetic acid to form a thin layer of liquid, allowing it to soak into the pores and then filling the funnel with distilled water before applying suction. A steady rate of filtration should be maintained and if the membrane is kept immersed in water when not in use it may be stored for repeated use. With filtration under suction it is important that the cobaltinitrite solution be collected in a large boiling tube inside the flask, or in some other manner so that it does not drip from the stem of the funnel and splash the walls of the receiving vessel. If exposed in thin films under reduced pressure evaporation of water will cause precipitation of crystal nuclei, and for this reason it is important that filtration be sufficiently rapid for this loss by evaporation to be negligible. It is a good plan to measure the volume of the filtrate, and then if necessary dilute to the right concentration The stock solution should be stored in a dark glass bottle preferably with a wide mouth and with a well-fitting screw cap. It may be stored for a year in the refrigerator without deterioration, and at room temperature for at least a month. Care should be taken when removing aliquots with a pipette to prevent liquid drying on the walls or neck of the flask and so contaminating the bulk with crystal nuclei.

It is convenient to measure the required 9 volumes of alcohol - glycol mixture into a large boiling tube or other suitable container that can be immersed in the water-bath at

15° C. The stock sodium cobaltinitrite solution, if stored in the refrigerator, should be warmed to room temperature or 15° C. before adding the necessary 1 volume to 9 volumes of the alcohol - glycol mixture. The stem of the pipette used for this purpose should be carefully wiped with a piece of clean filter paper to remove any drops of cobaltinitrite solution from the outside before running the contents directly into the alcohol - glycol mixture, so that no films of cobaltinitrite liquid are exposed to evaporation and deposition of crystal nuclei. Mixing should be completed with a glass plunger having a bulb blown at one end. A rise in temperature occurs on mixing and the reagent mixture should be allowed to remain suspended in the bath for 5 or 10 minutes before use.

The reagent mixture is not stable for more than 2 hours at 15° C., but this is sufficient time for conducting a large number of tests. It should not be exposed to direct sunlight.

## Some applications of the method

(*a*) *Cloud formation in solutions of Morgan's acetate mixture, 0·5 N acetic acid, 0·5 N calcium acetate, 1 per cent. citric acid and its equivalent of calcium citrate, water and 22 per cent. sodium perchlorate solution*—Fig. 2 shows that the turbidity was closely similar throughout.

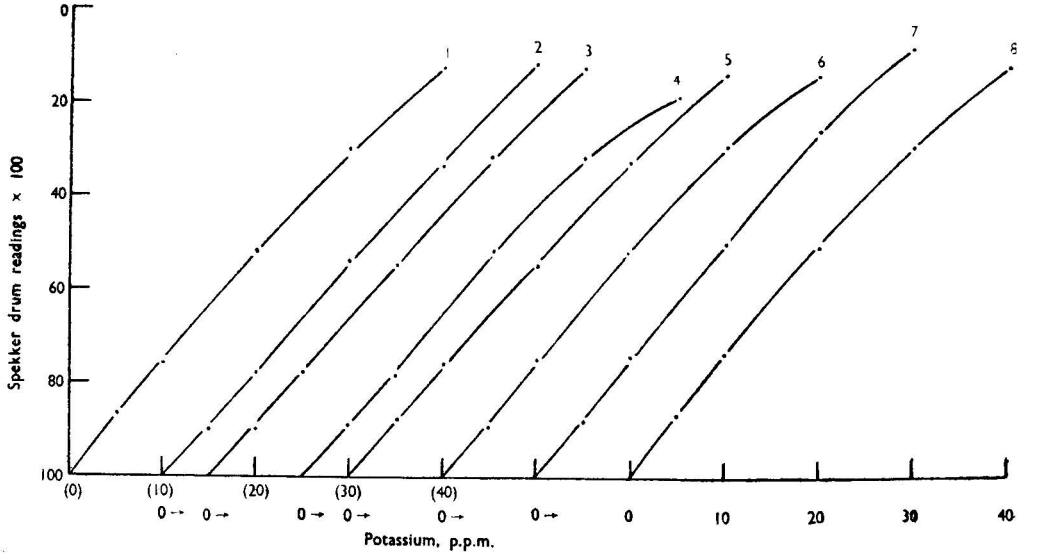



Fig. 2. Turbidity curves for potassium standards in various solutions.

1. Morgan's acetate solution.
2. 0·5 *N* Acetic acid.
3. 0·5 *N* Ca acetate.
4. 1% Citric acid.
5. Ca citrate solution
6. Water.
7. $NaClO_4$ solution.
8. $CCl_3COOH$ solution.

The potassium graduations for all these curves are similar but only those for curves 1 and 8 are set out in full, the former in brackets.

Other tests showed that sodium hypochlorite was effective for removing ammonium in each case. These results suggested that potassium may readily be determined turbidimetrically in soil extracts prepared with these solutions.

(*b*) *Determination of potassium in soil extracts*—(i) *With Morgan's acetate solution*—Ten soil samples of differing texture, and contents of organic matter, calcium carbonate and exchangeable potassium were extracted with solution in the ratio 1 : 2. The potassium was precipitated from 100 ml. of each extract by adding 10 g. of sodium cobaltinitrite and storing in the refrigerator overnight. The precipitate was then filtered on to a Gooch crucible packed with asbestos, washed with 0·01 *N* nitric acid and re-dissolved in hot 5 *N* nitric acid and the solution evaporated to dryness. The potassium was re-precipitated and determined by the volumetric cobaltinitrite method of Wilcox.[10] 4-Ml. portions of each soil extract were also measured in duplicate into test tubes for the turbidimetric determination of the potassium by the macro-procedure. The results recorded in Table VIII show that the mean difference between turbidimetric results and those by the Wilcox method was —0·65 per

cent. of the latter, with a standard deviation of 2·21 per cent. The greatest difference was −7·6 per cent., obtained with the smallest amount of potassium, and although this might have been better, the results are reasonably good on the whole, for the direct turbidimetric determination of potassium in soil extracts.

TABLE VIII

DETERMINATION OF POTASSIUM IN SOIL EXTRACTS

| Soil extract | Method of Wilcox p.p.m. | Turbidimetric method p.p.m. | Difference p.p.m. | Difference % of Wilcox figure |
|---|---|---|---|---|
| | (i) In Morgan's Acetate Extracts | | | |
| 1 | 34·8 | 33·3 | − 1·5 | − 4·3 |
| 2 | 41·9 | 39·5 | − 1·4 | − 3·3 |
| 3 | 9·2 | 8·5 | − 0·7 | − 7·6 |
| 4 | 14·5 | 15·0 | + 0·5 | + 3·4 |
| 5 | 18·9 | 19·0 | + 0·1 | + 0·6 |
| 6 | 51·7 | 51·0 | − 0·7 | − 1·3 |
| 7 | 29·4 | 29·5 | + 0·1 | + 0·3 |
| 8 | 17·6 | 18·5 | + 0·9 | + 5·1 |
| 9 | 28·0 | 29·0 | + 1·0 | + 3·6 |
| 10 | 26·3 | 25·5 | − 0·8 | − 3·0 |
| | (ii) In 1 per cent. Citric Acid Extracts | | | |
| 11 | 7·1 | 7·8 | + 0·7 | + 9·9 |
| 12 | 4·8 | 5·0 | + 0·2 | + 4·2 |

(ii) *With 1 per cent. citric acid solution*—Two soils, one acid and one calcareous were extracted with 1 per cent. citric acid in the usual manner and the potassium was determined turbidimetrically directly on the extract and also by the normal analytical method of Wilcox after extracting the ash. The results recorded in Table VIII show that agreement is fair and, though not numerous enough to warrant definite conclusions, they suggest that a direct turbidimetric determination in citric acid extracts of soils may prove sufficiently accurate for much routine advisory work.

(*c*) *Cloud formation in solutions of trichloroacetic acid*—A 4 per cent. solution of trichloroacetic acid is commonly used for the precipitation of proteins in blood serum, and milk. Many different methods have been used for the determination of potassium in the filtrates, and a reliable turbidimetric method would prove very useful. In earlier experiments procedure (*c*) did not give full precipitation of the potassium, and it was confirmed by the macro-method that procedure (*d*) was satisfactory. The solution has an initial pH of 0·8,

TABLE IX

TURBIDITY WITH SOLUTIONS OF POTASSIUM IN 4 PER CENT. TRICHLOROACETIC ACID

(i) With standard solutions

| | P.p.m. potassium | | | | |
|---|---|---|---|---|---|
| Macro-procedure (*d*): | 5 | 10 | 20 | 30 | 40 |
| Mean reading × 100 .. .. | 86·6 | 73·6 | 50·8 | 29·8 | 11·8 |
| 100 − Mean .. .. .. | 13·4 | 26·4 | 49·2 | 70·2 | 88·2 |
| Standard deviation .. .. | 1·93 | 1·74 | 2·13 | 2·31 | 2·61 |
| Coefficient of variation .. .. | 14·4 | 6·6 | 4·3 | 3·3 | 2·9 |

(ii) Determination of potassium in blood and milk filtrates

| Filtrate | Method of Wilcox p.p.m. | Turbidimetric method p.p.m. | Difference p.p.m. | Difference % |
|---|---|---|---|---|
| Blood .. .. | 60·2 | 60·0 | + 0·2 | + 0·3 |
| Milk .. .. | 195·0 | 190·0 | − 5·0 | − 2·6 |

which is very acid, but no advantage was gained by buffering it at pH 5 to 6 with sodium acetate solution before adding the reagent mixture. In the normal way the blood serum is diluted five-fold to give a concentration of potassium in the filtrate ranging between 35 and 60 p.p.m., and the milk is diluted ten-fold to give a concentration of potassium ranging between 150 and 200 p.p.m. Therefore it is generally necessary to dilute the blood and

milk filtrates further before a turbidimetric determination can be made, and this dilution can be done with water.

Readings were obtained with a series of potassium standards in 4 per cent. trichloroacetic acid replicated sixteen times on different occasions. The mean values together with the statistical information are given in Table IX. The calibration curve plotted from these values is included in Fig. 2.

(*d*) *Determination of potassium in blood serum and milk*—A sample of ox blood was obtained and the serum separated. The proteins were precipitated from 200 ml. to give a total volume of 1 litre. In the same way the proteins were precipitated from 100 ml. of separated milk, with the aid of heating to 70° C., to give a total volume of 1 litre. Duplicate 200-ml. samples of blood filtrate, and duplicate 50-ml. samples of milk filtrate were evaporated in silica dishes. In the final stages the residues were moistened with a few ml. of concentrated sulphuric acid and further evaporated on sand-baths before completing the ignition in a muffle furnace. The potassium was extracted from the residues with hot water and determined by the volumetric method of Wilcox. Aliquots of the original filtrates were diluted 1 in 2 and 1 in 10 respectively, and the clouds were formed in duplicate tubes by the macro-turbidimetric procedure. Readings were made on the "Spekker," 30 minutes after mixing, and the potassium content read off from the calibration curve. The results recorded in Table IX show a good agreement between the standard analytical method and the rapid turbidimetric determination.

## Summary

Aqueous sodium cobaltinitrite solution may be used together with an alcohol and a glycol in a single reagent mixture for the precipitation of potassium cobaltinitrite as a finely divided cloud suitable for turbidimetric determination. The most convenient temperature for precipitation is 15° C. The reagent mixture that appeared to give best results contained 10 volumes of sodium cobaltinitrite solution (20 per cent. + 20 per cent. of sodium nitrite), 50 volumes of methyl alcohol, 30 volumes of propylene glycol and 10 volumes of glycerol, but a fair variation in the relative amounts of alcohol and glycol is possible without affecting the turbidity seriously, provided the true limit of solubility of sodium cobaltinitrite is not exceeded. For reliable results crystal nuclei must be absent from the reagent mixture and potassium solutions.

The cloud may be formed in a small volume of 2·5 to 3 ml. in a cylindrical glass tube and the turbidity measured directly with the aid of a test tube holder designed for use on the "Spekker"; or the precipitation may be done in larger volume in a test tube and the reading made in a rectangular glass cell.

The turbidimetric method gave good quantitative estimations of potassium in various soil extracting solutions and also in trichloroacetic acid filtrates from blood and milk. Compared with the volumetric cobaltinitrite method of Wilcox the results normally did not differ by more than 5 per cent.

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*August*, 1947

# The Colorimetric Estimation of Thiols

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In the course of another investigation the need arose for a method for the rapid estimation of small quantities of

(I) 2-hydroxyethanethiol, monothioethyleneglycol, $HO.CH_2.CH_2.SH$

in solution in

(II) 2 : 2′-dihydroxydiethyl sulphide, thiodiglycol, $(HO.CH_2.CH_2)_2S$.

The iodine titration method of Klason and Carlson,[1] the mercuric chloride method of Sampey and Reid[2] and the copper oleate method of Bond[3] were all found to be capable of giving accurate results, although none was applicable to the analysis of small quantities of the thiol - sulphide mixture. Efforts were therefore made to develop a colorimetric micro-method.

Tasker and Jones,[4] Lecher and Seifken[5] and Rheinboldt[6] have demonstrated that primary, secondary and tertiary thiols react with nitrosyl chloride to form coloured thionitrites, *viz.*—

$$R.SH + NOCl = R.SNO + HCl.$$

Later, Rheinboldt[7] showed that ethyl mercaptan, thiophenol and triphenyl thiocarbinol react similarly with nitrous acid in aqueous alcohol solution; he claimed that the colours develop gradually on standing and are stable in concentrated but evanescent in dilute solution.

It has been found that the cherry red 2-hydroxyethane-1-thionitrite, $HO.CH_2.CH_2.SNO$ obtained in alcoholic solution by interaction between (I) and aqueous sodium nitrite in presence of glacial acetic acid is rapidly developed and is perfectly stable for at least 10 minutes both in dilute and concentrated solution, and that the coloured solution obeys Beer's law over a wide range (0·5 to 5 mg. per ml.) and consequently is ideal for the purposes of quantitative colorimetric estimation.

The Hilger Spekker photo-electric absorptiometer was used in the development of the method, and the 1-cm. cuvette (10-ml. capacity) and No. 5 filter were found to combine maximum sensitivity with maximum range.

### Development of colour—

The best method for making the coloured solutions required for the standard curve was found to be as follows: $x$-ml. of a solution of the pure thiol (I) in absolute alcohol (containing 0·02516 g. per ml.) were pipetted into the cuvette by means of a graduated pipette; (6-$x$) ml. of absolute alcohol were then added from a burette, followed by exactly 1 ml. of 4 $N$ aqueous sodium nitrite and finally exactly 2 ml. of glacial acetic acid A.R., making a total of 9 ml.

The contents of the cuvette were then mixed immediately by shaking, and readings taken on the absorptiometer after 2, 3, 5, 8, 11 and 15 minutes. The results obtained are recorded in Table I.

Table I

Filter: 5. Cuvette: 10 mm. Range of curve: 5 to 150 mg. of (I)

| Volume of thiol (I) solution taken ml. | Wt. of thiol (I) g. | Absorptiometer readings after 2 min. | 3 min. | 5 min. | 8 min. | 11 min. | 15 min. |
|---|---|---|---|---|---|---|---|
| 0·2 | 0·0050 | 0·055 | 0·057 | 0·057 | 0·057 | 0·056 | 0·051 |
| 0·5 | 0·0126 | 0·121 | 0·125 | 0·124 | | | |
| 1·0 | 0·0252 | 0·245 | 0·261 | 0·267 | 0·267 | 0·267 | 0·267 |
| 1·5 | 0·0378 | 0·342 | 0·369 | 0·369 | 0·369 | 0·369 | 0·366 |
| 2·0 | 0·0503 | 0·371 | 0·440 | 0·450 | 0·460 | 0·465 | 0·467 |
| 3·0 | 0·0755 | 0·480 | 0·560 | 0·575 | 0·595 | 0·600 | 0·605 |
| 4·0 | 0·1006 | 0·568 | 0·645 | 0·675 | 0·699 | 0·703 | 0·710 |
| 5·0 | 0·1258 | 0·658 | 0·710 | 0·738 | 0·770 | 0·775 | 0·778 |
| 6·0 | 0·1509 | 0·665 | 0·740 | 0·780 | 0·790 | 0·805 | 0·815 |

From these results it is apparent that the more dilute solutions rapidly reach the point of maximum colour intensity, and maintain their maximum colour for about 5 minutes

only before beginning to fade; with increase in concentration, both the time taken to reach maximum intensity and the stability increase. Provided the timing is made accurately it is possible to get exactly reproducible results, and for the purposes of the proposed method the standard curve is made from the 3-minute readings; only the reading after 3 minutes is required when carrying out the analysis.

It is essential that the sodium nitrite solution used should be exactly 4 *N*, as any variation in the strength directly affects the intensity of the coloured solution. A 4 *N* solution has been found to be the best in practice, as more dilute solutions give less intense colours, whilst from more concentrated solutions solid sodium nitrite tends to be precipitated on the addition of alcohol, and even if this does not occur the final increase in colour intensity is very small compared with that obtained from a 4 *N* solution, although rapidity of colour development increases with increase in concentration of the sodium nitrite solution. This is shown by the findings recorded in Table II, where the only variable is the concentration of the aqueous sodium nitrite solution used.

TABLE II

Filter: 5. Cuvette: 10 mm. (I) in 6 ml. of EtOH + 1 ml. of aq. $NaNO_2$ + 2 ml. of AcOH

| Concn. of aq. $NaNO_2$ used | Wt. of thiol (I) in 9 ml. | Absorptiometer readings after | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | g. | 2 min. | 5 min. | 8 min. | 11 min. | 15 min. | 18 min. | 22 min. | 30 min. |
| 2 *N* | 0·0693 | 0·273 | 0·320 | 0·340 | 0·350 | 0·364 | 0·370 | 0·387 | 0·410 |
| 4 *N* | " | 0·550 | 0·590 | 0·618 | 0·618 | 0·620 | 0·620 | 0·620 | 0·620 |
| 6 *N* | " | 0·610 | 0·630 | 0·630 | 0·630 | 0·630 | | | |

TABLE III

| Percentage of (I) in mixture of (I) and (II) | Wt. of mixture per 50 ml. of Solution A g. | Absorptiometer readings | Wt. of (I) per 5 ml. of Solution A, found from standard curve g. | Percentage of (I) in mixture, found |
|---|---|---|---|---|
| 0 | 10·5420 | 0·0; 0·0 | 0·0 | 0·0 |
| 3·98 | 10·2110 | 0·415; 0·410 | 0·0440 | 4·31 |
| 6·48 | 6·2885 | 0·398; 0·402 | 0·0420 | 6·68 |
| 10·1 | 4·1129 | 0·398; 0·400 | 0·0420 | 10·2 |
| 15·6 | 2·6113 | 0·400; 0·400 | 0·0420 | 16·0 |
| 49·8 | 0·8174 | 0·395; 0·388 | 0·0410 | 50·2 |
| 100 | 0·4058 | 0·392 | 0·0405 | 100 |

TABLE IV

Filter: 5. Cuvette: 10 mm.

| Percentage of (I) in mixture of (I) and (II) as made up | Weight of (I) in 9 ml. of solution used in cuvette g. | Absorptiometer readings | | Percentage increase in absorptiometer reading due to presence of (II) 100(A − B)/B |
|---|---|---|---|---|
| | | A With (II) | B Without (II) | |
| 1·02 | 0·0209 | 0·235 | 0·222 | 5·9 |
| 1·02 | 0·0419 | 0·426 | 0·401 | 6·2 |
| 2·01 | 0·0417 | 0·409 | 0·386 | 6·0 |
| 3·23 | 0·0207 | 0·225 | 0·213 | 5·6 |
| 3·30 | 0·0417 | 0·408 | 0·386 | 5·7 |
| 4·02 | 0·0417 | 0·406 | 0·386 | 5·2 |
| 5·18 | 0·0417 | 0·402 | 0·386 | 4·2 |
| 5·86 | 0·0209 | 0·228 | 0·222 | 2·7 |
| 5·86 | 0·0419 | 0·412 | 0·401 | 2·7 |
| 7·21 | 0·0209 | 0·223 | 0·222 | 0·4 |
| 7·21 | 0·0419 | 0·406 | 0·401 | 1·2 |
| 8·16 | 0·0419 | 0·406 | 0·401 | 1·2 |

EFFECT OF EXCESS OF THIODIGLYCOL—

The efficacy of the method as a means of estimating (I) quantitatively in the presence of (II) was assessed by analysing mixtures of (I) and (II) in various known proportions. An amount of the thiol - sulphide mixture containing about 0·4 g. of the thiol (I) was accurately weighed, and made up to 50 ml. with absolute alcohol ( solution A). Five ml. of this solution

thus obtained, containing approximately 0·04 g. of (I), were then examined by the colorimetric method. The results are shown in Table III.

From these results it is apparent that the presence of the sulphide (II) tends to enhance the colour intensity, but this increase never represents an error of more than 0·4 unit in the percentage of (I) in the mixture, and as an error on the amount of (I) present it only becomes serious when the concentration of (I) in the mixture is less than about 6 per cent. Even in these circumstances, however, the method is applicable, as the percentage increase in colour intensity is constant for a given thiol - sulphide ratio irrespective of the dilution of the mixture in the cuvette. These percentage increases in colour intensity were determined experimentally, with the following results.

Neither 2 : 2′-dihydroxyethyl sulphide (II), chloroethylchlorovinyl sulphide, 2 : 2′-di(β-chloroethylthio)diethylether nor dithian gives coloured solutions with nitrous acid.

The method finally developed for the estimation of (I) in an inert diluent or admixed with (II) is as follows.

## Method

*Reagents required*—Glacial acetic acid A.R., absolute alcohol, and aqueous sodium nitrite solution, exactly 4 *N*.

*Procedure*—Weigh accurately an amount of the material to be examined containing not more than 0·4 g. and not less than 0·1 g. of monothio-ethyleneglycol into a 50-ml. graduated flask and dilute with absolute alcohol to exactly 50 ml.

Transfer 6 ml. of this solution by means of a graduated pipette into a Hilger 10-mm. (10-ml. capacity) cuvette and add exactly 1 ml. of the 4 *N* aqueous sodium nitrite solution from a burette, followed immediately by exactly 2 ml. of glacial acetic acid A.R. from another burette. Start a stop-watch at once and intimately mix the contents of the cuvette by shaking. Measure the intensity of the coloured solution on the Spekker photo-electric absorptiometer exactly 3 minutes after the addition of the acetic acid.

Use No. 5 green filters on both sides of the lamp.

*Standard solution*—It is necessary to check the standard curve from time to time, and for this purpose a standard solution is prepared containing exactly 0·5 g. of pure monothio-ethyleneglycol per 100 ml. of alcohol solution, and 6 ml. of this are treated exactly as described above. A standard solution thus prepared will keep for several weeks in the dark in a stoppered bottle filled to the neck, and can be checked by iodine titration.

*Calculation*—

If the weight of sample originally taken = $w$ g.
If the reading of abscissa on the gauging curve corresponding to absorptiometer reading of the unknown = $a$
and that of the standard = $b$
Then the percentage of monothioethyleneglycol in the sample

$$= \frac{0{\cdot}03 \times a \times 50 \times 100}{6 \times w \times b} = 25a/bw \qquad \text{(i)}$$

The result thus obtained is accurate in the examination of monothioethyleneglycol alone or in presence of thiodiglycol in concentrations greater than 6·5 per cent. If the monothioethyleneglycol content thus found is less than 6·5 per cent. then the percentage of monothioethyleneglycol = $25af/bw$, where $f$ is a correction factor obtained from the following table, based on the results shown in Table IV, corresponding to the approximate monothioethyleneglycol content found by (i).

| Percentage of (I) found by equation (i) | Correction factor $f$ |
|---|---|
| 0 to 4·0 | 0·945 |
| 4·0 to 5·0 | 0·954 |
| 5·0 to 5·5 | 0·962 |
| 5·5 to 6·0 | 0·973 |
| 6·0 to 6·5 | 0·985 |

## Applications—

The method appears to be of general applicability to thiols. Standard curves for 2 : 2′-dimercaptodiethyl ether, $O(C_2H_4SH)_2$, ethyl mercaptan, $C_2H_5SH$, and 2-hydroxypropane-1-thiol, $HO.CHMe.CH_2.SH$, were made in addition to that for 2-hydroxyethane-1-thiol. The data obtained for them are recorded in Table V.

TABLE V

DATA FOR STANDARD CURVES FOR ETHYL MERCAPTAN, 2-HYDROXYETHANE-1-THIOL, 2-HYDROXYPROPANE-1-THIOL AND 2 : 2′-DIMERCAPTODIETHYL ETHER

Filter: 5. Cuvette: 10 mm.

Normal quantities of EtOH, 4 *N* $NaNO_2$ and acetic acid

| $C_2H_5SH$ | | $HO.C_2H_4.SH$* | | $HO.CHMe.CH_2.SH$ | | $O(C_2H_4SH)_2$ | |
|---|---|---|---|---|---|---|---|
| g. | absorption | g. | absorption | g. | absorption | g. | absorption |
| 0·01829 | 0·225 | 0·01 | 0·130 | 0·0118 | 0·108 | 0·0354 | 0·382 |
| 0·03658 | 0·390 | 0·02 | 0·243 | 0·0236 | 0·227 | 0·0708 | 0·588 |
| 0·05487 | 0·515 | 0·03 | 0·345 | 0·0354 | 0·335 | 0·1062 | 0·708 |
| 0·07316 | 0·598 | 0·04 | 0·424 | 0·0472 | 0·410 | | |
| 0·09145 | 0·655 | 0·05 | 0·492 | 0·0590 | 0·495 | | |
| | | 0·06 | 0·529 | 0·0708 | 0·522 | | |
| | | 0·07 | 0·580 | 0·0826 | 0·565 | | |
| | | 0·08 | 0·613 | 0·0944 | 0·580 | | |
| | | 0·10 | 0·668 | 0·1180 | 0·650 | | |
| | | 0·12 | 0·700 | 0·1416 | 0·685 | | |

* These absorptiometer readings differ from those recorded in Table I on account of differences between the galvanometers used.

Thanks are due to the Chief Scientist, Ministry of Supply, for permission to publish.

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H.M. RESEARCH ESTABLISHMENT
SUTTON OAK

*November*, 1947

# The Polarographic Estimation of Inorganic Nitrates in Propellants

BY J. PEARSON AND A. J. HOWARD

POTASSIUM nitrate is sometimes incorporated in propellants and has been estimated by a polarographic method, which depends upon a determination of potassium in the ashed propellant and calculation to nitrate. The potassium content found must be corrected for the amount of sodium also present. An estimation of nitrate, which is specific to the added ingredient, would be much sounder in principle.

## EXPERIMENTAL

The method employed for the estimation of nitrate was that proposed by Kolthoff, Harris and Matsuyama,[1] and a calibration graph relating the diffusion current to nitrate concentration was prepared by the method to be described further on.

To each of six 250-ml. beakers was added 0·3 g. of nitroguanidine and 0, 2, 4, 6, 8 and 10 mg. of potassium nitrate in aqueous solution. The contents of the beakers were made up to approximately 80 ml. and heated just to boiling to bring all the nitroguanidine into solution. They were then allowed to cool to room temperature, whereupon most of the nitroguanidine crystallised out. They were filtered through No. 41 papers into 100-ml. measuring flasks and washed with water so that the volume of filtrate and washings was 100 ml. Twenty ml. of each solution were pipetted into glass evaporating basins and 4 drops of methyl orange indicator and 4 ml. of *N* aqueous potassium hydroxide were added. The solutions were evaporated to dryness on a steam-bath and the residues washed into 50-ml. measuring flasks with about 20 ml. of water. These solutions were then titrated with *N* hydrochloric acid to the first permanent pink coloration, 10 ml. of the supporting electrolyte

were added and the volumes made up to the mark. Portions of the resulting solutions were then polarographed, and when the diffusion currents were plotted against the amount of nitrate the points fell on the calibration graph previously prepared. This indicated that the separated crystalline nitroguanidine had not adsorbed any measurable amount of nitrate and that no nitrate was produced or lost during the alkaline destruction of the dissolved nitroguanidine.

