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See Notices on p. 122

THE ANALYST

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MINISTRY OF SUPPLY invite applications for following post at the ATOMIC ENERGY RESEARCH ESTABLISHMENT, Harwell:—

(1) Senior Industrial Chemist.
(2) Junior Industrial Chemist.

Duties for post (1) are responsibility to the Maintenance Engineer for control of water supply to the Establishment, effluent disposal arrangements and general chemical service to the Chief Engineer, including control of boiler house, fuel economy, water treatment and large water distillation plants. The Junior Industrial Chemist will assist in these duties.

Candidates must be of British nationality (see detailed regulations); must hold an honours degree in metallurgy, engineering, physics, chemistry or chemical engineering, or have associateship of the Royal Institute of Chemistry, the Institution of Metallurgists or the Institute of Physics, and have had at least 3 years approved experience in a factory or industrial laboratory; or have corporate membership of the Institution of Chemical Engineers.

For post (1) candidates should be not more than 45 years of age and have had extensive experience in a responsible position in a chemical works, including control of technical staff. For (2) they should be not more than 35 years of age and have had good experience in chemical works operations and be used to routine analysis of water supplies. Favourable accommodation prospects exist for the successful candidate for post (1).

The appointments will be temporary in the first instance. The successful candidates will be eligible to compete for permanent appointment in the pensionable establishment in the event of such a competition being announced.

Salaries will be assessed with the following ranges—post (1), £720–£960; post (2), £448–£720—according to age, qualifications and experience.

Write, quoting F.215/48A, to Ministry of Labour and National Service, Technical and Scientific Register, Room 669, York House, Kingsway, London, W.C.2, for application form, which must be returned by 12th April, 1948.

BOOTS PURE DRUG CO., LTD., invite applications from Chemists with administrative experience of Analytical Control work, for appointment to a position leading directly to that of Head of the Analytical Control Division of the Company. The division comprises laboratories engaged in Chemical, Physical, Pharmacological and Bacteriological methods of control, with a staff of upwards of 120. Its principle function is the routine control of raw materials, drugs and galenicals, and the medical and pharmaceutical products issued by the Company.

The applicant must have a sound knowledge of the basic principles governing methods of control in the above fields, possess high professional qualifications, including the Fellowship of the Royal Institute of Chemistry, and administrative and organisational ability. He should be between 35 and 45 years of age, but due consideration will be given to applicants of outstanding ability and experience outside this age group.

Salary, conditions of employment, pension, housing, etc., will be dealt with on interview.

Applications, giving age, qualifications and experience should be addressed to The Secretary, Boots Pure Drug Co., Ltd., Station Street, Nottingham, marked "Personal."

CHIEF ASSISTANT to Head of large Research and Analytical Laboratory of national manufacturing firm with wide ramifications required. Laboratories comprise Cereal, Technological, Technical and Textile, etc., units.

Applicants must possess Honours degree and F.R.I.C. (Branch E), also experience in industry or post-graduate research essential. Preference will be given to candidates of not over 40 years of age, but the age limit will not be rigidly applied.

Initial salary according to qualifications and experience.

Applications will be treated in strict confidence. Write, giving full details of career, to Box No. 3677, THE ANALYST, 47, Gresham Street, London, E.C.2.

TECHNICAL ASSISTANT required for metallurgical reseach laboratory on Northern Rhodesian Copper Mine. Basic starting salary £42 10s. per month, rising to £45 within one year if satisfactory. In addition there is a fluctuating cost of living allowance, and benefits of Pension and Cash Bonus schemes, and low Income Tax rates. Outward passage paid. Work would involve routine chemical analysis which, if satisfactory, would be followed by training n metallurgical analysis, and use of microscope. Laboratory also employs lady microscopist and research chemist with whom successful applicant would have to work. Please write giving details age, qualifications and experience to Box No. 342, Foster Turner & Everetts Ltd., 11, Old Jewry London, E.C.2.

URGENTLY required, copy of THE ANALYST for February, 1943. Please reply Box No. 3676, THE ANALYST, 47, Gresham Street, London, E.C.2.

HIS MAJESTY'S COLONIAL SERVICE

There is a vacancy for a Government Chemist in the Public Works Department of the Gold Coast. The selected candidate must have had a general training in Chemistry, Physics, etc., equivalent to that required by the Institute of Chemistry, and should have had at least four years' experience in general work connected with engineering materials, and should be familiar with the methods of analysis and testing of the materials, and of work connected with water purification. Maximum age limit 40.

The appointment is permanent and pensionable after a probationary period, at a salary ranging from £600 to £1,300, including pensionable expatriation allowance. The selected candidate's war service and experience will be taken into account in determining the initial salary to be offered.

Government furnished quarters are normally provided at a low fixed rental. Free passages provided for the officer and his wife on appointment and on leave after each tour of service of approximately 18 months.

Application forms may be obtained from the Directon of Recruitment (Colonial Service), 15, Victoria Street, London, S.W.1. Closing date 30th April, 1948.

HIS MAJESTY'S COLONIAL SERVICE

There is a vacancy for a Government Chemist in Mauritius. Duties include analytical work for Government departments and private firms. Biochemist analyses for hospitals and private practitioners. Toxicological work, and supervision of customs department laboratory. Qualifications: 1st or 2nd class honours degree in Chemistry, or Associate of the Institu.e of Chemistry, with subsequent analytical experience. Possession of the Certificate of the Institute of Chemistry in Branch E an advantage.

The appointment is permanent and pensionable with a probationary period of three years. The salary ranges from Rs.6300 to Rs.9000 (approximate rate of exchange—1 rupee = 1s. 6d.) War service will be taken into consideration in determin ng the point of entry into the scale.

Free passages are provided on first appointment for the officer and his family (not exceeding five in all) and for the officer when proceeding on leave. Leave is granted at the rate of four days for each completed month of resident service. Local taxation is low and United Kingdom income tax is not payable. Quarters are not provided.

Intending candidates should write giving brief particulars of age, qualifications and experience to the Director of Recruitment (Colonial Service), 15, Victoria Street, S.W.1.

ASSISTANT RESEARCH CHEMIST with experience in Analytical Chemistry and preferably with degree or equivalent qualification required, mainly for development work in Polarography. Apply, stating salary required, to Research Department, Cambridge Instrument Company Ltd., Cambridge.

STAFFORD ALLEN & SONS LTD. require an Assistant Analyst of B.Sc., A.R.I.C. or equivalent standard. Previous experience of the analysis of drugs and galenicals, including alkaloidal assays, necessary. Knowledge of essential oil analysis an advantage. Salary according to qualifications and experience. Apply by letter to Department D/WM, Wharf Road, London, N.1.

ANALYTICAL CHEMIST. Young graduate with some general analytical experience required for progressive post in Research and Analytical Laboratory. Applications in writing only giving particulars to the Staff Manager, London Co-operative Society, Ltd., 54, Maryland Street, Stratford, E.15. Envelopes to be endorsed "Analyst."

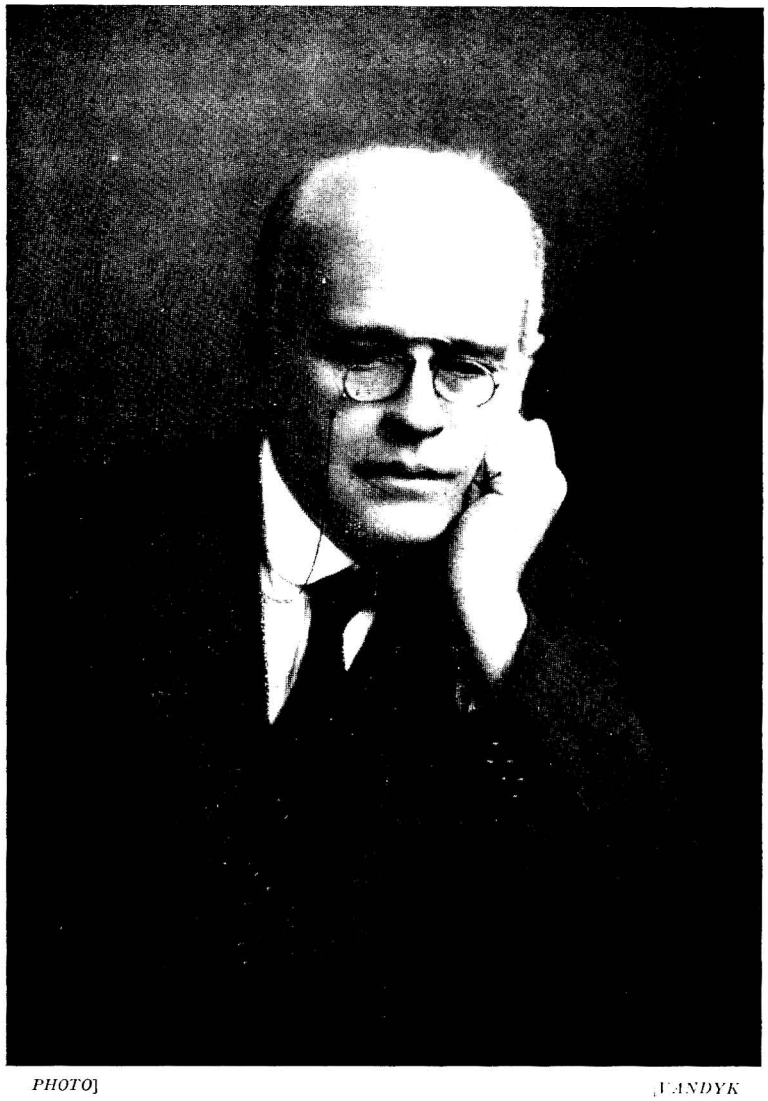
ANALYTICAL CHEMIST, 25 to 30 years of age, required for Works Laboratories of important industrial concern in West London district. Preference given to candidate accustomed to control of junior staff who is able to organise rapid and correct analyses of resins, waxes and pigments. The position is permanent and offers good prospects of advancement to the right man. Salary £350–£400 per annum. Write stating age, qualifications, experience, etc., to Box 3675, THE ANALYST, 47, Gresham Street, London, E.C.2.

APPLICATIONS are invited for the following posts in the Analytical Laboratories, British Chemicals and Biologicals Ltd., Loughborough.

(1) ANALYST possessing Honours Chemistry Degree or A.R.I.C., and preferably with industrial experience with examination of fine chemicals and medicinals or in biochemicals and microbiological methods of analysis. Familiarity with modern physical methods of analysis would be an advantage. Salary to be commensurate with qualifications and experience.

(2) Two Junior Analysts of Inter. or Subsid. B.Sc. standard and keenly interested in analytical chemistry as a career.

Applications, giving full particulars and stating salary required, should be addressed to· The Technical Director British Chemicals and Biochemicals Ltd., Derby Road Loughborough, Leics.



PHOTO] *[VANDYK*

Ainsworth Mitchell

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 4th, at the Chemical Society's Rooms, Burlington House, London, W.1, with the President, Mr. Lewis Eynon, in the chair. The following paper was presented and discussed: "The Micro-analytical Test for Purity in Food, with Special Reference to Cereals," by D. W. Kent-Jones, A. J. Amos, P. S. Elias, R. C. A. Bradshaw, and G. B. Thackray.

NEW MEMBERS

Judson William Airan, M.Sc., Ph.D. (Bombay); Professor Harold Burton, M.Sc. (Sheff.), Ph.D., D.Sc. (Lond.), F.R.I.C.; William Edwin Drinkwater, A.R.I.C.; John Stuart Harrison, B.Sc. (Lond.), M.Sc. (Leeds); John Wilfred Lowry, B.Sc., A.R.I.C.; John Webster Murfin, B.Sc. (Lond.), A.R.I.C.; Miss Joan Davena Peden, B.Sc. (Liv.), F.R.I.C.; Edward Donald Yardley, A.R.I.C.

DEATH

WE deeply regret to report the death of Dr. Bernard Dyer.

MICROCHEMISTRY GROUP

THE Fourth Annual General Meeting of the Microchemistry Group was held at Imperial College, South Kensington, on Friday, January 30th, 1948. The following new members of Committee were elected. Messrs. A. F. Colson, D. F. Phillips, and G. Ingram. The Committee and office bearers for 1948 are: *Chairman*—Mr. Norman Strafford; *Vice-Chairman*—Dr. Janet W. Matthews; *Hon. Secretary*—Mr. Ronald Belcher; *Committee*—Professor, H. V. A. Briscoe, Dr. A. F. Colson, Mr. G. Ingram, Dr. D. F. Phillips, Mr. J. T. Stock, and Mr. E. J. Vaughan.

After an interval for tea, Mr. J. T. Stock read a paper on "The Microchemical Aspects of Electrolytic Conductivity," and the following papers by Messrs. J. T. Stock and M. A. Fill were read: "A Micro Blowpipe for the Manipulation of Capillaries"; "A Diaphragm Pump for Air and Other Gases"; "A Melting Point Indicating Device"; and "A Transmitting Manometer for Micro Oxygen Uptake Experiments."

An Exhibition of new microchemical apparatus designed by the above authors was also held.

Obituary

CHARLES AINSWORTH MITCHELL

1867–1948

THE Society has lost one of its valued pillars—a member who for fifty years has been intimately associated with its every phase and who contributed in a rare degree to its enlargement and success. Every member for many years must have known Mitchell, his works and his friendly personality; his history is bound up with that of the Society.

Son of a Norfolk doctor—T. R. Mitchell, M.D.—Charles Ainsworth was born at Thetford on November 20th, 1867; in due time he went to school at King William's College, Isle of Man, and thence in 1887 to Exeter College, Oxford, graduating in 1889. One does not think of him as a footballer, but, in fact, he played half-back for his College. After a short time at Heidelberg he became assistant to Otto Hehner in Billiter Square, and there joined other well-known members of our Society, including L. K. Boseley, W. P. Skertchly, S. Aston, G. N. Huntly, A. Ashby, and H. Droop-Richmond. In 1894 he was elected a member, and it remained his first love all his days. He was elected a Fellow of the Institute of Chemistry in 1897 and of the Chemical Society in 1916.

Mitchell's life's work began in 1894, and thereafter there appeared a succession of scientific papers in THE ANALYST, the *J. Soc. Chem. Ind.* and other journals, and a number of books and translations which in themselves pay tribute to his literary and linguistic skill and scientific ability. Under the influence of Hehner, oils and fats became a dominant interest and the subject of several memoirs; we all know the joint names of Hehner and Mitchell in this connection. In 1897 Mitchell joined Beaufoy's vinegar brewery; he was probably the first qualified chemist in that industry and remained with the company until 1932, when it became part of British Vinegars, Ltd., and he retired to the less active role of consultant to the combine.

All these years Mitchell's activities with the Society and his horizons enlarged. More than forty original memoirs appeared on inks, on handwriting, on oils and fats, on finger prints and identification of the person, on documents and their examination, on tannins, on pencils and pigments, and on a wide variety of medico-legal topics in the more popular journals. Besides all these he wrote, edited or translated about thirty text books.

In 1920 Mitchell was appointed Editor of THE ANALYST in succession to Julian Baker, who had occupied that office with conspicuous ability for thirteen years. Under Mitchell's guidance and literary skill our journal went from strength to strength and has achieved an honoured place among the scientific journals of the chemical world. This is in very large measure due to Mitchell, and the Society owes him a very great debt of gratitude for his devoted service till he retired in 1945. The analytical chemist might say of Mitchell as others did of Wren:

"Si monumentum requiris, circumspice;"

he has only to look on his bookshelves.

Space does not permit even mention of all Mitchell's scientific and historical investigations. In 1905 he examined and adapted from the German the story of Dr. Armyons wife (P. Hoecker) and in 1927 the evidence of the Casket letters came under review in a series of pamphlets. Mitchell's scientific work was suitably recognised in 1929 by his old college in the award of the degree of D.Sc. (Oxon.).

All these literary activities plus the ordinary laboratory work might have sufficed many men, but not one of Mitchell's ability and industry. He also excelled as an expert witness and had unrivalled skill in the presentation of scientific evidence in the courts. In this department he set an example of fairness and accuracy and was called upon to assist in many famous cases connected with forgery, handwriting, identification of the person and similar matters. His unrivalled knowledge in this department led to his appearance during the 1914–18 war in many cases arising from the Postal Censorship and in some famous spy trials. Some of these are described in his books *The Scientific Detective* and *Science in the Criminal Courts*; others are still matters of secrecy.

Arising from this work for the Censorship came Mitchell's long co-operation with our only woman Honorary Member, the late Miss M. B. Elliott, who was engaged in the same department and it was her acumen that led to the discoveries of the treasonable documents which Mitchell examined. Miss Elliott's valuable work in assisting the Editor in indexing and in other ways will long be remembered with gratitude by our Society.

Outside the range of our Society, Mitchell's next interest was the Medico-Legal Society, of which he became a member in 1911. He read many papers there and was successively member of Council, Joint Editor of the Medico-Legal Journal, vice-president and, in 1935–1937, President—a great distinction for one who was neither a medical man nor a lawyer by profession. He was also a vice-president of the Medico-Legal Society of France. He was active in the Royal Institute of Chemistry, served on its Council and was a vice-president in 1937–1940.

What of the personality of Mitchell? He was of a kind and cheery disposition—a delightful companion; his fund of knowledge, breadth of interests and, above all, artistic temperament made him a most lovable and agreeable person. He was devoted to the interest of this Society; he maintained a life-long affection for Hehner and for all those associated with him at Billiter Square. He loved to help the younger men and, indeed, to befriend any who sought his advice or help—and this sometimes to his cost. In a friendship of many years the writer never knew him speak unkindly of anyone.

Mitchell also loved nature and produced under the name of Michael Fancourt some delightful verse on flowers and a few short stories.

In 1899 Mitchell married Miss Edith Boyle Keely, an artist of some distinction; though he had no family he took a lively interest in the children of his friends and followed their development with kindly interest.

After a long life of great activity, in which he achieved much honour, Mitchell retired on account of health in 1945; he was ill for about two years and passed away peacefully in his sleep on January 5th, 1948, at Ealing. At the funeral at Golders Green on January 8th, many members of the society were present to pay tribute to a beloved colleague and to express sympathy to Mrs. Mitchell.

"A good life hath but few days, but a good name endureth for ever."

H. E. Cox

The Photometric Determination of Tungsten

By C. H. R. GENTRY and L. G. SHERRINGTON

The available methods for the photometric determination of tungsten are not all that might be desired; they lack precision and are not readily applicable to routine determinations such as that of tungsten in steels or non-ferrous alloys. There is a definite need, therefore, for a method that will permit the rapid determination of small quantities of tungsten in a variety of products with an accuracy comparable with that of the procedures available for most other metals.

Of the known reagents for tungsten, thiocyanate in conjunction with a reducing agent such as stannous chloride,[1] and toluene-3 : 4-dithiol[2] deserve special consideration. The latter reagent offers the only known method of determining traces of tungsten in molybdenum compounds, but it has so far been applied only on a qualitative or semi-quantitative basis; the present work has therefore been concerned with the use of potassium thiocyanate and a reducing agent.

If thiocyanate and stannous chloride are added to an acid tungstate solution a weak greenish colour is produced. If, however, these reagents are added to a tungstate solution containing a small amount of sodium hydroxide, a yellow colour forms slowly in the final acid solution.[3] This is the basis of the most widely used method for the determination of traces of tungsten. The colour intensity develops slowly at room temperature, and readings are usually taken after 1 hour under standard conditions,[4,5] although the intensity still increases after 2 hours. Warming the solution hastens the development of the colour,[6] but too high a temperature or too prolonged heating results in precipitation of finely divided sulphur and stannous sulphide. A method has been reported in which the stannous chloride is replaced by titanous chloride and the transmittancy of the solution is determined after 10 minutes.[7] Despite the several investigations made on the thiocyanate method, none of its modifications constitutes an entirely satisfactory method for the photometric determination of tungsten.

The object of the present paper is to describe a method which avoids many of the disadvantages of previous methods. It differs from the older methods in that the tungsten is reduced to a definite lower valency state prior to the formation of the thiocyanate compound. Investigations by the present authors have shown that this difference in procedure is essential if precise results are to be obtained. The proposed method has other fundamental advantages; it is no longer necessary that the initial tungsten solution should be alkaline (but see ref. [8]); rapid reduction can be effected without danger of decomposition of the thiocyanate and consequent formation of precipitates; and the yellow compound that forms the basis of the procedure is of a definite composition and not the indefinite substance previously obtained.

Experimental

A number of preliminary experiments were made using in general the conditions established by previous workers, but replacing the stannous chloride with other more powerful reducing agents in the hope that the colour would be developed more rapidly. Particular attention was paid to methods in which the acidified tungstate solution containing potassium thiocyanate was shaken with mercury and the liquid amalgams. Typical results on a constant weight of tungsten with mercury and tin amalgam are given in Table I.

The effect of shaking time on the formation of the yellow colour was thus shown to be very critical. The results were explained by assuming that the yellow tungsten thiocyanate

compound was fairly rapidly formed on shaking, but that side reactions, presumably due to reduction of the thiocyanate, occurred simultaneously, and the products of the side reactions reacted in turn with the yellow tungsten thiocyanate compound and caused fading. This explanation was consistent with the rather smaller maximum intensity of colour obtained when tin amalgam was used for reduction and with the slower fading when mercury was used.

TABLE I

PRELIMINARY EXPERIMENTS USING MERCURY AND TIN AMALGAM

(*a*) Reduction with mercury.		(*b*) Reduction with tin amalgam.	
Time of shaking min.	Absorptiometer reading	Time of shaking min.	Absorptiometer reading
0·25	0·093	0·25	0·233
0·5	0·178	0·5	0·278
1	0·255	1	0·264
2	0·307	2	0·217
4	0·332	4	0·172
8	0·320		
12	0·298		

A series of experiments was made in which various factors were changed and organic solvents were added to extract the yellow tungsten compound. These experiments led to a method that did not suffer from the rapid fading of the yellow colour, possibly because the thiocyanic acid largely dissolved in the organic layer during the shaking and was thus removed in some degree from the action of the liquid amalgam. However, this method was liable to sudden inexplicable variations, and was finally abandoned in favour of the method in which the tungsten was reduced before the addition of the thiocyanate. The final form of the latter method may be described as follows.

METHOD

Place the tungsten solution, the volume of which must be between 5 ml. and 10 ml., in a 50-ml. graduated flask. Add 16 ml. of 12 *N* hydrochloric acid from a measuring cylinder and 1 ml. of a 10 per cent. stannous chloride solution in 6 *N* hydrochloric acid. Cool under a tap to room temperature. Add 2 ml. of tin amalgam (see below) from a short-pattern pipette. Shake for 2 to 5 minutes. Add from a pipette 5 ml. of 12 per cent. potassium thiocyanate solution and swirl to mix. Dilute to the mark with distilled water and gently mix (aqueous volume, 48 ml.). Carry out a blank by the same procedure but omitting the thiocyanate. Fill 2-cm. cells with the solution and the blank and take absorptiometer readings,[9] using the mercury vapour lamp in conjunction with Chance No. 8 (OV.1) and Ilford 601 filters.

The initial tungsten solution must not contain sufficient free acid or alkali to affect markedly the acid strength of the solution during reduction. Up to 1 ml. of 12 *N* hydrochloric acid or the equivalent amount of alkali would be permissible. This method was established after investigations of the several factors involved, a detailed description of which follows.

The reduction—Earlier experiments were concerned with the choice of a reducing agent; mercury, and zinc, cadmium, lead, tin and bismuth amalgams were tried. Tin amalgam was finally chosen, chiefly because the presence of stannous chloride in the solution was found to be essential (*vide infra*). The tin amalgam was prepared by heating mercury with an excess of tin in presence of dilute hydrochloric acid. The amalgam was subsequently kept over a little solid tin. After use, the amalgam was washed and returned to the stock bottle, which contained a dilute hydrochloric acid solution.

The effect of the acidity on the reduction of tungsten solutions was investigated using the method described on identical quantities of a standard solution of alkali tungstate, always adding the same volume of hydrochloric acid prior to reduction, but at the same time adding different amounts of water. By this means the acidity during reduction was varied, but the final acidity of the solution was kept constant. The results, shown in Fig. 1, indicated that the concentration of hydrochloric acid in the solution during reduction had to be more than 6 *N*.

The reduction was carried out by shaking the stoppered flask on a simple shaking machine built for this purpose. Hand-shaking was used in some of the earlier experiments, and was

quite satisfactory if only a few determinations were to be made. The mode of shaking was immaterial provided that good agitation was obtained without very violent motion, which led to formation of some turbidity and consequently to unreliable results. In a series of experiments on identical quantities of the standard tungsten solution, shaking was carried out at a uniform rate, but for different times. The results, plotted in Fig. 2, showed that under the conditions used complete reduction was attained by 2 minutes shaking.

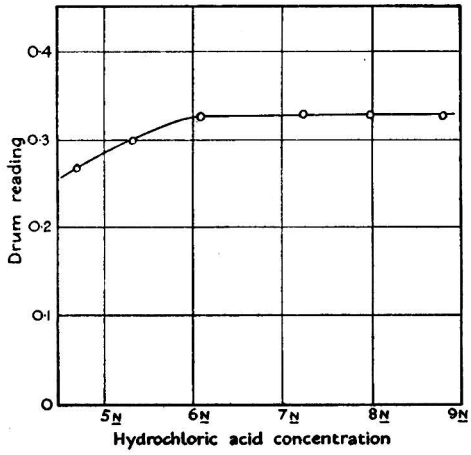


Fig. 1. Effect of acidity during reduction.

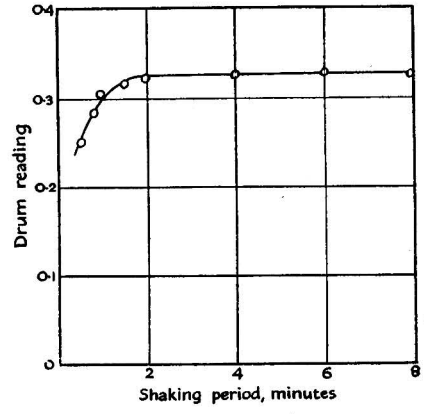


Fig. 2. Effect of shaking period.

Stannous chloride was added in some of the preliminary experiments in the hope that it would prevent fading, which was thought to be due to atmospheric oxidation. It was subsequently found that the presence of some stannous chloride was essential for other reasons. Thus, in absence of stannous chloride, slightly low and somewhat variable results were obtained, apparently owing to incomplete reduction. Again, atmospheric oxidation of traces of iron was found to be more significant than the re-oxidation of the tungsten, and it could be prevented by the presence of stannous chloride. The quantity of stannous chloride used was shown to have no effect on the results over the range investigated, *i.e.*, 0·1 to 10 ml. of the 10 per cent. stannous chloride solution.

Under the conditions finally adopted for the reduction of the tungsten, the reduced solution was quite stable. In a number of experiments the reduced tungsten solution was left in the flask for periods up to 80 minutes before addition of the thiocyanate solution, but the final results were unaffected.