Nitrocellulose is not reducible at the dropping mercury cathode, but when decomposition of nitroguanidine has to be effected it is of importance that nitrocellulose should not gain access to the solutions to be so treated, as it is decomposed by alkali to give compounds such as nitrates and nitrites which would interfere with the estimation desired. In the determination of nitrate in normal propellants the nitrocellulose is purposely precipitated in a very fine form to facilitate removal of inorganic salts, a condition which favours the colloidal suspension of nitrocellulose. Fortunately, this precipitation is not necessary when dealing with propellants containing nitroguanidine in relatively large proportions. These have a more "open" structure and water-extraction removes all soluble material leaving the nitrocellulose behind in a coarse form. To test whether any interference would arise from suspended nitrocellulose the following experiment was carried out. A sample of a nitroguanidine propellant, which contained potassium sulphate and no nitrate, was submitted to the procedure described below under Method. No nitrate was found. To a similar sample, potassium nitrate equivalent to 1·2 per cent. was added. On carrying out an estimation the same proportion was found. This indicates that in spite of the use of rapid filter papers (No. 41) the amount of nitrocellulose that gained access to the aqueous extract was, for all practical purposes, negligible.

## Method

### Reagents—

*Supporting electrolyte*—This contains 0·5 *M* potassium chloride, 0·05 *M* hydrochloric acid and 0·001 *M* uranyl acetate.

*Potassium hydroxide*—Normal solution.

*Hydrochloric acid*—Normal solution.

### Calibration—

Prepare an aqueous solution containing 0·200 g. of pure dry potassium nitrate per litre. Place 0, 2, 4, 6, 8 and 10 ml. of this in six 50-ml. measuring flasks. Add exactly 10 ml. of the supporting electrolyte to each flask and make up to the mark with water. Mix well and polarograph a portion, after flushing out with nitrogen or hydrogen, at appropriate galvanometer sensitivity, between −0·75 and −1·5 volts *versus* the internal mercury anode. Measure the diffusion currents and plot against the amount of added nitrate.

### Estimation of potassium nitrate in propellants containing nitroguanidine—

Extract 1·000 g. of the ground propellant with carbon tetrachloride for 6 hours in a Soxhlet apparatus, to remove nitroglycerine, etc. Dry the residue at 100° to 105° C. and transfer it as completely as possible to a dry 150-ml. beaker. Add about 50 ml. of boiling water and stir vigorously. Decant the liquid through a No. 41 Whatman paper into a 250-ml. conical flask, retaining the nitrocellulose in the beaker. Continue the washing by decantation with hot water until a total volume of about 150 ml. has been used. Wash the thimble in which the propellant had been extracted with about 30 ml. of boiling water, adding the washings to the conical flask. Allow the liquid to cool to room temperature and to crystallise. Filter through a No. 41 Whatman paper into a 200-ml. measuring flask, wash the residue with water and make up the volume of the filtrate and washings to the mark. Mix well, pipette 20 ml. of the solution into a glass evaporating basin, add a few drops of methyl orange indicator and 4 ml. of *N* potassium hydroxide, and evaporate to dryness on a steam-bath. Wash the residue with about 20 ml. of water into a 50-ml. measuring flask and add *N* hydrochloric acid slowly from a burette until a permanent pink coloration just appears. Add exactly 10 ml. of the supporting electrolyte, make up to the mark with water and mix well. Polarograph a portion of the solution as described above. Measure the diffusion current and calculate the content of potassium nitrate by reference to the calibration graph.

ESTIMATION OF POTASSIUM OR OTHER INORGANIC NITRATE IN PROPELLANTS NOT CONTAINING NITROGUANIDINE—

Extract 1·000 g. of the ground propellant with ether (sp.gr. 0·720) for 6 hours in a Soxhlet apparatus and remove most of the ether from the residue by drying in an oven. Transfer the nitrocellulose to a 150-ml. conical flask and dissolve it in 20 to 25 ml. of acetone. Add, slowly and with constant agitation, 70 ml. of water to precipitate the nitrocellulose in a finely divided form. Heat cautiously, with frequent swirling, on a steam-bath until all the acetone is evaporated and then dry completely. Add 100 ml. of water from a pipette, stopper the flask and shake it vigorously to dissolve the inorganic salts. Filter through a dry No. 42 Whatman paper, rejecting the first runnings. Alternatively the nitrocellulose may be washed and the complete water extract made up to 100 ml. To a 25-ml. measuring flask add 5 ml. of the supporting electrolyte and a measured volume of the filtered water extract. Make up to the mark with water, mix, polarograph as described above, and calculate the nitrate content. Since there is a limit to the concentration of nitrate, beyond which the diffusion current is no longer proportional to the concentration,[1] if the diffusion current is greater than can be read from the calibration graph take another polarogram on a solution made up with a smaller volume of the water extract. If the diffusion current is too small to be conveniently measured, use a correspondingly larger volume of the water extract.

## RESULTS

Some typical results of the estimation of potassium nitrate in propellants are given in Tables I and II.

TABLE I

PROPELLANTS CONTAINING NITROGUANIDINE

| Propellant | $KNO_3$ found polarographically % | $KNO_3$ calculated from potassium content determined as $KClO_4$ % |
|---|---|---|
| 1 | 1·20; 1·16 | 1·16; 1·17 |
| 2 | 1·14; 1·16 | 1·17; 1·19 |
| 3 | 1·13; 1·13 | 1·13; 1·14 |
| 4 | 1·10; 1·08 | 1·09; 1·13 |
| 5 | 1·10; 1·08 | 1·12; 1·10 |
| 6 | nil | $K_2SO_4$ only present |
| 6 + 1·2% of added $KNO_3$ | 1·22 | — |

TABLE II

| Propellant | $KNO_3$ found polarographically % | $KNO_3$ calculated from analysis of of sulphated ash % |
|---|---|---|
| 7 | 1·40 | 1·33 |
| 8 | 0·18 | 0·20 |
| 9 | 0·76; 0·78 | 0·84 |
| 10 | 2·40 | 2·37 |
| 11 | nil | No $KNO_3$ present |
| 11 + 0·5% of added $KNO_3$ | 0·50; 0·53 | — |
| 12 | 1·14 | 1·16 |
| 13 | 0·92 | 0·91 |
| 14 | 2·33 | 2·36 |
| 15 | 1·48 | 1·44 |

NOTES—(1) Pairs of figures are for duplicate estimations.
(2) The other essential constituents of the propellants were nitrocelluloses, nitroglycerine, diphenylamine, diethyldiphenylurea, phthalates, mineral jelly and graphite.

## SUMMARY

The method proposed by Kolthoff, Harris and Matsuyama for the polarographic estimation of nitrate has been adapted to the estimation of nitrate in propellants. A method has been developed for destroying nitroguanidine, present in some propellants, which would otherwise interfere.

The methods described offer simple, comparatively rapid and accurate means for the estimation of inorganic nitrates in a wide range of propellants. With suitable choice of procedure they could be used in routine specification analysis, for individual determinations and, if necessary, on extremely small samples.

The authors wish to thank the Chief Scientist, Ministry of Supply, for permission to publish this communication.

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EXPLOSIVES RESEARCH AND DEVELOPMENT ESTABLISHMENT
MINISTRY OF SUPPLY
WOOLWICH, LONDON, S.E.18

*April*, 1948

# The Determination of Small Amounts of Hydroquinone in Styrene

BY S. M. A. WHETTEM

(*Read at the Meeting of the Society, on Wednesday, November 3rd*, 1948)

HYDROQUINONE is used to stabilise certain organic liquid monomers, such as styrene, which would otherwise polymerise to solids at ordinary room temperatures. It is often necessary to know precisely how much hydroquinone there is present in the stabilised material, or how much remains in the purified material.

The reduction of phosphomolybdate solutions by hydroquinone under certain specified conditions is the basis of a method by Straub[1] for the determination of phosphates in water and it seemed likely that this reaction could be applied to the determination of small amounts of hydroquinone. When this was found to be unsuccessful, attention was directed to the possible reduction of phosphotungstate solutions by the hydroquinone. The uric acid reagent of Folin and Denis,[2] as originally prepared, was found to be suitable. According to Wu[3] this reagent is phospho-18-tungstic acid.

## EXPERIMENTAL

Solutions of hydroquinone were prepared containing from 0·01 to 0·10 mg. in 50 ml. of water. The first attempt to develop the molybdenum blue colour was made by adding to these solutions 2 ml. each of 20 per cent. v/v sulphuric acid, 10 per cent. w/v solution of A.R. ammonium molybdate and 10 per cent. w/v solution of A.R. diammonium phosphate, $(NH_4)_2HPO_4$. The colours produced were pale green and increased only slightly in intensity with the concentration of hydroquinone.

It was observed that if these solutions were made alkaline with sodium carbonate, the colours became more bluish and more intense. A second series of hydroquinone solutions was prepared containing the same amounts of hydroquinone in 40 ml. of water instead of 50 ml., to allow for subsequent addition of sodium carbonate solution. The same reagents were added as before and the green colour was developed for 15 minutes. At the end of that time the solutions were made alkaline with 10 ml. of 10 per cent. w/v sodium carbonate solution, and the colours measured in a Lovibond Tintometer, using 4-inch cells. The results, which are given in Table I, were promising in that the increase in colour intensity with the concentration of hydroquinone was more marked and the colours themselves more bluish and better to match.

It was considered, however, that the intensities were still not good enough to form the basis of a colorimetric method and attention was turned to the possibility of obtaining better results by using the phosphotungstate reaction. The sodium phosphotungstate reagent[2] was prepared as described below under REAGENTS.

To the same range of hydroquinone solutions, that is, 0·01 to 0·10 mg. of hydroquinone in 50 ml. of water, were added 2 ml. of the sodium phosphotungstate reagent and 4 ml. of 10 per cent. w/v sodium carbonate solution, making the total bulk of solution 56 ml., as in the previous experiments. The blue colours that developed were measured in the Tintometer, using 4-inch cells as before. The results are shown in Table I.

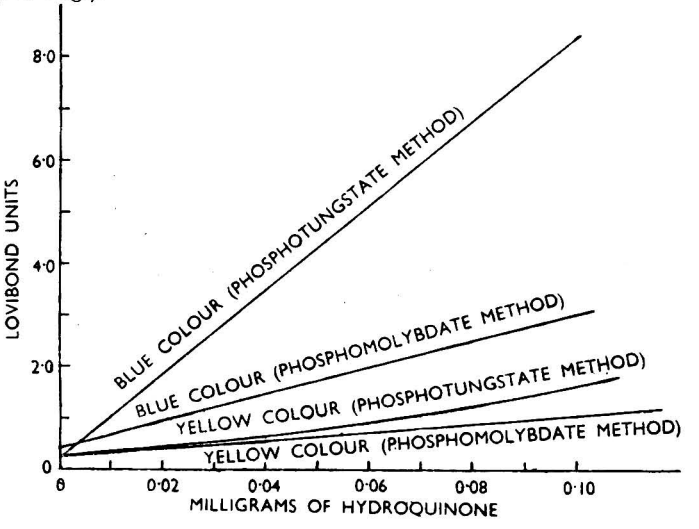
TABLE I

| Hydroquinone mg. | Lovibond units: Phosphomolybdate (a) Blue | Phosphomolybdate (a) Yellow | Phosphomolybdate (b) Blue | Phosphomolybdate (b) Yellow | Phosphotungstate (a) Blue | Phosphotungstate (a) Yellow | Phosphotungstate (b) Blue | Phosphotungstate (b) Yellow |
|---|---|---|---|---|---|---|---|---|
| Blank | 0·4 | 0·2 | 0·4 | 0·2 | 0·2 | 0·2 | 0·2 | 0·2 |
| 0·01 | 0·6 | 0·4 | 0·6 | 0·4 | 1·0 | 0·3 | 1·0 | 0·3 |
| 0·03 | 1·1 | 0·5 | 1·1 | 0·5 | 2·6 | 0·5 | 2·6 | 0·5 |
| 0·05 | 1·6 | 0·6 | 1·7 | 0·6 | 4·3 | 0·7 | 4·3 | 0·7 |
| 0·07 | 2·1 | 0·7 | 2·1 | 0·7 | 6·1 | 1·0 | 6·0 | 1·0 |
| 0·10 | 3·0 | 1·0 | 3·1 | 0·9 | 8·4 | 1·6 | 8·0 | 1·4 |

(*a*) = readings taken 15 minutes after the addition of the reagents; (*b*) = readings after 3 hours.

These results show that, for the same concentrations of hydroquinone, the blue colour produced by the sodium phosphotungstate reagent is much more intense than that produced by the ammonium phosphomolybdate reagent. The yellow component is of about the same order in both methods and is comparatively small. The optimum time for colour development is approximately 15 minutes and the colours after 3 hours show only slight fading in the maximum part of the range.

By plotting graphs showing Lovibond units against milligrams of hydroquinone, it is seen that, in the range investigated, approximately linear relationships are obtained by both methods (see Fig.).




APPLICATION TO THE DETERMINATION OF HYDROQUINONE IN STYRENE—

The above experiments indicated that the Folin - Denis reagent, employed in the manner described, was suitable for the colorimetric determination of hydroquinone and might be applied to determining hydroquinone in styrene.

Solutions of freshly distilled styrene were prepared containing 0·01 to 0·10 mg. of hydroquinone per ml. of styrene. The hydroquinone in these solutions was extracted with water. The method used and the results obtained are given below.

## METHOD

REAGENTS—

*Sodium phosphotungstate solution*—Boil together, under reflux, 10 g. of sodium tungstate (B.D.H. re-crystallised), 8 ml. of phosphoric acid (AnalaR) and 90 ml. of water for 1½ hours. Cool the solution and, if necessary, make up to 100 ml. with water.

*Sodium carbonate solution*—Prepare a 10 per cent. w/v aqueous solution of sodium carbonate A.R., filtering if necessary.

*Standard solution of hydroquinone*—Weigh out 1 g. of hydroquinone and dissolve in water in a 1 litre measuring flask. Make up to the mark with water and mix well. Dilute 100-fold for use. One ml. of this final solution ≡ 0·01 mg. of hydroquinone.

PROCEDURE—

Pipette 1 ml. of the sample of the styrene into a 50-ml. separating funnel. Extract the hydroquinone with 40 ml. of water, using 20 ml. for the first, 10 ml. for the second and 10 ml. for the third extraction. Collect the aqueous extracts in a 50-ml. Nessler cylinder and dilute with water to 50 ml. In similar Nessler cylinders prepare standards containing 1·0, 3·0, 5·0, 7·0 and 10 ml. of the standard hydroquinone solution and dilute each to 50 ml. To the sample and standards, add 2 ml. of the sodium phosphotungstate solution and 4 ml. of the sodium carbonate solution, *in this order*, mixing well after the addition of each reagent. Allow the solutions to stand for 15 minutes and compare the colour of the sample with that of the standards.

TABLE II

RESULTS ON STYRENE CONTAINING KNOWN AMOUNTS OF HYDROQUINONE

| Hydroquinone added to 1 ml. of styrene mg. | Hydroquinone found mg. |
|---|---|
| 0·010 | 0·010 |
| 0·030 | 0·030 |
| 0·050 | 0·047 |
| 0·070 | 0·070 |
| 0·100 | 0·096 |

Table II indicates that the method described for the extraction and determination of hydroquinone in styrene in these concentrations gives results within the experimental error and that the method is sensitive to 0·01 of a milligram of hydroquinone in 1 ml. of styrene or 10 mg. per litre. It can be made to detect 1 mg. per litre by extracting 10 ml. of styrene instead of 1 ml. with water.

RAPID METHOD FOR PROCESS CONTROL—

To avoid the necessity of making up standards for each determination, a colour disc is obtainable from Tintometer Ltd. containing 9 glasses, representing the range 0·01 to 0·09 mg. of hydroquinone, for use with the B.D.H. Lovibond Nessleriser. A "brightness screen" is also supplied with the disc, which is so placed on the top of the Nessleriser that the sample solution is viewed through the screen.

Treat the sample exactly as before, using one of the Nessleriser cylinders for the development of the blue colour. Prepare a reagent blank in the second cylinder by filling with water to the 50-ml. mark and adding 2 ml. of the sodium phosphotungstate solution and 4 ml. of the sodium carbonate solution. Place the sample solution in the right-hand side and the blank in the left-hand side of the Nessleriser. After 15 minutes have elapsed from the time of adding the reagents, match the colour of the sample with the colour glasses and read off the number of milligrams of hydroquinone shown on the disc.

INTERFERING SUBSTANCES—

Aqueous solutions of the following phenolic substances were prepared such that 1 ml. of each was equal to 0·01 mg. Five ml. of each solution were taken and made up to 50 ml. with water and the colour was developed in the same way as for hydroquinone. These colours were matched against the usual series of hydroquinone standards, with the following results.

| Milligrams of substance present | | Colour expressed as milligrams of hydroquinone |
|---|---|---|
| Phenol | 0·05 | None |
| *ortho*-Cresol | 0·05 | None |
| *meta*-Cresol | 0·05 | None |
| *para*-Cresol | 0·05 | None |
| Resorcinol | 0·05 | Trace (less than 0·01) |
| Pyrocatechol | 0·05 | 0·09 |
| Tannic acid | 0·05 | 0·02 |

It appears, therefore, that of these substances, only pyrocatechol and tannic acid interfere seriously with the method.

HYDROQUINONE IN METHACRYLIC ESTERS—

Although the above investigation was carried out for the determination of hydroquinone in styrene, the method has been applied with success to methyl methacrylate monomer and other methacrylates.

SUMMARY

A method has been described for the determination of hydroquinone in styrene, which will determine concentrations down to 10 parts of hydroquinone per million of styrene w/v and can be modified to detect 1 part per million. Interference to be expected by related substances is detailed. The method has been applied with success to methacrylic esters. A rapid method using a Lovibond Nessleriser disc is also described.

The author wishes to thank Mr. Haslam for his valuable assistance in preparing this script, and Mr. Chamberlain of Messrs. Tintometer Ltd., for co-operating and supplying a suitable colour disc for the rapid method.

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IMPERIAL CHEMICAL INDUSTRIES LTD.
PLASTICS DIVISION
RESEARCH DEPARTMENT
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DISCUSSION

The PRESIDENT asked how much hydroquinone is used to stabilise styrene.

Mr. W. C. WAKE said that hydroquinone and allied substances, which presumably could be estimated, are occasionally used, for research purposes, as anti-oxidants in rubber. One would like an assurance that oxidation products do not interfere.

Dr. W. ROMAN pointed out that other reducing agents would give the same reaction, and interfere; it was unlikely, however, that they would be present in styrene.

Dr. A. J. AMOS enquired as to the effect of changing the order in which the reagents are added to the test solution.

Mr. WHETTEM, in reply to the President, said that the amount of hydroquinone used to stabilise styrene was of the order of 0·01 per cent. In answer to Mr. Wake's question he said that quinone did not interfere with the reaction. Replying to Dr. Amos, he stated that if the sodium carbonate reagent was added before the phosphotungstate reagent no blue colour was produced.

# Notes

## THE USE OF REDUCED COPPER IN THE MICRO-DETERMINATION OF CARBON AND HYDROGEN

THE purpose of this note is to describe some preliminary attempts to avoid the use of lead dioxide in the combustion of nitrogenous organic compounds.

Niederl and Whitman[1] have described a method in which the substance, after being mixed with copper oxide, is burned in a stream of nitrogen. The following experiments were suggested by the modification of the micro-Dumas method devised by Dr. J. Unterzaucher[2] and successfully used in the laboratories of I.G. Farben, A.-G., Leverkusen. In this method the substance, contained in a platinum boat, is burned in a stream of carbon dioxide that has been bubbled through hydrogen peroxide to which a few scraps of platinum wire have been added to catalyse the production of oxygen. The oxygen transported by the carbon dioxide is sufficient to burn the sample, the excess being removed by a layer of reduced copper the greater part of which is not heated. In adapting the method to the determination of carbon and hydrogen, nitrogen was used as the transport gas; this was bubbled through a wash-bottle containing about 50 ml. of hydrogen peroxide and a few pieces of platinum wire. After passing through a flow-meter and a U-tube containing Ascarite and Anhydrone the gas was led into the side-arm of a silica micro-combustion tube.

The combustion tube contained an asbestos choking plug followed by 180 mm. of reduced copper and finally 150 mm. of copper oxide. The portion of the tube containing the reduced copper was covered by a metal sleeve and heated with a bunsen burner fitted with a flame spreader; the copper oxide was heated to redness by means of a small electric furnace.

The absorption tubes were of the normal Pregl type containing Anhydrone to absorb water and Ascarite to absorb carbon dioxide, and were weighed while filled with nitrogen.

Three to 5 mg. of material were weighed into a platinum boat and burned by the normal Pregl technique, taking about 20 minutes, during which time 120 ml. of gas were passed through the tube.

The introduction of silver into the combustion tube should make possible the analysis of substances containing halogen or sulphur, but owing to pressure of other work it has not yet been tried.

It is hoped to return to this investigation in the near future.

RESULTS

| | Found | | Calculated | |
|---|---|---|---|---|
| | % C | % H | % C | % H |
| Strychnine | 75·66 | 6·69 | 75·45 | 6·58 |
| | 75·61 | 6·66 | | |
| Nitrobenzoic acid | 50·61 | 2·98 | 50·30 | 2·98 |
| | 50·46 | 2·87 | | |
| | 50·56 | 3·14 | | |
| Aminoacetic acid | 32·17 | 6·66 | 32·00 | 6·66 |
| | 32·19 | 6·48 | | |
| Azobenzene | 79·11 | 5·70 | 79·11 | 5·48 |
| | 79·29 | 5·64 | | |

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WELLCOME CHEMICAL RESEARCH LABORATORIES
BECKENHAM, KENT

A. BENNETT
*August*, 1948

## ESTIMATION OF CYANIDE IN PLANT MATERIAL BY MEANS OF CONWAY UNITS

THE material on which this estimation was performed was a "feeding cake" imported from Turkey which had caused the death of several cows. The sample was found to be capable of producing considerable quantities of hydrocyanic acid and was most probably a bitter almond cake. Routine examination gave the following percentage figures: moisture at 100° C., 9·0; crude fat, 7·8; ash, 5·4; crude fibre, 5·8; protein ($N \times 6{\cdot}25$), 43·1; carbohydrates (as difference from 100), 28·9.

An attempt to determine the hydrocyanic acid by digestion at 60° C. for from 3 to 6 days, followed by aspiration of air in accordance with J. H. Roe's method,[1] proved unsatisfactory. Other methods found in the literature consulted[2] did not appear to be any more promising, and it occurred to us to try the use of Conway units,[3] which had already been found satisfactory for cyanides in general by Dr. Hirsch in these laboratories. The method finally adopted gave very satisfactory results and seems ideal for plant material, as the hydrocyanic acid liberated in the outer chamber of the unit is constantly absorbed in the inner chamber, thus enabling the decomposition of the glucoside to proceed to completion. The whole method is extremely simple, requiring no incubation and no aeration. Addition of tartaric acid was found unnecessary, though this might depend on the material analysed. At the end of an estimation all the units were kept for a further 24 hours to confirm that liberation of hydrocyanic acid was complete. The results obtained on the sample were 0·409 to 0·432 g. per cent. of hydrocyanic acid.

*Recommended method*—0·5 to 1·0 g. of the well-mixed and finely ground material is covered with 6 ml. of hot water at 60° C. If the material is too bulky for the Conway unit a squat weighing bottle (30 mm. by 60 mm., *Gallenkamp*, 11th Ed., No. 12,234) may be used, with a small Petri dish inserted in place of the middle chamber; it is advisable to keep the proportion between the sizes of the inner and outer chambers similar to that in Conway units so that the absorbing surface should be adequate. With the original Conway unit the maximum quantity of sample to be taken is 1 g., as the chamber is rather small. Into the central chamber 1 ml. to 2 ml. of 10 per cent. potassium hydroxide solution are introduced, and then the cover is smeared with yellow vaseline and the unit closed. It is allowed to stand at room temperature for 3 days, after which we found it advisable to add 2 ml. more of hot water at about 80° C. and allow it to stand for one more day. After addition of a few crystals of potassium iodide the liquid in the central chamber is titrated with 0·1 *N* or 0·02 *N* silver nitrate, according to the quantity of hydrocyanic acid expected. The end-point is sharp; 1 ml. of 0·1 *N* silver nitrate ≡ 0·0054 g. of HCN.

The accompanying table gives the results of a series of determinations.

| Time allowed for decomposition, hours | Wt. of sample taken, g. | 0·1 *N* $AgNO_3$ used, ml. | HCN found, % | Remarks |
|---|---|---|---|---|
| 48 | 2 | 0·86 | 0·232 | |
| 48 | 2 | 1·0 | 0·270 | |
| 72 | 2 | 1·32 | 0·356 | |
| 72 | 2 | 1·34 | 0·361 | |
| 72 | 2 | 1·25 | 0·337 | |
| 96 | 2 | 1·43 | 0·386 | |
| 96 | 2 | 1·43 | 0·386 | in squat weighing bottles |
| 96 | 2 | 1·41 | 0·380 | |
| 96 | 2 | 1·43 | 0·386 | |
| 96 | 1 | 0·76 | 0·410 | |
| 96 | 0·5 | 0·38 | 0·410 | |
| 96 | 0·5 | 0·38 | 0·410 | |
| | | 0·02 *N* $AgNO_3$ used, ml. | | |
| 96 | 1 | 3·78 | 0·409 | |
| 96 | 1 | 3·78 | 0·409 | |
| 96 | 0·5 | 2·0 | 0·432 | |
| 96 | 0·5 | 1·96 | 0·423 | |
| 96 | 0·5 | 1·96 | 0·423 | |
| 96 | 0·5 | 1·94 | 0·419 | |
| 96 | 0·5 | 1·96 | 0·423 | in Conway units |
| 96 | 0·5 | 1·94 | 0·419 | |
| 96 | 0·5 | 1·98 | 0·427 | |
| 96 | 0·5 | 2·0 | 0·432 | |
| 96 | 0·5 | 1·96 | 0·423 | |
| 96 | 0·5 | 1·96 | 0·423 | |
| 96 | 0·5 | 1·96 | 0·423 | |

As we decreased the quantity of the cake our results became higher, perhaps because of increased exposure of surface. At the same time, however, the experimental error also increased, a difference of only 0·04 ml. of 0·02 *N* $AgNO_3$ changed the results by 0·010 per cent. The mean figure obtained was 0·414 per cent. of hydrocyanic acid.

*Summary*—A method for estimating the cyanide content in plant material by means of the Conway unit is described. Almost constant results were obtained when 0·5 to 1 g. of samples was allowed to decompose for 96 hours in the closed unit.

Our thanks are due to Mr. H. R. Binns, M.R.C.V.S., for the loan of literature.

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DEPARTMENT OF HEALTH
GOVERNMENT OF PALESTINE
JERUSALEM

G. W. BAKER
S. TAUBES-STEINFELD

# Official Appointments

## PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1948, **73**, 679).

| *Public Analyst* | *Appointments* |
|---|---|
| JAFFÉ, Frederick William Moore .. | County Borough of Dewsbury. |
| MCLACHLAN, Thomas (Deputy) .. | Royal Borough of New Windsor. |

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1948, **73**, 680).

| *Official Agricultural Analyst* | *Appointments* |
|---|---|
| BRANSON, Victor Cecil (Deputy) .. | Administrative County of West Sussex. County Borough of Hastings. |
| JAFFÉ, Frederick William Moore .. | County Borough of Dewsbury. |
| SMITH, Arthur (Deputy) .. .. | County Borough of Kingston-upon-Hull. |

# Ministry of Food

STATUTORY INSTRUMENTS*

**1949—No. 614. The Mineral Oil in Food Order, 1949.** Price 1d.

*This Order prohibits, from April 9th, 1949, the use of mineral oil in the composition or preparation of any food sold or intended for sale for human consumption but does not apply where any article of food contains not more than 0·2 per cent. by weight of mineral oil and its inclusion is due to its use as a lubricant or greasing agent on some surface with which the article of food has necessarily to come into contact in the course of its preparation.*

"Mineral oil" means any hydrocarbon product, whether liquid, semi-liquid or solid, derived from any substance of mineral origin and includes liquid paraffins, white oils, petroleum jellies and hard paraffins.

Proceedings in England and Northern Ireland for an infringement of this Order may be brought by the Food and Drugs Authority without the consent of the Minister.

**—No. 762. The Coffee Essence (Amendment) Order, 1949.** Price 1d.

*This Order, as from April 24th, 1949, amends the Coffee Essence (Control) Order, 1942, as amended (S.R. & O., 1942, No. 560; S.I., 1948, No. 1098), by confining its application to dry coffee essences.*

**—No. 781. The Chocolate, Sugar Confectionery and Cocoa Products Order, 1949.** Price 3d.

*This Order, as from April 24th, 1949, revokes, consolidates and re-inacts the Chocolate, Sugar Confectionery and Cocoa Products (Control and Maximum Prices) Order, 1944 (S.R. & O., 1944, No. 451), as amended (S.R. & O., 1945, No. 598; 1946, Nos. 1365, 1639 and 2233; 1947, No. 1927; S.I., 1948, Nos. 233, 1962, 2470 and 2612; 1949, No. 190).*

*The principal changes, apart from prices, are—*

*Medicated chocolate and sugar confectionery is brought within the scope of the Order except for products that*

(a) *comply with recognised medical formulae; or*

(b) *consist of compressed tablets each containing one or more of the following ingredients in not less than the amounts stated;*

(i) *phenolphthalein, 0·5 grain;*

(ii) *santonin, 0·1 grain;*

(iii) *calomel, 0·05 grain; or*

(c) *are specifically exempted by the Minister.*

**—No. 894. The Oils and Fats Order, 1949.** Price 3d.

*This consolidating Order revokes and replaces the Animal Fats (Control and Prices) Order, 1946, as amended (S.R. & O., 1946, No. 2042; 1947, Nos. 557 and 2840), the Edible Oils and Fats (Control of Sales) Order, 1944, as amended (S.R. & O., 1944, No. 672; 1947, No. 701), the Oils and Oilseeds (Control) Order, 1946 (S.R. & O., 1946, No. 2043), the Lard (Control and Maximum Prices) Order, 1943, as amended (S.R. & O., 1943, No. 1767; 1947, No. 2841) and the Margarine and Cooking Fats (Requisition) Order, 1940, as amended (S.R. & O., 1940, Nos. 1107 and 1415).*

*The principal changes made are—*

(a) *Olive oil and sperm oil have been freed from all restrictions;*

(b) *Sunflower seed and sunflower seed oil have been added to the list of oilseeds and oils that are subject to control;*

(c) *A new definition of technical tallow has been included, viz.*

"Technical tallow" means any fat, grease or oil, other than premier jus, oleo oil, beef or mutton stearine, neatsfoot oil, or dripping, separated or substantially

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

separated by any process from the tissue or bones or from both tissue and bones of any quadruped animal other than the pig, and includes any mixture (other than any mixture containing more than 25 per cent. of mineral oil) of such fat, grease or oil with any other fat, grease or oil; but for the purpose of Articles 4 and 5 of this Order shall not include horse fat as defined herein, and for the purpose of Article 5 of this Order shall not include any oil having a melting-point which when determined by the method specified in the Fourth Schedule to this Order does not exceed 25 degrees centigrade.

*Article 4, referred to in this definition, forbids the sale, etc., or use of any technical tallow or rendered pig fat other than lard, or any emulsion of technical tallow or rendered pig fat with any other substance, for use as human food or in the manufacture, preparation or cooking of human food.*

*Article 5 relates to maximum prices.*

(d) *Among other definitions given in the Order are the following—*

"Cooking fats" means any fat or mixture of oils and fats fit for human food or for use in the manufacture, preparation or treatment of human food, having a melting-point which when determined by the method specified in the Fourth Schedule to this Order is not less than 30 degrees centigrade, but does not include dripping, premier jus, lard, margarine, butter, vegetarian butter, cocoa butter, horse fat, technical tallow, or any unrendered animal fat.

"Horse far" means unbleached, unadulterated oil or fat—(*a*) produced from, or by the rendering or processing of, the fat or bones of horses slaughtered for human consumption on premises licensed for such slaughter; (*b*) of sweet taste; (*c*) of sweet smell; (*d*) untreated by any chemical process; and (*e*) containing not less than 99 per cent. saponifiable matter and not more than 1·5 per cent. free fatty acids.

"Dripping" means unbleached, unadulterated fat—(*a*) produced from, or by the rendering or processing of, the fat or bones of sheep or oxen; (*b*) of a sweet taste; (*c*) of a sweet smell; (*d*) untreated by any chemical process; and (*e*) containing not less than 99 per cent. saponifiable matter and not more than 1·5 per cent. free fatty acids; but does not include imported premier jus.

"Lard" means rendered fat derived from a pig and having a melting point which when determined by the method specified in the Fourth Schedule to this Order is not less than 25 degrees centigrade, but (except for the purpose of Articles 2 and 9 of this Order) does not include unrefined or unbleached hog grease.

"Margarine" includes any vegetarian butter other than peanut butter or paste or sunflower butter or paste; it does not include cocoa butter.

"Vegetarian butter" means any soft fatty paste fit for human consumption consisting of vegetable oils or fats or a mixture of both, and containing also other material derived from oilseeds, nuts or kernels.

(e) *A list of oils, fats and mixtures that may be obtained only against a permit has been substituted for the general definition of the term "edible oil."*

(f) *The Margarine (Prepacking) Order, 1940 (S.R. & O., 1940, No. 898) is revoked and not re-enacted in this Order.*

*Schedule IV states that for the purpose of this Order melting-points shall be determined with the apparatus and by the method of determining "drop-points" specified in British Standard No. 894: 1940— "British Standard Specification for the Determination of the Flow and Drop Points of Fats and Allied Substances (Apparatus and Method of Use)" as amended by Amendment No. 1, dated February, 1947; provided that for the purpose of Section B of the said British Standard, as amended, the cup referred to therein shall be filled, at a room temperature, with the fat to be treated without the application of heat to the sample.*

# ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## Food and Drugs

**Routine Method for the Determination of Diacetyl in Butter. P. C. Den Herder** (*Netherlands Milk and Dairy J.*, 1947, **1**, 110–113)—The method of Prill and Hammer (*Iowa State Coll. J. Sci.*, 1938, **12**, 385) for determining diacetyl in butter, based upon steam distillation, is satisfactory but is too cumbersome for routine work. In molten butter diacetyl, if present, is dissolved partly in the fluid fat and partly in the serum. By passing a stream of carbon dioxide through the molten butter, first at 65° C. and then at 100° C. for a sufficiently long time, the whole of the diacetyl, together with the water present in the butter, is volatilised and can be collected in a receiver cooled in melting ice.

*Procedure*—The apparatus, with ground glass joints, consists of a distillation flask, into which a stream of carbon dioxide can be led, connected to two tubes in series acting as receivers. The carbon dioxide inlet tube reaches to the bottom of the flask and the delivery tube from the flask reaches to the bottom of the receiving tube. The second tube is always included, although it is unnecessary unless the diacetyl content of the butter is very high. The butter (25 g.) is placed in the flask, which has previously been filled with carbon dioxide. The first tube is charged with 1 ml. of the hydroxylamine solution described by Prill and Hammer (*loc. cit.*) and the second tube with 1·5 ml. of water. Both tubes are placed in melting ice. After connecting the carbon dioxide supply, the flask is placed in a water-bath maintained at 60° to 65° C., and a slow stream of carbon dioxide is passed through for 45 min. During this time most of the diacetyl with some of the water distils over. The water in the bath is now gently boiled and the carbon dioxide stream is accelerated. After about 30 min. the last traces of the diacetyl and practically all the water will have distilled over into the hydroxylamine in the first tube. The carbon dioxide stream is now stopped and the inlet tube of the first tube is rinsed with the water in the second receiver. The contents of the first receiver are transferred to a test tube, the second tube is rinsed with a little more water, which is then used to rinse the first tube and is added to the liquid in the test tube. The contents of the test tube are treated as described by Prill and Hammer (*loc. cit.*).

The accuracy of the method was established by adding known amounts of diacetyl to samples either prepared from sweet cream or freed from original diacetyl by steam distillation and determining the diacetyl content. Also, the results by this method were compared with those found by the steam distillation method (Prill and Hammer, *loc. cit.*). A. O. JONES

**Determination of Alcohol-Insoluble Solids and Sugar Contents of Vegetables. J. C. Moyer and K. C. Holgate** (*Anal. Chem.*, 1948, **20**, 472–474)—During a study of the losses of soluble constituents in the processing of vegetables, the need arose for a rapid method for determining the reducing and total sugars, and the desirability of combining the sugar determinations with that of the alcohol-insoluble solids became apparent.

The extraction of sugars from plant tissues with alcohol in sufficient concentration to precipitate polysaccharides and protein material and to inhibit enzymic action is greatly aided by maceration of the tissues in a Waring Blendor. The procedure gives a final alcoholic concentration of at least 75 per cent. according to the water content of the tissues.

*Procedure*—Freeze and store the material at −23° C. and, to obtain a representative sample, grind 1 lb. or more at this temperature twice in a food chopper. Weigh 20 g. of the finely ground sample and, at room temperature, wash it with 150 ml. of 85 per cent. alcohol into a Blendor cup of 500-ml. capacity having a rubber gasket under the screw top. After macerating for 5 min., wash the blended mixture into a 600-ml. beaker with 85 per cent. alcohol from a wash bottle, allow the solids to settle, and decant the liquid on to a weighed 5·5-cm., No. 40 Whatman filter paper in a Buchner funnel inserted through a two-holed rubber stopper in the mouth of a Kohlrausch sugar flask, to which suction can be applied by means of a tube passing through the other hole in the stopper. Transfer the mixture to the funnel and wash the solid matter on the filter three or four times with 85 per cent. alcohol, draining the residue after each washing, but avoiding thorough drying. When nearly 500 ml. of filtrate have been collected, allow the precipitate to dry, and place the paper in a weighing dish for complete drying overnight at 95° C. Finally, weigh the alcohol-insoluble solids. Adjust the volume of the filtrate to 500 ml. with 85 per cent. alcohol and mix thoroughly.

For clarification of the sugar solution the following modification of the procedure of Somogyi (*J. Biol. Chem.*, 1945, **160**, 61; *Analyst*, 1946, **71**, 85) was found suitable. Mix 56 g. of barium hydroxide with 2 litres of hot boiled water and filter the mixture into a storage bottle through a small Buchner funnel. The storage bottle should carry a soda lime tube and a siphon tube to a burette having a three-way tap. Dissolve 100 g. of zinc sulphate heptahydrate in 2 litres of water and adjust the concentration of the solution so that 10 ml. mixed with 50 ml. of water require 9·5 ml. of the barium hydroxide solution to give a faint pink end-point to phenolphthalein that is stable for 1 min. (The zinc sulphate and barium hydroxide solutions recommended by Somogyi leave zinc in the filtrate, which subsequently interferes with the colorimetric readings.) Evaporate 10 ml. of the alcoholic extract nearly to dryness on the steam-bath, rinse down the walls of the beaker with 5 ml. of water, add 2 ml. of barium hydroxide solution followed by 2 ml. of zinc sulphate solution with constant agitation during the addition of each

reagent. Wash the liquid into a funnel of diameter 50 mm. and collect the filtrate in a graduated tube. Wash the precipitate with a fine stream of water until the filtrate measures 35 ml. For determination of reducing sugars use a 2-ml. aliquot of this filtrate, and for the determination of total sugars invert a 5-ml. aliquot.

An enzymic method of inversion was found better than an acid method. To prepare the buffer solution dissolve 13·6 g. of sodium acetate trihydrate in water, add 8 ml. of glacial acetic acid, and dilute to 500 ml. Dissolve 200 mg. of invertase scales (Wallenstein Laboratories, Blue Label) in 100 ml. of water and store the solution in an ice-box under a layer of toluene. To a 5-ml. aliquot of the clarified extract in a graduated tube add 2 drops of the acetate buffer and 5 drops of the invertase solution and invert overnight at 35° C. Dilute to 35 ml. with water and use a 2-ml. aliquot for colour development. The overnight incubation period has been used for convenience; a much shorter period at a higher temperature would doubtless accomplish the same degree of inversion.

The colorimetric procedure found suitable was that of Nelson (*Ibid.*, 1944, **153**, 375; *Analyst*, **1944, 69**, 313) in combination with Somogyi's new copper reagent (*loc. cit.*), but it was found that variations in the excess of arsenomolybdate reagent had less influence on the colour readings at 600 mμ. than at 500 mμ. as recommended by Nelson.

To prepare Somogyi's copper reagent add 56 g. of anhydrous disodium phosphate slowly with stirring to 1400 ml. of water, and then with continued stirring 80 g. of Rochelle salt and finally 200 ml. of *N* sodium hydroxide. Dissolve 16 g. of copper sulphate in 160 ml. of water and add this solution to the first. Finally, add slowly, 360 g. of anhydrous sodium sulphate with stirring, dilute to 2 litres, and set the solution aside for 2 days before filtering. To prepare Nelson's arsenomolybdate reagent, dissolve 100 g. of ammonium molybdate with stirring in 1800 ml. of water, add 84 ml. of concentrated sulphuric acid with continued agitation and 12 g. of sodium arsenate heptahydrate. Finally, dilute the solution to 2 litres, set it aside for 48 hr. at 37° C., filter, and store it in a brown bottle.

*Procedure*—Place a 2-ml. aliquot of the clarified extract (for reducing sugars) or of the inverted extract (for total sugars) in a Folin-Wu blood sugar tube by means of an Ostwald pipette. Then add 2 ml. of Somogyi's copper reagent from a 25-ml. burette and place the tube in boiling water for 20 min. Cool the tube in water at room temperature, add 2 ml. of Nelson's arsenomolybdate reagent and mix the solutions by moderate agitation with a glass rod having a small knob at the end. Wash the rod and dilute the liquid to 25 ml. before shaking to ensure thorough mixing. Allow the solution to stand for 15 min. and read the colour intensity at 600 mμ. in a photo-electric colorimeter or spectrophotometer that has been adjusted to give 100 per cent. transmittance with water. With each series of unknown samples treat tubes containing 2 ml. of water for the blank value and 2 ml. of standard solutions containing 0·10 and 0·20 mg. of glucose in a similar manner to obtain a standard reference curve.

The precision of the analyses was determined with ten replicate samples of ground frozen lima beans, in which the reducing sugar content was found to be 0·068 per cent. and the standard deviation of a single determination ±0·003. After inversion of aliquots of the same extracts the total sugar content was 2·43 per cent. with ±0·07 as the standard deviation of a single determination. A. O. JONES

**Specific Surface of Wheat Flours. I. Determination by Air Permeability Method. L. Lyon, N. Pennington, and A. Boley** (*Cereal Chem.*, 1947, **24**, 394–407)—The method used is based on the determination of the rate of flow of air through a plug of the sample under a measured difference of pressure between the ends of the plug. The theory of the method has been worked out by Carman (*Trans. Inst. Chem. Eng.*, 1937, **15**, 150–166; *J. Soc. Chem. Ind.*, 1938, **57**, 225–234T; 1939, **58**, 1–7T) and by Sullivan and Hertel ("*Advances in Colloid Chemistry*," Vol. I, pp. 37–80).

The specific surface is said to be expressed by the equation

$$S = \frac{14}{d_s}\sqrt{\frac{A\Delta P t}{Q\eta L}\cdot\frac{\epsilon^3}{(1-\epsilon)^2}} \qquad (1)$$

in which $S$ = Specific surface of the powder in sq. cm. per gram.
$d_s$ = Density of the particles of the powder.
$A$ = Cross-sectional area of the plug in sq. cm.
$L$ = Length of the plug in cm.
$W$ = Weight of the plug in grams.
$\epsilon$ = Porosity of the plug = $1 - (W/d_s AL)$.
$\eta$ = Viscosity of the air in poises.
$Q$ = Volume of air flowing through the plug in $t$ seconds.
$\Delta P$ = Pressure difference of air between ends of plug, in grams weight per sq. cm.

*Apparatus and Procedure*—The essential features of the apparatus are shown in Fig. 1. The kerosene manometer F measures the difference of air pressure at the two end of the plug, A, and the system B, C, D, E, measures the rate of air-flow through the plug. The sequence of operations is as follows. With the three-way stopcock B open to the air, a pressure difference is established across A. After steady flow has been attained, usually in 3 min., B is closed and air from system D is drawn through A. As the pressure in D is lowered, kerosene from C flows into D, replacing the air. The kerosene level in C is adjusted so that a lowering of pressure approximating to 1 mm. of kerosene in D causes the liquid in C to flow into chamber D. Thus the pressure measurement by manometer F is not affected appreciably by the volume measurement. The rate of flow of air through A is determined by measuring the time taken for the kerosene to fill the space in container D defined by two etched marks on a capillary side-arm. In the apparatus

used that space was 4·977 ml. When the determination is complete the kerosene is drained from D by stopcock E and returned to reservoir C.

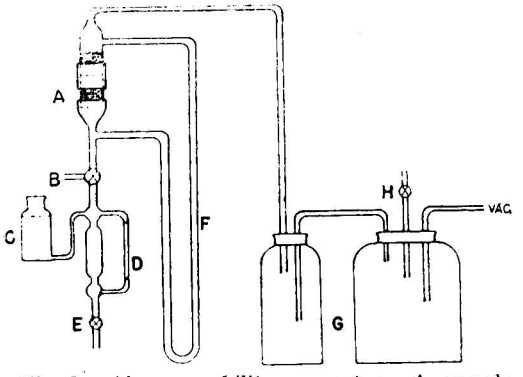



Fig. 1. Air permeability apparatus. A, sample tube. B, three-way stopcock. C, kerosene reservoir. D, volume measurement device. E, two-way stopcock. F, kerosene manometer. G, surge bottles. H, vacuum regulator.

The plugs of flour to be tested are formed in machined brass tubes of accurately known dimensions in which they remain during the test. Ten tubes, ranging in length from 20 to 40 mm. and in internal diameter from 4 to 12 mm., were in use. To ensure uniformity of packing throughout the length of a tube a tamping apparatus (Fig. 2) is

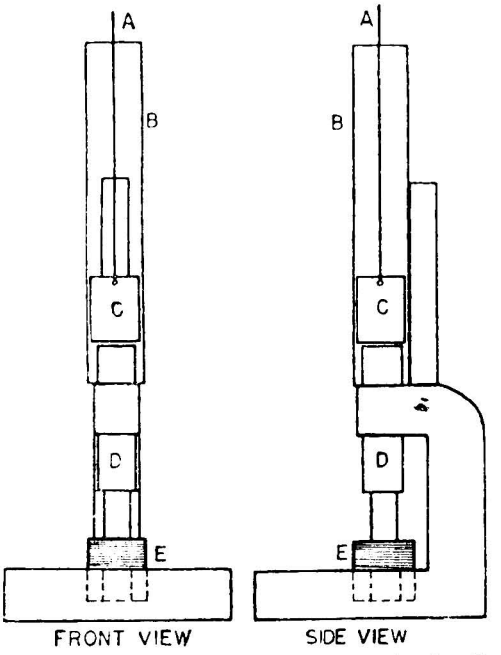



Fig. 2. Tamping apparatus. A, wire handle. B, glass guide tube. C, bucket containing lead shot. D, steel plunger. E, machined sample tube.

used. The sample tube is screwed into its base, about 0·1 g. of the flour is introduced at a time and each portion pressed by means of a plunger, of diameter 0·002 in. less than the inner diameter of the tube, on which a gravity-hammer is allowed to fall a certain number of times from a predetermined height, according to the degree of packing required. The weight of the plug is found by weighing the tube before and after filling.

If necessary the density of a flour is determined in a 25-ml. pycnometer with xylene as liquid, at 25° C., but it is rarely outside the range 1·42 to 1·45, and for routine tests the latter figure is usually taken.

*Influence of experimental factors*—In equation (1), which may be written in the form

$$\frac{\epsilon^3}{(1-\epsilon)^2} = \frac{S^2 d_s^2 QL}{14^2 A} \cdot \frac{\eta}{\Delta Pt} \quad .. \quad .. \quad (1a)$$

it was found that, for a range of $\Delta P$ from 0·2 to 100 cm. of kerosene, the product $\Delta Pt$ is constant to within 1 per cent. if all other factors are kept unchanged.

The influence of other factors was studied in experiments with a hard winter wheat. By adding to the right-hand side of equation (1*a*) a correction term, *b*, better agreement with experimental results was obtained for plugs of the same size, the standard error of the $S$ values calculated from the modified equation (equation 2) being 2·6 per cent. compared with 10 per cent. for those calculated from equation (1) or (1*a*).

By varying the size of plug used it was shown that $S$ was dependent on the length ($L$) and cross-sectional area ($A$) of the plug, a minimum value for $S$ being obtained when $A/L$ was approximately 0·45. The equation was accordingly modified into its final form,

$$\frac{A}{L} \cdot \frac{\epsilon^3}{(1-\epsilon)^2} = S^2 \cdot \frac{d_s^2 Q\eta}{14^2 \Delta Pt} + C \qquad (3)$$

where $C$ is a correction term, which depends on the size of plug (*i.e.*, of tube) used and the value of $S$ to be determined. For a given sample (*i.e.*, $S$ constant) and a given size of plug, it may be determined by carrying out several determinations and plotting the values of $A\epsilon^3/L(1-\epsilon)^2$ and $10^9 \times d_s^2 Q/14^2 \Delta Pt$ The graph is a straight line, whose intercept on the axis of $A\epsilon^3/L(1-\epsilon)^2$ is $C$ and slope is $S^2$. The accompanying table shows the specific surface of a patent flour determined with ten sizes of plug (tube) and calculated by use of equation (3) and the appropriate values of $C$: for each tube (plug size) the first figure is the length and the second the diameter, in mm.

| Tube | $\sqrt{A/L}$ | $S$, cm.$^2$ | $C$ |
|---|---|---|---|
| 20–4 | 0·253 | 2170 | 0·0016 |
| 40–6 | 0·264 | 2180 | 0·0018 |
| 20–5 | 0·297 | 2010 | 0·0032 |
| 30–6 | 0·305 | 2020 | 0·0038 |
| 20–6 | 0·373 | 1980 | 0·0047 |
| 20–8 | 0·504 | 1960 | 0·0115 |
| 40–12 | 0·530 | 1980 | 0·0138 |
| 30–12 | 0·612 | 1960 | 0·0175 |
| 20–10 | 0·616 | 1950 | 0·0200 |
| 20–12 | 0·745 | 1950 | 0·0340 |

Average of last 6 tubes: 1963

Provided that $\sqrt{A/L}$ is greater than about 0·375, $S$ is independent of $\sqrt{A/L}$ to within 1 per

cent. When $\sqrt{A/L}$ is greater than 0·4, results are obtained with a standard error of 0·5 per cent.

At least two determinations are necessary to find $S$ by this method. To evaluate $S$ by one determination the dependence of $C$ on $S$ must be known, for the plug size used (*cf.* Fig. 3); an approximate value of $S$ is first calculated by means of equation (1) and the corresponding correction term estimated from a graph such as Fig. 3 and inserted in equation (3) to enable a corrected value of $S$ to be obtained.

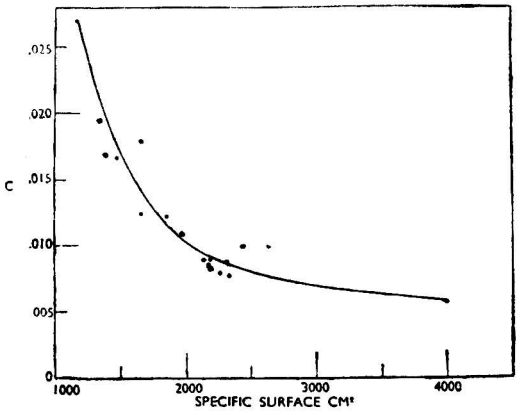



Fig. 3. Variation of correction term C with specific surface. Sample tube 20–8.

Comparison of the corresponding diameter, $D$, of equivalent smooth spheres ($D = 6/Sd_s$) with the particle size results obtained by sedimentation methods indicates that the equivalent diameters found by surface area measurements are smaller than those obtained from sedimentation or microscopical measurements. This is undoubtedly due to the irregular shape of flour particles.

*Results*—The following table shows the results of specific surface determinations on fractions of patent flour obtained by using A.S.T.M. wire sieves (in Ro-Tap shaker for 10 min.).

| A.S.T.M. sieves | Weight, grams | Specific surface, $S$ in cm.² | Diameter, $D$ in microns | Total surface area, grams × $S$ |
|---|---|---|---|---|
| Over 80 .. .. .. | 0·136 | — | — | — |
| Over 100 .. .. | 10·958 | 1380 | 29·9 | 15·120 |
| Over 140 .. .. | 8·239 | 1350 | 30·6 | 11·100 |
| Over 180 .. .. | 5·339 | 1660 | 24·9 | 8·850 |
| Over 300 .. .. | 23·400 | 2440 | 16·85 | 57·300 |
| Under 300 .. .. | 0·459 | — | — | — |
| Totals .. .. | 48·531 | | | 92·370 |

Total weight exclusive of "over 80" and "under 300" = 47·936 g.

$S = \frac{92{\cdot}370}{47{\cdot}936} = 1930$ cm.², the average specific surface from the above sieve analysis.

$S = 1960$ cm.², the average specific surface as determined on original flour.

W. MARTIN

**Methods for Determining Flour Particle-Size Distribution. F. W. Wichser and J. A. Shellenberger** (*Cereal Chem.*, 1948, **25**, 155–167)—The three methods dealt with in the present paper are those depending on the use of sieves, air elutriation, and sedimentation, respectively. All results were obtained with the same flour. Sieve methods using special wire sieves having accurately known apertures (*e.g.*, Tyler standard screen scale testing sieves) can give reproducible and accurate results for particles of size greater than 37 mμ. with the Ro-Tap shaker. The curve marked "sieve" in Fig. 1 shows a typical result for wheat flour by this sieve method (single sieve procedure), operated according to the procedure of Wichser *et al.* (*Ibid.*, 1947, **24**, 381–393).

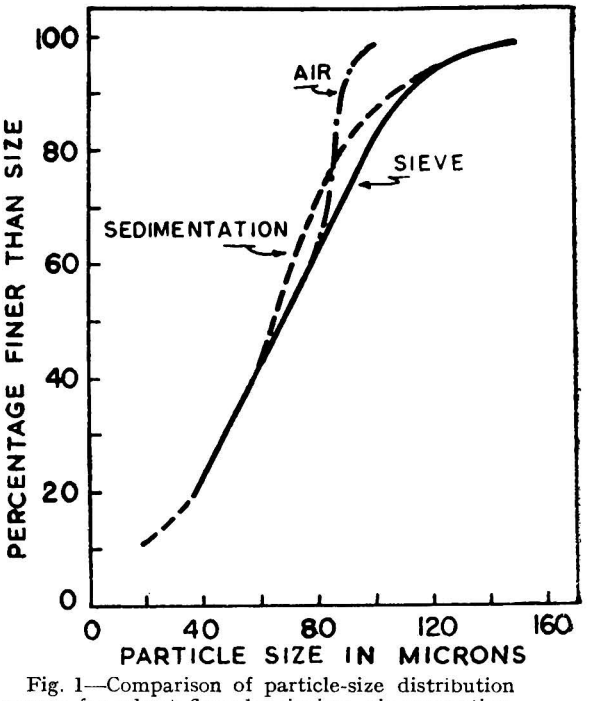



Fig. 1—Comparison of particle-size distribution curves for wheat flour by sieving, air separation, and sedimentation.

The air elutriation method employed was the Roller Particle Size Air Analyser described by Wichser *et al.* (*loc. cit.*). This method depends on the systematic removal of particles by increasing

air velocity in a suitable expansion chamber. Stokes's law in the form given below can be applied to this method.

$$V = 10^{-8}gDd^2/18\eta = 0{\cdot}00299\ Dd^2 \text{ cm. per sec.},$$

where $V$ = terminal velocity of fall in cm. per sec. in stationary fluid, $g$ = gravitational constant (980·3 dynes), $D$ = density of particles in g. per ml., $\eta$ = viscosity of fluid in c.g. units = $1{\cdot}82 \times 10^{-4}$ for air, and $d$ = diameter of particles in microns. The curve marked "air" in Fig. 1 shows the result obtained by this method.

The sedimentation method investigated made use of the Andreasen pipette shown in Fig. 2. Andreasen

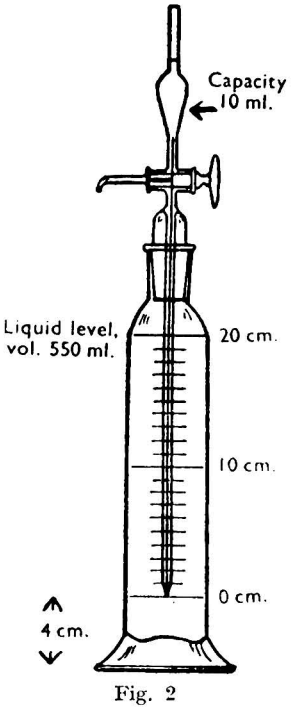



Fig. 2

(*Kolloid-Z.*, 1929, **48**, 175) showed that Stokes's law could be applied to angular particles of the same weight as spherical particles. By calculating the particle size as the edge length of a cube of the same volume as a sphere of radius $r$, his particle size conformed to the result of sieve analysis. This expression is shown as

$$r = \left[2739\sqrt{\frac{nh}{(D-d)tg}}\right]1{\cdot}612,$$

where $r$ = edge-length of particle in microns, $n$ = viscosity of suspending medium in poises, $h$ = height in cm. between liquid surface and pipette tip when sample is drawn, $t$ = time in minutes of settling, $D$ = density of flour, and $d$ = density of suspending medium.