Formation of the colour with thiocyanate—It was found necessary, for reasons that will be discussed later, to add the thiocyanate solution to the reduced tungsten solution and mix gently before dilution to the mark. The mixing was always made carefully, in order to minimise reduction of the thiocyanate by the amalgam. A series of experiments was made by the method described, on 5 ml. of the standard sodium tungstate solution, and reduction was carried out in 7·2 *N* hydrochloric acid. A varied amount of the thiocyanate solution was added, and the solution diluted to the mark after mixing, giving a final acidity of approximately 4 *N*. The results, shown in Fig. 3, indicated that the readings were independent of the amount of thiocyanate present provided that this exceeded a certain minimum amount.

The effect of the final acidity on the method was investigated by carrying out a series of experiments with the standard tungstate solution. The initial reduction was made in presence of different amounts of acid but with sufficient water to keep within the range 6 *N* to 8 *N*. By this means the final acidity of the solution was varied from 2 *N* to 8 *N*. The thiocyanate addition was kept constant at 5 ml. of a 12 per cent. solution of the potassium salt. The results, plotted in Fig. 4, show that the absorptiometer readings were sensibly the same over a range of 2 *N* to 4 *N* but were lower at higher acidities.

The final coloured solution was quite stable. In a number of determinations, after dilution to the mark and mixing, the flask was stoppered and left; no measurable change in the colour took place in 1 hour and only slight changes in 2 hours. However, when such solutions were left overnight decomposition occurred and a marked odour of hydrogen

sulphide was observed. The stability of the solutions when exposed to the air, as for example after pouring into the cell, varied with the tungsten concentration; but atmospheric oxidation caused no interference provided that the Spekker readings were made in a reasonable time, say within 5 minutes of filling the cell.

Preparation of standard graphs—A stock tungsten solution (1 mg. of W per ml.) was prepared by dissolving 1·261 g. of pure ignited tungsten trioxide in an excess of sodium hydroxide solution in a platinum dish, and diluting to 1 litre. This was diluted further to

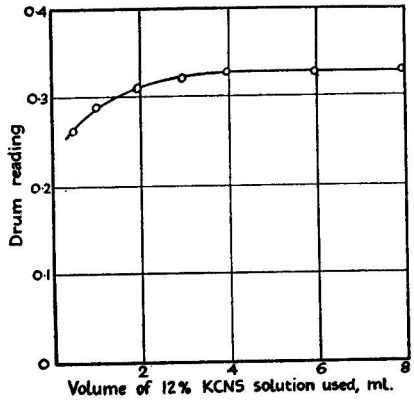


Fig. 3. Effect of thiocyanate concentration.

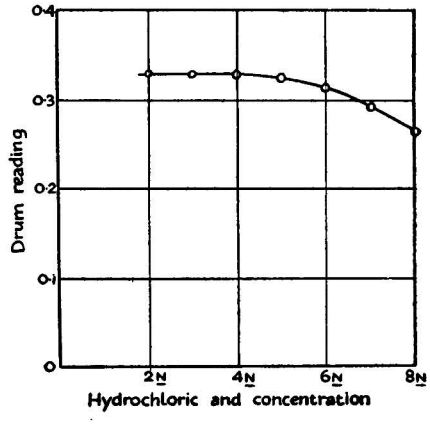


Fig. 4. Effect of final acidity.

give two standard solutions as required, containing 1 mg. of W per 10 ml. and 0·25 mg. of W per 10 ml. respectively. Selected volumes of these two solutions were measured from a microburette into 50-ml. standard flasks and diluted to approximately 10 ml. with water. The tungsten determinations were completed by the method previously described (p. 58). Absorptiometer readings were taken in 1-cm., 2-cm. and 4-cm. cells. After correction for the blank readings the graphs shown in Fig. 5 were plotted. The blank reading was constant; for 2-cm. cells it was 0·030.

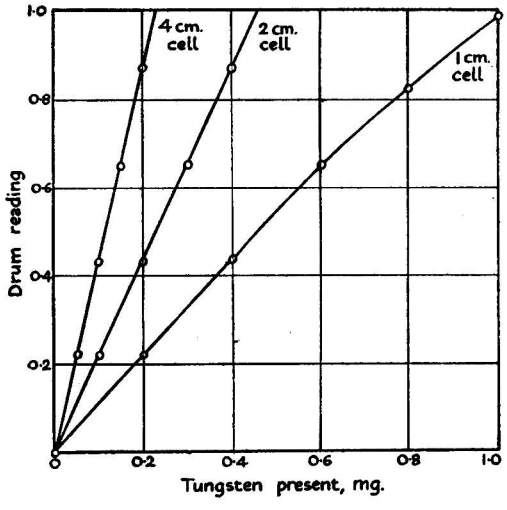


Fig. 5. Calibration graph.

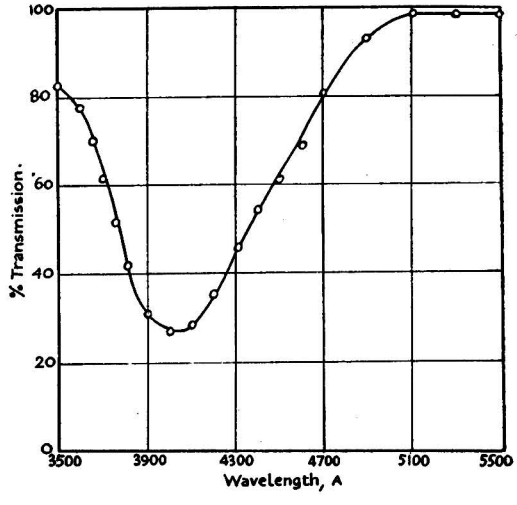


Fig. 6. Transmission curve of tungsten-thiocyanate complex; 0·5 mg. of tungsten in 48 ml., 1-cm. cell.

The graphs obtained with 2-cm. and 4-cm. cells were straight lines over the useful instrument range, but with 1-cm. cells the graph curved for quantities of tungsten greater than 0·5 mg. It was found that with longer shaking time the departure from linearity was

less marked, suggesting that reduction had been incomplete. For this reason it was thought advisable to limit the method to quantities of tungsten less than 0·5 mg. per 50 ml. of final solution.

The choice of the particular filter combination used was made after an examination of various others, including some liquid filters. It was desired to use light of wavelength 380 to 420 mμ., as the absorption peak was in this range (Fig. 6). However, the filter combination of Chance No. 8 and Wratten No. 2 recommended by the makers for the isolation of the 4077·8 A. and 4046·6 A. mercury lines, always gave curved graphs of drum readings against concentrations of tungsten. The difficulty was finally resolved when it was discovered that this recommended filter combination transmitted in the red region. Replacement of the Wratten No. 2 filter by the Ilford 601 filter gave a filter pair that transmitted only the two mercury lines, and this resulted in linear graphs. The improved selectivity of the filters was somewhat offset by their rather poor transmission and the resulting lack of sensitivity of the absorptiometer for readings above 0·8.

Precision of the method—Thirty determinations were made on a known amount of tungsten, 0·25 mg., over a period of several days and under conditions likely to be met in routine practice. The absorptiometer readings ranged from 0·570 to 0·601 with an average value of 0·583 and an average deviation of ±0·0054. The readings were distributed around the central value in a manner consistent with truly random errors. The standard deviation was calculated as ±0·0069.

Interferences

The preliminary investigations had indicated the possible existence of another form of the reduced tungsten thiocyanate complex, which was brown and not yellow in colour. As the formation of this brown form was to be regarded as a serious interference, a brief investigation of its properties was made.

If the hydrochloric acid used to obtain the required acidity was replaced by sulphuric acid, the reduced tungsten solution had a weak purple colour which gave a brown coloration on the addition of thiocyanate. This brown colour changed to the normal yellow colour when left for 2 or 3 minutes. If, however, a more dilute thiocyanate solution was used, a purple complex was formed which only slowly changed its hue. The addition of hydrochloric acid to this purple complex caused a more rapid change to the yellow form. A stable purple solution was obtained by measuring into a 100-ml. standard flask 5 ml. of alkali tungstate solution (≡ 1 mg. of W), adding 12 ml. of diluted sulphuric acid (1 + 1), 1 ml. of 10 per cent. stannous sulphate solution in 10 per cent. sulphuric acid and 2 ml. of tin amalgam, shaking for 3 minutes and then adding 40 ml. of 4 per cent. potassium thiocyanate solution and making up to 100 ml. with water.

Table II

Anionic interferences

Anion	Amount added	Absorptiometer reading	Remarks
—	Nil	0·583	—
Tartrate	1 g. of tartaric acid	0·589	—
Citrate	1 g. of citric acid	0·567	—
Perchlorate	1 ml. of 60% perchloric acid	0·580	Ppt. of potassium perchlorate
Oxalate	1 g. of oxalic acid	0·585	—
Sulphate	0·5 g. of potassium sulphate	0·580	—
	1·0 g. of potassium sulphate	0·581	—
Fluoride	2 g. of ammonium fluoride	—	Large ppt.

The brown colour was therefore attributed to a mixture of the purple and yellow forms of the complex, the purple form changing to the yellow at high acidities, especially in presence of high concentrations of thiocyanate. For this reason the technique of mixing the thiocyanate before dilution to the mark was adopted as previously mentioned.

Anionic interferences were studied by additions of the compounds shown in Table II to 5 ml. of the standard tungsten solution (≡ 0·25 mg. of W) in 50-ml. flasks. The method was completed in the normal manner. Blanks were carried out in absence of the tungsten, and the absorptiometer readings corrected accordingly. The results are shown in Table II.

The precipitate of potassium perchlorate consisted of large crystals which settled rapidly and caused no interference. By the use of a 10 per cent. solution of ammonium thiocyanate

in place of the potassium salt, the perchlorate precipitation was eliminated. The precipitate given by fluoride, which was thought to be potassium silico-fluoride, would not settle readily and no readings were taken, although the yellow colour of the tungsten thiocyanate was visible. As the presence of fluoride in the absorptiometer cells was undesirable, further work on the possible interference of fluoride was not carried out.

The interference of phosphate was of special interest, as the solubility of phosphotungstic acids makes phosphoric acid a very useful reagent for the solution of samples containing tungsten. Various amounts of a phosphoric acid solution were added to known amounts of tungstate solution and the determination of tungsten was attempted. With 250 μg. of tungsten and from 6 mg. to 0·3 g. of phosphoric acid, all the results, which were somewhat variable, were about 80 per cent. of the true value. If, however, the tungsten solutions, after the addition of phosphoric acid, were made just alkaline to phenolphthalein with either ammonia or sodium hydroxide and the 16 ml. of hydrochloric acid were then quickly added, the phosphate interference could be eliminated. Accordingly, in the presence of phosphate, citric acid followed by ammonia in slight excess was added to the aliquot prior to the addition of hydrochloric acid; iron, aluminium, etc., were held in solution by the citric acid, which was otherwise omitted.

As the use of mixtures of phosphoric acid and perchloric acid was significant in the application of the method to steel analysis, some experiments were made to determine the possible interference under these conditions. In these experiments a known amount of tungsten solution was evaporated to fuming with a mixture of equal parts of phosphoric and perchloric acids; the resulting solution was diluted to a known volume and a suitable aliquot was used for the determination of tungsten. Phosphoric acid was found to interfere with the tungsten determination unless the aliquot taken was made alkaline. With this modification, the presence of phosphoric acid in quantities up to 0·2 ml. of the concentrated acid in the aliquot was permissible.

Cationic interferences were studied in a similar manner to that used for investigating anions. Ammonium (1 ml. of "0·88" ammonia), calcium (3 g. of calcium chloride), zinc (1 g. of zinc acetate), cadmium (1 g. of cadmium sulphate), magnesium (3 g. of magnesium chloride), manganese (1 g. of manganese sulphate), aluminium (1 g. of potassium alum), beryllium (2 g. of beryllium sulphate) and zirconyl (1 g. of zirconium sulphate) were without effect on the method. Large amounts of sodium, potassium and ammonium salts caused precipitation of their chlorides during reduction, but these re-dissolved during the final dilution and were without effect on the results. Barium and strontium also gave precipitates of their chlorides, but tungsten was apparently co-precipitated for slightly low results were obtained; limited amounts up to about 0·1 g. of the chlorides were permissible. Large amounts of copper and lead gave thiocyanate precipitates, but small quantities were without effect on the results. Uranyl salts interfered when present in more than a limited amount, about 0·1 g. of uranyl acetate, by reaction with the tin and formation of a precipitate of mercurous chloride.

The more noble metals, platinum, gold, arsenic, antimony and bismuth, interfered by reaction with the tin amalgam to give the respective metals which sometimes formed precipitates and sometimes contaminated the amalgam. Silver and thallium interfered owing to the insolubility of their chlorides.

The metals that form coloured thiocyanates were investigated in detail. The results of experiments, made in absence of tungsten, are shown in Table III.

TABLE III

CATIONIC INTERFERENCES

Element	Amount present g.	Absorptiometer reading (2-cm. cells) Reading	Blank	Difference
Co	0·1	0·063	0·050	0·013
Ti	0·025	0·053	0·038	0·015
Cr	0·053	0·462	0·432	0·030
Ni	0·10	0·475	0·346	0·129
Mo	0·013	0·310	0·213	0·097
	0·026	0·584	0·405	0·179
V	0·001	0·400	0·045	0·355
Nb	0·0004	0·100	0·043	0·057

These results were obtained by the same procedure as that given for tungsten and illustrated the importance of the blank reading due to the colour of the reduced ions in the absence of thiocyanate. The determination of 250 μg. of tungsten was undertaken in presence of the following amounts of these elements: 0·1 g. of cobalt, 2 mg. of nickel, 25 mg. of titanium, 5 mg. of titanium with 0·2 ml. of phosphoric acid in alkaline citrate solution, 8 mg. of chromium, 4 mg. of molybdenum, 0·1 mg. of vanadium and 0·4 mg. of niobium. In each case a small correction calculated from the results shown in Table III was made for the interfering element. The results for the tungsten were within the permissible error of the method, and showed that the use of the small subtractive corrections was justified.

The final interference to be studied in detail was that of iron. Small amounts of iron were known to be without effect, as traces of iron were present in some of the reagents used, but large amounts gave rather surprising results. Ferrous iron was expected to undergo some oxidation and give the red ferric thiocyanate colour, so a series of experiments was made to determine the effect of more stannous chloride on the estimation of 250 μg. of tungsten in presence of 0·1 g. of ferrous iron added as ferrous sulphate. The results, shown in Table IV, indicated that even in presence of large amounts of stannous chloride, 0·1 g. of ferrous iron caused serious interference.

TABLE IV

EFFECT OF STANNOUS CHLORIDE ON THE INTERFERENCE OF FERROUS IRON

Absorptiometer reading in absence of ferrous iron 0·580.

0·1 g. of ferrous iron and 250 μg. of tungsten were present in all tests.

Stannous chloride present, g.	Absorptiometer reading
0·1 (Usual method)	0·460
0·4	0·465
0·8	0·518
1·6	0·538
3·2	0·522

Further experiments were concerned with increasing the thiocyanate concentration and with standing for short periods prior to the final dilution. It was shown that the low results were eliminated by the use of 0·4 g. of stannous chloride and 2 ml. of 60 per cent. potassium thiocyanate solution instead of the usual quantities, combined with a standing period of 2·5 minutes after mixing with the thiocyanate. Blanks in the absence of tungsten showed that the formation of a small amount of ferric thiocyanate could not be avoided, and the use of a small correction (0·013 Spekker unit for 0·1 g. of iron) was necessary.

EXTRACTION PROCEDURE

During the preliminary phases of the present investigation the use of an organic solvent for the extraction of the tungsten complex was considered. It was desirable to have a solvent that would extract the tungsten complex almost quantitatively in one operation, was denser than and immiscible with water, was not highly volatile and would separate readily after shaking with the aqueous solution. Ethyl ether, which has been previously used,[8] was considered to be too volatile.

A range of ethers and alcohols was therefore investigated. Mixtures of alcohols with carbon tetrachloride or chloroform produced suitable solvents which were heavier than water and therefore more convenient to use. A 1 : 1 mixture of *iso*-amyl alcohol and chloroform was adopted for determinations in which a single extraction was to be made. The procedure was as follows.

Carry out the reduction of the tungsten under the conditions previously used, in a 65-ml. separating funnel. After adding the thiocyanate and diluting to 50 ± 2 ml., run off the amalgam. Add from a pipette 10 ml. of the solvent mixture. Shake for 30 seconds and allow the layers to separate. Run the organic layer off through a dry cotton wool filter (previously well washed with 6 *N* hydrochloric acid and water, and dried by acetone and suction) into a dry 1-cm. Spekker cell. Take absorptiometer reading as before. Carry out a blank, omitting the thiocyanate.

The cotton wool filter removed droplets of water from the organic layer, which would otherwise have given rise to a slight turbidity. It was advisable to let the first few drops of the extract pass through the filter, before collecting in the cell. With 100 μg. of tungsten

a drum reading of 0·720 was obtained, compared with 0·531 which would have been obtained by the usual procedure with 500 μg. of tungsten, *i.e.*, the same concentration.

The distribution of the tungsten thiocyanate between the aqueous and organic layers was such that almost complete extraction was obtained by the single operation. However, in order to remove a trace of tungsten from an aqueous solution of more than 50 ml. it was preferred to carry out a double extraction and to use an extractant consisting of a 2 : 3 mixture of *n*-butyl alcohol and chloroform. This mixture extracted the yellow tungsten complex more readily than the amyl alcohol - chloroform mixture, but the greater miscibility of *n*-butyl alcohol with water precluded its use for a single-extraction procedure. In carrying out a determination, a double extraction with an intermediate and final washing with 0·5 ml. of solvent was made and the extracts were collected in, for example, a 10-ml. graduated flask, and finally diluted to the mark with further solvent. It was unnecessary to filter the extracts, as the final solution after dilution was not cloudy.

Applications of the method

Several applications of the method have been made and tested.

(*a*) *Tungsten filaments and deposits*—Thin tungsten filaments and tungsten deposits found on the bulbs of lamps and valves were dissolved in mixtures of hydrogen peroxide and ammonia solutions.[10] After boiling to destroy the excess peroxide, the solution, or a suitable aliquot of it, was analysed by the method as previously described (p. 58).

(*b*) *Tungsten-containing steels*—The following procedure is typical of several that have been tried.

Weigh 0·1 g. of the sample into a 100-ml. beaker. Add 10 ml. of acid mixture (20 ml. of 60 per cent. perchloric acid, 10 ml. of phosphoric acid, 10 ml. of nitric acid and 60 ml. of water) and warm gently until all attack ceases. Evaporate just to fuming and cool slightly. Add 4 or 5 drops of hydrofluoric acid and resume fuming for 2 minutes. Cool, and dilute to 250 ml. in a graduated flask. Pipette a 5-ml. aliquot (for samples containing 10 to 20 per cent. of tungsten) or a 10-ml. aliquot (for samples containing 1 to 10 per cent.) into a 50-ml. graduated flask. Add 1 ml. of ammoniacal citrate solution (70 ml. of "0·88" ammonia and 50 g. of citric acid diluted to 100 ml.). Mix and add rapidly 16 ml. of 12 *N* hydrochloric acid, 1 ml. of 20 per cent. stannous chloride solution and 2 ml. of tin amalgam. Shake for 2·5 minutes. Add 5 ml. of 12 per cent. potassium thiocyanate solution, mix, and allow to stand for 30 seconds. Dilute to the mark and mix gently by rotating the contents of the flask and inverting four or five times. Prepare a blank solution on a similar aliquot, omitting the thiocyanate. Take absorptiometer readings and calculate the percentage of tungsten from the difference.

Results obtained with British Chemical Standard high-speed steels No. 167 (16·12 per cent. of W) and No. 220 (6·75 per cent. of W) were 16·2 and 16·2 for the former and 6·83, 6·75 and 6·70 for the latter. With the steel No. 220 it was necessary to apply a small correction for the presence of vanadium, but the other alloying elements were without measurable effect on the results.

Alloy steels containing less than 1 per cent. of tungsten can be analysed by modifying the above procedure to increase the size of the aliquot. The phosphoric acid in the aliquot should be less than 0·2 ml. of the concentrated acid, and the other conditions must be adjusted so that they fall within the limits prescribed. In particular, when the aliquot is made alkaline, which is best done by adding 1 ml. of ammoniacal citrate solution followed by concentrated ammonia added dropwise until the phenolphthalein end point is reached, the total volume must not be so great that the acidity of the solution is subsequently upset. In presence of large amounts of iron the conditions discussed under iron interference should be adhered to. The use of a 10 per cent. ammonium thiocyanate solution instead of the potassium thiocyanate solution will prevent the precipitation of potassium perchlorate, which would otherwise occur when the aliquot contains sufficient perchloric acid.

(*c*) *Non-ferrous alloys*—As an example of the application of the procedure to non-ferrous alloys a procedure that was used for the analysis of N.93 alloy (about 2 per cent. of aluminium, 2 per cent. of tungsten and the remainder nickel) is described.

Weigh 0·1 g. of the sample into a 100-ml. beaker. Add 10 ml. of *aqua regia*, warm until dissolved and evaporate to moist dryness. Add 5 ml. of concentrated hydrochloric acid and evaporate nearly to dryness. Take up in 10 ml. of 10 per cent. citric acid solution, and add

dilute ammonia solution until alkaline. Dilute to 100 ml. in a standard flask. Determine tungsten and blank on 5 ml. aliquots by the standard method.

A small correction may be applied for the 5 mg. of nickel present in the aliquot, or alternatively in routine practice the method may be standardised in presence of this amount of nickel. The agreement between results carried out by the above method and check analyses by the usual gravimetric method was within the experimental error.

Discussion

The method that has been described differs from all previous methods for determining tungsten, with the exception of that of Sandell,[8] in that the initial tungsten solution may be acid, neutral or alkaline; it is only necessary that the tungsten should be completely in solution. It is reasonable to assume that the green colour that previous workers obtained when not using alkaline solutions was due to the presence of blue quinquevalent tungsten compounds, probably formed by reduction of precipitated tungstic acid. Previous procedures that required the solution to be alkaline necessitated the prior removal of metals that formed insoluble hydroxides or gave insoluble tungstates under these conditions; such separations are not only tedious but often lead to errors through co-precipitation of the tungstates. In the present method the absence of any interference from tartaric, citric and oxalic acids is very useful, as these acids will hold tungsten in solution in presence of dilute mineral acids, while citrate and tartrate will prevent the precipitation of insoluble hydroxides under alkaline conditions. Similarly, the formation of soluble complex phosphotungstates enables mixtures of phosphoric and mineral acids to be used to obtain solution of certain samples, provided that phosphate interference is overcome by the procedure given (p. 62).

It was beyond the scope of the present work to carry out a detailed investigation of the nature of the yellow tungsten thiocyanate complex, but the results of some preliminary experiments are of interest. Under the reducing conditions used it would be expected that tungsten would be reduced to the tervalent state. Reduction of a strong hydrochloric acid solution of sodium tungstate with cadmium amalgam was shown volumetrically to give tervalent tungsten; a similarly reduced solution gave the characteristic yellow colour on addition of thiocyanate. It is reasonable to assume that the thiocyanate complex is a compound of tervalent tungsten, which can exist in two forms, one purple and the other yellow in colour. The latter is the stable form and may be produced from the former on standing for some time. Measurement of the optical densities of solutions containing a fixed amount of tungsten and a varied small quantity of thiocyanate, suggested that in the yellow complex one thiocyanate radical is combined with each tungsten atom. By analogy with tervalent molybdenum halides[12] the formula $WOCNS.4H_2O$ is tentatively proposed, although this differs from the formulae put forward for the tervalent tungsten chlorides.[13] Whatever the formula of the tungsten complex, one of the original aims of the present investigation has been achieved, for the yellow complex that forms the basis of this method would appear to be a definite compound in comparison with the unknown variable tungsten thiocyanate previously used.

Of the interferences with the method, many, such as those due to the noble metals or silver, could easily be overcome by preliminary separations. The most significant interferences are those due to molybdenum, vanadium and niobium. The interference of molybdenum is negligible when the tungsten and molybdenum are present in equal amounts, and it would be possible to determine tungsten in presence of a hundred-fold excess of molybdenum, provided that suitable corrections were made. Vanadium and niobium, however, can only be tolerated when present in amounts much smaller than the tungsten. The vanadium interference is rather larger than was expected on the basis of the results reported by previous workers, and it is concluded that in the present method the vanadium is quantitatively reduced to the bivalent state and then reacts in a quantitative manner with thiocyanate. The reduction of quinquevalent niobium is usually regarded as being difficult and incomplete; but most attempts at its volumetric estimation have been made in sulphuric acid solution. In the present method, in hydrochloric acid solution, reduction with tin amalgam proceeds readily, and the thiocyanate complex of the reduced niobium has an intense yellow colour which is critically dependent on the final acidity, but may well prove to be the basis for an accurate photometric method for determining niobium. The use of the photo-electric absorptiometer permits a considerable increase in the permissible amounts of interfering elements. Thus, cobalt and titanium both give a colour on addition of the thiocyanate,

but the resulting solutions do not absorb the filtered light strongly; nickel and chromium absorb strongly in absence of the thiocyanate, but this is corrected for by the blank determination.

Ferrous iron tends to interfere by reason of the readiness of its oxidation and also by the marked tendency of the purple thiocyanate to form when a large quantity of ferrous iron is present. Although means of overcoming these difficulties have been detailed, the effect of ferrous iron is not completely understood. Recently ferrous iron has been reported to play a considerable part in the formation of the orange quinquevalent molybdenum thiocyanate.[14] Both copper and iron have been recommended as catalysts in the oxidation of reduced tungsten, but iron (0·1 g.) had a negligible effect on the fading of the colour in the present method. Copper (0·1 g. of copper sulphate) caused the colour to fade by approximately 5 per cent. in 1 hour, both with and without thiocyanate present.

To summarise the extent of the more important interferences, the following amounts of the several elements cause an error of 0·01 in the absorptiometer reading with 2-cm. cells, equivalent to 5 μg. of tungsten: 100 mg. of iron, 75 mg. of cobalt, 18 mg. of chromium, 15 mg. of titanium, 5 mg. of nickel, 2 mg. of molybdenum, 0·07 mg. of niobium and 0·05 mg. of vanadium.

The studies of methods of extraction of the tungsten complex have not been extensive, but several findings have been made. In some preliminary experiments it was found that sexavalent tungsten can be extracted as a colourless solution with the *iso*-amyl alcohol - chloroform solvent, from strong mineral acid solution in the presence of thiocyanate. This fact, although not entirely pertinent to the present work, may be of value as a preliminary means of separating tungsten from other metals. There would not appear in general to be any great advantage to be gained by the extraction of the yellow tungsten complex if the Spekker absorptiometer is to be used, but for trace determinations in which the final comparison would be made visually with a long column of the extract, the particulars given may be of value. It should be emphasised that the extent of the interferences undergoes considerable modification when extraction methods are used, *e.g.*, nickel, chromium and cobalt cause less interference, whilst molybdenum and titanium have a more marked interference.