Substituting in the above equation the constants of the test, it is reduced to the form $r = k\sqrt{h/t}$, where $k$ is a constant for all the known values.

The medium used was a mixture of naphtha and carbon tetrachloride. 6-g. portions of flour were added to the suspending medium in the pipette and the whole was well shaken to get a uniform mixture. An initial sample was taken and the apparatus kept at 30° C. Subsequently, 10-ml. portions were removed at suitable time intervals and the solids determined by evaporating the liquid. The curve marked "sedimentation," obtained by this method, is shown in Fig. 1.

The results obtained show that the sieve method using single sieves as opposed to stack sieves is the most accurate and trustworthy method for the purpose. The air elutriation method is comparably rapid, but is expensive in outlay and is limited to the smaller size particles. The sedimentation method has too many disadvantages both in operation and in determining the necessary constants.

W. MARTIN

**Determination of Theobromine and Caffeine in Cacao Materials. R. G. Moores and H. A. Campbell** (*Anal. Chem.*, 1948, **20**, 40–47)—THEOBROMINE—For the greatest accuracy, sample weights should be adjusted so that the materials in the clarification and adsorption steps contain about 40 mg. of theobromine. *Procedure*—To 2 to 3 g. of the sample add 1 g. of heavy magnesium oxide (U.S.P.) and about 4 g. of Celite 545, make into a smooth paste with hot water, mix thoroughly, and transfer the mixture to an extraction tube made by attaching a 100-ml. length of 8-mm. glass tubing to a 25 × 200-mm. test tube containing a glass wool plug and a Celite 545 filter bed (about 1 g.) fitted into a litre filter flask. Connect the extraction tube to a supply of boiling water and apply suction to the flask so as to draw boiling water through at such a rate that 500 ml. of extract will be collected in 30 to 40 min. The tube should not become dry during the percolation, and, to maintain the temperature of the sample at 80° to 90° C., the water should not be more than 2·5 cm. above the sample. Adjust the pH of the extract to about 6 by means of $N$ sulphuric acid and Universal Indicator paper and concentrate it to about 150 ml. Rinse the concentrate into a 200-ml. flask with small amounts of water until the volume is about 170 ml. Add 7 ml. of zinc acetate reagent (219 g. of zinc acetate dihydrate dissolved in water with 30 ml. of glacial acetic acid and diluted to 1 litre) and then immediately, with swirling, add 7 ml. of potassium ferrocyanide solution (106 g. of potassium ferrocyanide trihydrate in 1 litre of water). Adjust the volume to 200 ml. and mix thoroughly. After not less than 3 and not more than 5 min. filter the liquid through a dry paper (Green's No. 4882) and collect all but the first 5 to 8 ml. of the filtrate. Place 150 ml. in a separating funnel equipped with a rubber stopper to fit the top of the adsorption tube, which is made by attaching 80 mm. of 6-mm. glass tubing to the bottom of an 18 × 100-mm. test tube.

Place a glass-wool plug firmly in the bottom of the adsorption tube, add about 0·5 g. of Celite 545 to make a bed about 10 mm. thick and above it place about 6 g. of an intimate mixture (1 + 1)

of Celite 545 (or 535) and English Superfine XL fuller's earth. The fuller's earth should be tested for its adsorptive capacity by the following test. Stir 2 g. for 10 min. with a neutral aqueous solution of 200 mg. of theobromine in 200 ml. Centrifuge and determine the theobromine in an aliquot by the silver nitrate titration procedure (*infra*). The fuller's earth should adsorb at least 75 mg. of the theobromine.

Compress the packing by drawing air through the dry bed, and ensure that the column is free from channels. Immediately connect the adsorption tube to the separating funnel containing the clarified solution, which should contain preferably 40 mg. and not more than 60 mg. of theobromine. Draw the solution through the column in 20 to 30 min. until only 4 to 5 ml. remain above the clay bed. Wash the column with 50 ml. of water, keeping some liquid above the column at all times.

Replace the suction flask by a clean one, place 75 ml. of *N* sodium hydroxide in the separating funnel, and elute the theobromine by connecting the funnel to the adsorption tube and drawing the alkali through the column by suction. The time for elution should be 10 to 20 min. Add 2 drops of a mixed indicator prepared by dissolving 0·625 g. of methyl red in 450 ml. of 95 per cent. alcohol, filtering the solution through asbestos, mixing it with 0·412 g. of methylene blue in 50 ml. of water and diluting the mixture to 500 ml. The pH range of the indicator is 5·34 to 5·65, and the colour change from reddish-purple to green. Neutralise the eluate with *N* sulphuric acid to the faint red end-point and transfer it to a 250-ml. beaker, rinsing the flask with water. In the beaker place a glass electrode and a calomel electrode making contact with the solution by means of a salt bridge made by sealing a thread of asbestos into the end of an 18 × 100-··m. test tube filled with a saturated solution of sodium nitrate. Adjust the pH to 6·4 ± 0·05, using 0·025 *N* sodium hydroxide for the final adjustment. The total volume should now be about 125 ml. Add 25 ml. of 0·01 *N* silver nitrate, mix thoroughly and, with vigorous stirring, titrate to pH 6·40 ± 0·05, thereby determining the nitric acid liberated by formation of silver theobromine. For best results the titration should be made without interruption and within 5 min. Submit a sample of water to the processes of clarification, adsorption, elution with sodium hydroxide, neutralisation, and titration in presence of silver nitrate exactly as described. This blank determination should require not more than 0·2 ml. of 0·025 *N* sodium hydroxide. Submit a sample containing 40 mg. of theobromine to the same procedure to establish the quantitative nature of all the steps involved. The percentage of theobromine is given by $100\,nf/w$, where $n$ is the difference between the titre of the sample and that of the blank, $f$ is the theobromine factor and $w$ is the weight of sample clarified. The theobromine factor is $0{\cdot}18\,Nv/a$, where $N$ is the normality of the sodium hydroxide solution, $v$ is the total volume clarified and $a$ is the aliquot analysed. For 0·025 *N* sodium hydroxide and a 150-ml. aliquot from a total volume of 200 ml. clarified the factor is 0·006. A survey of the results of comparative analyses, recovery experiments, and about 10,000 determinations by the method indicates that for the highest accuracy an empirical correction of about 3 per cent. should be added to all results from samples containing soluble cacao materials. The correction for pure solutions of theobromine carried through the entire procedure should be 5 per cent.

With pure or crude theobromine samples the clarification and adsorption steps can be omitted. To 0·1 g. of the pulverised sample in a 500-ml. Erlenmeyer flask add 250 ml. of water, place a small funnel in the neck, and boil for 30 min. Filter while still hot through coarse paper into a 400-ml. beaker. (Filtration may be omitted with samples containing 90 to 100 per cent. of theobromine.) Wash the filter paper thoroughly with 50 ml. of hot water, cool the filtrate and washings to room temperature, adjust to pH 6·4 with 0·025 *N* sodium hydroxide, add silver nitrate, and titrate back as already described. Correct the result by means of a blank, using 250 ml. of water and 5 ml. of silver nitrate solution.

Chlorides and sulphates do not interfere with the titration unless they are present in amount sufficient to alter the concentration of the silver nitrate. If a large amount of chloride is present, the silver nitrate concentration should be increased by an equivalent amount. Carbonates interfere by formation of silver carbonate with consequent liberation of nitric acid which appears as an apparent increase in the theobromine content. The sodium hydroxide solutions should therefore be prepared from a 50 per cent. solution that has been allowed to stand for 10 days. Traces of carbonate occurring in the reagents will be compensated for in the blank determination.

CAFFEINE—*Procedure*—Mix 4 g. of the sample with 2 g. of heavy magnesium oxide and 8 g. of Celite 545 and extract it by the procedure described for theobromine. Neutralise the extract, concentrate it to 150 ml. and clarify it with zinc ferrocyanide. Add 10 ml. of 0·5 *M* sodium phosphate (190 g. of trisodium phosphate dodecahydrate in a litre of water) to 150 ml. of the filtrate, dilute to 200 ml., and filter through fluted paper.

Extract 150 ml. of the filtrate five successive times for 1 min. each with 30-ml. portions of chloroform and to the combined extracts in a separating funnel add 5 ml. of *N* sulphuric acid, mix thoroughly, allow to stand for 10 min., and draw off the chloroform layer through a cottonwool plug in the stem of the separating funnel into a 650-ml. Kjeldahl flask. Wash the residual acid layer with 30 ml. of chloroform and run the washing into the Kjeldahl flask. Remove all but 10 to 15 ml. of the solvent by slow distillation and determine the total nitrogen in the residue by the Kjeldahl-Gunning method. Make a blank determination with the reagents used, starting with 180 ml. of chloroform. The percentage of caffeine is given by $4{\cdot}85\,nN/w$, where $n$ is the volume of sodium hydroxide solution of normality $N$ (corrected for the blank titration) used and $w$ is the weight of sample.

The recommendations of Wadsworth (*Analyst*, 1921, **46**, 32; 1922, **47**, 152) for the preparation of cacao materials for analysis are satisfactory. Samples of whole beans or nibs containing more than 15 per cent. of fat should be treated with a fat solvent (*e.g.*, hexane or pentane) to facilitate extraction and subsequent clarification and adsorption. A. O. JONES

**Use of Low-Temperature Crystallisation in the Determination of Component Acids of Liquid Fats. IV. Marine Animal Oils. Component Acids and Glycerides of a Grey Atlantic Seal. T. P. Hilditch and S. P. Pathak** (*J. Soc. Chem. Ind.*, 1947, **66**, 421–424)—The component fatty acids of grey seal blubber oil were separated into four groups: A, insoluble in ether at −30° C.; B, soluble in ether at −30° C.; C, soluble in acetone at −40° C.; D, soluble in acetone at −60° C. The following results were obtained:—

| Component acids (increment per cent. wt.) | A (11·4) | B (17·1) | C (28·3) | D (43·2) | Total | %, excluding unsaponifiable (wt.) | %, excluding unsaponifiable (mol.) |
|---|---|---|---|---|---|---|---|
| Myristic | 1·3 | 1·3 | 0·7 | 0·4 | 3·7 | 3·7 | 4·6 |
| Palmitic | 8·3 | 1·4 | 0·8 | — | 10·5 | 10·5 | 11·5 |
| Stearic | 1·4 | 0·6 | — | — | 2·0 | 2·0 | 2·0 |
| Unsaturated $C_{14}$ | — | 0·1 | 0·6 | 0·9 | 1·6 | 1·6 | 2·1 |
| " $C_{16}$ | 0·1 | 1·6 | 5·8 | 7·9 | 15·4 | 15·5 | 17·2 |
| " $C_{18}$ | 0·3 | 10·1 | 12·3 | 8·0 | 30·7 | 30·8 | 30·9 |
| " $C_{20}$ | — | 1·9 | 7·1 | 7·4 | 16·4 | 16·5 | 15·2 |
| " $C_{22}$ | — | — | 0·9 | 17·0 | 17·9 | 18·1 | 15·5 |
| " $C_{24}$ | — | — | — | 1·3 | 1·3 | 1·3 | 1·0 |
| Unsaponifiable matter | — | 0·1 | 0·1 | 0·3 | 0·5 | — | — |
| Iodine value (per cent.) | 2·8 | 75·6 | 103·1 | 294·4 | | | |

Mixed glycerides fractionated from acetone were:—

| | Description | Per cent. (wt.) | Iodine value | Saponification value |
|---|---|---|---|---|
| A | Insoluble at −10° C. | 16·4 | 73·9 | 282·4 |
| B | Soluble at −10° C. or −15° C., insoluble at −40° C. | 28·9 | 119·7 | 287·7 |
| C | Soluble at −40° C.; insoluble at −40° C. | 30·4 | 189·6 | 295·1 |
| D | Soluble at −60° C. | 24·3 | 241·8 | 303·5 |

| | A | B | C | D | Total |
|---|---|---|---|---|---|
| Glycerides per cent. (mol.) | 17·0 | 29·5 | 30·1 | 23·4 | 100·0 |

The composition of these was:—

| | Component acids per cent. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | | B | | C | | D | | Total | |
| | (wt.) | (mol.) | (wt.) | (mol.) | (wt.) | (mol.) | (wt.) | (mol.) | (wt.) | (mol.) |
| Myristic | 6·0 | 7·1 | 4·3 | 5·2 | 4·1 | 5·0 | 1·8 | 2·3 | 3·9 | 4·7 |
| Palmitic | 29·8 | 31·3 | 12·8 | 13·6 | 6·2 | 6·9 | 2·2 | 2·5 | 11·0 | 12·1 |
| Stearic | 6·3 | 6·0 | 2·2 | 3·1 | 0·9 | 0·9 | 0·6 | 0·6 | 2·1 | 2·0 |
| Arachidic | 0·5 | 0·4 | 0·1 | 0·1 | — | — | — | — | 0·1 | 0·1 |
| Unsaturated $C_{14}$ | 1·2 | 1·4 | 3·0 | 3·6 | 2·8 | 3·5 | 3·4 | 4·3 | 2·7 | 3·3 |
| " $C_{16}$ | 13·0 | 13·8 | 20·7 | 22·2 | 17·0 | 18·9 | 17·3 | 19·7 | 17·5 | 19·2 |
| " $C_{18}$ | 17·9 | 26·6 | 38·2 | 37·0 | 32·5 | 32·6 | 27·3 | 28·1 | 32·2 | 31·8 |
| " $C_{20}$ | 15·1 | 13·2 | 12·8 | 11·4 | 15·8 | 14·6 | 12·5 | 11·9 | 14·0 | 12·9 |
| " $C_{22}$ | 0·2 | 0·2 | 5·9 | 4·8 | 19·5 | 16·7 | 34·0 | 29·8 | 15·9 | 13·4 |
| " $C_{24}$ | — | — | — | — | 1·2 | 0·9 | 0·9 | 0·8 | 0·6 | 0·5 |

The glycerides are the extremely "mixed" type characteristic of marine animal oils. Liver tissue examined contained less than 3 per cent. of lipids, consisting of phosphatides (29 per cent.), unsaponifiable matter (21 per cent.), and glycerides (50 per cent.); the liver glycerides contained a less unsaturated mixture of fatty acids than the blubber glycerides, and also a higher content of saturated fatty acids. E. B. DAW

**Content of Morphine and other Alkaloids in Poppy Heads of Different Varieties. J. F. Reith, A. W. M. Indemans, and W. H. Becker** (*Pharm. Weekblad.*, 1948, **83**, 449–459)—In 1946 an examination was made of the alkaloidal content of poppy capsules of different varieties grown in Holland. The results were reported in the *Pharm. Weekblad*, 1947, **82**, 582. The plan of the work comprised the following points. (1) Observation and analysis of 51 foreign varieties. (2) Comparison of the yields of seed, capsule, and alkaloids of four Dutch, four German, and eight French varieties. (3) Comparison of the results obtained with four Dutch varieties grown at four different places. (4) Continuation of the selection tests of

the preceding year. The table below summarises the results of these tests and gives figures for the outstanding varieties.

| Origin | | Per cent. Morphine | Per cent. Other alkaloids |
|---|---|---|---|
| *Series I*: | | | |
| Denmark | Mean of 10 varieties | 0·54 | 0·11 |
| | "Aesbo 9" | 0·62 | 0·09 |
| Germany | Mean of 3 varieties | 0·49 | 0·16 |
| Lithuania | Mean of 3 varieties | 0·42 | 0·31 |
| | "Lithaus red" | 0·28 | 0·45 |
| Turkey | "Amasya" | 0·73 | 0·21 |
| Czechoslovakia | Mean of 3 varieties | 0·43 | 0·11 |
| | "Dubsky Stribosedy" | 0·59 | 0·16 |
| Hungary | "Hongaars 2" | 0·42 | 0·12 |
| Austria | "Prohasha" | 0·35 | 0·07 |
| Sweden | Mean of 28 varieties | 0·43 | 0·21 |
| | Pap. somn. alb. Kaunas × Pap. somn. var. opif. Kaunas | 0·33 | 0·56 |
| | Cross P36 | 0·62 | 0·28 |
| *Series II*: | | | |
| Holland | Mean of 4 varieties | 0·50 | 0·09 |
| Germany | Mean of 4 varieties | 0·49 | 0·14 |
| France | Mean of 8 varieties | 0·55 | 0·29 |

The yields of seed in the second series were low, but weather conditions were abnormal. The best figure was 930 kg. per hectare for the Dutch "Noordster" strain. Differences were observed in the alkaloidal contents of plants grown in different parts of the country, but some strains appeared to be affected more than others by a change in locality. The morphine contents in 1947 were higher than in the preceding year, possibly owing to the drought. The figures indicate that selection of seed from capsules with a high alkaloidal content has favourable results. Considerable differences were found in the molecular weights of the "other alkaloids" obtained in the analysis. This mean molecular weight decreased after purification by silicotungstic acid. G. MIDDLETON

## Biochemical

**Determination of Aspartic Acid. B. Warshowsky and M. W. Rice** (*Anal. Chem.*, 1948, **20**, 341–344)—In view of the difficulty of carrying out a quantitative isolation of aspartic acid from protein hydrolysates, a method for its determination in presence of other amino acids has been worked out. The aspartic acid is converted to a mixture of maleic and fumaric acids which are determined polarographically (*cf. Analyst*, 1948, **73**, 45).

*Procedure*—To a sample containing between 2 and 10 mg. of aspartic acid in 2 to 5 ml. of solution, in a 50-ml. conical flask, add about 2 ml. of dimethyl sulphate followed by several drops of phenolphthalein indicator solution. Make the solution just alkaline by adding 40 per cent. sodium hydroxide solution drop by drop, and place the flask in a water-bath below 25° C., adding more sodium hydroxide at frequent intervals to keep the solution alkaline. It is necessary to add further amounts of phenolphthalein during this time as the indicator is gradually destroyed. After 1½ hr., add 1 ml. of concentrated sulphuric acid and allow the mixture to stand for a further ½ hr. Neutralise the solution with the sodium hydroxide solution, and transfer it to a 100-ml. volumetric flask. Add sufficient 6·0 *N* hydrochloric acid to reduce the pH to 1·5 (about 2 ml.), dilute to 100 ml. and, after removing dissolved oxygen, examine a portion of the solution polarographically over the range −0·5 to −1·1 v. *versus* the mercury pool. The wave-height obtained gives the aspartic acid content of the solution by reference to a calibration curve obtained by treating solutions of various known concentrations of aspartic acid by the same technique.

Experiments in which known amounts of aspartic acid were added to protein hydrolysates gave an average recovery of 99·5 per cent. J. G. WALLER

**Estimation of Creatinine. J. A. Barclay and R. A. Kenney** (*Biochem. J.*, 1947, **41**, 586–589)—A nephelometric method that is more selective than the Folin technique is described for the estimation of creatinine. The method is a modification of that suggested by Barrett (*Lancet*, 1936, **1**, 84) using Nessler's solution to which 25 ml. of 10 per cent. potassium iodide solution has been added per 100 ml.

*Procedure*—Dilute the sample to contain less than 3 mg. of creatinine per 100 ml. Add 3 ml. of this solution to 1 ml. of the reagent. Mix and allow to stand for 5 min. The precipitate remains dispersed for 30 min. Estimate nephelometrically in a photo-electric absorptiometer with a Chance O.B.2 glass light filter. Compare with standard solutions covering a suitable range not exceeding 3 mg. of creatinine per 100 ml., treated similarly.

*Blood and plasma samples*—De-proteinise by the zinc hydroxide method of Somogyi (*J. Biol. Chem.*, 1930, **36**, 655) or the tungstic acid method of Folin

and Wu (*Ibid.*, 1919, **38**, 81). Estimate creatinine in the filtrate as described above.

*Tissues*—Mince finely and mix with a known volume of water. Mix by shaking, filter, and treat the clear solution in the same manner as a plasma sample. For the estimation of total creatinine in tissue, autoclave the suspension of the material in water at 30 lb. pressure for 1 hr. and treat as above.

*Results*—Recovery experiments in which known amounts of creatinine were added to human and dog plasma showed that the zinc hydroxide gave rather better recoveries than the tungstate method. Creatine, ammonia, guanidine, methyl guanidine, sarcosine, and uric acid gave no reaction with the reagents and the hydantoin reaction was so slight as to make the reagent almost specific for creatinine in biological fluids. The specificity of the reagent was compared with that of the Folin method by estimating the apparent creatinine content of autoclaved rat tissue before and after treatment with a specific creatinine-destroying bacillus. The ratio of apparent to true creatinine was consistently smaller with this method than with the Folin method. The results for true creatinine agreed well with those of other workers. When creatinine was estimated in blood and urine by this method the creatinine clearance gave a true measure of filtration rate. J. S. HARRISON

**Micro-determination of British Anti-Lewisite. G. H. Spray** (*Biochem. J.*, 1947, **41**, 360–361)—A method for the micro-determination of British anti-lewisite, 2 : 3-dimercaptopropanol (BAL), is described with its application to plasma, blood, and urine.

*Method—Micro-determination in simple aqueous solution*—Mix 1 ml. of 0·5 per cent. cobalt nitrate solution with 0·5 ml. of 2 per cent. gum arabic solution and sufficient 0·1 *N* borate buffer at pH 9 to bring the final volume, including the BAL, to 10 ml. Heat to 45° C. in a water-bath, add the BAL solution, set aside at 45° C. for 10 min., and measure the colour intensity with a Pulfrich photometer and light of wavelength 4700 A. There is linear proportionality between colour intensity and BAL concentration in the range 10 to 200 μg. A precipitate forms if the solutions are heated to a temperature higher than 45° C. Acetic acid is added to stabilise the BAL in biological fluids and this causes a slight fall in colour intensity. Physiological concentrations of sodium chloride do not interfere with the colour development. Recoveries of 96 to 102 per cent. are obtained with aqueous solutions of known strength.

*Selectivity of the method*—Other thiols give colours with cobalt nitrate under the conditions described, and although the colours are often indistinguishable by the naked eye from the colour given by BAL the points of maximum light absorption are usually slightly different from that of the BAL colour. The intensities of the colours, calculated on a basis of SH content, vary widely.

*Application of the method to plasma, blood, and urine*—Added BAL could not be recovered quantitatively from plasma, blood or urine, probably owing to destruction of part of th BAL as a result of the highly reactive nature of the 1 : 2-dithiols, and not to any defect of the method. Recoveries varied from 30 to 88 per cent.

J. S. HARRISON

**Microbiological Assay of Subtilin. J. C. Lewis, E. M. Humphreys, P. A. Thompson, K. P. Dimick, R. G. Benedict, A. F. Langlykke, and H. D. Lightbody** (*Arch. Biochem.*, 1947, **14**, 437–450)—The turbidimetric bacteriostatic method described is based on McMahan's assay for penicillin (*J. Biol. Chem.*, 1944, **153**, 249).

METHOD—*Cultures and inoculum—Micrococcus conglomeratus* (MY), *Staphylococcus aureus* (H), and, occasionally, *Streptococcus faecalis* (N.R.R.L. B–537, A.T.C.C. 7080) are used as test organisms. Maintain cultures on Schmidt and Moyer's medium II (*J. Bact.*, 1944, **47**, 199) containing 0·5 per cent. of peptone, 0·15 per cent. of yeast extract, 0·15 per cent. of beef extract, 0·35 per cent. of glucose monohydrate, and 0·4 per cent. of potassium hydroxide with the addition of agar. Transfer weekly, incubate at 37° C., and store in the refrigerator. For the inoculum, transfer a loop of cells from a slant to 150 ml. of liquid medium and incubate overnight at 37° C. To the medium add 4 per cent. of the suspensions when *M. conglomeratus* and *S. faecalis* are used, and 2·5 per cent. for *S. aureus* in the final assay cultures.

*Assay medium*—For *S. aureus* and *S. faecalis* use medium II above. For *M. conglomeratus* use a medium containing 2 per cent. of trypsin-digested casein and 0·5 per cent. of yeast extract with the same amounts of glucose and potassium dihydrogen phosphate as medium II. Prepare the medium double strength to allow for additions. Cool to refrigerator temperature before use.

*Subtilin standard*—An arbitrarily chosen, partially purified lot of subtilin was chosen as standard. This was isolated by a method described by Dimick *et al.* (*Arch. Biochem.*, in press). It was stored in the refrigerator. A solution containing 40 μg. per ml. and adjusted to pH 2·5 with hydrochloric acid was stored in the refrigerator for routine use. It was renewed every 2 to 3 months.

*Samples*—Dilute aqueous culture samples with 3 volumes of 95 per cent. ethanol and shake for at least 1 hr. before use. Dilute butanol extracts with 70 per cent. ethanol. A concentration of 0·5 per cent. of ethanol in the final assay culture has little effect on the response. A 1 : 1 mixture of ethanol and butanol can only be tolerated at 0·1 per cent. or lower. Dissolve lyophilised subtilin preparations in dilute hydrochloric acid of pH 2·5. Triturate insoluble fractions with glacial acetic acid. If, on dilution, these give colloidal suspensions, dilute to about 40 μg. of activity per ml. with dilute hydrochloric acid at pH 2·5 and rock gently overnight in the cool room. Unneutralised acetic acid interferes at 0·05 per cent. concentration in the final assay culture. Store samples in the refrigerator pending assay.

*Procedure*—Use unsterilised, 18 × 150-mm. Pyrex culture tubes with 15 ml. of total medium. Measure triplicate aliquots of standard and sample

dissolved in dilute hydrochloric acid at pH 2·5 to give a volume of 5 ml. Add 10-ml. portions of cold, inoculated medium in essentially the same order as that for the subtilin solutions. Also prepare tubes with no subtilin and with sufficient to give complete inhibition of growth. Immediately incubate in a water-bath at 37° C. without disturbance for 4 hr. with *S. aureus* and *S. faecalis* and for 5 hr. with *M. conglomeratus*. After incubation cover the tubes with cotton-lined, stainless steel covers and sterilise under steam pressure. Measure the turbidity on the following day in an absorptiometer with a red filter. If readings are required on the same day steam at atmospheric pressure, otherwise there are marked changes in turbidity during the reading period.

*Calculations*—Convert the readings to a percentage of the maximum turbidity obtained with control not containing subtilin and plot the standard response on probability paper. This gives approximately linear standard curves for *M. conglomeratus* and *S. aureus*. An approximately linear curve for *S. faecalis* is obtained with logarithmic probability paper.

Considerable trouble was experienced in maintaining constancy of response to the standard throughout the assay experiments. To correct for this the standard solution was inserted in several positions throughout the assay. Progressive shifts in the activity of the standard as large as 15 per cent. were frequently observed in experiments with about 20 samples. An arbitrary standard curve, approximating the mean curve, was drawn and the apparent activities of the standard as well as the unknowns were estimated. The assays of unknowns were then corrected proportionately to the apparent activities of the adjacent standards.

Additional evidence is presented that the antibiotic activity of crude culture extracts of *Bacillus subtilis* and of partially purified lots of subtilin is not homogeneous. The activity of subtilin under assay conditions is unaffected by various compounds, including the common amino acids and the vitamins of the B group. J. S. HARRISON

**Re-examination of Halibut-Liver Oil. Relation between Biological Potency and Ultra-Violet Absorption due to Vitamin A. R. A. Morton and A. L. Stubbs** (*Biochem. J.*, 1947, **41**, 525–529)—A re-examination of the two halibut-liver oils used in the co-operative assays of 1936 has been carried out by photo-electric spectrophotometry. In 1936 it was calculated that the estimated biological activity of the mixed oils expressed in i.u. per g. divided by the intensity of absorption at 328 m$\mu$. expressed in terms of $E_{1\,cm.}^{1\%}$ was 1570. At that time it was believed that if the absorption curve obtained for a solution of a rich oil was the same as that obtained for an equivalent solution of its unsaponifiable fraction there was no irrelevant absorption. It is now shown that the belief is not usually justified. In a later co-operative experiment a solution of vitamin A $\beta$-naphthoate gave a conversion factor of 1770 and a similar assay with cod-liver oil gave 1820. A re-examination of the two samples of halibut-liver oil has been carried out to explain the discrepancy between the factors of 1570 and 1770.

The oils were dissolved in *cyclo*hexane and their absorption curves determined by means of a Beckman photo-electric spectrophotometer. The antimony trichloride colour test was carried out by measuring intensities of absorption at 617 and 583 m$\mu$. with a Hilger - Nutting visual spectrophotometer. Using the method of Morton and Stubbs (*Analyst*, 1946, **71**, 348), the irrelevant absorption at 328 m$\mu$. was estimated and the corrected intensities of absorption were calculated. From the known complete absorption curve for vitamin A and the observed values for the two oils, complete subtraction curves for irrelevant absorptions were constructed. From the irrelevant absorptions so obtained a corrected value of $E_{1\,cm.}^{1\%}$ 328 m$\mu$. for the original mixed oils was calculated, and from this a conversion factor of 1830 was obtained; this falls in line with the value of 1770 obtained from the assays on crystalline vitamin A $\beta$-naphthoate and 1820 obtained using cod-liver oil.

The results of fractionation experiments on the oils support the method of correction for irrelevant absorption. The significance of the results in the spectrophotometric assay of vitamin A is discussed.

J. S. HARRISON

**Chemical Estimation of the Oestrogens in Urine. A. E. Bender and A. Wilson** (*Biochem. J.*, 1947, **41**, 423–425)—The method of Talbot *et al.* (*J. Biol. Chem.*, 1940, **134**, 319) for the chemical estimation of oestradiol in normal urine is compared with the biological assay. The method consists essentially of the isolation of the weakly phenolic ketones in urine, coupling with diazotised dianisidine, and measurement of the colour produced. Analyses were carried out on human urine, with and without added oestrone and on rat urine before and after ovariectomy. The results show that Talbot's method gives figures many times higher than those obtained by the biological method. The values obtained for the urine of totally ovariectomised animals were not lower than those for normal animals, which indicates that it is not oestrogenic material that is producing the colour reaction. No chemical method is thus available for estimating oestrogens in normal urine, and the biological assay remains the only trustworthy method.

J. S. HARRISON

**Estimation of Oestrogens in Human Pregnancy Urine. New Method of Correcting for the Brown Colour Developed in the Kober Reaction by Non-Oestrogenic Substances. M. F. Stevenson and G. F. Marrian** (*Biochem. J.*, 1947, **41**, 507–511)—The determination of oestrogens in human pregnancy urine by the Kober method (*Biochem. Z.*, 1931, **239**, 209) is complicated by the fact that urinary oestrogen concentrates may contain substances that yield a brown colour in the reaction with the phenolsulphonic acid reagent. A simple procedure for correcting for the non-oestrogen brown colour has been worked out.

*Procedure—Hydrolysis and extraction of urine*—Collect urine samples and add toluene as preservative. Dilute 24-hr. specimens to a volume of 2·5 litres. Heat 100 ml. of the diluted urine to boiling-point under a reflux, add 15 ml. of concentrated hydrochloric acid down the condenser, and continue boiling for 30 min. Cool rapidly, extract once with 100-ml. and twice with 50-ml. portions of ether. Wash the combined ethereal extracts three times with 25-ml. portions of 5 per cent. sodium bicarbonate solution and back-extract the combined washings once with 20 ml. of ether. Warm the residue with about 3 ml. of ethanol and add 100 ml. of benzene. Extract the benzene solution once with 50 ml. and twice with 25-ml. portions of *N* sodium hydroxide. Acidify the combined alkaline extracts with 15 ml. of concentrated hydrochloric acid and extract once with 100 ml. and twice with 50-ml. portions of ether. Wash the ethereal extracts twice with 20-ml. portions of 5 per cent. sodium bicarbonate solution and back-extract the washings with 20 ml. of ether. Wash the combined ethereal extracts three times with 20-ml. portions of water and evaporate to dryness. Dissolve the residue, consisting of the total ether-soluble phenolic fraction of the hydrolysed urine, in ethanol and pipette out suitable samples for the colorimetric assay.

*Technique of the Kober reaction*—Prepare the phenolsulphonic acid reagent according to the method of Cohen and Marrian (*Biochem. J.*, 1934, **28**, 1603). The technique of colour development is essentially that of Venning *et al.* (*J. Biol. Chem.*, 1937, **120**, 225). Evaporate to dryness in a stream of air measured samples of the solution of urinary phenolic fraction containing between 10 and 80 μg. of oestrogen, and equivalent to not more than 2 per cent. of the 24-hr. urine specimen, contained in test tubes of 2 cm. diameter and bearing graduation marks at 8 and 15 ml. To the residues add 3-ml. portions of the Kober reagent and heat in a boiling water-bath for 20 min. Cool in an ice-salt freezing mixture, add 3 ml. of water to each tube, and mix thoroughly. Heat the tubes for 3 min. in boiling water, cool to room temperature by immersing in water, and dilute to 15 ml. with 10 per cent. v/v sulphuric acid. Measure the intensities of absorption at 520 mμ. on 7-ml. portions of the final solutions in a Spekker photo-electric absorptiometer, using an Ilford spectrum green No. 604 light filter. Heat the 8-ml. portions of the solutions remaining in the tubes in boiling water for 1·5 hr. in order to cause the pink colour produced by the oestrogen to fade. Cool, make good the water lost by evaporation, and again measure the absorption at 520 mμ. The amount of oestrogen as oestriol originally present in each tube is obtained by referring the difference between the initial and final absorptiometer readings to an oestriol calibration curve. The brown solutions yielded by ether-soluble phenolic fractions of human male urine in the Kober reaction undergo little change, as judged by absorption at 520 mμ., when heated at 100° C. for 1·5 hr.

Recovery experiments in which oestrone and oestriol were added to hydrolysed human male urine showed that when less than 10 mg. of oestrogen per 24 hr. was present, necessitating the carrying out of the colour reaction on the equivalent of 1 per cent. or more of the 24 hr. urine specimen, the recoveries obtained by the uncorrected Kober method were grossly high, whereas those obtained by the fading technique were mostly within the range 80 to 110 per cent. When the oestrogen per 24 hr. was more than 15 mg., a correction was hardly necessary. J. S. HARRISON

# Agricultural

**Fluorimetric Method for Estimating Small Amounts of Chlorophyll *a*. R. H. Goodwin** (*Anal. Chem.*, 1947, **19**, 789–794)—Purified chlorophylls *a* and *b* were prepared by the following procedure (Zscheile *et al.*, *Bot. Gaz.*, 1941, **102**, 463; 1934, **95**, 529). Grind 800 g. of fresh spinach leaves in 100-g. portions in a mortar with cold acetone, filter each extract, and transfer the pigment from each batch to light petroleum. Wash the petroleum solution with water, methyl alcohol, and again with water until the chlorophyll is precipitated. Dry the suspension over anhydrous sodium sulphate, collect the precipitate on a 3-cm. layer of powdered sucrose, wash it with light petroleum to remove carotenoids, and extract it from the sucrose with ether. Adjust the crude chlorophyll solution to contain 70 per cent. of light petroleum and 30 per cent. of ether and adsorb the chlorophyll on a sucrose column of diameter 5 cm. and length 35 cm. Develop the chromatogram for about 1·5 hr., adding during this time 500 ml. of fresh mixed solvent. Remove mechanically from opposite ends of the column the central portion of the upper green zone and the lower three-quarters of the lower blue zone and elute the pigment from each separately with ether. Wash the blue ether solution of chlorophyll *a* thoroughly with water, dry it over sodium sulphate, filter, and store it on solid carbon dioxide until immediately before spectroscopical examination.

To purify the green fraction adjust the ether eluate to the 70/30 ratio (*supra*) and re-adsorb the pigment on another sucrose column. After 2 hr. remove the central portion of the green zone and elute the pigment. Repeat the process once more with a third column. If spectroscopical examination of the eluate reveals the presence of chlorophyll *a* (as may occur in a very humid atmosphere), store the eluate in ether on solid carbon dioxide and develop a fourth column 3 days later. Elute the green band with ether, wash, dry and filter the eluate, dilute to volume and store it on solid carbon dioxide as with the chlorophyll *a* preparation. Conduct all these experiments in dim light.

Evaporate aliquots of the preparations in tared volumetric flasks in a vacuum desiccator, dry for 1 hr. at 103° C., cool, and weigh. Meanwhile make fluorimetric determinations with other portions of the solutions. Obtain absorption spectra (an automatic recording spectrophotometer, as described by Hardy, *J. Optical Soc. Amer.*, 1935, **25**, 305, is recommended) with a band-width of 10 mμ. and calculate the specific absorption coefficients (α) for

chlorophylls *a* and *b* thus, $\alpha = (\log_{10} I_0/I)/cx$, where *c* is the concentration of the chlorophyll in g. per litre and *x* is the cell-thickness in centimetres.

For fluorimetric determinations, a Klett fluorimetric colorimeter is suitable (Kavanagh, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 108) with a Leeds and Northrupp Type R galvanometer. The unknown solutions of chlorophyll should be in pure acetone, and the standard fluorescent solutions, shielded by a 5970 lamp filter, may contain 1 or 0·2 mg. of quinine sulphate per litre of 0·1 *N* sulphuric acid. Measurements are made at room temperature (22° to 26° C.). Although chlorophyll fluorescence decreases with rise of temperature (Zscheile *et al.*, *J. Phys. Chem.*, 1943, **47**, 623) errors due to temperature fluctuation within this range are less than 3 per cent. Readings should be made as rapidly as possible to minimise photo-decomposition. Decay of fluorescence is most pronounced when filters transmitting ultra-violet light are used, but is negligible with blue and violet filters. Fluorescence, as measured by potentiometric readings, is proportional to the chlorophyll present at low concentrations.

Chlorophyll *a* is much more fluorescent than chlorophyll *b*; it may also be somewhat less stable on storage in ether in the dark at 0° C. The difference between the fluorescence of *a* and *b* is greatest when the fluorescence is excited by the violet line 404·7 m$\mu$. Experiments showed that the two chlorophylls fluoresce independently, and it is thus possible to compute the fluorescence of various mixtures containing a constant total amount of chlorophyll. Pure chlorophyll *a* can be distinguished from chlorophyll *b* by the ratio $R = F_{404\cdot7}/F_{435\cdot8}$, a ratio that can be determined with considerable precision even when the concentration of the preparation is unknown, since the relative fluorescence at these wavelengths is the determining factor. For a mixture of the two chlorophylls the following relation should hold

$$R = \frac{Fa_{404\cdot7}(X)}{Fa_{435\cdot8}(X)} + \frac{Fb_{404\cdot7}(1-X)}{Fb_{435\cdot8}(1-X)},$$

where *Fa* is the fluorescence of a given concentration of chlorophyll *a*, and *Fb* the fluorescence of an equal concentration of chlorophyll *b* when excited by light of the wavelength indicated, and *X* is the proportion by weight of chlorophyll *a* in the mixture. An estimate of the proportion of *a* and *b* in an unknown mixture can be made by determining *R* and finding this value on a calibration curve showing the relation between the value of *R* and the composition of the mixture of *a* and *b*. The curve is rather flat for high percentages of chlorophyll *a* and the accuracy of the estimate is therefore low in this portion of the curve. Spectrophotometric methods (Comar, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 877; Griffiths *et al.*, *Ibid.*, 1944, **16**, 438) for obtaining the *a/b* ratio are preferable when the quantity of material permits. Since values of *R* for chlorophylls *a* and *b* will vary somewhat with the filter transmission, the spectral sensitivity of the photo-cells and other experimental conditions, a calibration curve must be computed for each new arrangement. This method is not applicable to crude extracts since the value of *R* is altered somewhat by the presence of other pigments. However, chromatographic separations of chlorophylls from the carotenoids can be carried out on small amounts of material (Goodwin *et al.*, *Plant Physiol.*, 1947, **22**, 197).

*Estimation of chlorophyll a*—Since chlorophyll *a* fluoresces about ten times as much as chlorophyll *b* when excited by the line 404·7 m$\mu$., the assumption can be made that all the fluorescence emitted by mixtures of the two components under these conditions is due to the fluorescence of *a*. For extracts of green tissues usually containing about 70 per cent. of chlorophyll *a*, the theoretical error should be about 4 per cent. If enough material is available a determination of the ratio *a/b* may be made either fluorimetrically or spectrophotometrically. Then, assuming constancy of the ratio for subsequent samples, the chlorophyll estimate can be corrected and a computation of the total chlorophyll content of the extract can also be made.

If only the relative concentrations are required and if the ratio *a/b* can be assumed to remain nearly constant among the samples to be tested, greater sensitivity will be attained by using ultra-violet light or blue light as excitants. With ultra-violet light, however, readings must be made as quickly as possible to minimise photo-decomposition, particularly of the more labile chlorophyll *a* component.

In mixtures of chlorophyll *a* with $\beta$-carotene the fluorescence of the chlorophyll is least affected by the presence of $\beta$-carotene when the solution is irradiated with ultra-violet light and most affected when irradiated with violet light. The reduction in fluorescence due to carotene becomes progressively more pronounced the higher is the concentration, but at low chlorophyll concentration the carotene does not appreciably reduce the fluorescence even when present in larger amounts than chlorophyll *a*, and for appreciable interference in dilute solution of chlorophyll the carotenoids must occur in concentrations considerably higher than that found in normal green tissues. Extracts of the shoots of dark grown oat seedlings containing flavones in amount equivalent to 100-mg. fresh weight of tissue when added to chlorophyll *a* solutions cause no decrease in fluorescence. Crude extracts of certain plant material, however, may contain sufficient amounts of flavones or other pigments to interfere.

*Determination of chlorophyll a in crude extracts*—Weigh the fresh tissue to be extracted on a microbalance to the nearest 0·1 mg., dip it for 30 sec. in boiling water and grind it finely with acetone in a suitable homogeniser. Centrifuge immediately and decant the clear supernatant liquid. Dilute the crude extract to 20 ml. with acetone. Determine the fluorescence of the extract or a dilution thereof immediately in a fluorimeter, using the 404·7 m$\mu$. line, and estimate the amount of chlorophyll *a* present by means of a calibration curve.

For details of the construction and manipulation of the instruments used in this investigation the original paper should be consulted. A. O. JONES

# Gas Analysis

**Determination of Acetylene in Gas. J. A. Shaw and E. Fisher** (*Anal. Chem.*, 1948, **20**, 533–536)—The gas is scrubbed with a 35 per cent. solution of silver nitrate, usually containing ferric nitrate. After diluting, filtering, washing, and drying, the $Ag_2C_2.AgNO_3$ formed is weighed.

*Procedure*—Pass a measured amount of gas at a rate of 0·5 cu. ft. per hr. successively through solutions of potassium hydroxide (20 to 30 per cent.), monochlorobenzene containing 5 to 10 g. of piperidine per litre, 10 per cent. sulphuric acid, and silver nitrate - ferric nitrate (twice). The last solution is made by dissolving 540 g. of silver nitrate in 1 litre of 3 *N* nitric acid with gentle heating and then dissolving the equivalent of 60 g. of anhydrous ferric nitrate in the cooled solution. Pass sufficient gas through to yield 100 to 150 mg. of $Ag_2C_2.AgNO_3$, and not more in view of explosion hazards. Use goggles and tongs in handling the dry precipitate and always dissolve residues in dilute hydrochloric acid. Dilute the contents of the two tubes to 300 ml., filter through a sintered-glass filter, wash with acetone, and complete the drying in a desiccator containing sulphuric acid.

The test was normally employed on coke-oven gas containing about 0·05 per cent. of acetylene, when about 0·5 cu. ft. were used. Other gases may need modifications in procedure. For example, if there is no hydrogen present, the acid ferric nitrate, which prevents the formation of metallic silver, is not necessary. The method is said to differentiate between acetylene and alkyl acetylenes.

W. J. GOODERHAM

# Organic

**Determination of Small Amounts of Ethyl Ether in Ethyl Alcohol. W. E. Shaefer** (*Anal. Chem.*, 1948, **20**, 651–652)—*Procedure*—Transfer 500 g. (approximately 630 ml.) of sample to a 1-litre, round-bottomed flask and add 250 ml. of water and a few particles of carborundum. If the sample is suspected to contain less than 0·2 per cent. of ether, add 2 ml. of ether from a 2-ml. pipette previously rinsed with ether and cooled by a current of air. Such a prepared pipette will deliver 1·38 g. of ether and will increase the ether content of the sample by 0·28 per cent. Attach a 3-bulb Snyder column bearing a thermometer to the flask, and weigh the unit and sample correct to 0·1 g. Connect the outlet of the column to a vertically arranged condenser and attach the latter to a suitable receiver.

Direct a strong jet of air against the base of the column and heat the sample rapidly, by means of an electric heater with a variable control, to incipient boiling. Adjust the heater until the condensed vapour just refluxes from the lowest bulb of the column. Re-adjust the heater and allow the distillation to proceed slowly (approximately 0·5 ml. in 5 min.) until the vapour temperature reaches 52° C. Maintain the vapour temperature at 52° ± 2° C. until the distillation rate falls below 3 drops per min., and then heat for an additional 10 min. at 52° C. If the initial rate of distillation never exceeds 3 drops per min. in the temperature range 52° to 54° C., continue the distillation within this range for 20 min. from the time the vapour temperature first reached 52° C. Remove the heater, disconnect the condenser, and weigh the system correct to 0·1 g.

The distillation rate is controlled by adjustment of either the heater or the air jet.

*Calculation*—If no ether was added before distillation, the ether content of the sample is calculated as follows:—loss in weight of sample × 100/weight of sample of alcohol = per cent. of ether (uncorrected). Apply the correction value read from a curve constructed from data obtained by distillation of 500-g. aliquots of ethyl alcohol to which known amounts of ether have been added, the percentage of ether added being plotted on one axis and the percentage found on the other.

If 2·0 ml. of ether were added to the sample before distillation, the ether content is calculated and corrected as described above and from the result so obtained 0·28 per cent. is deducted to give the ether content of the original sample.

Water, and up to 0·5 per cent. of benzene, do not interfere.

A. H. A. ABBOTT

**Spectrophotometric Determination of Diacetyl. J. C. Speck, jun.** (*Anal. Chem.*, 1948, **20**, 647–648)—The purple colour formed by diacetyl with chromotropic acid and sulphuric acid is nearly indistinguishable from that formed by formaldehyde under the same conditions, and is probably due to the production of formaldehyde from diacetyl by oxidation with sulphuric acid according to the scheme

$$CH_2 : \underset{\displaystyle OH}{\overset{\displaystyle |}{C}}.COCH_3 + 2[O] = CH_2O + CH_3.CO.COOH$$

The formation of this colour has been utilised as the basis of a rapid spectrophotometric determination of diacetyl. Formaldehyde, methyl ethyl ketone, and all substances that interfere in the determination of formaldehyde with chromotropic acid, interfere with the proposed method. Acetyl propionyl, the next higher homologue of diacetyl, gives no colour with chromotropic acid.

*Procedure*—Transfer 1 ml. of a solution containing 0·03 to 0·1 mg. of diacetyl to a test tube provided with a glass stopper, add 0·5 ml. of 10 per cent. chromotropic acid solution, mix well, and gradually add 5 ml. of 98 per cent. sulphuric acid. Immerse the stoppered tube in a water-bath so that the level of the contents is below the water level and heat at 100° C. for 1 hr. Cool, dilute the mixture to 50 ml. with water, and determine the extinction at 570 mμ. Carry out a blank determination on the reagents and deduce the diacetyl content of the sample by reference to a standard curve constructed from data obtained by application of the above procedure to samples containing known amounts of diacetyl.

A. H. A. ABBOTT

**Polarographic Determination of Methacrylic Ester. M. B. Neyman and M. A. Shubenko** (*Zavod. Lab.*, 1948, **14**, 394–396)—Methyl methacrylate can be determined polarographically in aqueous alcoholic solutions containing lithium chloride or tetramethyl ammonium iodide as supporting electrolyte. The method can be used for studying the polymerisation of the ester.

In 0·1 *N* lithium chloride in 25 per cent. alcohol at 20° C. well-defined waves were obtained for methyl methacrylate in concentrations of 8, 16, and 24 mg.-mol. per litre, and the wave-heights were proportional to concentration. The reduction potential and the half-wave potential were −1·7 and −1·92 v., respectively, *versus* the normal calomel electrode. Calculations from the Ilkovic equation gave the diffusion coefficient $D = 3{\cdot}8 \times 10^{-6}$ cm.$^2$ per sec.

Slightly better waves were obtained in 0·1 *N* tetramethyl ammonium iodide because of the more negative reduction potential of the supporting electrolyte. The diffusion coefficient is $2{\cdot}5 \times 10^{-6}$ cm.$^2$ per sec. G. S. SMITH

**Amperometric Micro-titration of Diamidines. J. B. Conn** (*Anal. Chem.*, 1948, **20**, 585–586)—Owing to the low degree of accuracy of the available methods of determining diamidines, an accurate amperometric method has been developed. It depends on the formation of the insoluble alizarin-sulphonates of the diamidines. Since alizarin-sulphonic acid is polarographically reducible, any excess is readily detected after the end-point has been reached by observing the current flowing between a dropping mercury cathode and a saturated calomel reference electrode immersed in the solution.

*Procedure*—Dissolve 6 to 10 mg. of the diamidine salt in 10 ml. of 0·1 *M* phosphate or borate buffer at pH 7·0, and transfer a 5-ml. portion of this solution to a polarographic cell. Pass nitrogen through the cell for 5 min. to remove dissolved oxygen and, after setting the applied potential at −0·9 v. *versus* the saturated calomel electrode, run in from a burette an approximately 0·08 *M* solution of recrystallised sodium alizarin sulphonate, dissolved in the buffer at pH 7·0. At approximately every 0·02-ml. addition pass nitrogen through the solution for 2 min. and then read the current, and plot it against the volume added. Continue this until the current is increasing linearly, and then read off the end-point at the intersection of the two straight lines obtained.

The results are accurate to within ±0·5 per cent. With phenamidine, the separation of the crystalline alizarin-sulphonate occurs slowly, and longer intervals must be allowed between additions of reagent; it is helpful to add a little solid phenamidine alizarin-sulphonate at the start of the titration to provide a seed for the crystals. J. G. WALLER

**Determination of Lignin in Plant Material of High Protein Content. E. R. Armitage, R. de B. Ashworth, and W. S. Ferguson** (*J. Soc. Chem. Ind.*, 1948, **67**, 241–243)—Extract 1 g. of ground (1/64-in. mesh) sample in a boiling mixture of alcohol and benzene (1 : 2) in a sintered-glass crucible placed in a fat-extraction apparatus, wash the residue with ether, and dry it by suction. Heat it under reflux for 1 hr. with 100 ml. of 5·0 per cent. (w/w) hydrochloric acid, filter it off through a fine cloth, wash it with water, and digest it with 100 ml. of 0·25 per cent. sodium carbonate solution and 0·1 g. of trypsin for 18 hr. at 38° C. Filter through cloth, wash the residue with alcohol and ether, dry it by suction, and stir it with 10 ml. of 72 per cent. sulphuric acid solution until no lumps remain. After 2 hr. at 15 to 20° C., transfer it to a 500-ml. flask with 240 ml. of water, and heat the mixture under reflux for 2 hr. Filter while hot through a tared asbestos Gooch crucible (alundum is untrustworthy), keeping the crucible full to accelerate filtration. Wash the residue well with hot water, dry it in a steam-oven until constant in weight, and weigh it quickly. Burn off the lignin in a furnace, and obtain its weight by difference, after reweighing the crucible. Make the determination in triplicate, treating two samples as above, but filtering the third through filter-cloth (instead of on a Gooch crucible) and then washing and drying it. Determine the nitrogen content and deduct this × 6·25 from the mean crude lignin content as obtained from the two other experiments. Data tabulated for grass, hay, oat straw, clover, and sheep's faeces show the effect of variations in the method of pre-extraction with solvent, enzyme treatment, protein content, and concentration of the acid hydrolysis. Comparison with the methods of Norman, of Crampton and Maynard, and of Davis and Miller show that the new method gives much lower results. They are, however, regarded as much nearer the true figure because the nitrogen correction is much less (2·0 per cent.), and because the treatment prior to acid hydrolysis removes most of the substances likely to form insoluble complexes with the strong acid used. J. GRANT

**Measurement of Enzymic Activity on Limit Dextrin. T. M. Back, W. H. Stark, and R. E. Scalf** (*Anal. Chem.*, 1948, **20**, 56–60)—When corn (maize) starch is treated with malt enzymes, 65 to 75 per cent. is converted to fermentable sugars in 15 min. The rate of reaction then decreases and the residual amylase-resistant "limit dextrin" is only slowly converted to fermentable sugar. The efficacy of enzymic preparations for the conversion of diastatically produced limit dextrin to fermentable sugar is of great technical importance, particularly when rapid and complete conversion is desired.

The method presented was devised to evaluate enzymic preparations on the basis of their power to convert diastatically produced limit dextrin to fermentable sugars (*i.e.*, their dextrinase activity) under standard conditions of time, temperature, pH, and substrate concentration. It is based on the difference in reducing power (after acid hydrolysis) in the blank medium (in which enzyme has been destroyed before limit dextrin has been added) and the enzyme-treated sample after complete, rapid removal of fermentable sugar from a dilute solution by a large amount of baker's yeast. A

2·5-hr. treatment of the dilute solution of sugars at pH 4·8 and at 30° C. with fresh baker's yeast (15 g. per 100 ml. of liquid) was selected. These conditions have been shown by Stark and Somogyi (*J. Biol. Chem.*, 1942, **142**, 579) to result in complete removal of maltose. If the fermentable sugar consists only of glucose, the amount of yeast may be reduced to 5 g. per 100 ml. and the fermentation period to 1 hr.

Since unwashed yeast contains a variable amount of reducing substances, Somogyi's method (*Ibid.*, 1927, **75**, 35; *Analyst*, 1927, **52**, 719) of repeatedly washing and centrifuging the yeast until the wash water remains clear was adopted. The slight dilution of the sugar solution that occurs when the washed yeast is added may be corrected for by determining the reducing power of the blank before and after yeast sorption.

Reducing power before and after removal of fermentable sugars was determined by the Shaffer - Hartmann micro-method (Shaffer *et al.*, *Ibid.*, 1921, **45**, 379) as described by Stiles *et al.* (*J. Bact.*, 1926, **12**, 429). The proposed method for the determination of limit dextrinase activity (mg. of fermentable sugar produced from limit dextrin by 1 g. of enzyme) is based on the fact that conversion of a standard solution of limit dextrin is proportional to the amount of enzyme employed up to a certain degree of conversion. This degree of conversion must be determined experimentally for the particular enzyme used, for it is essential that the reaction should occur within the range where a linear relation exists between the conversion and the amount of enzyme used.

To prepare diastatically produced limit dextrin, first prepare malt extract for use as "pre-malt" and as the saccharifying agent by extracting 550 g. of finely ground barley malt with 2 litres of water for 1 hr. with frequent shaking followed by centrifuging. To 12 litres of water add 1·8 kg. of corn starch dispersed in water, warm to 68° C., and add 320 ml. of the malt extract, also at 68° C. Agitate the dispersion at 80° C. for 2 hr. and then pressure-cook at 60 lb. per sq. in. for 7·5 min. (or at 15 to 20 lb. per sq. in. for 1 hr.). Cool to 58° C., adjust the pH to 5·5, and add 1280 ml. of the malt extract. After hydrolysis for 45 min. at 55° to 58° C., autoclave the hydrolysate for 1 hr. at about 16 lb. per sq. in. to destroy the enzymes. Cool to 30° C., add 123 g. of baker's yeast, adjust the volume to 16 litres, and maintain at 30° C. for 44 hr. Centrifuge to remove solid matter, reduce the volume to about 1 litre by evaporation under reduced pressure, filter the concentrate, and add the filtrate to 2 to 4 times its volume of methyl alcohol, stir, and allow to stand overnight. Remove the gummy residue from the main bulk and purify it by re-dissolving it in a small amount of water, filtering, and re-precipitating it with methyl alcohol. Wash it repeatedly with methyl alcohol and dry it in a vacuum oven at 50° C. Finally, powder the dried limit dextrin and pass it through a fine sieve.