A detailed discussion of the possible applications of the present procedure cannot be undertaken, but reference should be made to the paper of Sandell[8] in which some of the difficulties encountered in the separation of tungsten in rocks prior to its photometric estimation are described. The applications quoted in the present paper have been concerned chiefly with metallic samples. The precision of the method is such that it can compete with routine gravimetric methods for the determination of tungsten in steels, and the resulting saving in time is considerable. Of the alloying elements present in the more common steels, only vanadium would be expected to cause any difficulties and then suitable small corrections could usually be applied. If the tungsten were present only in small amounts, less than 0·5 per cent., it would be necessary to modify the procedures that have been described, probably by the incorporation of a preliminary separation of tungsten. The determination of 2 per cent. of tungsten in a nickel alloy by the photometric procedure, besides being more rapid than the gravimetric procedure, has the further advantage that it requires considerably less sample, an important consideration when the sample to be tested is in the form of very fine wire.

Summary

A precise procedure for the photometric determination of tungsten has been described, based upon reduction in hydrochloric acid solution by tin amalgam and subsequent addition of thiocyanate. The conditions of acidity required for complete reduction, the effect of thiocyanate concentration, conditions for the stabilising of the reduced state and the optimum conditions of final acidity have been systematically investigated. Interferences to be expected from diverse cations and anions have been detailed and instructions given for the application of the method to typical steel and non-ferrous metal analyses.

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A Review of Electrolytic Methods of Microchemical Analysis

BY A. J. LINDSEY

(*Read at the Annual General Meeting of the Microchemistry Group, January 31st*, 1947)

IN this review the field of study covered is the gravimetric determination on a microchemical scale of electrolytically deposited substances. Qualitative detection and "internal electrolysis" are not discussed. Other applications of electrochemistry to analysis are excluded because in these methods emphasis is not laid upon electro-deposition although it may occur.

The development of electrolytic analysis on a microchemical scale has taken a closely similar course to that of determinations on the ordinary scale. It has been subsequent to ordinary scale work, and it is therefore surprising that some of the electrochemical processes so firmly established on the larger scale were not used from the beginning on the smaller scale. Most investigators of electro-deposition on the microchemical scale were interested in the determination of single metals only. Later work has shown, and indeed it could have been forecast from theoretical principles, that many of the processes used by them would, if applied to mixtures of metals, give mixed deposits on the cathode. The work of Torrance[1,2] upon the deposition of arsenic from arsenious solutions containing a cupric salt and hydrochloric acid is an example. He has shown that arsenic in amounts up to 25 per cent. of the total weight of copper present is quantitatively deposited with the latter. Single-metal methods therefore cannot be applied to substances of unknown identity without the risk of weighing as a single element a mixture of two or more. If these facts are borne in mind and the result of a qualitative examination is available, the single-metal procedures will give good service.

The outstanding advantage of electrolytic methods, whether on the normal or the micro scale, is rapidity; but on the micro scale there is also a very great saving in cost of apparatus owing to the smaller platinum electrodes employed.

REVIEW OF PRINCIPLES OF QUANTITATIVE ELECTRO-DEPOSITION—

A metallic element is not deposited upon an electrode until the potential between the latter and the solution is sufficiently negative. In general this potential, which is well defined after the first few seconds, may be expressed as

$$E_D = E_o - V + \frac{RT}{nF} \log_e a_M$$

where E_D is the deposition potential, E_0 is the standard electrode potential for the metal ion being deposited, V is the overvoltage, n is the valency of the metal and a_M is the activity

of the metal ions in the solution. R, T and F have their usual significance. For most metals other than iron, nickel and cobalt, V is small, although it varies with current density.[3] At the beginning of electro-deposition, therefore, the potential is governed by the only variable, *i.e.*, the activity or concentration of the metal in solution. After a certain interval of time the electrolyte close to the cathode will have become exhausted to a considerable extent and

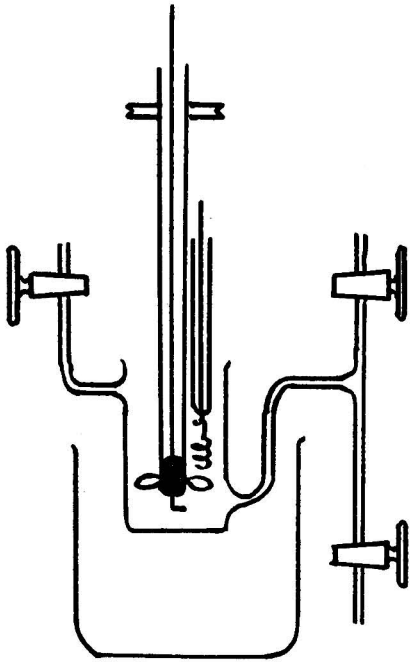


Fig. 1

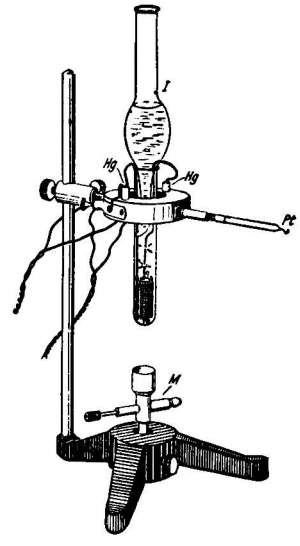


Fig. 2

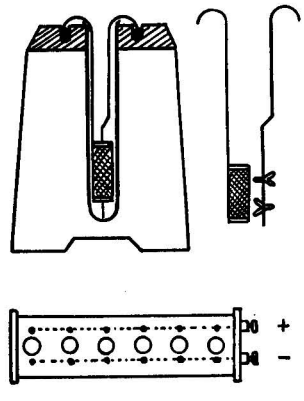
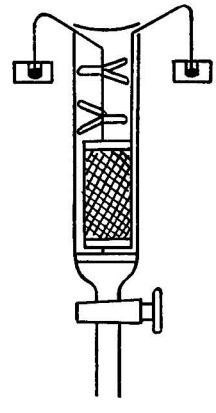
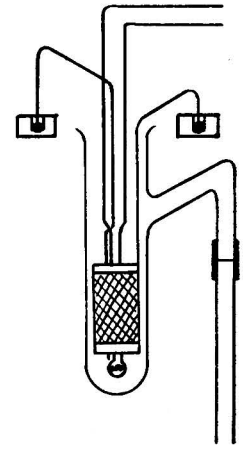


Fig. 3



(a)



(b)

Fig. 4.

the activity of the ions there may decrease to a very small fraction of the original value. Deposition can continue after this stage only at a rate determined by the rate of diffusion of ions up to the electrode surface, and the potential will be governed by this rate of diffusion. Rapid stirring will have the effect of keeping the concentration at the electrode almost the same as that throughout the bulk of the electrolyte. It is therefore necessary, in separating metals by potential control, to have vigorous stirring.

As the stirred solution becomes exhausted by deposition, the activity of the metal ions becomes less and the deposition potential becomes more negative in accordance with the equation given. In practice this change is about 0·058 volt for every ten-fold decrease in activity of a univalent ion or 0·029 volt for a bivalent ion. Account must be taken of this change in quantitative separation of metals.

Vigorous stirring also greatly decreases the time required for quantitative deposition and decreases the tendency for hydrogen to be liberated with the metal. If hydrogen is deposited simultaneously with certain metals there is a considerable tendency for the latter to become spongy and poorly adherent. Deposits of this kind tend to occlude electrolyte. The purity of the deposit is thus usually dependent upon control of potential.

EARLY DEVELOPMENT OF QUANTITATIVE METHODS—

The first application of micro-scale experiments to quantitative electrolytic analysis was made by Jänecke,[4] who determined mercury in urine by electro-deposition from a solution upon a gold wire cathode weighing about 25 mg., and used a Nernst[5] quartz fibre balance for the weighings. The advantage of using so small a cathode was that a balance of great sensitivity could be employed. The anode was a platinum crucible and the time of deposition was 24 hours. With no stirring, so long a time was unavoidable.

Another early application on a micro scale was that of Brill and Evans[6] who, with a Nernst balance and a small cathode weighing from 5 to 10 mg., examined small quantities of material extracted from thorianite, in order to measure the electrochemical equivalent of a supposed new element which later was shown to be antimony.

In 1909, Emich and Donau,[7] in a paper on the handling of microchemical precipitates, described the use of a small platinum crucible as a cathode upon which metals were deposited to separate them from other material.

Heinze[8] also described an apparatus in which metals were determined on a gold or platinum wire cathode.

Typical of these earlier methods is that of Riesenfeld and Möller,[9] who used an apparatus shown in Fig. 1. Determinations of copper, mercury and silver were made upon a small platinum spiral hung upon a hook. An ingenious system of taps allowed the electrolyte to be removed and the deposit to be washed without interrupting the current. These workers made the first attempt to control potential by inserting a capillary attached to a standard electrode into the electrolyte. The time for quantitative deposition was up to 6 hours.

The well known apparatus of Pregl, which was described by him in the first edition of his book,[10] has been the standard apparatus for many years and is shown in Fig. 2. The cathode is a platinum gauze cylinder of diameter 10 mm. and height 30 mm., with three glass beads sealed on to each end to prevent contact with the walls of the test tube used as the electrolysis cell. The anode is a platinum wire with two glass spacers of Y-shape to hold it centrally in the vessel. A condenser containing cold water stands in the neck of the vessel and prevents loss by spurting. The stand is arranged to allow adjustment of the micro-burner and other parts. Electrolysis is carried out at the boiling point of the solution and anodic evolution of oxygen prevents bumping. The time of deposition for a few milligrams of copper is from 10 to 20 minutes. Pregl's apparatus owes its continued success to the rapid stirring which takes place at the boiling point and the consequent rapidity of determination; it is far less satisfactory at lower temperatures and is not suitable for controlled-potential separations.

Very many modifications have been described, one of which, due to Bennedetti-Pichler,[11] is shown in Fig. 3. This is a battery of six Pregl type units that was used for determination of copper in its alloys. Interference from zinc, cadmium, iron and lead were avoided by using nitric acid in the sulphuric acid electrolyte. Determinations were completed in 20 minutes by first heating the vessels to boiling point and then electrolysing. The results were confirmed, with an accuracy of 0·005 mg., by redissolving in sulphuric acid by current reversal and redepositing.

RAPID STIRRING METHODS—

In 1932, Okáč[12] and Clarke and Hermance[13] devised apparatus for micro-electrolytic analysis in which efficient stirring is effected by means of gas bubbles. Both of these equipments are satisfactory for rapid determinations. Figs. 4*a* and 4*b* show Okáč's apparatus.

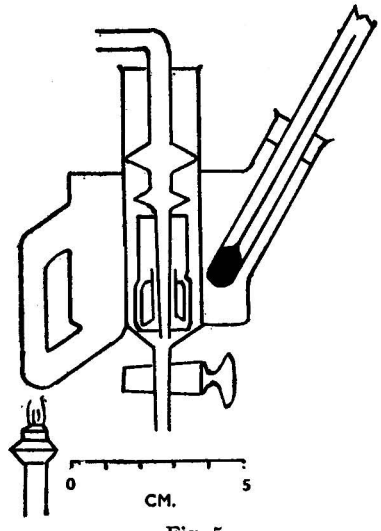


Fig. 5.

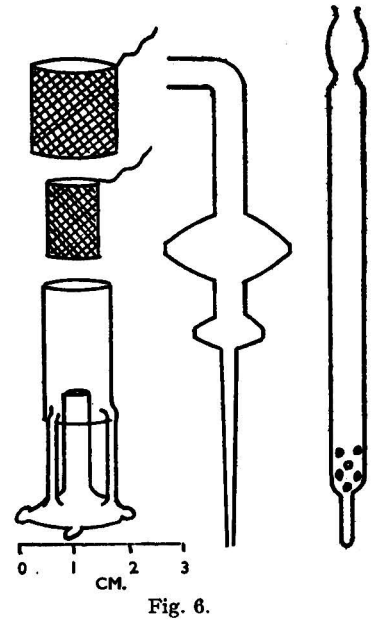


Fig. 6.

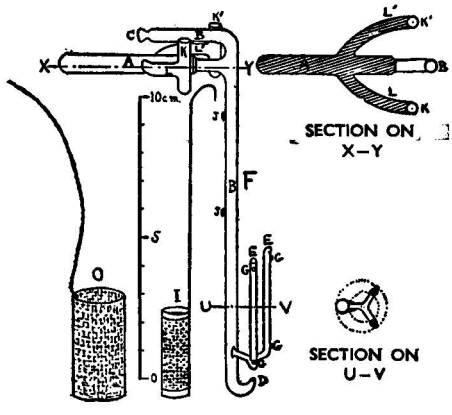


Fig. 7.

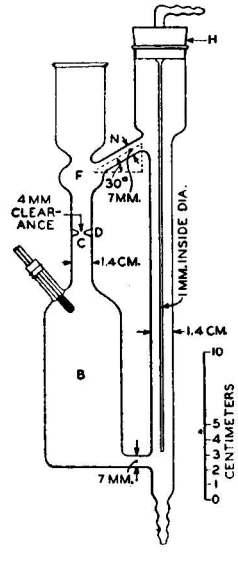


Fig. 8.

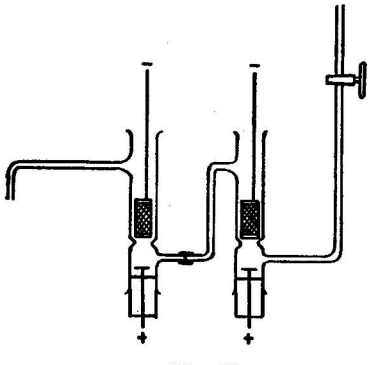


Fig. 10.

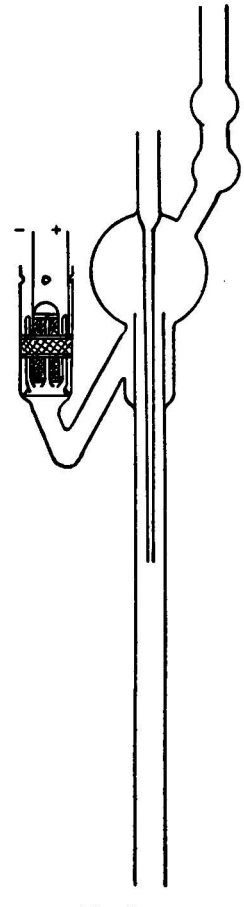


Fig. 9.

The first of these employs a small cylindrical tap funnel with a coarse sintered glass plate. The electrodes are of the same pattern as those of Pregl and gas from a cylinder is passed through the plate to stream upwards and stir the liquid. Termination of the electrolysis is not so satisfactory as in the second apparatus. This employs a similar type of cathode but the anode is sealed into the centrally placed inlet tube, through which carbon dioxide generated from a Kipp's apparatus may be bubbled into the electrolyte. The inlet tube may also be connected to a vessel full of distilled water and at the end of the determination the electrolyte may be syphoned away without disconnecting the current. The electrodes may be washed thoroughly by this water stream.

The cell of Clarke and Hermance shown in Figs. 5 and 6 has the advantage that a liquid bath around the cell vessel can be held at any predetermined temperature. A cylindrical anode surrounds the cathode and the whole apparatus may be taken apart rapidly. The gas stirring tube can be removed at the end of the electrolysis and replaced by a washing tube connected to a water supply. Both electrolyte and washings are run away through the stop-cock. Details of the analyses possible with these cells are reviewed later.

THE SEPARATION OF METALS BY POTENTIAL CONTROL—

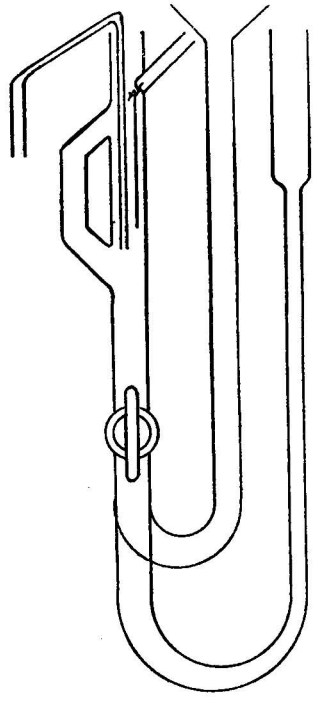
Apart from the early attempt of Riesenfeld and Möller to apply potential control to electro-deposition, there was no work published upon this subject until 1935, when the late Dr. H. J. S. Sand and the present author described a method for effecting quantitative separations on a micro scale. A brief description only is given here, as the method is fully described in the ANALYST.[14] The essential details are that an auxiliary electrode may be omitted if a large anode surrounding the cathode at a small distance is used and the potential drop due to resistance of the electrolyte is kept as low as possible by using a small current and an electrolyte of high conductance. The addition of considerable quantities of strong anodic depolarisers ensures that the anode - solution potential difference is constant throughout the determination and therefore that a measurement of anode - cathode potential difference is an adequate means of controlling the cathode - solution potential difference. Fig. 7, reproduced from the earlier paper, shows details of construction. The development of a series of analytical methods employing potential control was begun with a view to establishing a systematic scheme for the commoner metals and their alloys.[2,15,16] The war interrupted this work but it is now being resumed. A description of an auxiliary electrode which can be introduced into the apparatus is the subject of another communication (see p. 99). This addition, although not essential for the execution of a quantitative separation, will enable analysts to quote figures for control potentials that are independent of variations in electrode systems and of electrolyte composition.

SPECIAL APPARATUS—

Ingenious devices have been described by Clarke and Hermance[13,17] and by Hernler and Pfeningberger[18] for determining small quantities of metals in large volumes of liquid. The former apparatus, shown in Fig. 8, provides a means of circulating a large quantity of liquid through a small electrolysis cell by means of an air lift pump. With it the authors were able to determine traces of lead in zinc, zinc in aluminium, lead in nickel and traces of heavy metals in reagent grade chemicals.

The second apparatus, shown in Fig. 9, was a considerable improvement on the first and may be inserted into any large vessel. By means of an air-lift pump the liquid is circulated through the electrode system consisting of a pair of platinum gauze cylinders, similar to those depicted in Fig. 6, on a frame of glass. A glass ball rests on the cathode and serves to hold it steady and to divert the circulating electrolyte through the gauze. The apparatus was tested with large volumes (up to 2 litres) of solution, and quantities of about 1 mg. of the metals copper, zinc and lead were recovered in about 4 hours with an average precision within 1 per cent. A cell due to Hernler and Pfeningberger, shown in Fig. 10, is in the form of two electrolysis vessels through which the liquid flows in succession. Traces of metal that escape deposition at the first cathode are collected on the second. With this apparatus it was possible to recover quantities of from 0·1 to 1 mg. of copper from a litre of acidified solution in 5 to 6 hours. These devices offer considerable possibilities for separating a mixture of metals from a large volume of solution preparatory to re-solution and quantitative separation in a small volume.

Wehrli[19] described an ingenious piece of equipment (Fig. 11) which he called "an apparatus for single-pole micro-electrolysis." It was devised for the deposition of lead anodically as dioxide and, although it was not very successful, it could probably be employed usefully for other work. The anode was a wire hanging on a hook; the anode solution (3 ml.) was stirred by a gas stream and floated upon a concentrated solution of ammonium nitrate. The cathode dipped into strong cupric sulphate solution. It should be possible to apply this apparatus to the separation of metals by potential control by reversing the polarity of the equipment and making the anode a thermodynamically reversible electrode of sufficiently great capacity to resist polarisation.



Extremely small quantities of electro-deposited materials have been measured by Weisenberger[42] with an electromagnetic balance.

Other useful apparatus has been described by Lassieur[52] and by Clarke and Hermance.[55]

REVIEW OF METHODS—

Analytical procedures have been described for the metals arsenic,[2] bismuth,[15] cadmium,[21,43] cobalt,[22,23] copper,[7,9,10,11,13,16,17,18,20,25,26,27,28,29,30,32,33,34] gold,[35,36,37] lead,[8,13,15,17,19,20,21,38,39,40,41] mercury,[4,8,9,36,42,43] nickel,[12,22,23] silver,[7,9,28,43,47,48,49] tin,[13] and zinc.[13,17,28,29,33] This bibliography is, as far as is known, complete, but on account of the very large number of papers it is considered best to review only certain of them that illustrate important principles or analytical procedures. Earlier reviews by Ashcroft[50] and Sand[51] may be of value to readers as will also the critical analysis of accuracy of microchemical electrolytic methods by MacNevin and Bournique[27] who, in an exhaustive examination of the errors in employing the Pregl and Clarke and Hermance equipment, decided that the over-all error was ±0·01 mg. and that the errors of the microchemical balance exceeded those of handling, sampling, etc.

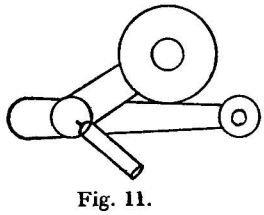


Fig. 11.

APPLICATIONS—

The analysis of alloys on a microchemical scale has been studied by Bennedetti-Pichler,[1] who determined copper in brasses and bronzes in sulphuric acid solutions containing nitric acid. Copper and zinc were determined by Nikitina[29] in aluminium and magnesium alloys by Bennedetti-Pichler's method. Wenger, Cimerman and Tschanun[33] analysed brasses by first depositing the copper and then, after weighing, the zinc from alkaline solution. In the absence of other metals this procedure is satisfactory but without potential control metals other than copper and zinc would be deposited. In this Institute, initial experiments have been made towards a comprehensive scheme of analysis, and the conditions of deposition for the metals copper, arsenic, bismuth, lead, mercury and tin have been defined.[2,15,16,53] Owing to the possibility of losses by evaporation or mechanically, it has been found advisable to deposit mercury on a gold cathode or to employ gold or copper-plated platinum. The amalgams are more readily handled without loss.

Metals have been determined in organic substances and in biological materials. In general, the organic matter is first destroyed by digestion with sulphuric acid assisted in suitable instances by nitric acid.[31] Wet oxidation with permanganate or ignition may also conveniently precede electrolysis. Traces of metals in foodstuffs, particularly canned products, have been determined. Typical methods are described by Pregl[10], Möhler and Hartnagel,[27] and Springer.[32] Methods for lead in blood[21] and mercury in urine and other body fluids[4,45] will interest the forensic analyst. Verdino[46] applied his method for mercury to the analysis of a number of organic mercurials used in modern therapy.

Strebinger and Pollak[49] have applied electrolytic silver determination to the analysis

of mixed halides. The halogens are precipitated and weighed together on a microchemical scale, and after solution in cyanide the silver is deposited. The proportion of the halides can then be calculated.

Another combination of methods has been used[54] for the determination of nickel, lead, zinc and mercury, in which the metals were deposited upon a copper wire that was later submitted to spectrographic analysis.

Very small quantities of gold (0·4 to 30 μg.) were determined in solution[35] by deposition upon a lead foil cathode which was cupelled in the normal manner and the small spheres of gold determined by measurement under a microscope.

In conclusion, it may be stated that the application of electrolytic methods on a microchemical scale has proved to be a rapid and accurate addition to the methods of analytical chemistry. Several constituents of an alloy can be determined in turn on a single sample weighing between 1 and 5 mg., using the microchemical balance with the rider only as a standard of mass. It is hoped that this review will assist in spreading the use of the methods amongst microchemical analysts.

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Discussion

The Chairman (Mr. Norman Strafford) mentioned that when a micro-balance was not available the minute amounts of metal deposited on the cathode could often be determined colorimetrically, with a precision at least equal to that obtained by weighing. He enquired how the determination of cations in a mixture by means of electrolysis compared in convenience and accuracy with that by polarographic analysis.

Mr. J. T. Stock said that apparatus for the automatic control of electrode potential had several uses in separations carried out on the macro scale and asked if the author considered that such apparatus would be useful on the micro scale.

Mr. D. F. Phillips said that the mercury cathode was useful for separating the heavy metals in micro-analysis.

Dr. Lindsey, in reply to the Chairman, said that electrolytic micro-determinations could be relied upon to give an accuracy within 1 per cent. and that considering the time taken in determinations and the convenience, both methods were equally valuable. In reply to Mr. Stock's question, he said that automatic control of electrode potential would undoubtedly be of value on a microchemical scale and he hoped to try such a method.

The Determination of Arsenic Pentoxide in White Arsenic

By D. A. LAMBIE

(*Read at the meeting of the Society on November 5th*, 1947)

As a result of the war-time need to utilise arsenious oxide from all available sources, analyses were necessary of material containing larger amounts of impurities—chiefly iron, lead, bismuth, copper, antimony oxides and sulphur trioxide—than usual.

The arsenic pentoxide in pure arsenious oxide is readily determined by treatment, in strongly acid solution, with potassium iodide and titration of the iodine liberated according to the equation:

$$As_2O_5 + 4HI \rightleftharpoons As_2O_3 + 2H_2O + 2I_2$$

But this method, due to Williamson,[1] is vitiated by the presence of iron, copper and the higher oxides of antimony, which also liberate iodine under the same conditions.

The development of a method for the determination of small amounts of arsenic pentoxide in "white arsenic" containing one or more of the impurities mentioned above is described in this paper.

Attempts to separate the arsenic pentoxide by precipitation with either magnesia mixture[2] or uranyl acetate[3] failed in the presence of so great an excess of the lower oxide. Removal of the arsenic trioxide by distillation as trichloride and determination of the residual arsenic (assumed to be pentoxide) is unsatisfactory since, even in the absence of reducing substances amongst the impurities, part of the pentoxide is reduced and lost as arsenic trichloride during distillation with hydrochloric acid.

In view of the failure to separate the arsenic pentoxide it was decided to attempt the elimination of interfering impurities and then use the iodimetric method.

Precipitation of the antimonic acid as sodium salt by saturating the alkali solution of the sample with sodium chloride was an attractive possibility, since all interfering elements could then be removed by one filtration. Although it was found possible to precipitate substantially all the antimony from potassium antimonate solution by sodium chloride, the precipitation was incomplete in presence of a large excess of sodium arsenite.

The ready solubility of arsenic pentoxide in water and the comparative insolubility of the other oxides suggested a possible method of separation, although the sparing solubility of arsenic trioxide, which forms the bulk of the material, would be a disadvantage. The use of alkali as a solvent was to be avoided owing to the liability of alkaline arsenite solutions to atmospheric oxidation. Tests were carried out on representative samples of white arsenic to decide upon a suitable solvent.

Extraction with 1 per cent. v/v hydrochloric acid was found to provide a satisfactory separation of arsenic pentoxide from the higher antimony oxides, but some iron was almost invariably dissolved also. All attempts to remove the iron without changing the state of oxidation of the arsenic oxides failed.

Water proved to be a satisfactory solvent, although the sparing solubility of the trioxide necessitated the use of a relatively large volume and fine grinding of the sample. Under the conditions adopted only a trace of iron was dissolved from samples in which substantial amounts were present (*e.g.*, 0·03 mg. of Fe from 5 g. of a sample containing 1·77 per cent.). Some samples with exceptionally high copper contents were found to yield a substantial amount of water-soluble copper, which, when present to the extent of more than a milligram or so in the strongly acid solution necessary for the iodimetric reduction of quinquevalent arsenic, catalyses the atmospheric oxidation of hydriodic acid, so vitiating the results. As it was not found practicable to remove the copper without affecting the arsenic pentoxide content a modified method was devised for samples containing sufficient copper to influence the arsenic pentoxide result.

For complete reduction of quinquevalent to tervalent arsenic according to the equation given above the solution must be strongly acid, according to Williamson (*loc. cit.*) 5 *N* in hydrochloric acid, whilst Pederson-Bjergaard[5] gives 4 *N*; the reaction is said to be reversed by dilution of the solution with water. Since titration of so strongly acid a solution with thiosulphate is unsatisfactory, tests were carried out to determine the effect of reducing the acidity and diluting the solution immediately before titration.