The buffer solution is an aqueous solution of 35·32 g. of disodium phosphate dodecahydrate and 9·73 g. of citric acid diluted to 1 litre, the pH being adjusted, if necessary, to 4·8 by addition of either component. To prepare washed yeast suspend baker's yeast repeatedly in fresh portions of water and centrifuge until the wash water remains clear. After the final wash water has been decanted press the yeast on absorbent paper.

If dry enzymic preparation is to be tested, first grind it thoroughly and then extract 0·9 g. with exactly 20 ml. of water for 1 hr. at 30° C. in a tightly stoppered flask with continuous agitation. After centrifuging, the decanted extract is ready to be diluted (*infra*). With liquid preparations extraction is unnecessary and the material is simply centrifuged or filtered through glass wool.

*Procedure for determining limit dextrinase activity* —To determine the range in which limit dextrin conversion is linear with respect to enzyme concentration it is necessary to determine the conversion produced with a series of different amounts of the enzyme. To do this add 5-ml. portions of diluted enzyme extract, the stock enzyme extract (*supra*) being diluted to give a series of enzyme extracts containing varying amounts of the stock extract, and then plot the degree of conversion of limit dextrin against the amount of enzyme used.

Place the required number of 50-ml. flasks, each containing a 20-ml. portion of limit dextrin solution containing 0·18 g., in a water-bath at 30° C., allow the contents to attain this temperature, and to each flask add 5 ml. of enzyme extract of the appropriate dilution to give an enzyme concentration within the linear range. Allow the action to proceed for 60 min., and then destroy the enzyme by adding 5 ml. of 1·5 *N* sodium hydroxide. After 30 min. adjust the pH to 4·8 with 1·5 *N* sulphuric acid, using methyl red as indicator. If the enzymic agent is buffered, the pH of the conversion mixture should be determined, as it is necessary that the conversion should be made at pH 4·8. It is desirable to determine limit dextrinase action with at least three different enzyme concentrations, but concentrations below 3 per cent. should be avoided.

For the blank determination place 5 ml. of the enzyme extract in a 50-ml. flask containing 5 ml. of 1·5 *N* sodium hydroxide. After 30 min. add 20 ml. of the standard limit dextrin solution and adjust the pH to 4·8 with 1·5 *N* sulphuric acid, using methyl red as indicator. Then treat the blank exactly as described for the samples. (If the enzymic agent contains no fermentable or reducing substance after acid hydrolysis, the blank can be run in exactly the same manner as the samples, except that no enzyme extract is added.)

After the samples and the blank have been adjusted to volume, place a 20-ml. portion from each in 40-ml. graduated centrifuge tubes. From the blank remove a second 10-ml. portion and add it to 10 ml. of 1·38 *N* hydrochloric acid. To each of the 20-ml. portions previously placed in centrifuge tubes add 3 g. (moist weight) of washed fresh baker's yeast, shake the tubes thoroughly, and maintain them at 30° C. for 2·5 hr., shaking them several times during that period. Centrifuge each tube, decant the liquid portion and add exactly 10 ml. to 10 ml. of 1·38 *N* hydrochloric acid. Hydrolyse all the samples that have been added to hydrochloric acid in boiling water for 2·5 hr., cool, neutralise

to the phenolphthalein end-point with 1 to 2 *N* sodium hydroxide, and dilute to 100 ml. Add triplicate 5-ml. portions from each sample to 5-ml. portions of sugar reagent and determine reducing sugar as described in the standard method (Stiles *et al., loc. cit.*). In the accurate determination of reducing sugar a curve is drawn on a large scale relating a series of 5 to 50 mg. per 100-ml. samples of dextrose to the thiosulphate titration. The result should be a straight line, or nearly so, not passing through the origin.

The percentage conversion is given by $100(B_2 - G_2)/B_2$, where $B_2$ is mg. of glucose in a 5-ml. sample of the blank after yeast sorption, the sample being removed after acid hydrolysis and final dilution, and $G_2$ is mg. of glucose in a 5-ml. portion of the enzyme-treated sample after yeast sorption, the sample being removed after acid hydrolysis and final dilution. Defining limit dextrinase units as mg. of fermentable sugar produced from limit dextrin by 1 g. of enzyme preparation in 1 hr. at 30° C., the number of dextrinase units is given by $100(B_2 - G_2)/E(B_2 \div B_1)$ where $B_1$ is mg. of glucose in a 5-ml. portion of the blank before yeast sorption, the sample being removed after acid hydrolysis and final dilution, and $E$ is g. of enzyme preparation extracted per 5 ml. of enzyme extract added to the 20-ml. portion of limit dextrin. The factor $(B_2 \div B_1)$ corrects for dilution due to the use of washed yeast.

By slight modifications of the standard method given, the effect of any variable on limit dextrinase activity may be studied. The method was applied to determination of the effect of the enzyme-limit dextrin ratio on conversion of limit dextrin over a wide range for Mylase bran and a 48-hr. submerged culture of *Aspergillus oryzae*. A. O. JONES

**Separation and Determination of Polyols by Selective Entrainment. Application to the Determination of Glycerol. G. Métayer** (*Chimie Analyt.*, 1948, **30**, 148–156)—*Apparatus*—This is a Dean and Stark apparatus with a 250-ml. flask. The receiver is of 7 to 10 ml. capacity, graduated in 0·1 ml., and is equipped with a draw-off stopcock. The apparatus is assembled with interchangeable ground joints and 14-cm. columns can be inserted between the receiver and the condenser.

*Procedure*—Mix 2 to 3 g. of the polyol mixture with 80 to 100 ml. of the selected entraining liquid and distil until no further increase in volume of the entrained liquid is apparent. Wash the condenser 5 or 6 times with 15 to 20 ml. of the entraining liquid, stir with a fine glass rod, and read the volume entrained.

*Determination of water in polyols*—Chloroform saturated with water is the recommended entrainer and for this procedure the stopcock is connected to a tube that leads the entrainer back into the flask. When employed for mixtures of ethylene glycol and water the latter is entrained quantitatively with about 5 per cent. of the glycol.

*Separation of polyols with aromatic hydrocarbons*—Benzene, toluene, and xylene are studied as entrainers, but the only successful separation is that of ethylene glycol from diethylene glycol and glycol by means of toluene.

*Separation of polyols with* cyclo*paraffins*—The behaviour of individual polyols is shown in the following table.

| | Ethylene glycol | Propylene glycol | Trimethylene glycol | Diethylene glycol | Glycerol |
|---|---|---|---|---|---|
| *Cyclo*hexane .. .. .. | + | + | + | — | — |
| Methyl*cyclo*hexane .. .. | + | + | + | O | — |
| Dimethyl*cyclo*hexane .. | + | + | + | + | + |

+ signifies quantitative entrainment, O partial entrainment, — no entrainment.

*Cyclo*hexane affords quantitative separation of ethylene glycol and/or propylene glycol from diethylene glycol and glycerol, but a 25-cm. column is necessary to prevent entrainment of diethylene glycol by ethylene glycol. The distillation requires up to 8 hr. Trimethylene glycol can be separated from diethylene glycol with *cyclo*hexane but not from mixtures containing a high proportion (70 to 80 per cent.) of glycerol unless ethylene or propylene glycol is also present to assist entrainment of the trimethylene glycol. Mixtures of trimethylene glycol and glycerol, containing less than 30 per cent. of the glycol, can be separated by entrainment with methyl*cyclo*hexane, but the distillation must not be prolonged beyond 8 hr. Decalin entrains glycerol quantitatively in 30 min. and diethylene glycol in 1 hr. It also entrains erythritol and quinitol, but not D-mannitol, D-sorbitol, inositol, or arabitol. Glucose, sorbose, and arabinose carbonise during the distillation.

In a mixture of polyols, water is determined by distillation with chloroform; ethylene, propylene, and trimethylene glycols are determined collectively by distillation of a second sample with *cyclo*hexane; total glycols and glycerol are determined by distillation of a third sample with decalin.

The polyol present, for example, in a cream, can be determined by distillation with decalin, followed by measurement of the refractive index and specific gravity of the polyol - water mixture. Gerlach's table (D. Holde, *Huiles et grasses*, 1929, 770) gives the percentages of water - glycerol mixtures in terms of the physical constants, and similar tables may be prepared for the glycols. For a table dealing with diethylene glycol, see Palfray, Sabetay, and Libmann-Métayer (*Industrie de la Parfumerie*, **2**, 325). W. C. JOHNSON

**Determination of Unsaturation of Synthetic and Natural Rubbers by Means of Iodine Monochloride. T. S. Lee, I. M. Kolthoff, and M. A. Mairs** (*J. Polymer Sci.*, 1948, **3**, 66–84)—The reaction of iodine monochloride with the unsaturated hydrocarbon polymers of isoprene and

butadiene, and butadiene-styrene co-polymer has been investigated. Three procedures for determining the unsaturation of these polymeric materials are given. In the first procedure the reaction-rate curve is obtained in which correction is made for substitution, the flat portion of the corrected reaction-rate curve giving the true unsaturation. A second procedure is applicable only to polymers actually covered by the present investigation for which empirical corrections are available, and involves a smaller number of iodimetric determinations. A third procedure is for the routine examination of certain types of polymer and is less accurate, errors of 2 per cent. being possible owing to lack of reproducibility in the amount of substitution occurring. The methods have been applied only to uncompounded, specially purified, raw rubbers.

*Nature of the reactions involved*—In the presence of an excess of iodine monochloride 90 to 95 per cent. of the double bonds in polymers react within a few minutes at room temperatures and, after this time, the remaining double bonds react only very slowly. The "splitting out" of hydrohalic acid, due, almost certainly, to some form of cyclisation, also occurs within the first few minutes of the reaction period. Any acid appearing after this time is the result of substitution. The acid formed is invariably hydriodic acid, which reacts with an excess of iodine monochloride as quickly as it is formed, to give iodine and hydrochloric acid. The iodine can therefore be estimated instead of the acid. This is done by titration with 0·02 *N* (0·005 *M*) potassium iodate solution, the end-point being indicated by the disappearance of the iodine colour from the organic phase. This reaction proceeds according to the equation

$$KIO_3 + 2I_2 + 6HCl = 3H_2O + KCl + 5ICl$$

*Preparation of the sample*—If the polymer contains more than 0·2 per cent. of anti-oxidant or other impurities, or if it contains gel, purification by precipitation is necessary. Cut 5 g. of the polymer into small pieces and place in 500 ml. of benzene. Allow to stand until dissolved or for 2 to 3 days. Decant the clear liquid and remove any gel from the solution by filtration through glass wool or a wire screen. Precipitate by the slow addition of the solution to 2 litres of absolute alcohol containing 0·2 per cent. of phenyl-β-naphthylamine. Stir the alcohol rapidly during the addition. Separate the polymer by decantation or filtration, wash with 100 to 200 ml. of 0·2 per cent. phenyl-β-naphthylamine in alcohol, remove the excess of washing liquid by blotting with filter paper, and dry at 80° C. in a vacuum oven for 1 hr., or, with an isoprene polymer, dry at room temperature for 24 hr. at a pressure of less than 10 mm. If the polymer is obtained from a latex, use a similar procedure, coagulating each 20 ml. of latex with 1 litre of alcohol.

*Procedure I*—Weigh 0·25 g. of the purified polymer (0·20 g. for polybutadiene) and transfer to a 250-ml. volumetric flask. Introduce the rubber through the neck of the flask with the aid of a tube of paper to prevent adhesion of the raw rubber to the glass. Add 50 ml. of chloroform and 135 to 140 ml. of carbon disulphide. Set aside or agitate gently until dissolution is complete (12 hr. to 2 days). Add 50 ml. of 0·11 *N* iodine monochloride solution in chloroform, dilute to the mark with carbon disulphide, and mix thoroughly.

After 10 to 15 min., pipette 25 ml. of the solution into 50 ml. of 6 *N* hydrochloric acid in an iodine flask. Titrate with 0·02 *N* (0·005 *M*) potassium iodate solution, the end-point of the titration being given by the disappearance of iodine from the organic phase. After successive periods of 0·5, 1, 2, and 4 hr., determine the total iodimetric titre as well as the free iodine. For the total iodimetric titre pipette 25 ml. of the reaction mixture into potassium iodide solution containing 40 ml. of water, 10 ml. of alcohol, and 0·5 g. of potassium iodide. Shake and titrate immediately with 0·05 *N* thiosulphate solution. Add starch near the end-point. Prepare a blank reaction mixture in exactly the same way as the sample reaction mixture and titrate, except that it is only necessary to carry out one titration against iodate and one against thiosulphate. Calculate the corrected unsaturation as,

$$\text{Per cent. unsaturation} = \frac{[a - (b - c)] \times \text{M.W.}}{w}$$

where $a$ = decrease in total iodimetric titre in mg.-mol. after 0·2 to 4 hr.
$b$ = number of mg.-mol. of iodine found after 0·2 to 4 hr.
$c$ = number of mg.-mol. of iodine found up to 0·2 hr.
M.W. = molecular weight of the monomer unit; *i.e.*, 54·1 for butadiene and 68·1 for isoprene polymers.
$w$ = weight of the sample in grams.

*Procedure II*—This is identical with Procedure I except that the number of the titrations is less. Titrate an aliquot portion with potassium iodate after 10 min. as before and determine the total iodimetric titre and repeat the iodate titre at the end of the time periods specified as necessary for complete addition; these are given in Table I.

*Procedure III*—Weigh into a 300-ml. iodine flask 0·100 g. of GR–S (butadiene-styrene copolymer) or isoprene, or 0·080 g. of polybutadiene. Add 20 ml. of chloroform and 60 ml. of carbon disulphide. Set aside or agitate gently until the sample is dissolved. Add 20 ml. of 0·11 *N* iodine monochloride solution in chloroform and allow to stand for the period specified for complete addition (see Table I). After this period, add 50 ml. of water containing 0·5 g. of potassium iodide. Shake and titrate with 0·1 *N* thiosulphate. Titrate also a blank prepared at the same time.

$$\text{Per cent. unsaturation} = \left[\frac{a \times \text{M.W.}}{10 \times w}\right] - d$$

where $d$ is the correction for substitution given in Table I, and the other symbols are as before.

*Presentation of results*—Percentage unsaturation as calculated is equivalent to the apparent percentage of polymerised butadiene or isoprene in the sample. To express these figures as iodine values, multiply by 4·696 for butadiene polymers or its copolymers with styrene, or by 3·728 for

isoprene polymers. Procedure I gives the corrected unsaturation for a number of reaction periods, the values for which can be plotted against time on a graph. The linear part of this graph is held to give the true figure for the unsaturation. The linearity of this curve is illustrated by reference to Table II, which gives results obtained by Procedure I for several types of polymer.

TABLE I

| Polymer | Temp. ° C. | Time needed for complete addition hr. | Approx. amount of substitution* |
|---|---|---|---|
| Emulsion butadiene-styrene .. .. | 25 | 1·0 | 1·2 |
| Emulsion polybutadiene .. .. | 25 | 1·0 | 1·0 |
| Emulsion polyisoprene .. .. | 25 | 0·2 | 1·0 |
| | 0 | 0·4 | 1·0 |
| Sodium butadiene-styrene .. .. | 25 | 1·0 | 2·3 |
| Sodium polybutadiene .. .. | 25 | 1·0 | 1·7 |
| Sodium polyisoprene .. .. .. | 25 | 0·2 | 2·8 |
| | 0 | 0·4 | 2·5 |

* Expressed as mol. per cent. of total number of double bonds, the value given being that required for *d* in Procedure III.

TABLE II

| Type of polymer | Temp. ° C. | Time hr. | Substitution % | Corrected unsaturation % |
|---|---|---|---|---|
| GR-S (64% conversion) | 25 | 0·35 | 0 | 81·5 |
| | | 1·08 | 1·6 | 80·9 |
| | | 2·07 | 2·3 | 80·9 |
| | | 4·42 | 3·5 | 80·9 |
| Emulsion polybutadiene | 25 | 0·17 | 0 | 95·6 |
| | | 1·0 | 0·9 | 97·7 |
| | | 4·0 | 1·6 | 97·7 |
| | | 6·0 | 1·9 | 98·1 |
| Emulsion polyisoprene | 0 | 0·1 | 0 | 96·3 |
| | | 1·2 | 1·4 | 96·8 |
| | | 4·0 | 3·0 | 96·8 |
| | 25 | 0·1 | 0 | 96·6 |
| | | 1·3 | 3·8 | 97·3 |
| | | 4·3 | 6·7 | 97·3 |
| Sodium polybutadiene | 25 | 0·15 | 0 | 88·8 |
| | | 1·0 | 1·7 | 92·1 |
| | | 2·6 | 3·4 | 92·2 |
| | | 5·0 | 4·6 | 91·9 |
| Natural rubber | 0 | 0·17 | 0 | 97·8 |
| | | 1·0 | 1·7 | 96·7 |
| | | 4·2 | 3·4 | 96·5 |

W. C. WAKE

**Determination of the Nitrosoamine Content of Propellant Explosive Stabilised with *sym*-Diethyldiphenylurea. T. C. J. Ovenston and C. A. Parker** (*J. Soc. Chem. Ind.*, 1947, **66**, 394–395)—In the approximate method of Lécorché and Jovinet (*Mem. Poudres*, 1928, **23**, 153) for the surveillance of double base propellant powder stabilised with *sym*-diethyldiphenylurea, N-nitroso-N-ethylaniline, formed gradually during storage, is estimated by means of the violet colour produced when it reacts with α-naphthylamine in presence of hydrochloric acid. Becker and Hunhold (*Z. Schiess. Sprengstoffw.*, 1938, **38**, 214) have since applied a modification of the method to the study of nitrocellulose powder stabilised with diphenylamine. The colorimetric reaction between aromatic N-nitrosoamines and α-naphthylamine hydrochloride has been shown by Parker (*J. Chem. Soc.*, 1946, 772) to be due to formation of the hydrochloride of 4-amino-1 : 1-azonaphthalene irrespective of the nitrosoamine employed. The method now presented is based on this reaction and serves to determine the total nitrosoamine content of cordite stabilised with *sym*-diethyldiphenylurea, and is applicable to the estimation of the thermal age of cordite and to the testing of materials for compatibility with cordite.

*Procedure*—Reduce the cordite to pass a B.S. Mesh Test Sieve No. 18. With artificially aged powder, allow the ground sample to stand for 24 hr.

at room temperature and then spread it on paper to aerate for 30 min. before weighing out the portion for the test.

Shake a small weighed portion of the prepared sample mechanically for 20 min. with purified 93 per cent. alcohol and adjust the mixture to a known volume. Allow the cordite to settle and take an aliquot containing up to the equivalent of 0·5 mg. of N-nitroso-N-ethylaniline. Owing to the photo-sensitivity of nitrosoamines the extraction and subsequent operations up to this stage should be carried out with the minimum exposure to light. After addition of 5 ml. of alcoholic 2 *N* hydrochloric acid and 5 ml. of 1 per cent. alcoholic α-naphthylamine solution, dilute the liquid to 90 ml. with 95 per cent. alcohol. Heat the liquid in a water-bath for 30 min. at 60° ± 2° C. and then place it in a thermostat at 20° C. for an hour. Finally, dilute the solution to 100 ml. with 95 per cent. alcohol and mix.

Determine the nitrosoamine concentration, calculated as N-nitroso-N-ethylaniline, by means of a Spekker absorptiometer (using a 1-cm. cell, a water-to-water setting of 1·00 and Ilford yellow-green filters, No. 605) by reference to a calibration curve obtained from known weights of N-nitroso-N-ethylaniline.

The method is rapid and convenient and determines 97 to 98 per cent. of the nitrosoamine present. Complete extraction can be made with hot ether in 2 hr. with a continuous extraction apparatus screened from light. Remove the ether carefully after extraction, blowing off the last few ml. at 20° C. Dissolve the residue in alcohol and proceed as already described. This modification is more suitable for compatibility testing, otherwise the more convenient extraction with alcohol is adequate.

Aged cordite SC contains brownish breakdown products partly soluble in alcohol. Interference from these is minimised by the filters used and is, in fact, negligible for samples having a thermal age not greater than 7 years at 49° C.

During the normal service life of cordite SC only two nitrosoamines are formed in noticeable amounts, *viz.*, N-nitroso-N-ethylaniline and its 4-nitro-derivative. After long periods of accelerated ageing very small amounts of the 2-nitro- and the 2 : 4-nitro-derivative are formed, and the full colour of these would not be developed in half an hour. The values obtained for "total nitrosoamine" content would then be slightly low, but not low enough to detract from the usefulness of the determination.

Compared with other stability tests the method described has the advantages that it is not empirical, it is unaffected by the presence of the distinguishing dyes sometimes added to cordite, it estimates directly a significant group of decomposition products and, with suitable modification, it can be applied to amounts of propellant far smaller than those used in other available methods.

A. O. JONES

# Inorganic

**New pH Indicator for Titration of Sodium Carbonate. Disodium 4 : 4′-bis-(2-amino-1-naphthylazo)-2 : 2′-stilbenedisulphonate. M. Taras** (*Anal. Chem.*, 1948, **20**, 680–681)—The dyestuff, known as Hessian Purple N extra and Direct Purple, is prepared by tetra-azotisation of 9·5 g. of 4 : 4′-diaminostilbene-2 : 2′-disulphonic acid (Eastman Kodak T4614), and coupling with 8·0 g. of β-naphthylamine, which is first dissolved in 50 ml. of glacial acetic acid and diluted to 100 ml. The solution of the disodium salt is formed by grinding a weighed amount of the dyestuff with the calculated volume of 0·05 *N* sodium hydroxide and diluting to the required concentration. Its sharpest colour change is at pH 3·8, so that it is suitable for 0·2 and 0·5 *N* sodium carbonate. The alkaline solution is red, changing to faint mauve at 4·0, sharply to purple at 3·8, and to bluish-purple near 3·0. No colour standard is necessary, and artificial light is adequate.

Using a final concentration of 1 drop of 0·1 per cent. indicator solution for 10 ml. of solution, results on six amounts of sodium carbonate ranging from 0·1 to 1 g. were correct to within ±0·2 per cent. Comparable results are obtained by titrating samples of solid sodium carbonate.

M. E. DALZIEL

**Determination of Hypochlorite in Presence of Chlorite. Determination of Hydrazine Sulphate. H. Caron and D. Raquet** (*Chimie Analyt.*, 1948, **30**, 163–164)—The iodometric method of Poncius (Treadwell and Hall, *Analytical Chemistry*, Vol. 2, 7th Ed., p. 598) for the determination of hypochlorite is subject to error when chlorite is present. The method now described depends upon the following reaction in presence of sodium bicarbonate

$$2NaClO + NH_2NH_2.H_2SO_4 \rightarrow 2NaCl + H_2SO_4 + N_2 + 2H_2O$$

It is not affected by chlorite, which may subsequently be titrated iodimetrically in the same solution after acidification.

*Procedure*—Prepare a standardised solution of hydrazine sulphate containing 14·5 g. per litre. To 20 ml. of this solution add 1 ml. of a saturated solution of sodium bicarbonate and 1 g. of potassium bromide, then add the hypochlorite solution from a burette until a faint yellow colour indicates the liberation of a trace of bromine. If $V$ = ml. of hypochlorite solution required, $100/V$ = strength of the solution in ml. of active chlorine per ml. If the strength is less than 5 or more than 10 ml. of active chlorine per ml., a correspondingly modified volume of hydrazine sulphate solution is used.

*Determination of chlorite*—Dilute the solution resulting from the hypochlorite determination to a suitable volume, take an aliquot, add a small amount of potassium iodide, acidify with dilute sulphuric acid, and titrate the liberated iodine with 0·1 *N* sodium thiosulphate. One litre of 0·1 *N* sodium thiosulphate is equivalent to 2·2625 g. of anhydrous sodium chlorite.

*Determination of hydrazine sulphate*—A solution of sodium hypochlorite of known strength is required and the procedure is the same as that used for the determination of hypochlorite.

W. C. JOHNSON

**Polarographic and Amperometric Determination of Barium. I. M. Kolthoff and H. P. Gregor** (*Anal. Chem.*, 1948, **20**, 541–544)—Barium ions give rise to polarographic reduction waves with a half-wave potential of −1·95 v. *versus* the saturated calomel electrode, when 0·05 *M* calcium chloride is used as the supporting electrolyte. For solutions that are less than $1{\cdot}0 \times 10^{-3}$ *M* with respect to barium ions, the wave-height is proportional to barium concentration. When 0·1 *M* lithium chloride or 0·05 *M* magnesium chloride is used as supporting electrolyte, the reduction waves obtained are not suitable for analytical purposes.

The amperometric titration of barium with chromate ions can be carried out at an applied potential of −1·4 v., using solutions containing from 20 to 50 per cent. of ethyl alcohol. The titration of solutions that are about 0·001 *M* with respect to barium gives results that are about 5 per cent. low, and if the barium concentration is 0·0001 *M*, the results are 10 per cent. low.

J. G. WALLER

**Precipitation of Oxalates from Homogeneous Solution. Application to Separation and Volumetric Determination of Magnesium. L. Gordon and E. R. Caley** (*Anal. Chem.*, 1948, **20**, 560–563)—As usually obtained, the precipitate is difficult to filter and wash, but special devices to ensure slow precipitation give better products. Precipitation of magnesium oxalate in 85 per cent. acetic acid solution by slow hydrolysis of ethyl oxalate requires higher temperatures to obtain the best separation from lithium and sodium, methyl oxalate, as used for thorium, being too easily decomposed under these conditions. The precipitate so obtained is dense and coarsely crystalline, and does not adhere to the walls of the vessel. It can be filtered on sintered-glass or porcelain of medium porosity, and the nature of the precipitate enables more than 100 mg. of magnesium to be dealt with. A buffer, ammonium acetate, is necessary to control the decrease in pH due to the hydrolysis.

Although the magnesium to oxalate ratio is good in the dehydrated precipitate, high values, probably due to occlusion, are obtained by weighing the precipitates. Ignition to the oxide is satisfactory, but is troublesome and long.

*Method*—Adjust the volume of the neutral magnesium solution to 14 or 15 ml., add 75 ml. of glacial acetic acid and stir until dissolution is complete; then add 10 ml. of glacial acetic acid containing 1 g. of ammonium acetate. Add 1·5 ml. of ethyl oxalate and stir thoroughly. Place the covered beaker on a hot-plate to raise the temperature of the solution to 100° C. and maintain that temperature for 2 hr. after precipitation begins for up to 50 mg. of magnesium, and for 3 hr. for 100 mg. As a precautionary measure add, 15 min. before filtration, 5 ml. of 85 per cent. acetic acid solution saturated with ammonium oxalate at room temperature. Filter while hot, and transfer the precipitate to the filter crucible by means of 85 per cent. acetic acid solution at 70° to 80° C. and a rubber-tipped stirring rod. Wash then with 4 to 5 additional portions of about 5 ml. each. Rinse the suction flask thoroughly with distilled water, replace it, and dissolve the precipitate and wash the crucible with a total of 200 ml. of 5 per cent. sulphuric acid solution at 80° C. in small portions. Titrate the solution at once with potassium permanganate solution standardised against sodium oxalate at 70° to 80° C.

The permanganate normality should be adjusted to give a convenient titre.

Interfering substances are essentially those listed by Elving and Clay (*Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 558), but sulphate interferes more in this procedure and oxalate must be absent. In general application, after precipitating calcium as the oxalate, ammonium salts and excess oxalate are removed by treatment with nitric and perchloric acids, the excess of perchloric acid then being eliminated by fuming. The ethyl oxalate must be stored out of contact with moisture, as the partly hydrolysed material decomposes too rapidly.

Between 1 and 101 mg. of pure magnesium can be determined to within 0·3 mg. In the presence of from 10 to 100 mg. of lithium results are up to 0·5 mg. high on a similar range. Sodium up to 100 mg. does not interfere. A similar procedure may be adopted for precipitating the oxalates of zinc, cadmium, and lead.

M. E. DALZIEL

**Rapid Determination of Small Amounts of Silicon in Magnesium Alloys. D. F. Phillips and S. E. Hermon** (*Metallurgia*, 1948, **38**, 179–180)—The method developed for the rapid determination of 0·02 to 0·25 per cent. of silicon in magnesium alloys containing up to 0·75 per cent. of zinc, 0·30 per cent. of manganese, 0·50 per cent. of copper, and 2 to 14 per cent. of aluminium is based on flocculation of the silica with gelatin. It gives good agreement with the usual sulphuric acid and perchloric acid methods. Twelve determinations can be made in about 2·5 hr. Highly oxidising conditions must be maintained during dissolution of the alloy or low results will be obtained.