A. THE IODIMETRIC DETERMINATION OF ARSENIC PENTOXIDE—

A solution of arsenic acid was standardised by distillation with hydrochloric acid and a reducing agent and subsequent iodine titration of arsenic in the distillate.[6] The following method was adopted, using aliquot portions of the arsenic acid solution containing the weights of As_2O_5 given in Table I. Dilute the arsenic acid solution in a 500-ml. glass-stoppered flask to 50 ml., add 10 ml. of 50 per cent. w/v potassium iodide solution followed by 20 ml. of concentrated hydrochloric acid, mix by rotating the flask and allow to stand in the dark for 5 minutes. Dilute the solution with 150 ml. of water and titrate the liberated iodine at once with 0·05 *N* thiosulphate, using starch solution as indicator. Make a blank determination and deduct. Table I shows that satisfactory results were obtained.

TABLE I

Experiment	As_2O_5 taken g.	As_2O_5 found g.
1	0·0396	0·0395
2	0·0198	0·0200
3	0·00990	0·00997
4	0·00495	0·00513
5	0·00396	0·00399
6	0·00198	0·00199
7	0·00099	0·00094

B. EFFECT OF COPPER ON THE IODIMETRIC DETERMINATION OF ARSENIC PENTOXIDE—

To determine the effect of small quantities of copper on the iodimetric determination of arsenic pentoxide, measured volumes of a copper sulphate solution diluted to 50 ml. were treated as under A. The results are recorded in Table II. In experiments 9 and 10 the starch iodide colour rapidly returned and in experiment 8 the end-point was even more indefinite.

TABLE II

Experiment	Cu taken g.	Volume of thiosulphate: Calculated ml.	Volume of thiosulphate: Required ml.
8	0·0032	1·00	1·37?
9	0·0013	0·40	0·39
10	0·0006	0·20	0·19
11	0·0003	0·10	0·09

In another series of experiments, solutions containing known amounts of arsenic pentoxide and copper were treated as under A. The volume of thiosulphate solution equivalent to the copper and blank was deducted from the titration. The end-point of each titration was definite but the blue colour rapidly returned, in experiment 13 in about 10 seconds. See Table III for results.

TABLE III

Experiment	As_2O_5 taken g.	Cu added g.	As_2O_5 found g.
12	0·0246	—	0·0246
13	"	0·0013	0·0241
14	"	0·0006	0·0243
15	"	0·0003	0·0243

Only a fraction of the copper present was extracted from any sample so far examined, and when the copper content was less than 0·01 per cent. its effect was negligible.

C. THE DETERMINATION OF ARSENIC PENTOXIDE IN "WHITE ARSENIC" CONTAINING LESS THAN 0·01 PER CENT. OF COPPER—

The following method was finally adopted. Grind the sample in an agate mortar to pass a 200-mesh sieve and weigh 2 to 5 g. (according to the anticipated As_2O_5 content) into a 250-ml. beaker. Since the fine powder is wetted with difficulty, moisten it with a few drops of water, stir to a paste with a glass rod and then add 100 ml. of water. Boil gently until the sample has dissolved, usually leaving a dark flocculent residue containing the heavy metals and oxides of antimony, etc. Add 1 g. of sodium chloride and filter through a 9-cm. Whatman No. 42 paper having a pad of filter paper pulp in the apex. Wash the residue with 1 per cent. w/v sodium chloride solution and receive the filtrate in a 250 ml. beaker

TABLE IV

	Taken			Found	
Experiment	Sb_2O_5	Sb_2O_4	As_2O_5	As_2O_5	Error
16	—	—	0·0491	0·0469	−0·0022
17	—	—	0·0246	0·0239	−0·0007
18	—	—	0·0196	0·0196	nil
19	—	—	0·0098	0·0095	−0·0003
20	—	—	0·0049	0·0049	nil
21	—	—	0·0020	0·0020	nil
22	0·05	—	0·0196	0·0198	+0·0002
23	0·05	—	0·0098	0·0101	+0·0003
24	0·05	—	0·0049	0·0049	nil
25	0·05	—	—	nil	nil
26	—	0·05	0·0244	0·0233	−0·0011
27	—	0·05	0·0098	0·0097	−0·0001
28	0·02	0·03	0·0244	0·0237	−0·0007
29	—	0·05	0·0049	0·0047	−0·0002
30	—	0·05	0·0020	0·0018	−0·0002
31	—	0·05	—	0·0005	+0·0005
32	—	—	0·0207	0·0197	−0·0010
33	0·02	—	0·0201	0·0197	−0·0004
34	0·03	—	0·0122	0·0121	−0·0001
35	0·03	—	0·0056	0·0055	−0·0001
36	0·006	—	0·0063	0·0063	nil
37	0·02	—	0·0202	0·0197	−0·0005
38	0·03	—	0·0235	0·0228	−0·0007
39	0·02	—	—	0·0003	+0·0003

marked at the 25-ml. level. Evaporate to 25 ml., cool, stir and allow to stand for several hours, preferably overnight, for the arsenic trioxide to separate. Collect the precipitate on a filter and wash with 1 per cent. sodium chloride solution, receiving the filtrate in a 500-ml. glass-stoppered flask. Dilute to 50 ml., add 10 ml. of 50 per cent. w/v potassium iodide solution followed by 20 ml. of concentrated hydrochloric acid, mix and allow the flask to stand in the dark for 5 minutes. Dilute to 200 ml. with water and titrate the liberated iodine with 0·05 *N* thiosulphate, adding 2 ml. of 0·5 per cent. starch solution near the end-point. Make a blank determination on 50 ml. of water, 10 ml. of potassium iodide solution and 20 ml. of concentrated hydrochloric acid, allowing the solution to stand in the dark for precisely the same period as in the assay.

Test of the method—Analyses were made of synthetic mixtures of As_2O_3, As_2O_5, Sb_2O_4, and Sb_2O_5. As the sample is initially almost completely dissolved in water it was considered legitimate to add measured volumes of a standardised arsenic acid solution after mixing the other oxides with a few ml. of water; the volume was then brought to 100 ml. and the

analysis continued as above. The results are given in Table IV. In experiments 16 to 31, 5 g. of arsenic trioxide were taken and the weights of the other oxides given in the table added. In experiments 32 to 39, in which 2 g. of arsenic trioxide were taken, the oxides, including As_2O_5 were ground together in an agate mortar, the mixture was transferred completely to a beaker with water and the analysis completed as before. The "AnalaR" arsenic trioxide used was found to contain 0·005 per cent. of As_2O_5, and this was deducted from the results recorded in the table. As may be seen from the table there is a tendency to obtain negative errors, especially with the larger quantities of As_2O_5. Although this is probably due to retention of the pentoxide by the large precipitate of arsenic trioxide, it is essential to remove the bulk of the latter to avoid formation of a yellow precipitate of arsenic tri-iodide, which would obscure the end-point of the titration. It is suggested that when a high arsenic pentoxide content is anticipated the smaller weight (2 g.) should be taken.

D. THE DETERMINATION OF ARSENIC PENTOXIDE IN "WHITE ARSENIC" CONTAINING MORE THAN 0·01 PER CENT. OF COPPER—

Treat 5 g. of the sample as under C to the point at which the re-crystallised arsenic trioxide is filtered off, but in this case collect the filtrate in a 250-ml. measuring flask. Make up to volume, mix and withdraw two 100-ml. aliquot portions. Evaporate one portion in a 250-ml. conical flask to about 25 ml., add a slight excess of saturated bromine water to oxidise the arsenious acid and boil down to about 5 ml. Cool, render alkaline with ammonia, re-acidify with acetic acid and determine the copper iodimetrically as usual.

If no more than 0·40 ml. of 0·05 *N* thiosulphate is required, evaporate the second aliquot portion to 50 ml. and complete the determination as under C. Deduct the amount of the copper titration and the blank.

Larger amounts of copper catalyse atmospheric oxidation of hydriodic acid and render the end-point indeterminate. In such circumstances transfer the second aliquot portion to a 500-ml. CO_2-flask and evaporate to 50 ml. Remove the flask from the source of heat and close it with a stopper fitted, as shown in Figure 1, with a lead-in tube and a loosely-stoppered wide tube. Pass a slow stream of carbon dioxide into the flask while it cools. When it is cold add 10 ml. of the iodide solution (prepared with freshly boiled water) by means of a pipette inserted through the wide tube with its tip almost touching the surface of the liquid. Replace the stopper, rotate the flask to mix the solutions and allow to stand for a minute or two, then add 20 ml. of concentrated hydrochloric acid in the same manner

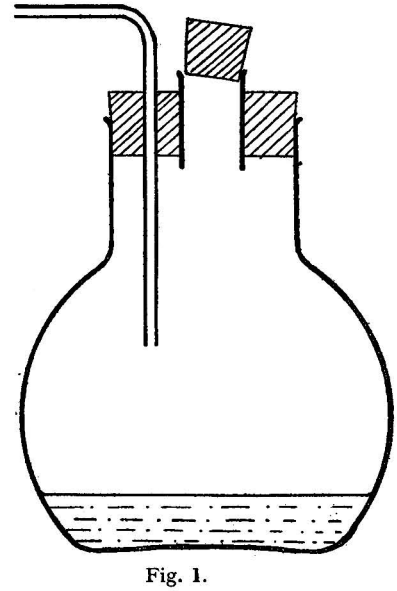

Fig. 1.

TABLE V

In each experiment 5 g. of As_2O_3 were taken.

	Taken				Found	
Experiment	Sb_2O_5	Sb_2O_4	Cu	As_2O_5	As_2O_5	Error
40	0·05	—	0·0032	0·0098	0·0097	−0·0001
41	—	0·05	0·0032	0·0098	0·0091	−0·0007
42	—	—	0·0032	0·0049	0·0049	nil
43	—	0·05	0·0064	0·0098	0·0098	nil
44	0·05	—	0·0064	0·0098	0·0098	nil
45	0·05	—	0·0064	0·0246	0·0251	+0·0005

by means of a pipette. Replace the stopper, mix the solutions and allow to stand for 5 minutes in the dark. Add, by means of a tap funnel the tap and stem of which are completely filled with water, 150 ml. of cold distilled water previously boiled to remove air. Introduce the burette tip through the wide tube and titrate the liberated iodine. Add 2 ml. of starch

solution by means of a pipette, towards the end of the titration. As in the previous instance a deduction is made for copper and blank on the reagents.

To avoid introduction of air the flow of carbon dioxide should be increased when the stopper is removed from the wide tube, and no attempt should be made to empty pipettes or tap funnel completely.

Test of method—As before, tests were carried out on synthetic mixtures of known composition. The results, which were satisfactory, are given in Table V.

SUMMARY—

A method for the determination of arsenic pentoxide in white arsenic has been described in which the pentoxide is separated from interfering elements (except copper) by extraction with water, so permitting its determination by iodimetry. A modification of the method suitable for material containing copper has also been described.

In conclusion, I desire to thank the Directors of Messrs. Cooper McDougall and Robertson for permission to publish this work.

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Iodimetric Methods of Estimating Peroxidic Oxygen

BY J. H. SKELLON AND E. D. WILLS

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SINCE 1929, when Marks and Morrell[1] published a method for determining organic peroxides, many variations of the iodimetric method have been suggested and many conflicting results recorded. Recent views[2,3] as to the structure of the initial peroxides formed on oxidation of mono-ethenoid fatty derivatives have again focussed attention on the need for reliable methods for ascertaining the extent of formation and transition of these initial products of fat oxidation.

Organic peroxides are difficult to determine accurately, since they are weak oxidising agents and—certainly for the small amounts that develop consequent on rancidity in edible fats—the influence of atmospheric oxygen is important.

Nakamura[4] recorded inconsistent results when determining peroxides in soya-bean oil by the iodimetric method, the active oxygen content apparently increasing in proportion to the time of contact of the peroxidic material with the potassium iodide solution. According to this author, full interaction between potassium iodide and peroxide did not occur under the conditions adopted, and peroxide reaction developed in the solvents. Taffel and Revis[5] considered that in oils blown at high temperature two types of peroxide develop, (*a*) those easily reducible and (*b*) those not easily reducible and therefore not accurately determined by normal iodimetric procedure. This accords with our experience to a certain extent, for peroxides developed in fatty material consequent on high temperature oxidation are reduced with difficulty and reasonable time is needed for quantitative interaction with potassium iodide; there is, in fact, growing evidence that the "complex peroxides" developed by powerful gaseous oxidations above 100° C. are completely non-reducible. A suggested structure for these complexes is discussed in a separate communication[6] by one of us (J. H. S.), now in course of publication. Lea,[7] in a new modification of his original procedure[8] for determining peroxides in edible fats, has taken account of such factors as the influence of dissolved oxygen and moisture in the reagent. Whilst de-aeration of the solvent is necessary for accurate determination of the very small amounts of peroxides developed in the early stages of rancidity, a scrutiny of the comparative figures obtained by Lea and other authors

indicates that the discrepancies due to dissolved oxygen may be offset by other factors. We have obtained consistent results with the Lea "cold method" using suitable modified apparatus, but when the method is applied to determination of the much larger quantities of peroxides developed in thermally oxidised fats, de-aeration of solvent does not appear to be essential. Recent work on photochemical and thermal oxidation of fats and fat components has shown that considerable amounts of peroxidic oxygen develop in a very short time—for example, independent gaseous oxidations of methyl oleate for 4 hours have given the following results for peroxide content expressed as active oxygen: 0·64 per cent. after photochemical treatment at 18° C.*(Farmer and Sutton, *loc. cit.*); 1·1 per cent. after thermal treatment at 120° C. (Gunstone and Hilditch[9]); 0·56 per cent. after thermal and catalytic treatment at 120° C. (Skellon, *loc. cit.*). (Calc. for $C_{19}H_{36}O_4$: 4·88 per cent. of active oxygen ≡ 6100 mg.-equivs. per kilogram.)

The object of the present work was to investigate factors that influence iodimetric methods of determining peroxides, whether the peroxides are formed by simple exposure to air and light or as a result of photochemical or thermal gaseous oxidation. A simplified method of determination is advanced as suitable for use in tracing the course of oxidation of oils and fats.

Benzoyl peroxide was chosen as a standard substance for the critical work because it can be obtained in a highly purified condition and determinations can be checked against its theoretical peroxide oxygen content.

The work to be described showed that benzoyl peroxide can be determined consistently and accurately by the following simplified iodimetric method:

> RECOMMENDED METHOD—Dissolve the sample (0·2 g.) in 25 ml. of glacial acetic acid contained in a stoppered bottle. Add 2 g. of pure sodium bicarbonate, and replace the stopper after evolution of gas begins to abate. Place the solution in the dark for 10 minutes, and then add 2 ml. of saturated potassium iodide solution and allow the mixture to stand for a further 15 minutes in the dark. Then dilute with 100 ml. of distilled water and titrate with accurately standardised sodium thiosulphate solution, using starch as indicator towards the end of the titration.

For a sample weight of 0·2 g. of benzoyl peroxide, 0·1 *N* sodium thiosulphate solution is suitable, but for smaller weights 0·01 *N* thiosulphate may be used.

The method is suitable for fatty acid and other organic peroxides. When it is used for determining peroxidic oxygen in thermally oxidised fats the standing time should be extended to at least 1 hour. For very small amounts of peroxides 0·01 to 0·005 *N* thiosulphate may be required.

When the time of standing is to be prolonged, the inert atmosphere is well maintained by using an initial stream of pure carbon dioxide. Addition of mineral acid is not necessary in the above method, and the size of the sample, within reasonable limits, has no effect. Under the conditions described, iodine does not appear to be re-absorbed at residual ethenoid linkages when the peroxidic oxygen content of oxidised fatty compounds is being determined. Organic peroxides, including fat peroxides, are largely insoluble in water and, since water-soluble reagents are used in their determination, it is highly desirable to use a solvent in which both inorganic reagents and organic peroxides will be soluble, so as to avoid separation of the reaction mixture into distinct phases. An ionising solvent such as acetic acid is preferable. Additional non-polar solvents such as carbon tetrachloride or chloroform should be employed only in minimum quantity to obtain solution of peroxidic material. Normal temperature is recommended for determinations, as initially-formed peroxides are liable to some decomposition at high temperature. It is desirable to carry out control determinations on a pure compound such as benzoyl peroxide as a standard. Peroxide transformations during high-temperature thermal oxidation of unsaturated fatty acids and esters vary considerably with conditions and are not comparable with the normal changes that occur during slow oxidation at moderate temperatures.

EXPERIMENTAL

I. INVESTIGATION OF THE FACTORS INFLUENCING PEROXIDE DETERMINATION IN A KNOWN STANDARD—BENZOYL PEROXIDE $(C_6H_5COO)_2$

The benzoyl peroxide used in the following experiments was purified according to the method described by Gattermann.[10] Sodium thiosulphate solutions were standardised against potassium dichromate and potassium iodate, both of analytical reagent quality.

The first experiments were carried out by the original method of Marks and Morrell (*loc. cit.*, p. 504), benzoyl peroxide being dissolved in 25 ml. of glacial acetic acid. Two ml. of concentrated potassium iodide solution were added and the mixture was allowed to stand for 10 minutes. It was then diluted with 100 ml. of distilled water and titrated with 0·1 *N* thiosulphate. Blank tests were made simultaneously. The results of eight determinations are set out in Table I.

TABLE I

IODIMETRIC DETERMINATION OF BENZOYL PEROXIDE

(Original method)

No.	Benzoyl peroxide taken g.	Sodium thiosulphate (0·0965 *N*) required ml.	≡ ml. for 0·2 g. of benzoyl peroxide
1	0·2145	18·50	17·25
2	0·1799	15·70	17·45
3	0·1999	17·50	17·51
4	0·2040	17·50	17·16
5	0·1883	16·00	16·99
6	0·1755	15·20	17·32
7	0·1963	17·00	17·31
8	0·2020	17·50	17·33
9	Blank	0·125	
10	"	0·200	
11	"	0·100	

Using 0·0097 *N* sodium thiosulphate, a further set of blanks under the same conditions required: 2·10, 1·00, 2·00 and 0·80 ml. These results show that considerable variation is possible under these conditions.

Factors influencing the precision of peroxide determination were next investigated.

(1) TIME OF STANDING AND QUANTITY OF SOLVENT—

Using the same method as for Table I, the effect of time of standing on the blanks is shown in Table II, *A*. Table II, *B* shows the effect when the volume of the solvent was varied also.

TABLE II

IODIMETRIC DETERMINATION OF BENZOYL PEROXIDE

(Original method)

A. Effect of time of standing on blanks

No.	Time of standing min.	Sodium thiosulphate (0·0097 *N*) required ml. (I)	(II)
1	0	0·20	—
2	5	1·25	1·20
3	10	2·20	1·80
4	15	2·65	5·70
5	20	2·10	3·20
6	25	8·80	—
7	30	11·05	3·20
8	35	10·75	—
9	40	11·00	—
10	70 hours	32·70	39·10

B. Effect of volume of solvent and time of standing on blanks

No.	Volume of solvent ml.	Time of standing min.	Sodium thiosulphate (0·0097 *N*) required ml.
11	25	10	2·00
12	50	10	2·30
13	25	20	3·2
14	50	20	2·0
15	25	30	3·2
16	50	30	2·1

The figures in Table II, *A*, indicate that for blank determinations under the conditions of the original method there is no strict correlation between increasing time of standing and increasing titration value, and that the results are not accurately reproducible; other factors such as shaking and time of titration have an effect. In the experiments of Table II, *B*, if oxidation were due to an impurity in the solvent, the effect should have been approximately doubled when 50 ml. were used in place of 25 ml. The results show that the variations in results are not due to oxidation of potassium iodide by impurity in the glacial acetic acid and must therefore be due to atmospheric oxidation.

(2) USE OF AN INERT ATMOSPHERE—

Blank determinations were next carried out, with an inert atmosphere, 2 g. of pure sodium bicarbonate being added before addition of the 2 ml. of potassium iodide solution; the stopper was removed to enable carbon dioxide to displace air from the bottle and then replaced.

The volumes of 0·0097 *N* sodium thiosulphate required after 5, 10 and 15 minutes standing time for three separate blanks were 0·075, 0·075 and 0·095 ml., respectively. Thus a standing time of 15 minutes in an atmosphere of carbon dioxide gave an error equivalent to 0·01 ml. of 0·1 *N*, well within the limits of experimental error. After 50 hours standing however, the volumes of 0·0097 *N* thiosulphate required in two blanks were 25·05 and 34·25 ml., *i.e.*, the blank results did not remain small indefinitely and were not reproducible for very long standing periods.

Precise results were obtained, as shown in Table III, when the procedure recommended in this paper (p. 79) was adopted.

TABLE III

DETERMINATION OF BENZOYL PEROXIDE. MODIFIED IODIMETRIC METHOD

No.	Benzoyl peroxide taken	Sodium thiosulphate (0·0965 *N*) required	
	g.	ml.	≡ ml. for 0·2 g. of benzoyl peroxide
1	0·2018	17·20	17·05
2	0·1521	12·95	17·03
3	0·1757	14·95	17·02
4	0·2371	20·20	17·04
5	0·1871	15·95	17·05
6	0·2006	17·15	17·10
			mean 17·05

The mean value of the last column corresponds to 6·583 per cent. of available oxygen in the benzoyl peroxide, which is 99·7 per cent. of the theoretical value.

Four independent workers in this laboratory, at random, recently recorded the following values for the peroxidic oxygen content of benzoyl peroxide:

(*a*) By the modified iodimetric method with carbon dioxide atmosphere and standing time 15 minutes: 6·58, 6·55, 6·58 and 6·60 per cent. of active oxygen.
(*b*) By the "cold" method using nitrogen atmosphere and de-aeration of solvent: 6·61, 6·50 and 6·55 per cent.
(*c*) By the original iodimetric method with no inert atmosphere and standing time over 20 hours: 7·84, 7·98, 7·85 and 7·84 per cent.

These figures confirm that for large amounts of peroxides good results are possible if an atmosphere of nitrogen or carbon dioxide is used with or without de-aeration of the solvent.

(3) NATURE OF THE SOLVENT—

A survey of the literature indicates that acetic acid is widely used as a solvent. Alone or mixed with chloroform, it is a good solvent for oxidised fats and derivatives. Benzoyl peroxide is not precipitated from glacial acetic acid solution on addition of water. Some workers (Stansby,[11] Stuffins and Weatherall,[12] Risby and Nisbet[13]) have observed discrepancies when the ratio of the constituents in chloroform - acetic acid solvent mixture is varied, but in our experience the use of acetic acid by itself gives consistent results. An ionising solvent is advantageous, for the oxidation $2I' + O + 2H^{\cdot} = H_2O + I_2$ is essentially ionic and would be retarded in a non-ionising solvent. Any error consequent on speeding up the

reaction $4I' + O_2(\text{atmospheric}) + 4H^{\cdot} = 2I_2 + 2H_2O$ can be minimised by use of an inert atmosphere and a comparatively short time of contact.

Solvents other than glacial acetic acid have been recommended by various authors, for example, acetone (Gelissen and Hermans[14]), acetic anhydride (Nozaki[15]) and acetic acid with chloroform (Lea, *loc. cit.*). Although acetone is a good solvent for fats, solutions of benzoyl peroxide in this solvent were found to be partially reprecipitated on addition of water and consequently gave low results for peroxidic oxygen content. Samples of benzoyl peroxide were dissolved in 10 ml. of acetone and placed in the dark. One ml. of 50 per cent. sulphuric acid and 2 ml. of saturated potassium iodide solution were added and the mixtures allowed to stand for times ranging from 5 to 15 minutes before titration with standard sodium thiosulphate. Results are shown in Table IV.

TABLE IV

DETERMINATION OF BENZOYL PEROXIDE

Use of acetone as solvent

No.	Benzoyl peroxide taken g.	Time of standing min.	Sodium thiosulphate (0·0965 *N*) required ml.	≡ ml. per 0·2 g. of benzoyl peroxide
1	0·1765	5	14·70	16·66
2	0·1507	5	12·45	16·50
3	0·1884	15	15·70	16·59
4	Blank	5	0	0
5	"	15	0	0

Theoretical volume of 0·0965 *N* thiosulphate for 0·2 g. of benzoyl peroxide = 17·11 ml.

With acetone as solvent, even under the favourable conditions described, the results were consistently low. The blanks were zero even in absence of an inert atmosphere—probably owing to the low concentration of hydrogen ions, ionisation being suppressed in acetone solution.

(4) PRESENCE OF MINERAL ACID—

The results detailed in Table III indicate that absence of mineral acid has little or no effect on the accuracy of the method when applied to benzoyl peroxide. The 25 ml. of acetic acid, even after partial neutralisation with sodium bicarbonate, are sufficient to provide a *p*H value low enough for the reaction to proceed rapidly and completely.

(5) USE OF IODIDE MIXTURES—

The use of a mixture of cadmium iodide and potassium iodide in place of potassium iodide was suggested by Green and Schoetzow,[16] and used by Lindgren[17] in determinations

TABLE V

DETERMINATION OF BENZOYL PEROXIDE WITH CADMIUM IODIDE - POTASSIUM IODIDE MIXTURE

No.	Benzoyl peroxide taken g.	Time of standing min.	Sodium thiosulphate (0·0965 *N*) required ml.	≡ ml. per 0·2 g. of benzoyl peroxide
1	0·1678	40	9·95	11·86
2	0·1576	35	9·70	11·76
3	0·1849	35	11·40	12·33
4	Blank	35	0	0
5	"	35	0	0
			Sodium thiosulphate (0·01930 *N*) required ml.	≡ ml. per 0·04 g. of benzoyl peroxide
6	0·0422	85	16·40	15·55
7	0·0315	85	9·95	15·91
8	Blank	55	0	0
9	"	55	0	0

Calc. for 0·2 g. benzoyl peroxide, 17·11 ml. of 0·0965 *N* thiosulphate.
" 0·04 g. " " 17·11 ml. of 0·01930 *N* "

of peroxides in ether. The reagent is prepared by dissolving 1 g. of an equimolecular mixture of cadmium iodide and potassium iodide in 5 ml. of 36 per cent. acetic acid and diluting with 20 ml. of ethyl alcohol. Determinations of benzoyl peroxide were made with this reagent. About 0·2 g. of benzoyl peroxide was dissolved in 15 ml. of acetone and placed in the dark. Five ml. of the iodide reagent were added, and the mixture was allowed to stand for times ranging from 35 to 85 minutes and then titrated with standard thiosulphate. Results are shown in Table V.

The figures indicate the consistently low results obtained when this mixture is used under the conditions described. The cause may possibly be suppression of ionisation of the potassium iodide by the cadmium iodide.

(6) SAMPLE SIZE—

Reimenschneider, Turer and Speck[18] consider that peroxidic oxygen content may possibly vary with size of sample. There seems little reason why such variation should occur in a good standard method and the results shown in Table VI support this view, being consistent for quantities of benzoyl peroxide from 0·02 to 0·2 g.