METHOD—*Reagent—Brominated nitric acid*: 1 volume of saturated bromine water and 2 volumes of nitric acid (d. 1·42), to be made up freshly and well cooled. *Sulphuric acid mixture*: 6 volumes of diluted sulphuric acid (1 : 1) and 1 volume of 20-volume hydrogen peroxide. *Gelatin solution*: 0·25 per cent. prepared with hot, but not boiling, water and sterilised with a pea-sized crystal of thymol in a litre of solution. *Wash solution*: 10 ml. of diluted hydrochloric acid (1 + 1) and 20 ml. of 0·25 per cent. gelatin diluted to 1 litre.

*Procedure*—Add 50 ml. of brominated nitric acid to a 2-g. sample in a 500-ml. conical beaker. After dissolution of the sample, wash down the sides of the beaker with water and add 35 ml. of the sulphuric acid mixture. Cool, wash round with

10 to 20 ml. of water and add 40 ml. of gelatin solution with 2 filter accelerators. Stir vigorously, set aside for 45 min., filter through a Whatman No. 540 paper containing an accelerator, and wash 10 times with the wash solution. Dry the precipitate and ignite finally at 1000° C. C. F. HERBERT

**Determination of Phosphorus in Hexa-ethyl Tetraphosphate and Tetra-ethyl Pyrophosphate. M. Jacobson and S. A. Hall** (*Anal. Chem.*, 1948, **20**, 736–737)—Alkali-nitrate fusion converts the phosphorus into orthophosphate, and a colorimetric method of determination is preferred to a gravimetric method to avoid repeated ignitions. The yellow molybdivanadophosphoric acid method of Misson (*Chem. Ztg.*, 1908, **32**, 633), as modified by Kitson and Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 379), is used as nitrate is present.

*Procedure—Standard graph*—Dissolve 43·9 mg. of twice-recrystallised potassium dihydrogen phosphate in water in a 100-ml. volumetric flask, dilute to volume to obtain a solution containing 0·1 mg. of phosphorus per millilitre. Measure into 50-ml. volumetric flasks volumes ranging from 1 to 10 ml. (to within 0·01 ml.) of the solution and add to each 5 ml. of diluted nitric acid (1 + 2), 5 ml. of 0·25 per cent. ammonium vanadate solution containing 20 ml. of concentrated nitric acid per litre, and 5 ml. of 5 per cent. ammonium molybdate solution, and dilute the solutions accurately to 50 ml. Shake thoroughly and allow to stand for at least 10 min. Pour a portion into a clean, dry photometer test tube and measure its colour in a photo-electric photometer with a No. 46 blue filter, comparing with a blank solution of the nitric acid, ammonium vanadate, and ammonium molybdate solutions as 100 per cent. transmittance. Plot a curve on semi-logarithmic paper as percentage transmittance against concentration of phosphorus, and use it for reference for all analyses made with a given set of apparatus and reagents.

*Fusion of sample*—Weigh 40 to 50 mg. of sample into a small 7-ml. platinum crucible by means of a weighing pipette consisting of a glass tube with a fine capillary tip and a rubber bulb, the whole being supported in a wire holder. Add about 0·9 g. of an intimate mixture (4 : 1) of powdered sodium hydroxide and potassium nitrate, cover the crucible, and heat it gently until foaming ceases and then more strongly until a clear melt is obtained; about 5 to 6 min. are required. Allow to cool, and then dissolve the fused mass from the crucible in water and cover it with 75 ml. of hot distilled water. Transfer it to a 125-ml. Erlenmeyer flask. Acidify with concentrated nitric acid to Congo red and allow to simmer gently to reduce the volume to 50 ml. Cool the solution and nearly neutralise to litmus by means of aqueous ammonia solution (28 to 29 per cent.), transfer it to a flask, and dilute accurately to 100 ml. Treat a 5-ml. portion with 5 ml. each of diluted nitric acid (1 + 2), ammonium vanadate solution, and ammonium molybdate solution and dilute accurately to 50 ml. Measure the percentage transmittance and refer to the standard graph.

Attack on the platinum crucible is only slight, representing a loss of a few milligrams in 35 fusions, but the use of a gold crucible may be preferable.

M. E. DALZIEL

**Polarographic Determination of Small Amounts of Arsenic [from Measurements of the Height of the "Maximum"]. N. Ya. Khlopin, N. A. Rafalovich, and G. P. Aksenova** (*J. Anal. Chem. Russ.*, 1948, **3**, 16–20)—Under certain conditions the height of the maximum of arsenic obtained at applied potentials of −1·2 to −1·5 v. is linearly related to the arsenic concentration. Besides arsenic, essential components of the solution are: a weak acid, cations of cobalt, iron, or nickel, and an indifferent electrolyte.

The effects of various weak acids were studied in concentrations from 1 to 50 mg.-mol. per litre. In the presence of the other essential components, anthranilic and boric acids failed to give a maximum but formic, acetic, oxalic, malonic, succinic, lactic, tartaric, citric, benzoic, salicylic, monochloroacetic, and orthophosphoric acids were all effective.

Of cobalt, iron, and nickel cations, within the limits of 0·05 to 0·5 mg.-mol. per litre, the most effective was cobalt, but iron was found most convenient for ordinary use.

Variations of drop-time within the range 2 to 6·5 sec., and of the geometrical characteristics of the capillary had no appreciable effect on the form of the maximum.

Media suitable for the determination of 0·01 to 0·05 mg.-mol. of arsenious anhydride per litre are (1) 0·5 *M* in sodium chloride, 0·015 *M* in acetic acid, and 0·00015 *M* in ferrous ammonium sulphate, (2) 0·4 *M* in sodium sulphate, 0·35 *M* in sodium chloride, 0·015 *M* in acetic acid, and 0·00015 *M* in ferrous ammonium sulphate, and (3) 0·4 *M* in magnesium sulphate, 0·35 *M* in magnesium chloride, 0·015 *M* in acetic acid, and 0·00015 *M* in ferrous ammonium sulphate. Medium (1) allows a polarographic determination to be made directly on an arsenic distillate after neutralisation with sodium carbonate and addition of sodium chloride, (2) requires neutralisation of the distillate with sodium carbonate, and addition of sodium sulphate, and (3) requires neutralisation of the distillate with magnesium carbonate, and addition of magnesium sulphate.

Tests carried out with 1 to 5 μg. of arsenious oxide per ml. of a solution containing 0·2 g.-mol. of magnesium sulphate, 0·15 mg.-mol. of cobalt sulphate, and 15 mg.-mol. of a weak acid, showed that the height of the maximum ($y$) and the arsenic concentration ($x$) could be related by the formula, $y = bx + a$, where the coefficients $b$ and $a$ varied with the acid used, *e.g.*, under similar conditions, they were 2·6 and 5 for acetic acid, 2·6 and 10·2 for orthophosphoric acid, and 3·7 and 0 for succinic acid.

Samples of flour, paper, and bread were treated with known amounts of arsenic and the distillates obtained by the usual method were examined polarographically by the maximum method. The amounts found agreed to within about 5 parts per 100 with the amounts added. [Details of the procedure are not given.] G. S. SMITH

**Reduction of Antimony Solutions with Metallic Nickel. H. Holness** (*J. Soc. Chem. Ind.*, 1948, **67**, 238–241)—In order to apply the method to the removal of antimony in the tannin method for estimating tin in antimony-rich alloys, experiments on the influence of time, temperature, acid concentration, antimony concentration, and weight or area of nickel on the reduction of antimony trichloride in pure hydrochloric acid solution have been carried out. The solution must be able to make contact with the metal-hydrogen interface in the presence of both metals for reduction to proceed. The antimony deposit must thus be kept loose and porous and all the hydrogen must not be detached from the metal-solution interface.

*Method* 1, *powdered nickel*—Dissolve up to 1·8 g. antimony trioxide in 100 ml. of diluted hydrochloric acid (1 + 3). Heat on a water-bath. Add 5 g. of nickel powder and continue heating 20 min. without stirring or ebullition.

*Method* 2, *sheet nickel*—Prepare a spiral giving 30 sq. in. of surface. Clean by immersion in diluted nitric acid (1 + 3) until gassing freely and wash thoroughly. Activate by immersion in a boiling solution of 10 g. of sodium chloride, 25 ml. of hydrochloric acid, and 0·1 g. of antimony trioxide in 75 ml. water; remove after 2 to 3 min. when freely gassing and coated with a loose black deposit of antimony. Proceed as in Method 1.

C. F. HERBERT

**Colorimetric Determination of Copper with Carbon Disulphide and Diethanolamine. W. C. Woelfel** (*Anal. Chem.*, 1948, **20**, 722–724)—A reagent prepared from carbon disulphide and diethanolamine reacts with cupric ions to form a water-soluble, brownish-yellow salt of *bis*-(2-hydroxyethyl) dithiocarbamic acid. Serious interference is caused by bismuth, chromium, cobalt, iron, mercury, nickel, silver, uranium, cyanide, dichromate, nitrite, and sulphite, but procedures are described for eliminating the interference of appreciable amounts of bismuth, chromium, iron, and uranium. It is suggested that the new reagent be named *cuprethol*.

*Reagents*—(*a*) *Acetate buffer solution pH* 5—Dissolve 96 g. of sodium acetate trihydrate and 17 ml. of glacial acetic acid in water and dilute to 1000 ml. (*b*) *Cuprethol reagent*—*Solution A*—Dissolve 4 g. of diethanolamine in 200 ml. of methyl alcohol. *Solution B*—Dissolve 1 ml. of carbon disulphide in 200 ml. of methyl alcohol. Prepare sufficient reagent for one day's use by mixing equal volumes of Solutions A and B. (*c*) *Standard copper solution*—Dissolve 0·1000 g. of pure copper in 5 ml. of 8 *N* nitric acid, add 1 ml. of concentrated sulphuric acid, and evaporate to dryness. Dissolve the residue in water and dilute to 1000 ml.; 1 ml. contains 0·1 mg. of copper. Dilute 50 ml. of this solution to 1000 ml.; 1 ml. of this solution contains 0·005 mg. of copper.

*Procedure for samples of low iron content*—The solution to be tested should contain between 2 and 10 mg.-equivalents of mineral acid and not more than 10 mg. of iron and 0·2 mg. of copper. Dilute to approximately 50 ml. with water. Add 1 ml. of a 3·0 per cent. solution of sodium pyrophosphate ($Na_4P_2O_7.10H_2O$) for each 2 mg. of iron present and adjust the reaction of the solution to a pH of 5 to 6 by adding 20 per cent. sodium acetate solution. If no turbidity develops in 5 min., add 1 ml. of cuprethol reagent, dilute to 100 ml. with water, and within 1 hr., measure the colour intensity in a photometer.

*Procedure for samples of high iron content*—If the sample contains large amounts of iron or other metals precipitated by pyrophosphate, remove them by careful precipitation with 15 *N* ammonia solution. Dilute an aliquot of the ammoniacal filtrate containing not more than 0·2 mg. of copper to approximately 50 ml. with water, and make just acid to litmus with 1·2 *N* hydrochloric or nitric acid. Add 10 ml. of acetate buffer solution, 1 ml. of cuprethol reagent, dilute to 100 ml. with water, and within 1 hr. measure the colour intensity in a photometer.

Determine the copper content of the aliquot by reference to a standard curve prepared from the standard copper solutions. Beer's law is valid for concentrations up to 0·2 mg. of copper per 100 ml. of solution.

*Interfering ions*—Drabkin (*J. Assoc. Offic. Agr. Chem.*, 1939, **22**, 320) has shown that potassium cyanide destroys the colour formed by cupric ions with diethyldithiocarbamate in that process for the estimation of copper. The principle has been utilised to nullify the interference of up to 10 p.p.m. of bismuth, 300 p.p.m. of uranium, and 100 p.p.m. of chromium by making photometer readings of the coloured solution before and after addition of a small amount of potassium cyanide: the copper is determined by difference.

The presence of tungsten causes a green colour to develop early, but this disappears after a few minutes and does not interfere in the copper determination.

With more than 200 p.p.m. of thiosulphate, interference is caused by the precipitated sulphur. Dichromate oxidises the cuprethol reagent and gives gross negative errors in concentrations above 2 p.p.m. Twenty p.p.m. of nitrite or 100 p.p.m. of sulphite or oxalate can be tolerated if the photometer readings are obtained within 10 min. Not more than 20 p.p.m. of cyanide may be present.

The presence of mercuric ions inhibits colour development. A. H. A. ABBOTT

**Ammonium Citrate in the Colorimetric Determination of Copper. A. J. Hall and R. S. Young** (*Anal. Chem.*, 1948, **20**, 776–777)—Because large amounts of mercury interfere with the determination of copper with sodium diethyldithiocarbamate, it is proposed to measure the blue colour of a copper ammonium citrate complex directly in presence of mercury, which is held in solution by ammonium citrate and an excess of ammonia. Under these conditions variations in the quantity of mercury present have no effect on the colour due to the copper, and an adequate amount of mercuric chloride is provided in the solutions used to prepare a standard curve.

*Reagent*—Dissolve 200 g. of citric acid in water, add 270 ml. of aqueous ammonia (S.G. 0·88) and dilute to 1000 ml.

*Procedure to obtain standard curve*—(*a*) *0 to 50 mg. of copper*—From a solution of copper sulphate containing 1 mg. of copper per ml., measure volumes containing up to 50 mg. of copper in 5- or 10-mg. increments, and to each add 50 ml. of 7 per cent. mercuric chloride solution, 50 ml. of ammonium citrate solution, and 50 ml. of aqueous ammonia (sp.gr. 0·88). Dilute to 200 ml. with water and measure the absorption of the solution in a 4-cm. cell. (*b*) *0 to 200 mg. of copper*—From a solution of copper sulphate containing 5 mg. of copper per ml., measure volumes representing up to 200 mg. of copper in 10-mg. increments, and to each add mercuric chloride solution, ammonium citrate solution, and aqueous ammonia, as above. Dilute to 200 ml. with water and measure the absorption in a 1-cm. cell.

To determine the copper content of unknown solutions containing mercury, add ammonium citrate solution and ammonia, dilute to 200 ml. with water, and compare the absorptiometer reading with the standard curves.

Copper may be similarly determined in presence of silver, zinc, cadmium, magnesium, aluminium, and lead. Solutions containing coloured ions, *e.g.*, cobalt, chromium, and nickel, cause interference and more than 10 mg. of iron imparts a green tinge. The presence of chloride, acetate, and nitrate ions has no effect on the copper colour.

A. H. A. ABBOTT

**1 : 2-*Cyclo*hexanedione Dioxime (Nioxime). Reagent for Nickel. R. C. Voter, C. V. Banks, and H. Diehl** (*Anal. Chem.*, 1948, **20**, 458–460; *cf.* Johnson and Simmons, *Analyst*, 1946, **71**, 554)—The sensitivity of the reagent for nickel is 1 in $10^7$, and quantitative precipitation occurs at pH values of 3·0 and greater. In gravimetric work, the precipitate is obtained in a readily filterable form by slowly raising the pH of the solution from a point where the nickel complex does not precipitate to a value of about 4·5. The precipitate contains co-precipitated reagent but the error arising from this cause under the standardised conditions, is a linear function of the excess of nioxime and is corrected by the application of a factor. The correction is small enough to be neglected when the quantity of nickel is less than 15 mg.

*Procedure*—To the solution containing about 25 mg. of nickel in 250 ml. add 8 ml. of a 0·8 per cent. aqueous solution of nioxime for each 10 mg. of nickel present. Add sufficient hydrochloric acid to re-dissolve any red precipitate formed and then ammonia until a faint red colour persists. Heat to 60° C. and add, dropwise with constant stirring, 25 ml. of 20 per cent. ammonium acetate solution. Maintain at 60° C. for 30 to 40 min. with occasional stirring, filter through a crucible of medium porosity, wash with hot water, and weigh. The volume of solution actually used to precipitate the nickel = weight of precipitate × 104. Correction in g. of nickel = 0·0002 × excess of nioxime per cent. × weight of precipitate × 0·1721.

Four determinations of quantities of nickel 1·8 to 14·4 mg. show in each an error of 0·1 mg. and a determination of 20·3 mg. shows no error.

The following ions present in solution do not interfere with the nickel determination: acetate, $Cl'$, $SO_4''$, $NO_3'$, $ClO_4'$, tartrate, sulphosalicylate, $UO_2''$, $Mn''$, $Na'$, $K'$, $Li'$, $Ba''$, $Ca''$, $Sr''$, $Mg''$, $Cd''$, antimonite (+ tartrate), aluminium (+ tartrate), $AsO_2'$, $Be''$, and $Zn''$. Copper gives a brownish-green colour and cobalt is co-precipitated. No satisfactory method can be recommended for the quantitative separation of nickel from iron by nioxime. Ferrous iron forms a stable complex with nioxime. Nioxime will also reduce ferric to ferrous iron.

W. C. JOHNSON

**Photo-colorimetric Determination of the $Co''$ Ion by means of α-Nitroso-β-naphthol and β-Nitroso-α-naphthol. A. L. J. Beguet** (*Anal. Asoc. Quím. Argentina*, 1946, **34**, 105–126)—Compounds containing the grouping –CO–C=N–OH have been proposed (Illinski and von Knorre, *Ber.*, 1885, **18**, 699) for the determination of small amounts of cobalt. The reagents most frequently used are the following: α-nitroso-β-naphthol (Bellucci, *Gazz. Chim. Ital.*, 1919, **49**, II, 294), β-nitroso-α-naphthol (Baudish *et al.*, *Ber.*, 1912, **45**, 1164; 1915, **48**, 1610), *o*-nitrosophenol (Feigl, *Specific and Special Reactions*, p. 109, Elsevier Publishing Co., Inc., New York, 1941), nitroso-resorcinol (Tonucek and Komarek, *Z. anal. Chem.*, 1932, **91**, 29B; Novelli, *Actas y Trabajos del II Congreso de Química* (I Sudamericano), Buenos Aires, 1924; *Idem.*, *Ibid.*, Vol. II, p. 117), 2-nitroso-1-naphthol-4-sulphonic acid (Sarver, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 378), nitroso-R-salt (*Anal. Asoc. Quím. Argentina*, **31**, 124), and *iso*-nitroso-acetophenone (Feigl, *Ibid.*).

The present author has determined the limits of the application of Beer's law in the photo-colorimetric determination of the $Co''$ ion with α-nitroso-β-naphthol and with β-nitroso-α-naphthol, both in the aqueous phase and in ethyl acetate, using Pulfrich and Hellige photometers. The interfering effect of the ions $Ni''$, $Cu''$, and $Cr'''$ in the aqueous solution, and of $Ni''$ and $Cr'''$ in ethyl acetate have been investigated.

*Reagents*—3 *M* aqueous ammonia; ammonium citrate solution, 0·6 g. per ml.; 0·1 per cent. solution of α-nitroso-β-naphthol (prepared according to Snell and Snell, *Colorimetric Methods of Analysis*, Vol. I, pp. 324 *et seq.*, D. van Nostrand, New York, 1936); 0·1 per cent. solution of β-nitroso-α-naphthol (*Idem.*, *Ibid.*); 0·1 *M* solution of cobalt, prepared from $CoCl_2.6H_2O$ and standardised by the electrolytic method.

*Determination of the $Co''$ ion by means of α-nitroso-β-naphthol. Aqueous solution*—The most suitable colour filter was determined by measuring the light transmission at different wavelengths through a solution of α-nitroso-β-naphthol and through one of the cobalt complex. Curves from which could be read the wavelength that gave the maximum sensitivity and the minimum interference by the reagent were then constructed. This wavelength was 530 mμ.

In the determination, the order of addition of the reagents is important. To prepare a solution of the complex that contains $x$ p.p.m. of $Co^{\cdot\cdot}$, add to $x$ ml. of a solution containing 10 p.p.m. of cobalt, 0·5 ml. of ammonium citrate solution and then 0·5 ml. of 3 *M* aqueous ammonia, dilute to 9 ml. with water, and then add 1 ml. of α-nitroso-β-naphthol solution, and shake. Beer's law is obeyed from 0 to 2 p.p.m. The lower limit of determination of the cobalt ion by this method is 0·025 p.p.m. The results obtained with a Pulfrich photometer differ very slightly from those of Burkhardt (Doctoral Thesis, F.C.E.F. y N., Buenos Aires, 1943), who used an Aminco photo-electric photometer, and who found Beer's law to be obeyed up to 0·05 p.p.m. With the Hellige photometer, the limits of conformity to Beer's law were 0 to 2·4 p.p.m. and the lower limit of cobalt that could be determined was 0·5 p.p.m.

*Determination of the $Co^{\cdot\cdot}$ ion by means of β-nitroso-α-naphthol. Aqueous solution*—The determination is carried out as before, the optimum wavelength being 530 mμ. Beer's law is obeyed between 0 and 1 p.p.m. of cobalt ion, and the lower limit of determination of the ion is 0·025 p.p.m.

*Determination of the $Co^{\cdot\cdot}$ ion by means of α-nitroso-β-naphthol. Ethyl acetate solution*—The reaction is carried out in a separating funnel, into which are put 1·25 ml. of ammonium citrate solution, $x$ ml. of cobalt salt solution of $z$ p.p.m., and 1·25 ml. of 3 *M* aqueous ammonia solution; the volume is brought to about 20 ml. with water, and, after shaking, 2·5 ml. of the alkaline 1 per cent. α-nitroso-β-naphthol solution are added, the volume is made up to 25 ml. with water, and the funnel is shaken. There are then added 7·5 ml. of ethyl acetate, and the funnel is shaken vigorously and set aside for the phases to separate. The aqueous phase is run off, the tap being opened carefully and only partially; the tap is closed and the funnel rotated horizontally until the last drops of water have separated, whereupon they are run off. The ethyl acetate phase is run out through the neck of the funnel, the first two portions being used to wash out the photometer cells and the third used for the measurement; the compensation liquid is also ethyl acetate. It is important in all these determinations that the cells be washed out at least twice with the liquid on which measurements are to be made. With ethyl acetate, which is volatile, the cells must be completely full and the measurements must be made quickly. The readings should not be taken in bright light.

Curves were constructed as before, one in which wavelength is plotted against the light transmitted by a 0·01 per cent. solution of the reagent in ethyl acetate, and the other similarly for a solution of reagent and 0·3 p.p.m. of cobalt ion. The wavelength giving maximum sensitivity and minimum interference is 530 mμ. Beer's law is obeyed between 0 and 1 p.p.m., and the smallest quantity of cobalt determinable is 0·01 p.p.m.

*Determination of the $Co^{\cdot\cdot}$ ion with β-nitroso-α-naphthol. Ethyl acetate solution*—The determination is carried out exactly as with α-nitroso-β-naphthol in ethyl acetate. The optimum wavelength is 530 mμ. Beer's law is obeyed from 0 to 0·3 p.p.m. of cobalt ion, and the smallest quantity of cobalt determinable is 0·01 p.p.m. With the Hellige photometer, Beer's law is obeyed from 0 to 0·14 p.p.m., and the smallest amount that can be determined is 0·02 p.p.m.

*Determination of the $Co^{\cdot\cdot}$ ion in presence of the $Ni^{\cdot\cdot}$ ion, with α-nitroso-β-naphthol. Aqueous solution*—The only additional reagent is a 0·06 *M* solution of nickel chloride, prepared from pure $NiCl_2.6H_2O$, and standardised electrolytically. The Hellige photometer is used. To determine the optimum wavelength for the measurements, curves are prepared, as before, relating transmission to wavelength for a 0·05-p.p.m. [0·5?] nickel solution, for the same nickel solution in the presence of a 0·01 per cent. solution of α-nitroso-β-naphthol, and for a solution containing nickel, α-nitroso-β-naphthol, and 1 p.p.m. of cobalt ion. [The optimum wavelength found is not stated.] The technique of the determination is the same as for the direct determination with α-nitroso-β-naphthol in aqueous solution, except that sufficient of the nickel solution to give a concentration of 0·5 p.p.m. is added. Beer's law is obeyed between 0 and 0·8 p.p.m., and the smallest quantity of cobalt determinable is 0·025 p.p.m., the same concentration as can be determined in absence of nickel.

*Determination by means of β-nitroso-α-naphthol in presence of nickel. Aqueous solution*—The optimum wavelength is 530 mμ. The Hellige photometer was used. Beer's law is obeyed between 0 and 1 p.p.m., in presence of 0·5 p.p.m. of nickel; the smallest quantity of cobalt that can be determined is 0·025 p.p.m.

*Determination by means of α-nitroso-β-naphthol in the presence of 0·5 p.p.m. of $Cu^{\cdot\cdot}$ ion. Aqueous solution*—The additional reagent required is a 0·024 *M* solution of copper sulphate, prepared from pure, recrystallised $CuSO_4.5H_2O$, and standardised by the electrolytic method in a medium containing sulphuric and nitric acids. Two curves were constructed, with the Hellige photometer, one for light transmission at different wavelengths by a 0·01 per cent. solution of the reagent plus 0·5 p.p.m. of copper, and the second for a solution containing 0·01 per cent. of the reagent, 0·5 p.p.m. of copper and 1 p.p.m. of cobalt. The optimum wavelength is 530 mμ. The technique is as before, with addition of sufficient of the copper solution to give a concentration of 0·5 p.p.m. Beer's law is obeyed between 0 and 0·8 p.p.m. of cobalt, and the smallest quantity of cobalt that can be determined is 0·05 p.p.m.

*Determination by means of β-nitroso-α-naphthol in the presence of the $Cu^{\cdot\cdot}$ ion. Aqueous solution*—Curves were constructed as in the previous example, with the Hellige photometer. The optimum wavelength is 530 mμ. Beer's law is obeyed between 0 and 1 p.p.m. of cobalt ion, and the smallest concentration that can be determined is 0·05 p.p.m.

*Determination by means of α-nitroso-β-naphthol in the presence of the $Cr^{\cdot\cdot\cdot}$ ion. Aqueous solution*—Re-crystallised chrome alum was used to prepare a 0·25 *M* solution, which was standardised by the

gravimetric method, as $Cr_2O_3$, calcined in a current of hydrogen. Three curves were prepared, with the Hellige photometer, the first relating the light transmission through a 25 p.p.m. chromium solution to the wavelength, the second for a 0·1 per cent. solution of α-nitroso-β-naphthol containing 25 p.p.m. of chromium, and the third for this same solution containing 1 p.p.m. of cobalt ion. The optimum wavelength was 530 mμ. Further increase in the chromium concentration causes a turbidity due to the formation of chromium hydroxide. Beer's law is obeyed between 0 and 1·4 p.p.m. of cobalt, and the smallest quantity determinable is 0·025 p.p.m.

*Determination by means of β-nitroso-α-naphthol in the presence of the $Cr^{\cdots}$ ion. Aqueous solution*—The Hellige photometer was used. Beer's law is obeyed between 0 and 1·2 p.p.m. of cobalt, and the smallest quantity of cobalt determinable is 0·05 p.p.m.

*Determination by means of α-nitroso-β-naphthol in the presence of the $Ni^{\cdot\cdot}$ ion. Ethyl acetate phase*—Curves are prepared as before, using a 0·01 per cent. solution of the reagent with 2 p.p.m. of nickel, and this solution with the addition of 2 p.p.m. of cobalt ion. The optimum wavelength is 530 mμ. The Hellige photometer was used. Beer's law is obeyed between 0 and 0·08 p.p.m. of cobalt, and the smallest quantity of cobalt determinable is 0·01 p.p.m.

*Determination by means of β-nitroso-α-naphthol in the presence of 2 p.p.m. of nickel. Ethyl acetate phase*—The optimum wavelength is not stated. The Hellige photometer was used. Beer's law is obeyed between 0 and 0·12 p.p.m. of cobalt, and the smallest quantity of cobalt that can be determined is 0·02 p.p.m.

*Determination by means of α-nitroso-β-naphthol in the presence of 10 p.p.m. of the $Cr^{\cdots}$ ion. Ethyl acetate phase*—Curves are prepared for solutions containing 0·01 per cent. of the reagent and 0·10 [10?] p.p.m. of chromium, and containing 0·01 per cent. of the reagent, 0·2 p.p.m. of cobalt and 10 p.p.m. of chromium. The Hellige photometer was used. The optimum wavelength was 530 mμ. Beer's law is obeyed between 0 and 0·2 p.p.m. of cobalt, and the smallest concentration of cobalt determinable was 0·02 p.p.m.