TABLE VI

DETERMINATION OF BENZOYL PEROXIDE. MODIFIED IODIMETRIC METHOD
VARIATION OF WEIGHT OF SAMPLE

No.	Benzoyl peroxide taken g.	Sodium thiosulphate (0·0965 *N*) required ml.	≡ ml. per 0·2 g. of benzoyl peroxide
1	0·2133	18·20	17·07
2	0·1563	13·35	17·08
3	0·2574	22·05	17·13
4	0·2331	19·95	17·12
			mean 17·10

Mean result ≡ 6·602 per cent. of active oxygen

		Sodium thiosulphate (0·00850 *N*)	(per 0·02 g.)
5	0·0160	15·60	19·50
6	0·0121	11·80	19·50
7	0·0244	23·65	19·39
			mean 19·46

Mean result ≡ 6·616 per cent. of active oxygen.

Determinations with various concentrations of oxidised mono-ethenoid fatty acids have confirmed our view that within reasonable limits the peroxidic oxygen content found appears to be independent of the concentration.

II. PEROXIDE - OXYGEN DETERMINATION IN OXIDISED FATTY ACIDS AND ESTERS
PROBLEM OF RE-ABSORPTION OF IODINE AT ETHENOID LINKAGES

Various workers have criticised the application of iodimetric methods of peroxide determination to unsaturated compounds, on the ground that loss of liberated iodine may occur owing to re-absorption of iodine by residual ethenoid linkages (Dastur and Lea,[19] Bolland, Sundralingham, Sutton and Tristram,[20] Young, Vogt and Nieuwland,[21] Yule and Wilson[22]).

Lea (*loc. cit.*) considered that variation in the balance of the opposing effects of slow liberation of iodine by products not true peroxides, on the one hand, and re-absorption of iodine, on the other, might perhaps account for the appreciably different values given by the "hot" as compared with the "cold" method of determining peroxidic oxygen, but no direct evidence of loss of liberated iodine due to re-absorption was obtained. In the present work, the possibility of such absorption has been tested; benzoyl peroxide was determined by the modified iodimetric method described, in the presence of known weights of a pure mono-ethenoid fatty acid, *viz.*, oleic acid. The results are shown in Table VII.

The mean value 17·08 ml. compares well with those in Table III (17·05) and Table VI (17·10), being almost the exact average of the two. As oleic acid is capable of absorbing nearly its own weight of iodine, and 0·2 g. of benzoyl peroxide liberates only 0·25 g. of iodine, the possibility of reduction of peroxide value was great, but the results show that it did not occur under the conditions described.

These results were confirmed by determinations of peroxidic oxygen in pure oleic acid by modifications of the method of Marks and Morrell and by the modified iodimetric method recommended in this paper, all the determinations being accompanied by blanks. After standing times of 15 to 60 minutes the amounts of iodine liberated were coincident with those liberated in the blanks. The oleic acid thus showed zero peroxide value, and the ethenoid linkage did not absorb iodine under these conditions.

TABLE VII

DETERMINATION OF BENZOYL PEROXIDE

MODIFIED IODIMETRIC METHOD IN PRESENCE OF OLEIC ACID

No.	Benzoyl peroxide taken g.	Oleic acid present g.	Sodium thiosulphate (0·0965 *N*) required ml.	≡ ml. per 0·2 g. of benzoyl peroxide
1	0·1943	0·6477	16·50	16·98
2	0·1592	0·5640	13·65	17·15
3	0·2035	0·7518	17·40	17·10
				mean 17·08

Theoretical volume of 0·0965 *N* thiosulphate for 0·2 g. of benzoyl peroxide = 17·11 ml.

Thus, it seems fairly conclusive that little or no re-absorption of iodine takes place with mono-ethenoid fatty derivatives under the prescribed conditions, and there is little direct evidence that it occurs in polyethenoid fatty acid chains; further systematic investigation of the behaviour of a wide series of ethenoid compounds seems desirable before the point can be cleared up with certainty.

The apparently anomalous changes in peroxidic oxygen content that occur during thermal oxidation of fats and component unsaturated acids and esters have little or no connection with possible re-absorption of iodine at ethenoid linkages during determination. The general trend of such changes is for the peroxidic oxygen content to rise to a maximum and then decrease—owing to transformation of the normal peroxides into compounds that have little or no effect on concentrated potassium iodide. The transformation may occur at different stages, depending on various conditions—temperature, presence or absence of catalysts, nature of the catalyst and nature of the unsaturated compound. Typical results illustrating these comparative changes are given in Table VIII.

TABLE VIII

CHANGES IN PEROXIDE CONTENT OF OLEIC ACID DURING CATALYTIC OXIDATION BY GASEOUS OXYGEN AT 120° C.

Peroxide oxygen found, expressed as per cent. of active oxygen.

Oxidation period hrs. . .	1	2	3	4	5	6	7	9	10	11	14
Catalyst (0·05 per cent. metal):											
Vanadium oleate . .	0·22	0·33	0·38	0·40	0·57	0·34	0·20				
Zinc oleate . .	0·28	0·44	0·47	0·51	0·76	0·61	0·59	0·55	0·46	0·46	0·39

Calc. for peroxide of oleic acid, $C_{18}H_{34}O_4$, peroxidic oxygen content 5·09 per cent. of active oxygen.

Thus, with catalysts at 120° C. the induction period is negligible and the time required to attain a maximum is very short; at lower temperatures (65° to 100° C.) peroxide development is much slower in the first 2 or 3 hours, but the maximum eventually reached is greater than that attained at higher temperature. Changes of this type have invariably been observed by one of us in catalytic gaseous oxidations of unsaturated fatty acids and esters, and are due to transformation of the initial peroxides into a variety of oxidation products which may include ketols, complex non-reducible peroxides and scission products.

SUMMARY

Benzoyl peroxide has been used as a standard to examine the reliability of several variations of the iodimetric technique for determination of peroxidic oxygen in fats and oxidised fatty acid components. Factors influencing the accuracy of the methods involved have been investigated and a modified technique for determination of peroxidic oxygen, applicable to oxidised fats and their components, is suggested. The complex character of peroxide transformation in high-temperature thermal oxidation of unsaturated fatty acids and esters is discussed in connection with peroxide determination.

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Discussion

Mr. C. B. Stuffins emphasised the importance of removing dissolved oxygen from the reaction mixture and pointed out that a paper published by him and Mr. H. Weatherall in 1945 had shown the necessity of passing inert gas through the mixture to prevent erratic results. The presence of as little as 0·1 ml. of dissolved oxygen would be equivalent to 1 ml. of 0·002 *N* sodium thiosulphate solution which represents a considerable error with edible fats of low peroxide values.

Dr. K. A. Williams congratulated the authors on their interesting paper. He confirmed the very slow absorption of iodine by unsaturated fats from solution in organic solvents and referred to work of J. P. K. van der Steur (*Rec. Trav. Chim. Pays-Bas*, 1927, **46**, 278, 409, 414; Analyst, 1927, **52**, 609) in which the stereo-configuration of long-chain fatty acids was shown to have a profound effect on the equilibrium constant of their reaction with iodine in carbon tetrachloride solution. In his experience it required from 1 to 3 days for equilibrium to be established, the time depending on the temperature of reaction. This being so, no appreciable absorption of iodine liberated in the determination of peroxide values was to be expected.

On the question of the various units that have been proposed for recording peroxide values, he mentioned that the International Commission for the Study of Oils and Fats at its recent meeting in London had adopted the definition: the peroxide value is the number of micrograms of peroxide oxygen in 1 g. of oil or fat.

Dr. J. R. Nicholls said that the paper was a valuable contribution to the iodimetric determination of benzoyl peroxide. But it must not be assumed that all other peroxides would behave similarly. Benzoyl peroxide was a very stable substance in some circumstances and a powerful oxidising agent in others. When dissolved in organic solvents such as acetone or alcohol it appeared quite stable, but the addition of alkali caused instantaneous reaction with oxidation of the solvent. The apparent stability in the absence of alkali would not exclude a slow rate of reaction, and the low results the authors had obtained by the iodimetric method in presence of such solvents might be accounted for by a partial oxidation of the solvent. The peroxides found in oils or fats were weak oxidising agents and the analogy with benzoyl peroxide should not be pressed too far.

Dr. D. C. Garratt asked the authors whether he had understood correctly that they discouraged the use of non-polar solvents in the titration. This would raise difficulty when the peroxide value of the fat from foodstuffs was to be determined as, for instance, in milk powder, and he asked the authors, if he had understood them aright, to suggest the most suitable extracting solvent to be employed.

Mr. H. N. Wilson said he was particularly interested in the authors' remarks on the non-interference of unsaturated compounds, as he had always been afraid that reactive unsaturated bodies would absorb iodine liberated by peroxide, but he had never obtained convincing evidence. He thought that the authors' method for investigating this effect—to add the unsaturated body in question to a known amount of benzoyl peroxide and ascertain whether it affected the amount of iodine liberated—was a very valuable one. Peroxidation was very important in other fields beside oils and fats, *e.g.*, in the manufacture of some high polymers, and in the petroleum industry, where gum formation is intimately connected with the peroxidation of unsaturated substances. Had the authors any experience at all in dealing with the more reactive kind

of unsaturated compound? From the literature and from the discussion, it was apparent that investigators had reached divergent views as to the necessity or otherwise of excluding air from the reaction mixture when the potassium iodide method was used. He thought that this might probably be due to the fact that reaction between atmospheric oxygen and iodide in acid solution was normally quite slow but was very susceptible to catalysis by substances sometimes present, *e.g.*, some aldehydes were very active in promoting this reaction.

Dr. B. S. EVANS said he had encountered what appeared to be evidence of catalysis of air oxidation of iodides in a widely different field, though one connected with peroxides. When trying to work out a method for determining lead as peroxide, finishing with liberation of iodine from potassium iodide and titration, he had found that the concentration of acid (mineral) had to be kept quite low, otherwise blank values became unmanageable. This seemed to indicate catalysis of air oxidation by hydrogen ions.

Mr. G. W. GODIN asked the authors (*a*) whether the 100 ml. of water added before the titration were de-aerated or not and (*b*) whether they had had any experience with the method of peroxide estimation given by Nozaki (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 583), in which acetic anhydride was used in place of the glacial acetic acid. Referring to Mr. Wilson's remarks about aldehydes, he suggested that their interference was probably due to the rapidity and ease with which they can form peroxides by atmospheric oxidation.

Mr. WILLS, replying to Mr. Stuffins, said that the dissolved oxygen was displaced from the reaction mixture by the carbon dioxide actually liberated in the solution. The water added before starting the titration was also de-aerated, since it caused a further rapid evolution of carbon dioxide. In reply to Dr. Garratt, he said that when the use of a non-polar solvent was unavoidable for extraction purposes, the proportion of this solvent should be kept as low as possible in the estimation, by the addition of a relatively large quantity of acetic acid. To Mr. Wilson's remarks, Mr. Wills replied that his own observations showed that the reaction between atmospheric oxygen and potassium iodide in acid solution was rapid, the velocity apparently increasing with increasing acidity. He had had no experience of the catalytic effect of aldehydes. In reply to Mr. Godin, Mr. Wills stated that (*a*) the 100 ml. of water were automatically de-aerated, since evolution of carbon dioxide recommenced with renewed vigour when the strong acetic acid solution was diluted, (*b*) he, personally, had not investigated Nozaki's method. Dr. Skellon mentioned that since completing this work with Mr. Wills, he had tried the method with benzoyl peroxide but not with oxidised fatty acids or fats.

Dr. SKELLON, replying to the point raised by Dr. Nicholls, referred to the necessity of using a reliable control when determining peroxidic oxygen in a series of oxidised fatty compounds; in contrast with the original process, the modified method invariably gave almost theoretical results with benzoyl peroxide so that the observed peroxide values of oxidised fatty acids, esters and fats carried out at the same time, were likely to be absolute; incidentally, peroxide values found by this method and also by the Lea "cold" method were in good agreement.

Dr. Skellon agreed with Mr. Wilson that peroxidation in the more complex unsaturated compounds found in certain other fields presented a special problem; so far, the authors' work had been confined to peroxidised fatty acids, esters and fats. In all the determinations carried out, there had been little or no positive evidence of absorption of iodine at residual ethenoid linkages and the results confirmed the observations of several other investigators that re-absorption of iodine (if any) was so slow as to be negligible. But, as pointed out in the paper, some systematic investigation of a wide range of the higher polyethenoid compounds seemed desirable before such a conclusion could be generally applied. With regard to the observation of Dr. Evans, they had obtained satisfactory results with benzoyl peroxide without use of sulphuric acid, and preferred to omit it. Finally, he was glad to note from Dr. Williams's observations that a uniform method of expressing peroxidic oxygen content had now been agreed upon.

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

Part 1. Review

BY J. TINSLEY

THE main demand for the reliable determination of potassium in small amounts is for biological materials, especially blood and other body fluids, and also for studies in plant physiology and soil fertility. This serves to emphasise the great biochemical importance of potassium in both plant and animal organisms. The micro determination of potassium has been reviewed previously by Burton,[1] by Cimerman and Rzymowska,[2] and by Heller, Haurowitz and Stary.[3] Of the various methods employed the cobaltinitrite group has proved the most popular to date, and in this review attention is mainly confined to this reagent. The meaning attached here to the term "micro-determination" follows almost exclusively the pattern of

the centrifuge technique first established by Kramer and Tisdall.[4] The quantity of liquid from which the potassium is precipitated may vary from 5 ml. or more, to 0·5 ml. or less, depending on the particular purpose of the method.

The extensive literature is considered under headings as follows.

1. Reagent

Most commonly an aqueous solution of sodium cobaltinitrite is used to precipitate potassium, following its initial use by Adie and Wood.[5] The stock solution may be prepared by mixing a solution of sodium nitrite with a solution of cobalt acetate, chloride, nitrate or sulphate, and then adding acetic acid, because the cobaltinitrite complex does not readily form in neutral solution. Oxides of nitrogen are then removed, generally by passing a slow stream of air through the solution or by evacuation. Kramer and Tisdall used a reagent prepared in this way; it was filtered or centrifuged each time before use, and found to keep satisfactorily for a month if stored in the refrigerator.

Various workers have used separate stock solutions of sodium nitrite and a cobalt salt for macro-determinations. These solutions may be stored satisfactorily and mixed in the precipitation vessel as required, but, except by Jendrassik and Takács,[6] this two-solution method does not appear to be favoured for micro-determinations.

Tischer[7] used a solution of sodium cobaltinitrite freshly prepared by dissolving the pure solid in water, for the micro-determination of potassium, and this method of preparing the reagent has since become increasingly popular for both macro- and micro-determinations. A 20 per cent. (w/v) solution is mostly preferred at the present time.

Potassium silver cobaltinitrite is even less soluble than the dipotassium sodium salt and, following the pioneer work of Burgess and Kamm,[8] it has been utilised for micro-determinations. Breh and Gaebler,[9] after precipitating proteins with tungstic acid, and chloride with silver nitrate, determined potassium in the filtrate from blood serum with a reagent prepared by adding silver nitrate to a solution of sodium cobaltinitrite.

Adams, Hall and Bailey[10] used zinc cobaltinitrite as a qualitative reagent for potassium when it was desired to avoid introducing sodium, and this reagent was later used quantitatively by Chen and Shen.[11] Sergienko[12] precipitated potassium with sodium lead cobaltinitrite. Apparently neither of these two reagents has been adapted for a micro-determination.

2. Precipitation

Continual controversy has centred around the variability in the composition of the precipitate. Adie and Wood[5] obtained a precipitate corresponding to the formula $K_2NaCo(NO_2)_6.H_2O$, but apparently the composition can vary between those of the mono- and the tri-potassium salts, depending on the relative proportions and concentrations of sodium, potassium and cobaltinitrite present.

Kramer and Tisdall[4] claimed that precipitation could be made directly from 2 ml. of blood serum by adding 1 ml. of sodium cobaltinitrite solution slowly, drop by drop with careful mixing, to prevent occlusion of the reagent with the precipitate. After the mixture had stood for 45 minutes at room temperature, 2 ml. of water were added and the precipitate was stirred before centrifuging. Jacobs and Hoffman[13] also followed this procedure for precipitation from blood serum, and Sobel and Kramer[14] described the precipitation of potassium from 0·2 ml. of serum in a specially designed 5-ml. centrifuge tube. Later, Sobel, Hanok and Kramer[15] using the procedure of Kaye,[16] obtained low recoveries of potassium from serum direct, and recommended a preliminary separation of potassium by electrodialysis. Several workers adapted the method of Kramer and Tisdall to solutions other than blood serum, but while the original method yielded precipitates of fairly uniform composition with normal sera in which the proportion of sodium to potassium does not vary greatly, it generally proved less satisfactory with wide variations in this ratio. Lewis and Marmoy[17] examined its use for aqueous solutions of potassium as the chloride, derived from citric acid and hydrochloric acid extracts of soils and also from plant ash. Kawe[18] also examined the sensitivity of the method and recommended it as being suitable for soil extracts.

Hubbard[19] proposed the addition of 1 ml. of 40 per cent. hydrated sodium acetate solution prior to the cobaltinitrite reagent, and also cooling the tubes in an ice-bath for 0·5 to 2 hours in order to secure more uniform precipitation. Eden[20] has described a modification of the method due to Jacobs and Hoffman[13] which may be applied to blood sera, to feeding stuffs or to soils. 0·5 Ml. of 50 per cent. sodium nitrite solution was added to a suitable aliquot

of potassium solution before diluting to a total volume of 4 ml. and then adding 2 ml. of cobaltinitrite reagent. No temperature control was exercised, the tubes being allowed to stand for 1 hour at room temperature before centrifuging. The addition of sodium nitrite followed the procedure described by Peters and Van Slyke,[21] and served to prevent the precipitate adhering to the sides of the tube. It also aided coagulation and compactness of the precipitate obtained by centrifuging, and the extra sodium tended to produce a precipitate of uniform composition. However, sodium nitrite usually contains a trace of potassium, which must be allowed for.

Bowser[22] introduced ethyl alcohol to increase the sensitivity of his qualitative test; for quantitative estimations he used a mixture of equal volumes of glacial acetic acid and alcohol, which gave a precipitate of coarser particles more suited to filtration. Taylor[23] used ethyl alcohol for the determination of potassium in the tungstic acid filtrate from blood serum. Five ml. of filtrate were first evaporated to dryness in a centrifuge tube by means of a current of air under reduced pressure, and the residue was dissolved in 1 ml. of water. One ml. of 95 per cent. ethyl alcohol was mixed with this solution and followed by 1 ml. of sodium cobaltinitrite reagent, and the tubes were then allowed to stand for 2 hours at room temperature before centrifuging. Lohse[24] examined this procedure and found that the composition of the precipitate varied under different conditions. Sideris[25,26] precipitated potassium from 0·5 ml. of a chloride solution at a *p*H between 3 and 6 by adding 5 ml. of a mixture of equal volumes of ethyl alcohol and a 12·5 per cent. aqueous solution of sodium cobaltinitrite. Alcohol throws the sodium cobaltinitrite out of solution as a suspension of fine yellow crystals, and this reagent mixture must be prepared immediately before use. The tubes were allowed to stand for 4 hours or overnight in a refrigerator. Tinsley and Pizer[27] used a similar procedure for precipitating potassium directly from sodium acetate - acetic acid extracts of soils, the alcohol being added to the sodium cobaltinitrite in the centrifuge tube before the potassium solution. Brown, Robinson and Browning[28] used alcohol in a procedure similar to Taylor's and their reagent contained sodium cobaltinitrite, sodium acetate and acetic acid. The tubes were allowed to stand for 1 hour at 20° C., and recovery of potassium was found to be higher than theoretical at lower temperatures, and lower at higher temperatures. Kelley, Hunter and Sterges[29] have recently adapted their method to a system of plant tissue analysis based on digestion with sulphuric acid - salicylic acid mixture.

Wander[30] adapted the macro procedure of Wilcox[31] and precipitated potassium from 0·1 *N* nitric acid solution by adding 5 ml. of 20 per cent. sodium cobaltinitrite solution to 10 ml. of potassium solution in a centrifuge tube, which was then allowed to stand for exactly 2 hours at 20° C. Parks, Hood, Hurwitz and Ellis[32] used Wander's method for potassium in their system of micro-analysis for plant tissues. Peech[33,34] evolved a system of rapid micro-analysis for exchangeable cations in soils, following their extraction with neutral 1 *N* ammonium acetate solution. The final solution was obtained in 0·1 *N* nitric acid and the potassium precipitated from 3 ml. with 1 ml. of 25 per cent. aqueous sodium cobaltinitrite solution. After standing for 1 hour at 10° C. in a refrigerator, 4 ml. of 70 per cent. aqueous ethyl alcohol were added and the precipitate was stirred immediately before centrifuging. This late addition of alcohol, presumably to aid separation of the precipitate, follows the procedure of Morris and Gerdel[35] for the determination of potassium in the sap expressed from plants. Reed, Mehlich and Piland,[36] after comparing a number of procedures for the determination of exchangeable potassium in soils, modified the method of Volk.[37] The precipitate was formed by addition of an equal volume of 20 per cent. aqueous sodium cobaltinitrite solution to the potassium solution in 0·16 *N* acetic acid, and the tubes were stored for 16 hours at 10° C. before centrifuging. No advantage was gained by addition of extra sodium as acetate provided the amount of potassium exceeded 0·05 mg. per ml., and although 0·1 *N* nitric acid in place of acetic acid appeared to give more rapid precipitation there was otherwise little difference between the two.

Regarding the quantitative precipitation of potassium silver cobaltinitrite, Breh and Gaebler[9] did not concentrate the tungstic acid filtrate from blood serum. Two ml. of reagent were added to 5 ml. of filtrate and the tubes were allowed to stand for 22 hours at room temperature before centrifuging. This procedure was modified by Truszkowski and Zwemer[38] for determinations on 0·1 to 0·2 ml. of serum. In their later method[39] the precipitate was stored overnight in a refrigerator, and before centrifuging 0·5 ml. of aqueous octyl alcohol was added to each tube to prevent the precipitate adhering to the walls. Chapman[40] added

a few drops of 70 per cent. ethyl alcohol saturated with camphor to bring about the settlement of any precipitate of sodium potassium cobaltinitrite adhering to the surface.

Robinson and Putnam[41] determined potassium in water samples by means of a reagent containing 1 per cent. of silver nitrate, 12·5 per cent. of sodium cobaltinitrite, 25 per cent. of sodium nitrite and 1 per cent. of acetic acid. Two ml. of this reagent were added to 1 ml. of potassium solution and the tubes were allowed to stand for 3 hours at room temperature or, for greater sensitivity, at 0° C. The composition of the precipitate obtained was best represented by the formula $K_{1\cdot35}Ag_{1\cdot65}Co(NO_2)_6$. Ismail and Harwood[42] developed a similar method for soil extracts, but precipitation was carried out in presence of acetone by adding to each tube 2 ml. of halogen-free potassium solution, 1 ml. of 0·7 per cent. silver nitrate solution and 1 ml. of acetone. After mixing, the tubes were cooled in an ice-bath before adding 1 ml. of cold 25 per cent. aqueous sodium cobaltinitrite solution drop by drop with stirring. The precipitate was centrifuged after standing for 2 hours in the ice-bath.

Harris[43] precipitated potassium from tungstic acid filtrates of blood sera in presence of ethyl alcohol. One ml. of alcohol was mixed with 4 ml. of the filtrate before addition of 2 ml. of sodium silver cobaltinitrite reagent. The tubes were kept at 20° C. in a water-bath and maintained at this temperature for 0·5 hour before centrifuging. If the temperature was too low (16° C.) silver nitrite was precipitated, and if too high (25° C.) the potassium/nitrite ratio in the precipitate changed. Weichselbaum, Somogyi and Rusk[44] made a detailed study of the precipitation of potassium silver cobaltinitrite from blood sera. They used copper sulphate in conjunction with sodium tungstate for protein precipitation in order to obtain a filtrate free from tungstate. The required amount of silver nitrate solution was added to 5 ml. of potassium solution and followed by 0·5 ml. of a freshly prepared 12·5 per cent. aqueous sodium cobaltinitrite solution. The tubes were placed for 10 to 20 minutes in a water-bath at 18° to 20° C. before centrifuging.

Because an excess of silver influences the composition of the precipitate, Pereira[45] removed halogens from biological materials by a wet ashing procedure involving nitric and perchloric acids, followed by evaporation with sulphuric acid to the fuming stage. The potassium was finally obtained in 1 ml. of solution, made slightly acid with acetic acid and precipitated with 1 ml. of cobaltinitrite reagent prepared according to Robinson and Putnam.[41] The tubes were allowed to stand for 2 hours at 4° to 6° C. before centrifuging.

3. Washing the precipitate

After centrifuging, the supernatant liquid is best drawn off with a siphon or suction tube without disturbing the precipitate, but in many procedures the precipitate settles completely and compactly so that the liquid may be carefully poured off without loss. Kramer and Tisdall washed the precipitate four times with 5-ml. portions of water, taking care to disturb the precipitate as little as possible and so reduce the extent to which it dissolved in the washing liquid. The same procedure was employed by Breh and Gaebler,[9] and also by Weichselbaum *et al.*[44] for washing the potassium silver cobaltinitrite, but if the precipitate is bulky soluble cobaltinitrite may not be completely removed in this way. Truszkowski and Zwemer[39] used only one washing with water for their small quantities of precipitate.

Taylor[23] washed the precipitate five times with 3 ml. portions of 30 per cent. aqueous ethyl alcohol, taking care to stir the precipitate into suspension each time to effect complete removal of the reagent, and this procedure was also followed by Sobel and Kramer.[14] The precipitate is much less soluble in aqueous alcohol and remains coagulated, so that there is less danger of mechanical loss during removal of the washing fluid. Jacobs and Hoffman[13] used water for the first washing when precipitation was made directly from serum, to prevent the precipitation of protein by alcohol, but Hoffman[46] later recommended a saturated solution of potassium sodium cobaltinitrite in 10 per cent. aqueous alcohol for the first washing. Eden[20] used water saturated with potassium sodium cobaltinitrite for the first washing and 70 per cent. alcohol for subsequent washings. Alcohol of this strength has been widely used as a washing fluid.[17,28,34,36,40] Brown *et al.*[28] washed the precipitate only once, taking care to stir it into suspension completely, and, after centrifuging and draining, the last traces of alcohol were removed by drying in an oven at 80° to 85° C. Reed *et al.*[36] stirred the precipitate by blowing air through the jet of a small pointed glass tube.

Lohse[24] used two 10-ml. portions of 48 per cent. ethyl alcohol, followed by absolute alcohol and acetone, and finally dried the precipitate in a vacuum desiccator. Hubbard[19]

recommended that the first washing be made with 25 per cent. aqueous acetone followed by pure acetone. Sideris used water for the first washing, followed by acetone, while Robinson and Putnam[41] washed the potassium silver cobaltinitrite first with water, then with 60 per cent. aqueous acetone and finally with pure acetone in preference to alcohol. Pereira[45] used a similar procedure, and Ismail and Harwood[42] employed 50 per cent. aqueous acetone for the first washing, taking care not to disturb the precipitate, and followed this by three further washings with 80 per cent. acetone during each of which the precipitate was stirred. If the precipitate was determined by titration with ceric sulphate a further washing with ether was made to remove the last trace of acetone. Harris employed a special washing fluid composed of 2 volumes of 95 per cent. ethyl alcohol, 1 volume of ether (peroxide-free) and 2 volumes of water.

Wander[30] used 0·01 *N* nitric acid as washing fluid.

4. Estimation of the precipitate

(i) Volumetrically—

Kramer and Tisdall[4] followed the customary method of the time and dissolved the precipitate in an excess of 0·02 *N* potassium permanganate solution in presence of sulphuric acid by heating the tubes in a boiling water-bath. A measured excess of 0·01 *N* sodium oxalate solution was then added and the titration completed with the original permanganate solution. This method has been widely used and has been recently employed by Kawe,[18] Cantani,[47] and also by Milton, Hoskins and Jackman[48] in their system of micro-analysis for the determination of the mineral contents of foods following wet oxidation with nitric acid and ammonium nitrate. However, most workers agree that dilute permanganate solutions are not stable, especially when hot, and if hydrated manganese dioxide precipitates the results are not accurate. Further, errors arising from the addition of three separate volumes of liquid accumulate in the final result. Dénes[49] determined the excess of permanganate iodimetrically with increased sensitivity, and earlier Leulier, Velluz and Griffon[50] dissolved the precipitate in hot sodium phosphate solution prior to acidification with sulphuric acid, addition of potassium iodide and titration of the liberated iodine with sodium thiosulphate solution. Chapman,[40] in his rapid method for the determination of potassium in leaf ash, dissolved the precipitate in 5 ml. of a hot 30 per cent. solution of hydrated disodium hydrogen phosphate and titrated the nitrite directly with permanganate solution in a test tube immediately after acidifying with dilute sulphuric acid. This was found to be far better than the direct macro-titration procedure of Schueler and Thomas.[50A]

Ceric sulphate offers several advantages in comparison with potassium permanganate. Harris[51] used it for macro-determinations, and Brown *et al.*[28] used it for micro-determinations. Kaye[16] made the titration procedure very sensitive by titrating the excess of ceric sulphate iodimetrically. Klein and Jacobi[52] applied a direct titration with ceric sulphate to the precipitate obtained by the method of Weichselbaum *et al.*[44] They dissolved the precipitate in hot *N* sodium hydroxide solution and, after cooling in ice water, acidified with *N* sulphuric acid and titrated immediately with ceric sulphate, using ferroin as indicator.

Ismail and Harwood also used ceric sulphate but employed erioglaucine as indicator. As an alternative to titration of the nitrite they dissolved the precipitate in dilute nitric acid and determined the silver with 0·01 *N* ammonium thiocyanate, using ferric alum as indicator. This method was not suitable for small amounts of potassium, less than 0·1 mg., but it was also used by Harris, and by Weichselbaum *et al.*,[44] for studies on the composition of the potassium silver cobaltinitrite precipitate.

(ii) Colorimetrically—

(*a*) *Determination of the cobalt in the precipitate*—This has the advantage that the cobalt is a stable constituent compared with the nitrite.

Leberman[53] used the procedure of Kramer and Tisdall[4] for precipitation and washing, and then dissolved the precipitate in hydrochloric acid. The green colour of cobalt chloride was compared with a series of potassium standards treated similarly, but the method was neither very sensitive nor accurate. Yoshimatsu[54] dissolved the precipitate in dilute nitric acid and produced a brown colour with dimethylglyoxime and sodium sulphide for comparison. Blanchetière and Pirlot[55] employed the blue colour of basic cobalt carbonate obtained with sodium bicarbonate solution, and this was also used by Steenkamp[56] for potassium in his

system of micro-analysis for soils. The method has recently been revived for biological materials by Albanese and Wagner.[57]

Breh and Gaebler[9] dissolved the potassium silver cobaltinitrite in dilute nitric acid and produced a green colour by adding a 2 per cent. solution of ammonium thiocyanate in alcohol, but the colour varied in intensity with temperature and concentration of alcohol, and was not directly proportional to the cobalt content. Tomula[58] obtained a blue colour of ammonium cobalt thiocyanate in presence of 75 per cent. acetone, and this was employed by Uhl,[59] and Zinzadze,[60] and also by Lewis and Marmoy[17] who found that the mixture of aqueous ammonium thiocyanate and acetone must be prepared fresh daily for use. Miethke and Finzenhagen[61] used a similar procedure with potassium thiocyanate and acetone for the micro-determination of potassium in milk after precipitation directly from the protein-free filtrate obtained with trichloroacetic acid. Durupt and Schlesinger[62] and also Gerschman and Marenzi[63] dissolved the precipitate in hydrochloric acid and evaporated the solution to dryness before adding aqueous ammonium thiocyanate. The blue colour was then extracted with a mixture of equal volumes of acetone and amyl alcohol.

Jacobs and Hoffman[13] dissolved the precipitate in hot water and added a 1 per cent. solution of choline hydrochloride and a 2 per cent. solution of potassium ferrocyanide. An emerald green colour resulted which reached a maximum intensity in a few minutes and remained stable for several hours. It is well suited to small quantities of cobalt and was used by Eden,[20] Lewis and Marmoy,[17] Morris and Gerdel[35] and Reitemeier.[64]

Sobel and Kramer[14] employed cysteine hydrochloride solution to form an olive green complex, which became bright yellow on addition of hydrogen peroxide. However, this colour reaction does not appear to have been widely used, probably because it is too tedious to produce.

Sideris used nitroso-R-salt (disodium salt of 1-nitroso-2-hydroxy-3 : 6-naphthalene disulphonic acid) for the colorimetric reaction with cobalt, after dissolving the precipitate in 2 *N* sulphuric acid and buffering the solution with sodium acetate. The red colour produced was very stable and has proved very suitable for photometric measurement, as shown by its recent use by Peech, and by Reed *et al.*[36] for the determination of exchangeable potassium in soils.

Pereira[45] dissolved the potassium silver cobaltinitrite in hydrochloric acid and evaporated to dryness on an air-bath. The residue was dissolved in water and treated with a 1 per cent. solution of dimethylglyoxime in ethyl alcohol, followed by a similar solution of benzidine. The colour obtained after suitable dilution was sensitive to small amounts of cobalt.

(*b*) *Determination of nitrite in the precipitate*—This is generally based on the formation of azo-dye derivatives, which, though highly sensitive, are not very stable.

The extremely sensitive Griess method was used by Briggs[65] for the determination of potassium in blood plasma, and has since been adapted by numerous workers.[6,17,23,39,41,43,66,67,68,69]

The cobaltinitrite precipitate is first dissolved in 0·1 *N* sodium hydroxide solution and then treated with solutions of sulphanilic acid and α-naphthylamine in acetic acid to form a red azo dye. Robinson and Putnam[41] observed that the two reagents should be freshly mixed together before being added to the nitrite solution, and that colour formation in presence of 10 per cent. acetic acid followed the Lambert-Beer law over a range of nitrite concentration corresponding to 1 to 10 μg. of potassium.

von Wrangell[70] used an alcoholic solution of indole to form nitroso-indole having a red to violet colour, but this was not considered reliable by Alten *et al.*[66] Tischer[7] used Riegler's naphthol reagent, and Mousserson[71] employed the green colour formed with antipyrine. Rosanov and Kazarinova[72] formed the yellow tropoeolin colour with sulphanilic acid and phenol.

Wander[30] measured, by means of a photo-electric apparatus, the colour produced when the precipitate partially reduced a solution of potassium dichromate in presence of sulphuric acid, a procedure somewhat similar to that of Marjarov and Matzkevitsch[73] who had previously determined the excess of dichromate iodimetrically.

Emmert[74] dissolved the precipitate in dilute sodium hydroxide and then oxidised the nitrite to nitrate with sodium chlorate and fuming sulphuric acid, after which the familiar yellow colour was obtained with phenoldisulphonic acid. This method appears tedious but may be useful where nitrate and potassium determinations are required simultaneously.

(iii) GASOMETRICALLY—

Weichselbaum *et al.*[44] dissolved the precipitate in sodium hydroxide solution and liberated nitrogen by mixing with a solution of urea followed by addition of sulphuric acid. Chambon[75] boiled the precipitate with a 5 per cent. solution of disodium hydrogen phosphate and measured the nitrogen liberated by addition of amido-sulphonic acid in a nitrometer.

(iv) ELECTROLYTICALLY—

Lohse[24] determined the cobalt content of the precipitate by electrolysis.

5. INTERFERENCE

Ammonium is most likely to cause errors in the micro-determination of potassium and every precaution should be taken to avoid contamination of solutions, reagents and glassware. If ammonium is present in the solution to be analysed it is generally removed by boiling with sodium hydroxide.

Kramer and Tisdall[4] ascertained that other organic nitrogenous constituents of blood serum, such as proteins and creatine, did not interfere and assumed that there was insufficient ammonium present to cause error. Subsequent workers have mainly followed this practice, but Truszkowski and Zwemer[38] state that blood samples should be deproteinised as soon as possible after drawing and the filtrates stored in a refrigerator if necessary.

Tischer[7] and Kawe,[18] among others, have reported on interference by other ions when sodium cobaltinitrite is used alone, and interference in presence of silver nitrate has been discussed by Burgess and Kamm.[8] Besides ammonium, rubidium and caesium give precipitates very similar to that given by potassium. Barium and several heavy metals such as mercurous mercury, thallium and lead also form sparingly soluble cobaltinitrites. Ferric iron and aluminium are likely to interfere if present in more than slight traces. The elements commonly found with potassium in biological fluids, and in the extracts of ash derived from biological tissues and agricultural materials are sodium, calcium and magnesium. These may be present in quite large excess without interference.

Nitrate and chloride do not interfere with precipitation by means of sodium cobaltinitrite, but all halogens must be previously removed from solution before potassium is precipitated with silver cobaltinitrite. Sulphate does not interfere unless present in fair excess, but more care needs to be exercised regarding the presence of phosphate.

The possibility seems at times to be overlooked that ionic associations that do not interfere in purely aqueous solution may interfere when alcohol or acetone is used to decrease the solubility of the potassium cobaltinitrite, because the solubility of other salts will also be decreased.

6. SENSITIVITY AND ACCURACY

The original method of Kramer and Tisdall[4] proved suitable for determining from 0·2 to 0·5 mg. of potassium dissolved in 1 ml. of solution when precipitated with sodium cobaltinitrite at room temperature. Taylor[23] obtained an increased sensitivity by precipitating in presence of 30 per cent. ethyl alcohol, and Robinson and Putnam[41] found that with this method 0·006 mg. of potassium per ml. (6 p.p.m.) is the minimum that can be quantitatively recovered at room temperature, although at 4° to 6° C. as little as 0·003 mg. per ml. can be estimated when the azo dye colour reaction is employed.

Breh and Gaebler[9] stated that as little as 0·083 mg. of potassium could be precipitated as the silver cobaltinitrite from 5 ml. of serum filtrate, with an accuracy to 3 to 5 per cent. Robinson and Putnam[41] claimed for their method that 0·01 mg. per ml. was the minimum quantity that could be precipitated at room temperature, and 0·002 mg. per ml. at 0° C. Thus it may be generally assumed that precipitation as potassium silver cobaltinitrite is slightly more sensitive than precipitation with sodium cobaltinitrite in the presence of alcohol.

Whilst Sideris[26] claimed that 0·5 to 15 μg. of potassium could be recovered quantitatively from 0·5 ml. of solution in the presence of alcohol at a low temperature, that is from 1 to 30 p.p.m., Reed *et al.*[36] estimated from 10 to 100 p.p.m. in a volume of 5 ml. by precipitation with sodium cobaltinitrite at 10° C. without alcohol. Both these estimations were based on the nitroso-R-salt colour reaction. As extremes in sensitivity for different purposes we may compare the method of Wander,[30] in which from 1 to 7 mg. of potassium were precipitated from 10 ml. of solution at 20° C., with the method of Wretlind[69] in which from 2 to 20 μg. of potassium were determined in 0·01 to 0·1 ml. of blood by means of the Griess azo-dye colour reaction.

Truszkowski and Zwemer,[38] in their potassium silver cobaltinitrite procedure for 0·2 ml. of blood, added 0·5 ml. of a standard potassium sulphate solution containing 20 p.p.m. to 5 ml. of protein-free filtrate prior to precipitation.

As a general rule for titration procedures such as that of Brown, Robinson and Browning,[28] 0·2 to 1·0 mg. of potassium should be precipitated, although Kaye[16] claimed for his iodimetric procedure that 0·03 to 0·1 mg. of potassium could be determined with an accuracy of 0·5 per cent. For colorimetric procedures based on the cobalt content, amounts of potassium within the range 0·1 to 0·3 mg. are more suitable, though the nitroso-R-salt method of Sideris is for smaller quantities. For the smallest quantities, of the order of 10 μg., the Griess colour reaction has proved most useful.

As for most colorimetric determinations, an accuracy to within ± 2 per cent. is considered generally satisfactory for potassium. Although some investigators claim greater accuracy, there is a lack of adequate statistical treatment of results in most published methods. In the writer's opinion there is a definite need for comparative information on the accuracy and suitability of the best known micro methods for the determination of potassium.

Other methods including sedimentation and turbidimetric procedures

Three other systems have been or are used, each of which is designed to give rapid estimation of potassium without the necessity of separating and washing the precipitate from the mother liquor.

(i) *Measuring the decrease in concentration of reagent following precipitation of the potassium as described by Emmert*[76]—The decrease in concentration of the sodium cobaltinitrite solution may be measured colorimetrically or photometrically. Since relatively large concentrations of sodium cobaltinitrite are required for the complete precipitation of potassium as a compound of constant composition, this decrease will be only very slight for small quantities of potassium and consequently such a method will not be very sensitive or accurate for micro-determinations. The principle has been applied with greater success to the precipitation of potassium with the lithium salt of hexanitro-diphenylamine (dipicrylamine) by Amdur,[77] Williams,[78] Cotton[79] and Lawton.[80]

(ii) *Measuring the volume of precipitate collected in a graduated centrifuge tube*—Hamburger[81] first used this method for the determination of 0·1 mg. of potassium in blood. Haematocrit tubes having a funnel shape with capillary stem were used for precipitation. Arrhenius and Riehm[82] simplified the method for the determination of 2 to 6 mg. of potassium with an accuracy within 2 per cent., and later Riehm[83] used the procedure with a large centrifuge tube holding 35 ml. of liquid, for the direct precipitation of potassium from soil extracts obtained with Krauss solution. More recently Hauser,[84] following the method of Riehm, has described corrections for various errors, together with details of the method. He claimed an accuracy within 2 per cent. for 4 mg. of potassium and 7 to 10 per cent. for 1 mg. when applied to soil extracts. Nowak[85] described the precipitation of amounts of potassium of the order of 50 mg. in a special centrifuge tube having a capillary at the tip. Vladesco[86] determined potassium in the filtrate from milk after precipitation of the proteins with trichloroacetic acid. Such a method is not suited to the determination of very small amounts of potassium because of the mechanical difficulty of securing sedimentation in a capillary tube of sufficiently small diameter to give a readable volume.

(iii) *Turbidimetrically*—Potassium has proved difficult to determine accurately in this way. Bray[87] first introduced a procedure for the rapid determination of replaceable and water-soluble potassium extracted from soils. The cobaltinitrite precipitate was thrown out of solution as a cloud of fine particles by adding alcohol after the aqueous sodium cobaltinitrite reagent. The turbidity of the cloud was measured against standard potassium solutions treated in the same way or by means of a calibrated "line chart." The advent of reliable photo-electric measuring instruments has provided a new stimulus to turbidimetric procedures, but a full discussion of the subject is not included here since it will be dealt with in a subsequent paper.

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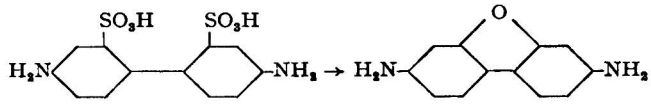
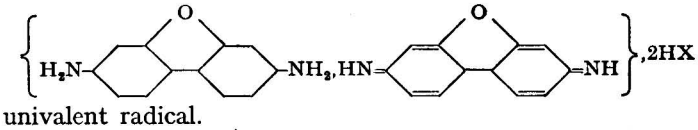
2:7-Diaminodiphenylene Oxide as a Reagent in Analysis

BY N. M. CULLINANE AND S. J. CHARD

2 : 7-DIAMINODIPHENYLENE oxide can be used with advantage in place of benzidine for the detection of radicals with oxidising properties. It may be prepared by treatment of benzidine 2 : 2′-disulphonic acid with sodium hydroxide[1] and crystallises from hot water in colourless needles, m.p. 151° C.



The blue coloration or precipitate produced in the tests is presumably due to the conversion of the amine, as occurs also with benzidine,[2] into an oxidation product of quinonoid structure, consisting of a molecular compound of the base with the corresponding di-imine, together with two molecules of an acid:



where X is a univalent radical.

Reagent—To prepare the reagent dissolve 0·375 g. of the amine by warming it with a mixture of 5 ml. of glacial acetic acid and 45 ml. of water.

Parallel tests were carried out with diaminodiphenylene oxide and benzidine (the latter reagent was made up in the same way) under identical conditions either in a small tube or on a white tile; the colorations are best observed against a white background. A blank experiment was also performed with each test. A drop or two only of the test solution is needed. The presence of a large excess of free mineral acid destroys the colour or inhibits its formation; hence the most satisfactory results are obtained when the test solution is neutral or contains a slight excess of acetic acid.

The reactions can be carried out in the form of "spot" tests; the usual procedure consists of adding a drop of the reagent to a drop of the test solution. A blue coloration is produced, but if the solution is concentrated a blue precipitate may be formed.

Results given by various ions are listed below and the corresponding results obtained with benzidine are indicated. The limits of identification refer to 1 drop of test solution.

Silver—Limit of identification 0·64 μg. of Ag (benzidine 63·5 μg.).

Ferric iron—Limit of identification 0·06 μg. of Fe (benzidine 0·12 μg.).

Platinum—Platinum salts must be present in the platinic state for this test. Oxidation is carried out where necessary by means of *aqua regia*, but most of the acid must be subsequently removed by evaporating almost to dryness and then diluting with water. Limit of identification 0·13 μg. of Pt (benzidine 12·5 μg.).

Gold—The test is given by auric salts, oxidation of aurous compounds being effected by a similar procedure to that adopted for platinous salts and excess of mineral acid being afterwards removed by evaporation. Limit of identification about 1 μg. of Au, the same as with benzidine.

Thallium—Thallic salts give a blue colour. Thallous salts may however be oxidised in the same way as aurous salts. The diaminodiphenylene oxide produces a deeper blue than benzidine in concentrated solutions, but the limit of identification, 0·04 μg. of Tl, is only slightly less than when benzidine is used.

Cerium—With ceric salts the blue colour is observed on allowing the solution to stand for a short time, but the colour may fade later. Limit of identification 0·09 μg. of Ce (benzidine 0·18 μg.).

Chromate—Limit of identification 0·003 μg. of Cr (benzidine 0·27 μg.).

Ferricyanide—Limit of identification 0·1 μg. of $Fe(CN)_6$ (benzidine 1·0 μg.). *Ferrocyanides* give a white precipitate but no coloration.

Periodate—The solution should be allowed to stand for about I minute. Limit of identification 0·23 μg. of IO_4 (benzidine 0·5 μg.).

Persulphate—Limit of identification 0·01 μg. of S_2O_8 (benzidine 0·1 μg.). The colour deepens on standing.

Vanadate—The solution should be allowed to stand for about 30 seconds. Limit of identification 0·05 μg. of VO_3 (benzidine 1·8 μg.). The sensitivity of this test is due not only to the production of the blue quinonoid compound from the amine but also to the formation of the blue vanadyl salt from the vanadate.

Bismuthate—Limit of identification 0·15 μg. of $NaBiO_3$, approximately the same as with benzidine.

Iodine—Limit of identification 0·035 μg. of I, about the same as with benzidine. Chlorine water and bromine water give similar results.

Lead—Add to a solution of a lead salt two drops of ammoniacal (2 *N* NH_4OH) hydrogen peroxide (3 per cent.), followed by a drop of the reagent. Limit of identification 0·65 μg. of Pb (the test with benzidine is slightly less sensitive).

Copper—To a drop of the copper solution add a drop of the reagent followed by a drop of a saturated solution of potassium bromide. Limit of identification 0·025 μg. of Cu (benzidine 0·25 μg.).

In this reaction the cupric halide appears to decompose into the cuprous salt and free halogen, which is thus responsible for the oxidation of the amine.

Manganese—To a drop of the test solution add a drop of 0·05 per cent. sodium hydroxide solution followed by a drop of the reagent. The addition of the alkali causes the formation of manganous hydroxide which is oxidised by aerial oxygen to a hydrated form of manganese dioxide. The test may also be applied to a suspension of manganese dioxide in water. Limit of identification 0·01 μg. of Mn (the test with benzidine is slightly less sensitive).

Cobalt—To a drop of the test solution add 2 drops of 30 per cent. hydrogen peroxide and 3 drops of 0·5 *N* sodium hydroxide. Shake for a few minutes, then warm with further shaking until the effervescence subsides. Next add 3 drops of 2 *N* acetic acid, warm to dissolve the precipitate, then cool, add the reagent and allow to stand. The blue colour which appears first deepens on standing and afterwards fades. Limit of identification 19·2 μg. of Co (benzidine 38·5 μg.). Nickel salts give no coloration under these conditions.

In this reaction the oxidation of the amine is caused by the cobaltic salt, which is reduced in the process to the cobaltous state.

Orthophosphate—Heat 1 drop of the solution cautiously with 1 drop of ammonium molybdate solution until the liquid just begins to bubble. Cool. Add 1 drop of the reagent and 1 drop of a saturated solution of sodium acetate. A blank experiment is especially advisable in this test. Limit of identification 0·002 μg. of PO_4 (benzidine 0·004 μg.). The molybdate solution is made by dissolving 5 g. of ammonium molybdate in 100 ml. of cold water, and pouring the solution into 35 ml. of nitric acid of density 1·2.

Here the oxidation appears to be caused by the fact that phosphomolybdic acid or its ammonium salt is capable of oxidising the amine. Two blue products are thus formed—the oxidation product of the amine and the reduction product of molybdic acid ("molybdenum blue"). Under the conditions specified arsenates do not yield a coloration.

Silicate—The procedure followed is exactly the same as in the phosphate test and a blank experiment is advisable. The oxidation is apparently due to the silicomolybdic acid, which is itself reduced to molybdenum blue. The test with diaminodiphenylene oxide is five times as sensitive as that with benzidine.

Cyanide—Moisten some absorbent paper with a freshly made mixture of equal parts of normal cupric acetate (47·5 ml. of a saturated solution diluted to 100 ml.) and the reagent. Add 2 *N* sulphuric acid to the cyanide solution in a test tube, fix the test paper at once over the mouth of the tube and warm. The hydrogen cyanide liberated gives a blue colour. Limit of identification 0·4 μg. of CN (benzidine 1·6 μg.).

In this test the oxidation appears to be caused by nascent oxygen, produced as indicated in the following equations:

$$Cu(CH_3COO)_2 + 2HCN = Cu(CN)_2 + 2CH_3COOH$$
$$2Cu(CN)_2 + H_2O = Cu_2(CN)_2 + 2HCN + O$$

Blood—To 1 drop of aqueous blood solution add 1 drop of the reagent followed by 1 drop of 3 per cent. hydrogen peroxide and allow to stand for 2 or 3 minutes. A blue colour is observed. Limit of identification, 1 part of blood in 200,000 parts of water (benzidine 1 in 40,000).

Milk—The blue colour obtained when the diaminodiphenylene oxide solution is added to milk weakly acidified by acetic acid and then treated with 3 per cent. hydrogen peroxide is much more pronounced than when benzidine is used as the reagent. Boiled milk gives no coloration.

In general the procedure adopted for the removal of interfering radicals is the same as with benzidine.[3]

SUMMARY—

The use of 2 : 7-diaminodiphenylene oxide for the detection of oxidising radicals is described. The analysis may be carried out in the form of "spot tests"; in no instance was the new reagent found to be less sensitive than benzidine and in many tests its sensitivity is much greater.

As well as blood and milk, the following radicals may be detected by this reagent: silver, ferric iron, platinum, gold, thallium, cerium, lead, copper, manganese, cobalt, iodine, chromate, ferricyanide, periodate, persulphate, vanadate, bismuthate, orthophosphate, silicate and cyanide.

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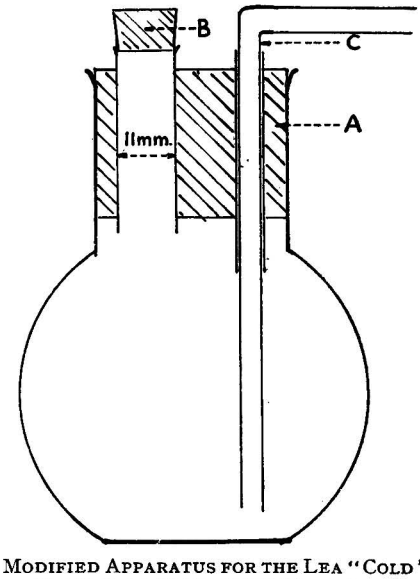
Notes

IODIMETRIC DETERMINATION OF PEROXIDES
MODIFIED APPARATUS FOR THE LEA "COLD" METHOD OF ESTIMATION OF PEROXIDE OXYGEN

IN a recent paper on iodimetric methods of estimating peroxidic oxygen,[1] Skellon and Wills refer to the use of "suitable modified apparatus" for the Lea "cold" method of estimating peroxidic oxygen.[2] The simple apparatus illustrated in the accompanying sketch was designed by one of us (M. N. T.) and has given satisfactory results when working with Lea's "cold" de-aeration method in this laboratory. The apparatus consists of a wide mouthed flat-bottom flask (capacity 150 to 200 ml.) fitted with a rubber bung (A) bored to take a short piece of glass tubing (11 mm. internal diameter) on one side, and a piece of ordinary glass tubing (6 mm. internal diameter) on the other. The wide tube is closed by a rubber stopper (B), and a right-angle delivery tube (C) passes easily through the narrow tube. The procedure recommended by Lea (*loc. cit.*) needs but slight modification for use with this apparatus.



MODIFIED APPARATUS FOR THE LEA "COLD" METHOD OF ESTIMATING PEROXIDES

Twenty ml. of the solvent (glacial acetic acid - chloroform, 3 : 2) are pipetted into a flask. A slow stream of pure nitrogen is passed through the solvent with the bung (A) in place and the stopper (B) loosely fitted. After 5 minutes de-aeration, 1·2 ml. saturated potassium iodide solution is added and de-aeration is continued for a further 10 minutes. The weighed sample, in a small glass container (piece of cut-down specimen tube) is quickly added *via* the wide tube, and the stopper (B) is replaced.

The tube (C) is removed with gas still passing, the resultant outlet being closed with a "policeman" or piece of stopped rubber tube, and the stopper (B) is pushed home tightly. The contents are mixed by

swirling and the flask is placed in the dark for 1 hour, 50 ml. of distilled water are added and the mixture is titrated with suitable accurately standardised sodium thiosulphate solution using starch as indicator towards the end of the reaction. As stated by Lea (*Ibid.*) the blank titration is reduced, usually to zero, by addition of the iodide solution 5 minutes after the beginning of de-aeration.