*Determination by means of β-nitroso-α-naphthol in the presence of 10 p.p.m. of chromium. Ethyl acetate phase*—Curves are prepared as before, both solutions containing 10 p.p.m. of chromium. The Hellige photometer was used. The optimum wavelength was 530 mμ. Beer's law is obeyed between 0 and 0·1 p.p.m. of cobalt, and the smallest concentration of cobalt that can be determined is 0·02 p.p.m.

E. M. POPE

**1 : 2-Cyclohexanedione Dioxime. A Reagent for Palladium. R. C. Voter, C. V. Banks, and H. Diehl** (*Anal. Chem.*, 1948, **20**, 652–654)—The reagent, which is water-soluble, yields with palladium an insoluble yellow compound that can be filtered from a hot solution after a brief period of digestion. Slight yellow colorations are obtained with 5, 7, or 10 parts per 10 million after 5 min., whilst dimethylglyoxime shows no reaction, although both reagents give slight precipitates on standing several hours.

Quantitative precipitation is obtained at pH values between 0·7 and 5 in presence of an excess of between 30 and 150 per cent. of the reagent. From 6 to 30 mg. of palladium can be determined to within 1 part in 135, but filtration of larger quantities is difficult.

*Procedure*—Adjust the volume of the solution containing 5 to 20 mg. of palladium to about 200 ml., and the pH to between 1 and 5 depending on other cations. Heat the solution to about 60° C. and add slowly from a pipette, with constant stirring, 0·43 ml. of a 0·8 per cent. solution of the reagent for each milligram of palladium present; this provides a 30 per cent. excess. Digest with occasional stirring for 30 min. at 60° C., filter through a medium-porosity crucible and wash the precipitate with 5 portions of hot water. Dry at 110° C. for 1 hr. The compound contains 27·43 per cent. of palladium.

In presence of platinum the same procedure applies, the platinum compound separating only after more than 24 hr. standing; spectrographic examination shows that separation is better than with dimethylglyoxime. Sulphate, nitrate, chloride, acetate, tartrate, and sulphosalicylate do not interfere, nor do uranyl, beryllium, ruthenium, aluminium, lanthanum, zinc, calcium, alkali metals, or alkaline earths, but aurous ions at 60° C. give a precipitate contaminated with metallic gold.

M. E. DALZIEL

**Mercaptobenzothiazole as a Reagent for Platinum, Rhodium, and Palladium. I. Ubaldini** (*Gazz. Chim. Ital.*, 1948, **78**, 293–301)—Mercaptobenzothiazole is a sensitive reagent for determining platinum, rhodium, and palladium, giving visible precipitates in solutions containing about 10 μg. of metal per 100 ml.

The reagent is prepared by dissolving the thiazole in alcohol, alkali, or acetic acid.

*Platinum* is precipitated quantitatively in acid solution. In alkaline solution no precipitate is formed but, on acidifying with acetic or hydrochloric acid and boiling, a canary yellow, flocculent precipitate that filters very readily is produced. It is washed with hot water, dried, and incinerated in a porcelain or quartz crucible and weighed as the metal. The precipitate is insoluble in boiling concentrated hydrochloric acid, or in 25 per cent. nitric acid.

*Rhodium* is completely precipitated in alkaline solution or in presence of acetic acid, but only imperfectly in presence of mineral acids. On boiling the alkaline solution the flocculent, red-brown precipitate forms immediately and filters very readily. The metal is obtained by incinerating in a porcelain crucible followed by ignition in a current of hydrogen.

*Palladium* behaves like platinum in acid solution, but can also be precipitated in presence of alkali.

A. H. BENNETT

# Physical Methods, Apparatus, etc.

**Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology** (*Anal. Chem.*, 1948, **20**, 683–684)—Data for two modifications of thiamine hydrochloride (vitamin $B_1$) are given (*cf. Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579). M. E. DALZIEL

**Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology** (*Anal. Chem.*, 1948, **20**, 779–780)—Crystal properties of α-pyridinesulphonic acid in three polymorphic forms, only one being well-defined, are described under the headings given in the original publication (*Ibid.*, 1948, **20**, 275; *Analyst*, 1948, **73**, 579). M. E. DALZIEL

**Storage and Titration with Oxygen-Sensitive Solutions. H. W. Stone** (*Anal. Chem.*, 1948, **20**, 747–749)—The system described uses nitrogen under a slight pressure and avoids the necessity of maintaining inert gas pressure by means of a Kipp's apparatus or a cylinder, and also of scrubbing the inert gas over a long period.

*Apparatus*—In Fig. 1 the gas pressure compensating systems for storage and the burette are separate.

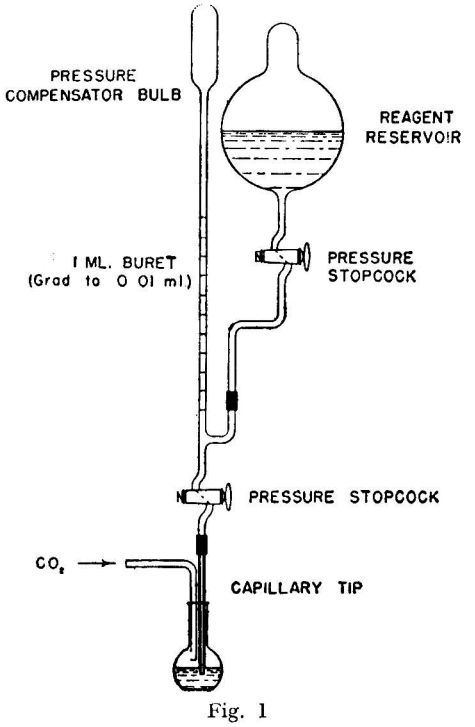



Fig. 1

The nitrogen pressure in the reservoir, made from a round-bottomed flask, must be just sufficiently greater than that in the burette to allow the burette to fill, and that in the burette must be high enough to expel the solution during titration. The apparatus shown in Fig. 2 requires a reservoir above the burette graduations, as gravity controls the flow of solution; thus, only one pressure adjustment is necessary in preparing the apparatus, but if the burette is allowed to empty, the nitrogen becomes contaminated and the whole system must be recharged.

*Introduction of reagent*—The necessary apparatus is shown in Fig. 3, the top stop-cock representing the burette tap in Fig. 2. At the start, screw-clamps B, C, and E are open and D is closed. Pass air at 2 atmospheres pressure and vent through B, which is gradually closed until the chromic chloride solution in hydrochloric acid is forced up

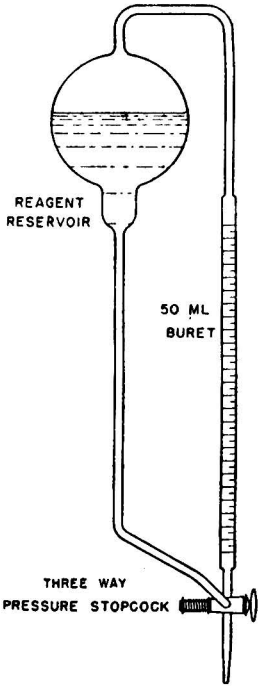



Fig. 2

through the reductor, through E to C, thus clearing air from the line. Open the burette stop-cock, close C, and regulate the flow into the reservoir (Fig. 2) by adjusting B. When enough solution is in the burette and reservoir to absorb all the oxygen, close the stop-cock and E and shake the apparatus, allowing nitrogen through D and the stop-cock to compensate for the decrease in pressure. The pressure of nitrogen should be reduced to about 1 atmosphere by means of a valve on the cylinder. Close the stop-cock and shake the apparatus again lest the nitrogen contains any oxygen. Clear the storage flask of the partly-spent solution and the excess of nitrogen by closing D and opening the burette stop-cock and C and adjusting the pressure to 1 atmosphere by venting through C. Then charge with the reagent by closing C, opening E and forcing the solution up with B closed until the reservoir is half full, the pressure then being 2 atmospheres.

To dispense oxygen-free water, pump out the system with an oil pump and fill with oxygen-free nitrogen alternately until the partial pressure of oxygen is adequately reduced, then admit distilled

water, previously boiled and cooled in a stream of nitrogen.

Nitrogen can be replaced by hydrogen, but carbon dioxide is not suitable as its solubility is too variable. Diffusion of oxygen through the

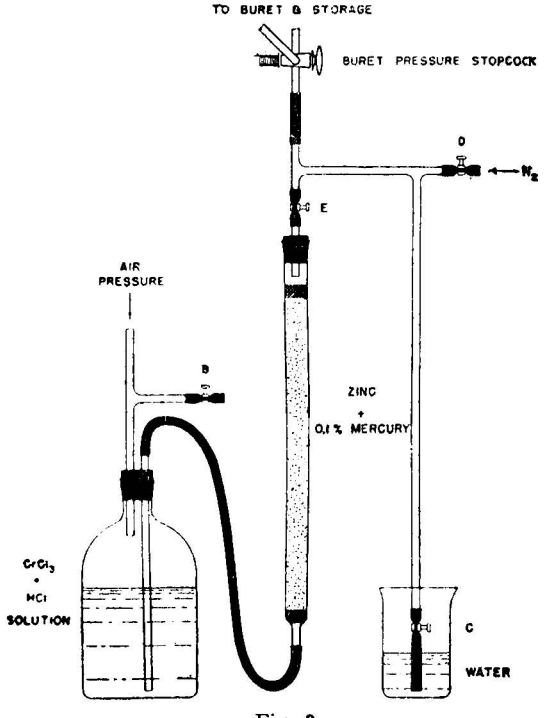



Fig. 3

rubber is compensated for by replacing the reagent in the lines with fresh solution before a fresh group of titrations. During titrations a capillary tip is fitted to the burette and allowed to dip below the surface, so restricting diffusion and oxidation, while commercial carbon dioxide is blown over the liquid surface at a rate of 200 to 300 ml. per min. The gas stream is started about 5 min. before titration to sweep out the vessel, but longer time is necessary to clear the line from the cylinder.

Solutions of 0·03 *N* chromous chloride in hydrochloric acid showed no variation in titre in 10 months. The apparatus requires no attention when not in use, and is ready at a moment's notice.

M. E. DALZIEL

**Automatic Potentiometric Titrations. J. J. Lingane** (*Anal. Chem.*, 1948, **20**, 285–292)—Potentiometric titrations applicable to any type of electrode reaction can be carried out with the apparatus described, which will either record the complete titration curve or stop the titration at the equivalence point. The titrant is delivered from a 50-ml. hypodermic syringe, the plunger being driven by a screw thread turned by a synchronous motor. The number of rotations of the screw are recorded by a revolution counter and the syringe is calibrated by weighing the water forced out by a definite number of complete rotations of the screw. Since the plunger moves at a constant speed, the volume delivered can also be related to the time of delivery.

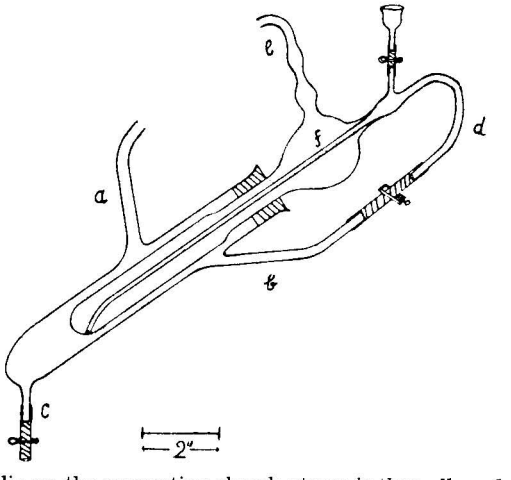
An indicator electrode and a saturated calomel reference electrode are coupled to a recording potentiometer fitted with a mercury switch, which can be set to stop the titration at the equivalence point, if this is desired. The nature of the indicator electrode depends on the electrode reaction taking place, and is usually a strip of an appropriate metal.

Since additions can be made more accurately from a syringe than from a burette of the same volume, this apparatus is capable of giving results that are more accurate than those obtained by the usual methods. By using a syringe of smaller volume, the method can be applied on the semi-micro scale.

For routine purposes in which the shape of the titration curve is known, the recording potentiometer is replaced by any potentiometer control of rapid response, set to stop the titration at the equivalence point.

J. G. WALLER

**"Micro"-Kjeldahl Distillation Apparatus. R. Johanson** (*J. Proc. Austral. Chem. Inst.*, 1948, **15**, 183–184)—In the apparatus shown the outer jacket serves as a steam jacket for the inner distillation tube and as a vacuum jacket for collecting the spent sample, the inner tube being suspended in it by means of a rubber stopper. The sample is admitted through the thistle funnel, and the

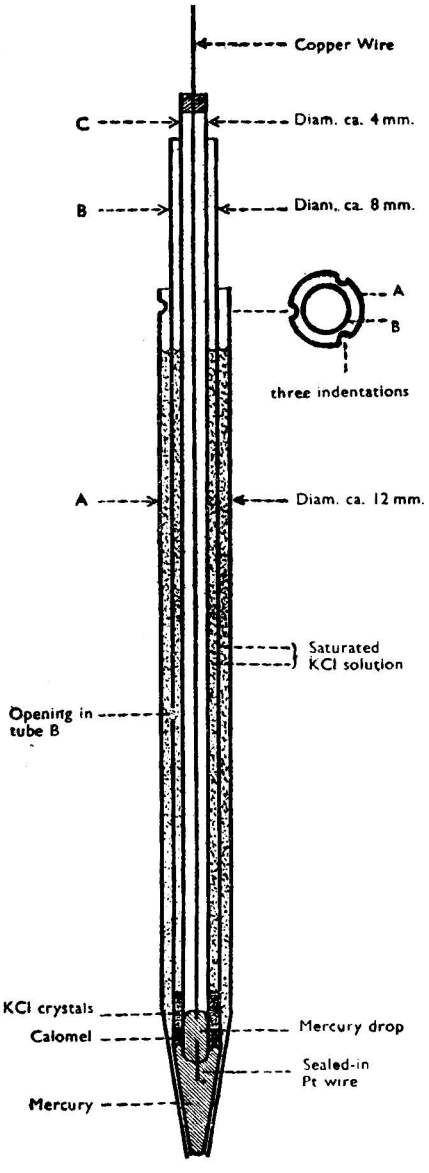



clip on the connection closed; steam is then allowed to enter through the tube *a*, is led through *b* into *d* and tube *f*, and bubbles through the sample liquor into *e* and the condenser, which is not shown. Distillation is rapid and smooth, and emptying out of the spent liquor into the outer jacket is automatic at the close of a determination.

M. E. DALZIEL

**Calomel Electrode. B. van der Burg** (*Chem. Weekblad*, 1948, **44**, 417–418)—The simple and easily made calomel electrode described is composed of a glass tube, A, of 140 mm. length, drawn out at the lower end and provided with three indentations at the upper end. A second tube, B, inserted

into the first one, has the lower end drawn out and sealed off, the drawn-out portion being ground into the corresponding part of the tube A. In B




is placed a small amount of re-distilled mercury, then some calomel and a layer of potassium chloride crystals, after which the innermost tube, C, which is closed at the bottom and has a platinum wire sealed through the lower end, is inserted into the tube B. Contact is made with the platinum wire by means of a bead of mercury in the tube C and an amalgamated copper wire. At the upper end this tube is closed by wax. Finally, the tubes A and B are filled with saturated potassium chloride solution. The prepared electrode is placed in the liquid of which the pH is to be determined. After use it is washed with distilled water and dried with filter paper, and a drop of potassium chloride solution is allowed to flow out. The level of the liquid in the electrode must always be above the hole connecting the two tubes. G. MIDDLETON

**Chemical Analysis Based on X-ray Absorption Measurements with a Multiplier Phototube. I. Solids and Liquids. H. A. Liebhafsky, H. M. Smith, H. E. Tanis, and E. H. Winslow. II. Gases. E. H. Winslow, H. M. Smith, H. E. Tanis, and H. A. Liebhafsky** (*Anal. Chem.*, 1947, **19**, 861–865, 866–867)—The use of X-ray absorption as a method of chemical analysis has long been appreciated, but simple and precise measurement of X-ray intensity has only recently become possible. These papers describe how the method may be used when X-ray intensity is measured by means of a phosphor and multiplier photo-tube coupled to an amplifier.

METHOD—Polychromatic X-rays are passed through sheets of the material being analysed or, for gases, liquids, or divided solids, through a cell in which a known weight of sample is placed. The absorption is then measured and the mass absorption coefficient deduced. Absorption, being an atomic property, independent in general of the physical state, depends upon the elements and their relative proportions present in the sample. The method of analysis is based upon this principle.

A constant-voltage source is necessary for accurate work.

RESULTS—**Solids and Liquids**—Preliminary experiments show that materials such as polystyrene, saflex, butvar, ethyl cellulose, cellulose acetate, cellophane, koroseal, and saran, can be distinguished from one another.

The uniformity and, very roughly, the quality of coal can be assessed in a few minutes, and the chlorine contents of chlorinated hydrocarbon polymers determined in about one-tenth the time required for the conventional analysis.

A limitation of the method is exemplified by methyl alcohol and sucrose which give identical absorption coefficients.

This ambiguity arises from the use of polychromatic X-rays, changes in "effective wavelength" of the X-rays occurring from one measurement to the other. The "effective wavelength" of a polychromatic beam is defined as that of a monochromatic beam absorbed to the same extent under the experimental conditions.

**Gases.**—Measurements made on hydrogen, methane, air, oxygen, methyl chloride, and chlorine, show that, as would be expected, the softest radiation is best for analytical work, but it is found that when longer wavelengths are used, particular attention must be paid to changes in the "effective wavelength."

The temperature and pressure of the gas should be known in order to calculate the density of the sample.

Curves for the absorption of methyl chloride at reduced pressures are given. E. G. STEWARD

# Reviews

TRACE ELEMENTS IN FOOD. By G. W. MONIER-WILLIAMS, O.B.E., M.C., M.A., Ph.D., F.R.I.C. Pp. viii + 511. London: Chapman & Hall Ltd. 1949. Price 30s. net.

It is not so long since "trace elements" in food would have been defined as elements known or suspected to occur naturally and thought to exert vital functions in the life cycle, but present in such minute amounts as not to be quantitatively assessable. The emphasis would have been on the word trace to indicate a lack of precise knowledge. Within recent times, however, analytical chemistry, particularly in its spectrographic and colorimetric branches, has advanced and developed to such purpose that it is now possible to obtain at least close approximations for the smallest amounts of these elements. In the preface to his book Dr. Monier-Williams remarks that there is for most trace elements a wide range between those quantities that are by common consent innocuous or even essential to health and those that show signs of being injurious; and that to decide on the limits within this range to which foods should conform is not easy. To any food chemist this remark applies with particular force. The difficulty arises in assessing the validity of much that may be regarded as having been accepted by "common consent." There may be little doubt of a minimum toxic amount, yet a grave doubt of the minimum amount properly to be regarded as potentially harmful in the circumstances of differing chemical combinations or under conditions of continuous absorption for varying periods of time. Thus, late in 1900 and early in 1901, it was observed that in parts of northern England cases of peripheral neuritis were becoming increasingly numerous, particularly among beer drinkers. At first this was attributed to alcoholism, but as cases were found to have occurred among moderate drinkers attention was directed to other possible causes, and late in 1901 analysis of samples of beer revealed the presence of arsenic in significant, though minute, amounts. A Royal Commission was appointed and the occurrence critically investigated, together with the related subject of arsenical poisoning, and in 1903 a full and detailed Report was published. In concluding the Report, the recommendation was made that solid foods and foodstuffs should not be contaminated with arsenic to an extent greater than 1/100th of a grain per pound. This recommendation is now forty years' old and has been generally accepted and stood the test of time in a remarkable fashion, yet it can still be stated authoritatively that more precise knowledge is required of the cumulative effect of repeated minute doses of arsenic, and that the relative toxicity of inorganic forms of arsenic and organic forms (such as occur in shell-fish) is still more or less unknown. Again, the toxicity of lead has probably been the subject of more investigation than that of any other metal, and by common consent a maximum safe limit has been accepted, yet the medical data of men working in lead industries frequently suggest that many of them must regularly absorb more than this maximum. Tolerance and idiosyncrasy confuse the issue, the former to a greater extent because it may characterise a group, whereas the latter is essentially individualistic. The real difficulty arises, however, from the comparative absence of data which are indispensible, namely, the results of direct experiments on human beings on a sufficiently comprehensive scale. To correlate animal experiments with human requirements is of doubtful value, particularly as a substantial proportion of such experimental work has been made by the administration of inorganic salts, either in solution or mixed with food but not, as far as our knowledge goes, in the forms in which the elements are likely to be present in foods. The requirement for direct experiments with human beings is impracticable and incapable of fulfilment. It remains therefore to seek an answer—even if only a tentative one—in another way. What amounts of these elements are daily ingested in food by the average person; what would be the expected effect on the health of the average person; and what medical evidence is there of significant or potential variations in average health? An example of such a method of attack is afforded by the work that led to goitre prophylaxis with iodine.

Dr. Monier-Williams's book is probably the first published attempt to survey critically the whole field, and, as would be expected from such an authority, it is a great achievement. It deals with 26 elements in separate chapters, a single chapter is devoted to barium and strontium, and the last chapter (of 7 pages) under the heading of "Lithium and Other Metals," contains references to lithium, beryllium, zirconium, cerium, germanium, tungsten, thallium and uranium. Each element is examined from all angles and the information goes far beyond what might be expected from the title: the occurrence and amounts in different foods, the biological functions of the element, its toxic amount and effect, its retention in or method of excretion from the animal system, and the analytical method of determination (in outline but not in working detail) are all dealt with, and of course a very full bibliography is given. Better perhaps than a mere catalogue is the following quotation of the sub-headings in the longest chapter (Copper—64 pages).

*Copper*—As an essential element in plant and animal life; content of copper in organs of the body; biochemical availability; absorption, excretion and retention; minimum requirements; deficiency effects in cattle and sheep; pigmentation; toxicity; in soils and plants; copper fungicides; corrosion by foods; in dairy products; copper and vitamin C; copper and fermentation; greening of vegetables; copper haze in wines; copper in tomatoes; copper in various foods; copper and public health; the determination of copper. The bibliography covers 8 pages.

On similar lines lead takes 43 pages; zinc, 31; tin, 24; arsenic, 45; antimony, 15; cobalt, 12; manganese, 19; iodine, 19; selenium, 16; iron, 33; fluorine, 32; boron, 16; chromium, 5; molybdenum, 7.

With the aid of Dr. Monier-Williams's work the foundations of future legal limits for the metallic contamination of foodstuffs will be laid, and all workers in this field will not only congratulate him, but will also thank him very sincerely for its publication. GEORGE TAYLOR

POLAROGRAPHISCHES PRAKTIKUM. By Professor J. HEYROVSKÝ. Anleitungem für die Chemische Laboratoriumspraxis. Band IV. Pp. vi + 118 with 90 Figures. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1948. Price DM 8.40.

The appearance of a new book on Polarography by Professor Heyrovský will be welcomed by many members of the Society who have met the distinguished founder of the subject, heard him lecture, or read his lecture in *The Analyst*. This monograph, as its title suggests, is a purely practical introduction to the subject. Those desiring information on the theory or wishing for an exhaustive compendium of methods and applications must look elsewhere. A distinct advantage of the present monograph is that it brings to one's notice numerous new practical devices in use in the Czechoslovakian school. English, and particularly American, practice is almost ignored. The pen recorders, so common in England and the U.S.A., are brushed aside as being only fit for "technical analysis." An excellent chapter on potential - time curves obtained by the oscillographic method may help to bring to a wider circle of polarographic workers the value of this method; the original paper, which appeared in the *Zeitschrift für physikalische Chemie* in 1943, has not been widely read in this country. An increase in sensitivity would make the oscillographic method quite important, but, even in its present state of development, it can give valuable information about the nature of electrode reactions.

*Polarographisches Praktikum* is excellently printed on good paper and the many illustrations are well produced. The paper binding is poor. W. CULE DAVIES

# British Standards Institution

NEW SPECIFICATIONS*

B.S. 391: 1948. Tung Oil (Types F and M). Price 2s. 6d.
B.S. 522: 1948. Ortho, Meta and Para Cresols. Price 2s.
B.S. 523: 1948. Phenol. Price 2s.
B.S. 524: 1948. Refined Cresylic Acid. Price 2s. 6d.
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B.S. 1917: 1948. Sampling and Analysis of Acid Casein. Price 2s. 6d.
B.S. 1482: 1948. Home-produced Technical Tallow. Price 2s. 6d.
B.S. 1983: 1948. Home-produced Technical Bone Grease. Price 2s. 6d.
B.S. 1499: 1949. General Guidance in Sampling Non-ferrous Metals. Price 1s.

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.

Draft Specification prepared by Technical Committee ISE/18—Sampling and Analysis of Iron and Steel—

CK(ISE)1883 (To supersede CK(ISE)635)—Method for the Determination of Aluminium Oxide in Permanent Magnet Alloys.

* Obtainable from the British Standards Institution, Publications Department, 28, Victoria Street, London, S.W.1.

ANALYSTS. May & Baker Ltd., Dagenham, Essex, require qualified Chemists (either sex) for vacancies in their Analytical Control Laboratories. Previous experience in analysis of Pharmaceuticals desirable but not essential. Progressive salaries commensurate with experience and abiilty. Staff Pension Fund (contributory). Please apply giving full details of education, experience, etc., and quoting reference No. 2631 to Personnel Officer.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED, Plastics Division, Black Fan Road, Welwyn Garden City, Herts., are now requiring a Micro-Analyst, preferably with Honours Degree or A.R.I.C. (by examination). Candidates should have several years' experience of Organic Micro-Analysis, and possess good organising ability. Age range 25–35. Salary according to age and experience. Write for Application Form to Staff Manager.

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BOOTS PURE DRUG CO. LTD. have vacancies in their Control Division for qualified Assistant Analysts, aged 27–35, with practical experience in the control of medicinal and pharmaceutical preparations. Applicants must be capable of taking responsibility; the positions will carry suitable salaries and offer good prospects of advancement. Applications to Personnel Manager, Station Street, Nottingham.

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VACANCIES for two Senior Analytical Chemists exist in the Research Division of a large manufacturing concern in the West London area. The interests of the company embrace a very wide variety of organic and inorganic materials, and the analytical work calls for initiative and ability to adapt modern methods of analysis to the specific needs of the Division. Experience in food chemistry would be an advantage for one of the posts to be filled. For the second vacancy an interest in physical methods and/or microchemistry would be an asset. Apply stating reference CR/1 to Box 3711, THE ANALYST, 47, Gresham Street, London, E.C.2.

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VACANCY in London for Chemist experienced in Base Metal Analyses, particularly tin in ores, residues, etc. Box No. 3710, THE ANALYST, 47, Gresham Street, London, E.C.2.

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SENIOR ANALYTICAL CHEMISTS required. Good general experience in organic analysis. Excellent prospects. Salary £500–£700 per annum according to experience, qualifications, etc. Apply for interview: Personnel Manager, Petrochemicals Limited, Urmston, Manchester.

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MICROANALYSIS ASSISTANT aged 21–27 required by large firm in South West London. Applicants should have experience in microanalytical work and some academic traning. Write Box No. U 4199, A. K. Advg., 212a Shaftesbury Avenue, W.C.2.

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ASSISTANT CHEMIST aged not more than 26 required to work on microanalysis. Candidates should have good degree or A.R.I.C., but experience in microanalysis is not essential. Write Senior Research Manager, British Oxygen Co., Ltd., R. & D. Dept., Lombard Road, Morden, S.W.19.

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APPLICATIONS are invited for the post of Analyst, Mineral Nutrition of Plants and Soils, at Long Ashton Research Station.

The appointment will be under a special scheme of the Agricultural Research Council and will be graded in accordance with the London Scale, less Provincial Differentiation, for Assistant Experimental Officers. The salary will be from £220 (at age 18) to £460 p.a. for men, and from £220 (at age 18) to £380 p.a. for women, with superannuation allowance.

Candidates should possess the National Certificate in Chemistry or an equivalent qualification.

Further particulars may be obtained from the Secretary, to whom applications should be addressed not later than 16th July, 1949.

The Secretary,
Long Ashton Research Station,
Long Ashton, Bristol.

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WANTED. Complete "Spekker" Absorbtiometer Outfit, S.P.A.I. Williams Alexandra Foundry Ltd., East Moors Road, Cardiff.

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WANTED: Single copy of THE ANALYST, No. 844, July, 1946, to complete year's issue. Please send particulars to Box T.873, Willings, 362, Grays Inn Road, W.C.1.

Volume 74, No. 876, Pages 153—222 March, 1949

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## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS



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

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