Typical values obtained in this laboratory (*a*) by the Lea "cold" method with the modified apparatus as above, and (*b*) by the Skellon - Wills method,[1] are appended for comparison:

Sample	Method	
	Lea (1946) (modified apparatus)	Skellon - Wills (1947)
	(Mg.-equivs. per kg.)	
"Blown" castor oil	28·7	27·5
(commercial specimen)	28·7	28·7
Groundnut oil (blown 2 hr. at 60° C.)	7·1	7·5
	(Per cent. active oxygen)	
Benzoyl peroxide	6·60	6·59

Concordant results are obtained by these methods provided that when cylinder nitrogen is used precautions are taken to ensure that the gas is free from oxygen.

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J. H. SKELLON
M. N. THURSTON

CHELSEA POLYTECHNIC

October, 1947

CONGO RED PAPER OF HIGH SENSITIVITY

A CONGO red indicator paper of particularly high sensitivity was developed some years ago in the laboratories of the War Department Chemist (now Chemical Inspection Department, Ministry of Supply), and as it has proved to be of considerable utility in certain directions, details of the production of the paper are presented in this note.

The sensitivity of the paper may be as high as 1 in 200,000, or even higher, *i.e.*, a colour change from red to blue which is reasonably non-transient (*e.g.*, permanent for some 10 seconds) is given when a drop of sulphuric acid of concentration 5×10^{-6} w/w is applied to a piece of the paper held horizontally. The colour develops as a definite, though maybe faint, blue or blue-black fringe round the periphery of the spot where the drop was originally applied to the paper.

In order to prepare the paper, a solution of 6 g. of congo red in 3 litres of boiled distilled water is prepared and 0·1 *N* hydrochloric acid is carefully added (*e.g.*, from a burette) until appreciable darkening occurs. The formation of any noticeable precipitate must be avoided; about 3 ml. of the acid will often suffice to bring about the darkening. A loosely coiled sheet of pure white absorbent paper, *e.g.*, Ford "J" filter paper, is then immersed on edge in the liquid and left undisturbed for 5 minutes, after which it is removed and hung up in the air to dry, surplus water being allowed to drain off without interference. When thoroughly dried the sheet is consolidated (for the purpose of lowering its rate of absorbing applied liquid) by passage to and fro between rollers with progressively increasing applied pressure, this operation being continued until the paper acquires a hard surface unaccompanied by any undue reduction in strength. The aim is to secure a drop absorption time of the order of, say, a second or so. The resulting rolled strips have a slight polish on both sides, their thickness being about half the original.

The quantities given above suffice for impregnating about ten 20 in. × 6 in. sheets of paper; the impregnation is, of course, accompanied by progressive removal of the dye from solution (so that, whilst each sheet will be uniformly coloured, consecutive sheets may show some reduction in colour intensity) and thorough stirring of the liquid before immersion of each fresh sheet is necessary.

Owing to the high sensitivity of the paper, unnecessary handling is to be avoided, and it should be touched only with well-washed hands.

The usefulness of the paper in the examinations of very small volumes of liquids (extracts) and of coloured solutions for acidity will be readily appreciated, as indeed will be the general utility of the paper in laboratory practice.

Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this note.

CHEMICAL INSPECTION DEPARTMENT
MINISTRY OF SUPPLY

THE CLOISTERS
BICKLEY, KENT

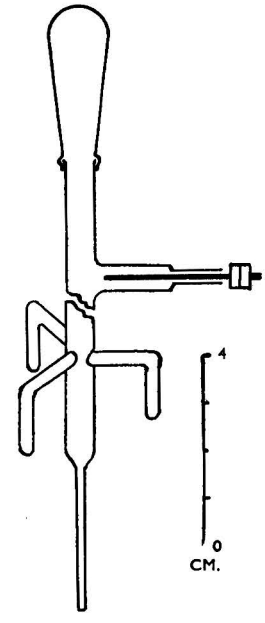
May, 1947

AN AUXILIARY ELECTRODE FOR CONTROL OF MICROCHEMICAL ELECTRO-DEPOSITION

CONTROL of cathode potential for quantitative deposition on a microchemical scale has not been widely used. Apart from the early unsatisfactory attempts by Reisenfeld and Möller[1] at control by means of a calomel electrode with a capillary side-arm, the only methods described are those carried out in the apparatus devised by the late Dr. H. J. S. Sand in conjunction with the present author.[2,3,4,5]

In these methods measurement and control of the anode to cathode voltage of the analysis cell gives adequate control of the cathode potential. Essential factors in the method are the use of a large anode surrounding, and separated by only a short distance from, the cathode and considerable concentrations of a strong anodic depolariser in the electrolyte. Although these analytical methods are completely satisfactory it is considered desirable to be able to quote cathode potentials with reference to a standard half cell and therefore to enable analysts to use apparatus differing somewhat in design and electrolytes varying in composition from the original.

The auxiliary electrode shown partly in section in the Fig. is satisfactory for this purpose. It is made in the form of a teat pipette with a frame to fit over the tubes used in the electrolytic apparatus previously described.[2] The capillary is made of sufficient length to terminate about half-way down the cathode. The electrode is of pure silver wire and is fitted into the glass by means of thin rubber tubing or resin cement. It is coated with silver chloride by electro-deposition in hydrochloric acid. Decinormal potassium chloride is used in the pipette, giving a potential on the normal hydrogen scale of +0·141 volt. The half cell is kept full of potassium chloride and is placed in the analytical solution just before electrolysis. When the metal is almost exhausted a drop or two of the solution is expelled and traces of metal ions that have diffused into the capillary are then deposited.



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SIR JOHN CASS TECHNICAL INSTITUTE
JEWRY STREET, LONDON, E.C.3

A. J. LINDSEY
February, 1947

Ministry of Health

TRANSFER OF FOOD FUNCTIONS

MANY of the functions at present exercised by the Ministry of Health under the Food and Drugs Acts will be transferred to the Ministry of Food on March 1st, 1948. This is in consequence of an Order in Council entitled the Transfer of Functions (Food and Drugs) Order, 1948, made under the Ministers of the Crown (Transfer of Functions) Act, 1946.

Broadly, the effect of the transfer will be that the Ministry of Food will be concerned with the composition, description and inspection of foods, while the Ministry of Health will continue to be concerned in measures for dealing with infected food or food poisoning.

All these changes affect solely departmental functions. The duties of enforcement and execution placed by the Acts and Regulations upon local authorities are not in any way affected.

The Minister of Health, Mr. Aneurin Bevan, has, in a circular, given local authorities details of the new division of powers. This sets out the duties of the two Ministries as follows:—

Ministry of Food—It will become the department concerned with the Preservatives and the Condensed and Dried Milk Regulations and other matters relating to the composition and description of food; with the Meat and Imported Food Regulations and other matters relating to food inspection and food hygiene generally; and with the Milk and Dairies and Milk (Special Designations) Regulations so far as they concern dealers and distributors. The Ministry will also become responsible for approval of terms of appointment etc., of public analysts.

Ministry of Health—It will remain the department primarily concerned with drugs and with matters directly related to the protection of the public from infected food—for example, food poisoning and regulations relating to ice-cream, shell fish and the stoppage of the sale of infected milk. Until the Food and

Drugs (Milk and Dairies) Act, 1944, comes into operation, the Ministry of Health will also continue to issue licences to local authorities who are producers of designated milk as well as deal with appeals from producers against refusal, suspension or revocation of producers' licences.

All Regulations under the Food and Drugs Act, 1938, which are at present made by the Minister of Health, will in future be made jointly by the Ministers of Food and Health. Certain changes are also being made regarding functions under the Act exercised now by the Minister of Agriculture and Fisheries, and certain Regulations relating to milk, for example, will be made jointly by the Ministers of Agriculture, Food and Health.

None of the changes involved in this transfer of functions affects the position in Scotland.

P.N. No. 19, Feb. 20th, 1948.

Order in Council

STATUTORY INSTRUMENT*

1948—No. 107. The Transfer of Functions (Food and Drugs) Order, 1948.

This Order transfers to the Minister of Health acting jointly with the Minister of Food the powers of the Minister of Health under the Food and Drugs Act, 1938.

The Order provides that the Minister of Agriculture and Fisheries be joined in the powers of making regulations relating to milk and dairies and to the use of special designations for raw milk when the Food and Drugs (Milk and Dairies) Act, 1944, *comes into operation.*

Certain other administrative functions at present exercisable under the Food and Drugs Act, 1938, *by the Minister of Health or, in some cases, by the Minister of Agriculture and Fisheries are also transferred to the Minister of Food by the Order. The duties of enforcement and execution placed by the Act upon local authorities are not affected by the Order.* (*See Notice above.*)

Ministry of Supply

IDENTIFICATION AND ESTIMATION OF NATURAL AND SYNTHETIC RUBBERS. Users' Memorandum No. U.9A. Pp. 37. The Services Rubber Investigations. 1947. Gratis.

The new edition of this booklet describes research carried out by Imperial Chemical Industries Ltd. (Dyestuffs Division) on behalf of the Director of Chemical Research and Development of the Ministry of Supply and the Director of Aeronautical and Engineering Research of the Admiralty. Memorandum No. U.9, which was published in 1944 under the auspices of the same authorities together with the Director of Scientific Research of the Ministry of Aircraft Production and the Research Association of British Rubber Manufacturers, was similar, but several new methods are described in the revised publication, some of them for the first time.

A list of fifty-three trade names of synthetic elastomers is given with their specific gravities and indications of their compositions; this is followed by a brief account of those properties of the main types that are of greatest diagnostic value, and a table which outlines a scheme of systematic qualitative analysis. Detailed instructions are given for carrying out colour tests for natural rubber and for styrene co-polymers, also specific reactions of butyl rubber and poly-isobutene and of thioplasts. Further diagnosis consists of the determination of the swellings of the rubber in aniline, benzene and petroleum spirit, as well as its rapidity of reaction with mixed nitric and sulphuric acids; these methods are described at length. Practical details are given for the following quantitative methods: the determination of natural rubber by oxidation to acetic acid, the estimation of rubber hydrocarbons by determination of the degree of unsaturation, the determination of poly-isobutene and the estimation of thioplasts by determination of the sulphur extractable by sodium sulphite solution. The booklet concludes with a review of recent developments which might find future application in routine examinations. G. H. W.

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* Italics signify changed wording.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Quaternary Ammonium Compounds in Foods. J. B. Wilson (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 311–327)—For a number of years quaternary ammonium compounds have been used extensively as antiseptics and germicides. More recently they have been used as preservatives in food and in the cleansing of milk cans and other dairy equipment. Two methods are recommended for these compounds; the ferricyanide method applicable to commercial strength preservatives and dilutions down to about 0·5 per cent. and the bromophenol blue method applicable to dilutions as low as 1 or 2 mg. per 100 ml.

THE FERRICYANIDE METHOD (for commercial preparations only)—The quaternary base is precipitated as a salt of ferricyanic acid and the excess of ferricyanide is titrated iodimetrically. To determine the approximate amount of quaternary ammonium salt, pipette 1 ml. of buffer solution (130 g. of sodium acetate dissolved in water, mixed with 42 ml. of acetic acid and made up to 500 ml.), 2 ml. of ferricyanide solution (6·6 g. of potassium ferricyanide per litre) and 20 ml. of water into each of four, small conical flasks. Pipette into them 0·5, 1·0, 2·0, and 4·0 ml. of sample, respectively, mix, and filter. To each filtrate add 2 ml. of the sample. If no precipitate appears in any of the liquids the sample contains 8·4 per cent. or more, a precipitate in the first flask only indicates 5 per cent., in the first and second, 2·5 per cent., in the first three, 1·25 per cent., and in all four, 1 per cent. or less. These figures refer to solutions of an alkyldimethylbenzylammonium chloride of molecular weight 357.

Procedure—Pipette into a 100-ml. Kohlrausch flask an aliquot of the sample containing 0·5 per cent. of the quaternary salt, dilute to 50 ml. if necessary, add 5 ml. of the buffer solution, and mix. Add from a pipette 30 ml. of the ferricyanide solution, rotate the flask during the addition, dilute to the mark with water, and mix. After 30 min., filter, discard the first 10 or 15 ml. of filtrate, pipette 50 ml. into a 500-ml. conical flask, add 100 ml. of water, 1 to 2 g. of potassium iodide and, after solution of the salt, 10 ml. of diluted hydrochloric acid (1 + 1), mix, and allow to stand for 2 min. Add 10 ml. of zinc sulphate solution (20 g. of the hydrated salt in 180 ml. of water) and titrate with 0·02 *N* sodium thiosulphate, starch being used as indicator. Make a blank determination. Calculate the content of quaternary ammonium salt from the difference between the two titrations: 1 ml. of 0·02 *N* sodium thiosulphate ≡ 0·02142 g. of alkyldimethylbenzylammonium chloride of molecular weight 357.

THE BROMOPHENOL BLUE METHOD—This depends upon the reaction of the quaternary nitrogen in weakly alkaline solution with bromophenol blue to form a product soluble in ethylene chloride, in which neither reactant is soluble.

Procedure for fruit juices—Centrifuge 100 ml. of fruit juice containing from 1 to 10 mg. of a quaternary ammonium salt, at about 1780 r.p.m. for 30 min., and decant. Reserve the supernatant liquid (*A*). Add 100 ml. of alcohol to the residue, shake thoroughly for 2 min., allow to stand for 10 min., centrifuge for 20 to 30 min., and decant. Repeat this procedure twice. Unite the alcoholic solutions in a steam distillation flask fitted with a spray tube reaching nearly to the bottom of the flask, pouring them through a filter if the pulp rises during decantation. Add 20 to 25 mg. of bromophenol blue, 15 ml. of diluted hydrochloric acid (1 + 1), about 250 ml. of water, and mix. Insert the spray tube, close the steam inlet, and distil rapidly until at least 250 ml. of distillate have been collected. Open the steam inlet and steam-distil rapidly until a litre has been collected. Certain interfering substances are thus removed. Cool the residue in the flask and wash it with three 100-ml. portions of light petroleum. The precipitate that forms during distillation contains quaternary ammonium compounds and must be preserved for the final extraction with ethylene chloride. Run the aqueous layer into the distilling flask, filter the petroleum layer, and reserve the filter. After the third extraction, measure 200 ml. of ethylene chloride into the separating funnel and run it thence into the flask. Shake the stoppered flask gently for 2 min. Pour the mixture into the separating funnel, rinsing the flask two or three times with water, and again shake for 2 or 3 min. Draw off the lower ethylene chloride layer into a flask through the filter paper reserved from filtering the petroleum extracts. Transfer the supernatant liquid *A* to a second distilling flask, add bromophenol blue, diluted hydrochloric acid (1 + 1) and water as before and steam-distil, collecting a litre of distillate. Wash the residue with light petroleum and extract with ethylene chloride as before, but use 100 ml. of each solvent.

Preparation of the standard curve—Standardise a 1 per cent. solution of the quaternary ammonium compound to be determined by the ferricyanide method, and ascertain the maximum and minimum concentrations of the compound that produce in 50 ml. of ethylene chloride colours of a density suitable for the colorimeter to be employed. Prepare a set of three or more standards containing, in 50 ml., amounts of the quaternary compound covering this range. Pipette 50 ml. of each standard into a series of separating funnels, to each add 3 ml. of an aqueous bromophenol blue solution containing 40 mg. per 100 ml., and 1 ml. of hydrochloric acid, and 50 ml. of ethylene chloride, and shake for 2 or 3 min. Run the clear lower layers first into a series of separating funnels containing 10 ml. of 1 per cent. sodium carbonate solution and, after shaking for 2 or 3 min., separate the clear ethylene chloride layers into glass-stoppered flasks containing 1 to 2 g. of granular, anhydrous sodium sulphate. After 30 min., measure the colour in a photometer with a

filter transmitting at 610 mμ. If the instrument indicates per cent. transmission, convert to colour density before plotting the readings against concentration.

Measurement of the colour of the sample extract—Treat each ethylene chloride extract separately as follows. Shake 30 to 50 ml. with 10 ml. of sodium carbonate solution, run the clear lower layer into a glass-stoppered flask containing 1 to 2 g. of anhydrous sodium sulphate and, after 30 min., read the colour in a suitable cell with a filter transmitting at 610 mμ. If the colour is too deep for accurate measurement, dilute an aliquot portion of the liquid with ethylene chloride and repeat the treatment with sodium carbonate and sodium sulphate. Calculate the amount of quaternary ammonium compound in mg. per 100 ml. of fruit juice by means of the standard curve.

Shorter procedure for fruit juices—Centrifuge 20 ml. of the juice for 15 min., decant the liquid into a 500-ml. steam-distillation flask, add 10 mg. of bromophenol blue, 2 ml. of diluted hydrochloric acid (1 + 1), and 80 to 100 ml. of water. Steam-distil, collecting about 100 ml. of distillate, and wash the cooled residue in the flask with one 40-ml. and two 30-ml. portions of light petroleum. Pipette 50 ml. of ethylene chloride into the separating funnel, shake for 3 or 4 min., and shake the clear lower layer in a second funnel with 10 ml. of 1 per cent. sodium carbonate solution for 3 or 4 min. If the lower layer is blue, a quaternary base is present. Judge from the colour whether or not it is suitable for reading in the photometer. If so, treat the lower layer with granular, anhydrous sodium sulphate, and after 30 min., read the colour as before. If the colour is too deep, acidify the contents of the second separating funnel with 1 or 2 ml. of diluted hydrochloric acid (1 + 1), shake until the contents become yellow, and replace the liquid in the first separator. Add a second 50-ml. portion of ethylene chloride, shake for 3 to 4 min. and, when the lower layer is clear, measure the colour as before. Meanwhile add 30 ml. of alcohol to the pulp in the centrifuge tube, mix, allow to stand for 10 min., centrifuge for 5 min. and decant into the steam-distilling flask. Make two more extractions of the pulp with 15 to 20 ml. of alcohol and treat the combined extracts as already described, beginning "Add 10 mg. of bromophenol blue . . ." but collect 200 ml. ofdistillate.

Bottled beverages containing fruit juice—Filter 50 ml. of the sample and dilute to 100 ml. with water (solution *A*). Extract the filter with small portions of alcohol until no more colour is extracted. Steam-distil the combined alcoholic extracts with 10 mg. of bromophenol blue, 2 ml. of diluted hydrochloric acid (1 + 1) and 100 ml. of water, collecting a volume of distillate at least 100 ml. greater than the volume of alcohol in the extract. Proceed by the shorter method beginning "and wash the cooled residue. . . ." Pipette a suitable aliquot of solution *A* in a separating funnel with 3 ml. of bromophenol blue solution and 1 ml. of diluted hydrochloric acid (1 + 1), and proceed as in the shorter method, beginning "Pipette 50 ml. of ethylene chloride. . . ."

Mayonnaise, salad dressing and salad spread—Mix 10 g. of the sample in a 250-ml. beaker with 100 ml. of acetone (or enough to dissolve separated oil) and knead any separated gummy matter until all oil is pressed out into solution. Filter with suction, wash two or three times with 20-ml. portions of acetone, transfer the combined acetone extracts to a separating funnel, rinsing out the suction flask with an equal volume of water. Rinse the flask with light petroleum, adding the rinsings to the acetone extracts and, after shaking and separation of the acetone - water layer, discard the petroleum layer and repeat the procedure with two portions of light petroleum. Evaporate the acetone - water layer on the steam-bath in a current of air until the volume is less than 75 ml. and the odour of acetone disappears. Dilute the cooled liquid to 100 ml., treat 25 ml. in a separating funnel with 3 ml. of bromophenol blue solution and 1 ml. of diluted hydrochloric acid (1 + 1). Proceed as in the shorter method for fruit juices, beginning "Pipette 50 ml. of ethylene chloride. . . ."

Milk—Agitate 25 ml. of milk in a 250-ml. flask with 10 mg. of bromophenol blue until the indicator has dissolved. Gradually add 50 ml. of acetone, then drop by drop, diluted hydrochloric acid (1 + 1) until a bright yellow colour appears and then 0·2 to 0·3 ml. more. Gradually, with mixing, dilute to the mark with acetone and, after 30 min., filter. To 200 ml. of the filtrate add an equal volume of water and wash the mixture with three 50-ml. portions of light petroleum. Pour each petroleum washing through a filter paper and reserve the paper for filtration of the ethylene chloride extract. Reduce the volume of the acetone - water solution to 100 ml. or less, cool, transfer to a separating funnel and proceed as in the shorter method for fruit juices beginning "Pipette 50 ml. of ethylene chloride, . . ." passing the ethylene chloride extract through the filter paper before developing the blue colour.

Beer—Steam-distil 100 ml. of decarbonated beer with 10 mg. of bromophenol blue and 2 ml. of diluted hydrochloric acid (1 + 1), collecting about 200 ml. of distillate. Cool the residue and wash it with a 100-ml. and a 50-ml. portion of light petroleum. Proceed as in the shorter method for fruit juices, beginning "Pipette 50 ml. of ethylene chloride. . . ."

Table syrup—Dilute 20 g. of the sample to 100 ml. and treat an aliquot in a separating funnel with 3 ml. of bromophenol blue solution, 1 ml. of diluted hydrochloric acid (1 + 1), and proceed as in the shorter method for fruit juices.

Dog biscuits—Mix 1 to 5 g. of the ground sample with 10 mg. of bromophenol blue and 30 ml. of acetone, stir occasionally during 10 min., and then centrifuge for 7 min. Decant the liquid into a separating funnel and repeat the extraction with two 20-ml. portions of acetone. Dilute the combined extracts with 100 ml. of water, add 5 ml. of diluted hydrochloric acid (1 +1) and wash with three 50-ml. portions of light petroleum. Remove the acetone and reduce the volume to 50 ml. by evaporation, transfer the liquid to a separating

funnel, and proceed as in the shorter method for fruit juices.

Pickles and relishes—Drain, slice and grind pickles and drain relishes, squeezing out as much of the liquid phase as possible. Treat 100 g. of the solid phase in a 100-ml. beaker three times as follows. Add 10 mg. of bromophenol blue, 100 ml. of acetone and 1 ml. of diluted hydrochloric acid (1 + 1), stir occasionally during 1 hr., and decant through a small cotton-wool plug. Mix the combined extracts with an equal volume of water and extract with 100-ml. portions of light petroleum until no more green colour is extracted. Remove the acetone from the acetone - water mixture and reduce its volume to about 100 ml. by evaporation, transfer to a separating funnel, and proceed as in the shorter method for fruit juices. Transfer the liquid phase to a steam-distillation flask and proceed as in the shorter method for fruit juices, beginning "add 10 mg. of bromophenol blue. . . ."

The blue complex with bromophenol blue has been formed with a considerable number of quaternary compounds. A strong colour was always obtained when a long side-chain such as lauryl or a cetyl group was attached to the nitrogen atom. When no such group is present, as in trimethylbenzylammonium salt, no colour appears in the ethylene chloride layer. With triethylbenzylammonium chloride, the colour of the ethylene chloride extract has only one-fiftieth of the intensity of that produced by the same amount of lauryldimethylbenzylammonium chloride. The relation between depth of colour and number of carbon atoms has not yet been established, and no means has yet been found of identifying the quaternary base when only small amounts of the salt are present. A. O. JONES

Determination of Quaternary Ammonium Compounds in Fruit Juices. T. H. Harris (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 310–311)—When the procedure described in the preceding abstract was being developed, a satisfactory alternative method for separating the quaternary compound from its inhibitors was devised. All the quaternary compound is associated with the insoluble pigment and pulp, and thus only one analysis is required.

Procedure—If the juice contains little pigment and pulp, add 100 ml. of orange juice (free from quaternary ammonium salts) to it, shake, and allow it to stand for 30 min. Filter 50 ml. of the juice with suction through an 11-cm. Buchner funnel, using two Whatman 41 filter papers and pouring the filtrate back until it is bright. When filtration is almost complete, wash the residue with a few millilitres of water, and maintain the suction until the residue contains no excess of liquid. Cut the filter papers and the residue into small pieces, place them in a Soxhlet extractor without a thimble, plugging the outlet lightly with cotton wool, and extract with 95 per cent. alcohol until all the pigment has been removed (about 1 hr.).

Concentrate the extract to about 35 ml., transfer it completely to a 100-ml. flask, add 5 ml. of concentrated hydrochloric acid, and heat the mixture under refluxing conditions for 1 hr. Transfer the hydrolysed material to a 1-litre beaker, add 200 ml. of water, and evaporate to about 100 ml. Transfer this solution to a separating funnel, add 40 ml. of alcohol, and extract the solution with five, 25-ml. portions of light petroleum (b.p. 33° to 38° C.). Extract the combined extracts once with 25 ml. of 30 per cent. alcohol, combine this with the first alcoholic residual layer from the light petroleum extracts, and evaporate the total alcoholic solution (which now contains the quaternary salts and is almost free from colour) on a steam-bath until the alcohol has been removed, cool, and dilute to 250 ml. Extract a 100-ml. aliquot with 25 ml. of ethyl ether and wash the ether layer, without shaking, with a few millilitres of water. Heat the combined aqueous layers on the steam-bath until the ether has been removed, cool, add bromophenol blue solution, make alkaline with solid sodium carbonate, extract the quaternary compound with ethylene chloride, and measure as described in the preceding abstract. A. O. JONES

Volumetric Determination of Nicotine as Picrate. N. S. Drozdov and N. P. Materanskaja (*J. Anal. Chem. Russ.*, 1947, **2**, 17–20)—Nicotine and other bases that form insoluble picrates may be determined by titrating the excess of picric acid with 0·001 *N* methylene blue. Picric acid is more selective than other reagents for nicotine (*e.g.*, silicotungstic acid is inapplicable in presence of other organic bases, alkaloids, proteins, and amino-acids), but the normal method of titrating the picrate itself is inaccurate (*cf.*, Staikoff, *Z. Unters. Lebensm.*, 1942, **84**, 492).

Procedure—For determination of nicotine in plant material, treat the sample with alkali, and distil with steam, testing for completion of distillation with silicotungstic acid. If significant amounts of other alkaloids are likely to be present use stepwise distillation (Koenig, *Handbuch der Lebensmittel. Chemie*, 1934, **6**, 296; Dawson, *Proc. Indian Acad. Sci.*, 1940, **49**, 91). To the distillate add 0·1 *N* acid until the solution is faintly acid to methyl red, transfer the solution to a 500-ml. graduated flask, make up to the mark, and take 50 ml. Mix with an equal volume of 0·01 *N* picric acid, or a smaller volume of 0·05 *N* solution, and leave the mixture for 1 to 2 hr. at 0° C. Collect the precipitated picrate on a 1-cm. diameter filter funnel and wash with 0·5 ml. of ice-cold water. The precipitate may be used for the identification of the alkaloid. Dilute the filtrate to 200 to 500 ml., take 10 to 50 ml. in a conical separating funnel, add to it 100 to 150 ml. of water, and a third of the volume of dichloroethane or chloroform. Run into the funnel measured volumes of 0·001 *N* methylene blue, shaking after each addition. The picrate of methylene blue dissolves in the organic solvent, which is renewed several times during the titration. The titration is completed when the aqueous layer possesses the pure blue colour of methylene blue and a fresh organic solvent layer remains colourless. G. S. SMITH

New Selective Reaction for Antipyrine. G. J. Vanag and M. A. Matzkanova (*J. Anal. Chem. Russ.*, 1947, **2**, 21–26)—2-Nitroindan-1 : 3-dione (Wanag, *Ber.*, 1936, **69**, 1066), easily soluble in water and in alcohol, giving yellow solutions, is a strong acid, the *p*H of a 0·01 *N* solution being the same as that of 0·01 *N* hydrochloric acid, and yields stable salts with weak bases. With primary and secondary amines, certain heterocyclic bases and alkaloids, the salts are difficultly soluble in water (*Ber.*, 1937, **70**, 547; 1942, **75**, 82). When antipyrine is boiled with a concentrated aqueous solution of nitroindandione an orange colour, which becomes pale on cooling, appears. Re-heating restores the orange colour. It can be transferred to chloroform, but not to ether. Aqueous ammonia solution destroys the colour. A similar colour appears when solutions of the substances in absolute alcohol or glacial acetic acid are mixed. With concentrated aqueous solutions, an orange-coloured oil separates in the cold.

With 1 g. of antipyrine, boiling for 1 min. with 5 ml. of aqueous saturated (approximately 6·5 per cent.) nitroindandione solution suffices to give an orange colour, but with 0·001 g., 10 min. are necessary. After being kept, however, for 24 hr., the colour of the latter solution is unchanged, whilst that of the former is pale orange. Tests carried out in test-tubes containing 1 ml. of saturated nitroindandione solution with small additions of antipyrine show that 15 min. heating in boiling water is adequate for colour development and that the limiting dilution for detection of antipyrine is 1 in 10,000, which may be increased to 1 in 20,000 by shaking the solution with a few drops of chloroform and observing the colour of the chloroform.

Most antipyretics other than antipyrine do not give the reaction, *e.g.*, pyramidon, bromural, phenacetin, aspirin, diplosal, etc., and these substances in 500-fold excess do not affect the detection of antipyrine.

Similar orange colours are obtained with—(i) Certain phenols, resorcinol, pyrogallol, phloroglucinol, and also neo-salvarsan, *para*-codeine, and hydrazine hydrochloride; these colours cannot be transferred to chloroform but some may be transferred to ether. (ii) Indole, the colour from which is transferable both to chloroform and to ether, and, furthermore, is obtainable in the cold. (iii) Codeine, dionine, strychnine, brucine, and papaverine, which react like antipyrine, except that the colours are intensified by addition of dilute aqueous ammonia solution, and are not destroyed as with antipyrine, and that they do not yield colours in absolute alcohol. (iv) *p*-Dimethylaminobenzaldehyde, which gives positive reactions in aqueous, alcoholic, and glacial acetic acid solutions, but the colour may be transferred to both chloroform and ether, and addition of aqueous ammonia solution intensifies it. G. S. SMITH

Biochemical

Estimation of Basic Organic Compounds in Biological Material. I. General Principles. B. B. Brodie, S. Udenfriend, and J. E. Baer (*J. Biol. Chem.*, 1947, **168**, 299–309)—In a programme for testing new anti-malarials it became necessary to devise methods of estimating a large variety of basic organic compounds in blood and tissues. The work was simplified by the preparation of a general scheme involving: (*a*) the isolation of the compounds by extraction; (*b*) a concentration step, where necessary; and (*c*) a method of estimating the substances based on fluorimetry or photometry.

The fluorimetric method of assay is preferred because of its simplicity, but is limited by the fact that the only instruments available are those using activating radiation at 365, 405, and 436 mμ. Some compounds, which do not themselves fluoresce, may be converted into fluorescent substances by oxidation, hydrolysis, or ultra-violet irradiation. Where a fluorescence technique cannot be used, a coupling reaction to form a dye may be tried; for example, some aromatic amines may be diazotized and coupled with N-1-naphthylethylenediamine, whilst others may be coupled with diazonium salts to form a coloured azo compound. Another method that may be used is based on the observation that most organic bases form salts with methyl orange, and these are very soluble in certain organic solvents, but relatively insoluble in water; they may, therefore, be extracted into an organic solvent and estimated photometrically. Finally, spectrophotometry in the ultra-violet region of the spectrum may sometimes be used.

The solvents generally used for extraction are ethylene dichloride, benzene, light petroleum, or heptane. Errors may be introduced by the adsorption of compounds from organic solvents on to glass surfaces, but these can be minimised by addition of an alcohol. Any procedure used should first be checked by measuring the recovery of known amounts of the compound added to blood or tissue extract. The general procedure in the extraction of plasma is as follows. To 10 ml., add 1 ml. of 2·5 *N* sodium hydroxide and extract with ethylene dichloride or benzene; centrifuge to separate the two phases, if necessary. When heptane or light petroleum is used for extraction, dilute the plasma with an equal volume of 0·1 *N* sodium hydroxide. In assaying tissues, homogenise up to 2 g. with 5 ml. of 0·1 *N* hydrochloric acid. With faeces, add 20 ml. of concentrated hydrochloric acid and dilute to 2 litres. Treat urine in the same way as plasma. In determining the specificity of the selected procedure the choice of the proper solvent is important. Basic organic compounds are generally metabolised to substances more polar than the parent compounds, so that they have lower partition coefficients between organic solvents and water than the parent drugs. This difference in behaviour can be used to facilitate their separation by choosing for extraction the least polar solvent that quantitatively extracts the parent drug. Thus, quinine can be separated completely from its metabolic product by extraction with benzene at *p*H 10 or higher; at this *p*H ethylene dichloride extracts an appreciable amount of the metabolic product, whilst light petroleum is not a sufficiently good solvent for quinine. Similarly, ethylene dichloride extracts both chloroquine and

its metabolic product from solutions between *p*H 9 and *p*H 13, whilst heptane effects some separation of the two compounds at the higher *p*H. The remainder of the metabolic product can be removed by washing the solvent extract with alkali.

In studying the specificity of any particular method, therefore, the results obtained by extracting a solution of the pure substance at various *p*H values should be compared with those obtained by extracting, over the same *p*H range, a plasma extract containing the metabolic product as well as the parent substance. The effect of washing the solvent phase with alkali should also be tested, as such a wash may be necessary to obtain satisfactory recoveries. F. A. ROBINSON

Estimation of Basic Organic Compounds in Biological Material. II. Estimation of Fluorescent Compounds. B. B. Brodie, S. Udenfriend, W. Dill, and G. Downing (*J. Biol. Chem.*, 1947, **168**, 311–318)—Fluorimetric analysis is the preferred method for estimating organic bases, where it can be used. The base is isolated from the sample, after the latter has been made alkaline, by extraction with an organic solvent, and any metabolic products not removed in the extraction are eliminated by washing the extract with alkali. The solution is then acidified and the *p*H is adjusted to that at which fluorescence is maximal and the fluorescence is then measured. A simplified method of assay can often be used. The method is illustrated by reference to the procedure with quinacrine.

Procedure—Double extraction—To 1 to 10 ml. of sample, containing 1 μg. of quinacrine, add an equal volume of 0·1 *N* sodium hydroxide and 30 ml. of light petroleum. Shake for 30 min., allow to separate, centrifuging if necessary, and mix 1 ml. of *iso*-amyl alcohol with the light petroleum solution in such a way as not to disturb the aqueous phase. Transfer 20 ml. of the petroleum extract to a tube containing 6 ml. of 0·1 *N* hydrochloric acid. Shake for 3 min. and centrifuge for 2 min. at low speed. Remove the supernatant organic phase, and transfer 5 ml. of the acid phase to a fluorimeter tube containing 1 ml. of 0·5 *N* sodium hydroxide and 2 ml. of a buffer solution *p*H 9·5 (add 5 volumes of 0·6 *M* boric acid in 0·6 *M* potassium chloride to 3·2 volumes of 0·6 *N* sodium hydroxide). Adjust the setting of the fluorimeter by means of a blank in which water is substituted for the plasma, and run through the same procedure. Evaluate the fluorescence of the samples in a Coleman photo-fluorimeter with a 2-mm., No. 5113 Corning filter to isolate the activating energy and Corning No. 3385 filter to transmit the fluorescent light. With each set of estimations, measure the fluorescence of a series of standard solutions prepared by adding a known amount of the drug in 5 ml. of 0·1 *N* hydrochloric acid to 1 ml. of 0·5 *N* sodium hydroxide and 2 ml. of buffer in a fluorimeter tube.

Procedure—Single extraction—To 1 to 5 ml. of the sample, containing 1 μg. of quinacrine, add an equal volume of 0·1 *N* sodium hydroxide and 15 ml. of heptane. Shake and separate as before. Add 0·5 ml. of *iso*-amyl alcohol to the heptane phase and transfer about 10 ml. of the latter to a fluorimeter tube containing 1 ml. of a 25 per cent. solution of trichloroacetic acid in ethylene dichloride. Adjust the setting of the instrument with a blank as before and calculate the results from the readings obtained with standard solutions. The double extraction method gave recoveries ranging from 95 to 110 per cent. and the single extraction method, recoveries from 96 to 105 per cent. F. A. ROBINSON

Estimation of Basic Organic Compounds in Biological Material. III. Estimation by Conversion to Fluorescent Compounds. B. B. Brodie, S. Udenfriend, W. Dill, and T. Chenkin (*J. Biol. Chem.*, 1947, **168**, 319–325)—Non-fluorescent organic compounds, *e.g.*, 7-chloro-4-amino-quinolines, may yield fluorescent derivatives on irradiation. Some irradiated compounds lose their fluorescence, however, on continued irradiation, but this may be prevented, and the fluoriphore stabilised, by irradiating anaerobically or in presence of cysteine. The method may be illustrated by reference to the method of estimating chloroquine, 7-chloro-4-(1-methyl-4-diethylamino-butylamino)quinoline.

Procedure—To 1 to 10 ml. of the sample, containing up to 1 μg. of chloroquine, add an equal volume of 0·1 *N* sodium hydroxide and 30 ml. of heptane. Shake for 30 min. and allow to separate, centrifuging if necessary. Mix 8 drops of ethanol with the heptane phase so as not to disturb the aqueous phase, and transfer as much of the heptane phase as possible to a 125-ml., glass-stoppered tube. Add about twice the volume of 0·1 *N* sodium hydroxide and shake for 5 min. Allow to settle, add 8 drops of ethanol to the heptane phase, and mix as before; remove the aqueous phase by means of a pipette. Repeat the washing with 0·1 *N* sodium hydroxide, add 5 drops of ethanol and transfer 20 ml. of the heptane phase to a centrifuge tube containing 6 ml. of 0·1 *N* hydrochloric acid. Shake for 3 min., and then centrifuge for 2 min. at low speed. Remove the supernatant organic phase and transfer 5 ml. of the acid phase to a fluorimeter tube containing 1 ml. of 0·5 *N* sodium hydroxide and 1·5 ml. of buffer solution *p*H 9·5 (see preceding abstract); add 0·5 ml. of cysteine reagent (dissolve 1 g. of cysteine hydrochloride in 20 ml. of water and add 0·8 ml. of 10 *N* sodium hydroxide immediately before use). Run a reagent blank, with water substituted for plasma, through the same procedure. Prepare standards by adding known amounts of the drug in 5 ml. of 0·1 *N* hydrochloric acid to 1 ml. of 0·5 *N* sodium hydroxide and 1·5 ml. of buffer solution in fluorimeter tubes. Add 0·5 ml. of cysteine reagent to each tube. After 30 min., irradiate all the tubes with ultra-violet light from a mercury arc lamp similar to that used in the Coleman photo-fluorimeter, with the samples in a circular rack so that they are equidistant from the lamp. The time of irradiation must be determined for each lamp, as too long an exposure results in a gradual diminution of fluorescence. Recoveries of chloroquine added to plasma ranged from 90 to 105 per cent.

F. A. ROBINSON

Estimation of Basic Organic Compounds in Biological Material. IV. Estimation by Coupling with Diazonium Salts. B. B. Brodie, S. Udenfriend, and J. V. Taggart (*J. Biol. Chem.*, 1947, **168**, 327–334)—The aromatic amine is isolated from the biological material by extraction with an organic solvent, after the solution has been made alkaline, and is then extracted into a small volume of acid containing diazotised sulphanilic acid, with which it reacts in the *para*-position to form a coloured compound. Amines possessing a free phenol group are coupled with diazotised *p*-nitroaniline-*o*-sulphonic acid in neutral or slightly alkaline solution. The procedure is illustrated by the methods used to estimate pamaquine, 6-methoxy-8-(4-diethylamino-1-methylbutylamino)-quinoline, and another anti-malarial, SN.5918, 4:4′-dihydroxy-3:3′-*bis*(diethylaminoethyl)diphenyl ether.

Procedure for pamaquine—To 1 to 2 ml. of the sample, containing up to 10 μg. of pamaquine, add an equal volume of 0·1 *N* sodium hydroxide and 30 ml. of light petroleum. Shake for 30 min. and allow to separate, centrifuging if necessary. Mix 2 ml. of *iso*-amyl alcohol with the petroleum phase, without disturbing the aqueous phase. Transfer 20 ml. of the petroleum solution to a centrifuge tube containing 0·5 ml. of coupling reagent (prepared by mixing, at least 10 min. before, 0·3 ml. of 0·5 per cent. sodium nitrite solution with 10 ml. of a solution of sulphanilic acid (0·5 g.) in concentrated hydrochloric acid (7·5 ml.) diluted to 500 ml.). Shake for 5 min. and centrifuge for 2 min. at low speed. Remove the supernatant organic liquid, transfer 0·3 ml. of the aqueous phase to a micro-colorimeter tube and measure the optical density of the dye solution at 480 mμ. Prepare standards by mixing 1 volume of a standard solution of pamaquine in 0·1 *N* sulphuric acid, containing 100 mg. per litre, with 9 volumes of coupling reagent. The coupling reagent is used for the blank setting of the instrument. Recoveries ranged from 91 to 101 per cent. of the theoretical.

*Procedure for SN.*5918—This compound, which contains a free phenol group, is coupled with diazotised *p*-nitroaniline-*o*-sulphonic acid in neutral or slightly alkaline solution. The absorption spectrum of the resulting dye indicates maximal absorption at 450 mμ. but, because the reagent itself absorbs a considerable amount of light at this wavelength, it is necessary to take readings at 520 mμ., where the blank is negligible and the absorption of light by the dye is still sufficient for precise measurements. To 1 to 10 ml. of the sample, containing up to 50 μg. of SN.5918, add 1 ml. of 2·5 *N* sodium hydroxide and 30 ml. of benzene. Shake for 10 min. and allow to separate, centrifuging if necessary. Mix 2 ml. of *iso*-amyl alcohol with the benzene layer without disturbing the aqueous phase, and transfer 20 ml. of the benzene solution to a bottle containing 6 ml. of 0·05 *N* hydrochloric acid. Shake for 5 min. and centrifuge for 2 min. at low speed. Remove the benzene layer by aspiration and transfer exactly 5 ml. of the aqueous phase to a colorimeter tube. Add 0·5 ml. of 0·5 *N* sodium hydroxide and 0·5 ml. of 0·5 *M* disodium hydrogen phosphate, followed by 0·5 ml. of coupling reagent (prepared by mixing, at least 10 min. before, 0·3 ml. of 0·5 per cent. sodium nitrite solution with 10 ml. of a solution of *p*-nitroaniline-*o*-sulphonic acid (1·26 g.) in 15·0 ml. of concentrated hydrochloric acid diluted to 1 litre; dilute the reagent 1 : 5) and, after 10 min., 0·5 ml. of *N* sodium hydroxide. Measure the optical density at 520 mμ. Use a mixture of the coupling reagent, the acid, and the buffer solution for the blank setting of the instrument. Prepare a standard curve using a standard solution of SN.5918 in 0·1 *N* hydrochloric acid containing 100 mg. per litre. The recovery of the anti-malarial ranged from 97 to 111 per cent. of the theoretical.

F. A. ROBINSON

Estimation of Basic Organic Compounds in Biological Material. V. Estimation by Salt Formation with Methyl Orange. B. B. Brodie, S. Udenfriend, and W. Dill (*J. Biol. Chem.*, 1947, **168**, 335–339)—The methyl orange salts of most organic bases are very soluble in certain organic solvents, but relatively insoluble in water. Most bases can therefore be estimated by extraction of their methyl orange salts into an organic phase, followed by photometric estimation. The applications of the reaction are limited when ethylene dichloride is used as solvent because methyl orange is soluble to some extent in this solvent and, in addition, certain organic bases extractable from the biological material may also form soluble complexes with the dye. The use of the less polar solvent, benzene, minimises the difficulties and increases the sensitivity of the method. The method is illustrated by the estimation of cinchonine.

Procedure—To 1 to 10 ml. of sample, containing up to 5 μg. of cinchonine, add 1 ml. of 2·5 *N* sodium hydroxide and 30 ml. of benzene. Shake for 10 min. and allow to separate, centrifuging if necessary. Mix 0·5 ml. of *iso*-amyl alcohol with the benzene phase without disturbing the aqueous phase. Transfer as much of the benzene phase as possible to a centrifuge tube containing 0·5 ml. of methyl orange reagent (dissolve 90 mg. of the sodium salt in 100 ml. of 0·5 *M* boric acid by gentle heating; cool, filter if necessary, and extract several times with an equal volume of ethylene dichloride. The solution when diluted 100-fold with *N* hydrochloric acid should give an optical density of about 0·5 at 515 mμ). Shake for 5 min. and centrifuge for 10 mins. at 2500 r.p.m. Transfer 20 ml. of the benzene layer to a centrifuge tube containing 0·5 ml. of *N* hydrochloric acid. Shake for 5 min. and then centrifuge. Carefully remove the benzene layer and transfer at least 0·3 ml. of the aqueous solution to a micro-colorimeter tube. Measure the optical density at 515 mμ. Set the instrument at zero by means of a reagent blank prepared by substituting water for plasma and carrying through the above procedure. This reagent blank should not give an optical density of more than 0·01 when *N* hydrochloric acid is used to set the instrument at zero. Prepare standard curves by carrying a solution of cinchonine (100 mg. per litre)

in 0·1 *N* sulphuric acid through the above procedure. Recoveries of cinchonine added to plasma ranged from 93 to 114 per cent. of the theoretical.

F. A. ROBINSON

Estimation of Basic Organic Compounds in Biological Material. VI. Estimation by Ultra-Violet Spectrophotometry. E. S. Josephson, S. Udenfriend, and B. B. Brodie (*J. Biol. Chem.*, 1947, **168**, 341–344)—Although ultra-violet spectrophotometry lacks the sensitivity of the assay methods previously described, it may be useful for compounds not capable of being estimated by other methods. It has been used for the estimation of bases in concentrations of the order of 1 mg. per litre. The organic base is isolated in the usual way, and interfering substances are removed by washing the solvent solution with aqueous alkali. The method is illustrated by the procedure for quinine.

Procedure—To 1 to 4 ml. of plasma add 1 ml. of 2·5 *N* sodium hydroxide and 15 ml. of ethylene dichloride. Shake for 10 min. and centrifuge for 5 min. at 3000 r.p.m. Remove the aqueous phase by aspiration and transfer as much of the solvent phase as possible to a glass-stoppered bottle. Add about 3 volumes of 0·02 *N* sodium borate and shake for 5 min. Remove the aqueous phase by aspiration and repeat the washing. Transfer 10 ml. of the solvent phase to a glass-stoppered test tube containing 4 ml. of 0·1 *N* sulphuric acid. Shake for 3 min. and centrifuge at low speed. Transfer about 3 ml. of the aqueous phase to a cuvette and measure the optical density at 250 mμ. in a spectrophotometer. Use as a blank the solution obtained by substituting water for plasma and carrying through the same procedure. This should have an optical density not greater than 0·005. A solution of quinine in 0·1 *N* sulphuric acid, containing 5 μg. per ml., has an optical density of 0·430. The recovery of quinine added to plasma ranged from 98 to 101 per cent.

F. A. ROBINSON

Rapid Estimation of Tyrothricin in Fermentation Liquors. S. C. Rittenberg, H. E. Sternberg, and W. G. Bywater (*J. Biol. Chem.*, 1947, **168**, 183–189)—Because the bacteriological methods of assaying tyrothricin are unsuitable for routine use, an attempt was made to develop a chemical method of analysis. It was assumed that if the proportion of gramicidin and tyrocidine is constant under a wide range of culture conditions, then the estimation of any amino acid present in either or both components of tyrothricin should give a satisfactory measure of the total. Tryptophan has been stated to account for 40 per cent. of the nitrogen of gramicidin and 15·4 per cent. of that of tyrocidine. The tryptophan method of Horn and Jones (*Ibid.*, 1945, **157**, 153; Abst. ANALYST, 1945, **70**, 266) was therefore applied to alcoholic extracts of material precipitated from fermentation liquors.

Assay procedure—Dissolve weighed samples of the standard in alcohol to give a suitable concentration. Add 0·5 ml. of a 5 per cent. solution of *p*-dimethylaminobenzaldehyde in concentrated hydrochloric acid and 0·5 ml. of alcohol to 5·0 ml. of concentrated hydrochloric acid. Add 1 ml. of the solution to be tested. If tyrothricin is present, a pink colour develops almost at once. After 5 min., add one drop of 0·2 per cent. aqueous sodium nitrite solution and evaluate the blue colour after 15 min. in a Klett-Summerson photoelectric colorimeter with a blue filter (400 to 465 mμ.). Use as a blank a tube containing 1·5 ml. of alcohol, 5 ml. of concentrated hydrochloric acid, 0·5 ml. of the 5 per cent. *p*-dimethylaminobenzaldehyde solution, and one drop of 0·1 per cent. sodium nitrite solution.

Assay of fermentation liquors—Transfer 5 to 10 ml. to a centrifuge tube and adjust the *p*H to 4·0 to 4·5 by addition of a pre-determined quantity of hydrochloric acid. Centrifuge and discard the supernatant liquid. Add exactly 5 ml. of alcohol to the tube, re-suspend the sediment, shake for 10 min., leave for 20 min., and again centrifuge. Use 1 ml. of the clear alcoholic extract for development of the colour. Treat in the same way 1 ml. of a solution containing 200 μg. of standard with each set of unknowns. Recoveries of tyrothricin added to fermentation liquors varied from 96 to 103 per cent. of the theoretical.

F. A. ROBINSON

Studies on Penicillinase. II. Manometric Method for the Assay of Penicillin and Penicillinase. R. J. Henry and R. D. Housewright (*J. Biol. Chem.*, 1947, **167**, 559–571)—The penicillins, which are mono-basic acids, when inactivated with the enzyme penicillinase, give di-basic acids, known as penicilloic acids. The amount of penicilloic acid formed can be estimated by measuring the amount of carbon dioxide evolved from a mixture of penicillin, enzyme, and bicarbonate buffer. Over a certain range, the rate of evolution of carbon dioxide is proportional to the concentration of the enzyme catalyst, which can thus be estimated. The total volume evolved is a measure of the penicillin present.

Penicillinase assay—In the main space of a constant-volume Warburg respirometer kept at 36° C. place 1 ml. of the enzyme solution and 3 ml. of sodium bicarbonate buffer at *p*H 7·0. In the side-arm, place 0·5 ml. of the same buffer containing 5000 units of penicillin. Start the shaking mechanism and, when equilibrium has been reached, close the system. As soon as constant readings are obtained, add the penicillin from the side-arm; when the evolution of carbon dioxide is steady, take readings of the volume of gas evolved and the corresponding time. The graph of these readings should be linear.

Make similar experiments with different concentrations of the enzyme and plot the respective graphs.

To prepare a calibration curve, plot the slopes of the graphs obtained as above against the enzyme concentrations, determined by tube dilution assay; the curve should be linear up to fairly large enzyme concentrations, beyond which it tends to a constant level and a preliminary dilution should be made. The calibration curve will vary with the particular penicillin used.

Penicillin assay—Place 3 ml. of penicillin solution

containing at least 300 units with 1 ml. of the buffer in the main space of the respirometer. Place 0·5 ml. of a potent enzyme solution in the side arm. Proceed as above, but measure the total volume of gas evolved. An accuracy of ±4·6 per cent. is claimed. The method is limited to solutions of relatively high concentration and of low buffering capacity. W. S. WISE

Collaborative Comparison of Three Rations for the Chick Assay of Vitamin D. C. I. Bliss (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 396–408)—A comparison was made of two experimental diets with the A.O.A.C. basal ration for the chick assay of vitamin D. The first diet was a modification of the A.O.A.C. ration and was designed to promote growth, whilst the second diet utilised ingredients present in chick starting diets supplied under wartime conditions. Several levels of vitamin D, in the form of the U.S.P. Reference Cod Liver Oil, were used, and the data submitted from thirteen laboratories were analysed statistically. The chicks fed the experimental diets were heavier than those on the A.O.A.C. ration, indicating that the new diets were nutritionally more adequate, but the variation in the individual bone ash data produced by the experimental diets was no more homogeneous than with the A.O.A.C. diet. A straight line relating percentage ash to the log dose of vitamin D was fitted to the data for each diet in each laboratory. Since, with the experimental rations, there was no relation between diet and slope and no significant reduction in the variability of the group ash about the fitted lines, these rations offered no advantage over the A.O.A.C. diet for purposes of assay.

F. A. ROBINSON

Agricultural

Determination of Carotene in Alfalfa. F. P. Zscheile and R. A. Whitmore (*Anal. Chem.*, 1947, **19**, 170–172)—A simple and rapid method for the routine determination of the carotene fraction in alfalfa is described, and the factors affecting the determination are discussed.

Method—Extraction—Hand-pick alfalfa leaves, retain a sample for dry-matter determination, and wrap 5 g. in a cheese-cloth package tied with wire or string. Blanch by immersing in boiling water for 5 to 10 min. and remove the excess of water by applying moderate pressure. Analyse soon or store in dry ice.

Blend for 5 min. in a Waring Blendor with 100 ml. of a 40 : 60 mixture by volume of acetone and light petroleum (Skellysolve B) containing 0·1 g. of magnesium carbonate to neutralise any plant acids. After 3 min. of blending, clean the sides of the blendor by wiping downward with filter paper. Shred this, add it to the mixture, and blend for 2 min. more. Filter under suction and wash the residue with two, separate 10-ml. portions of acetone, followed by light petroleum until the filtrate is colourless. Separate the aqueous layer in a funnel and make up the other phase to 200 ml. with light petroleum. No drying is needed. Take 15 ml. of this solution, mix with 15 ml. of light petroleum, and take a chromatogram with 10 to 20 ml.

Dehydrated or dried alfalfa meal—Grind the dry meal to 40 mesh and place 2 g. between layers of cotton in a Coors porcelain extraction crucible, size 3, suspended in an A.S.T.M. extraction apparatus. Into the 400-ml. flask of the apparatus, place 60 ml. of 30 per cent. acetone - light petroleum and extract for 3 hr on a steam-heated bath, with glycerol or mineral oil for rapid heat transfer, at a drip rate of 100 to 150 drops per min. Dilute the extract to 100 ml. with light petroleum. The solution is usually turbid. Take a chromatogram with 5 to 20 ml. of this solution.

Chromatography—Using a wooden tamper, dry-pack a column, 19 mm. in diameter and about 6 cm. long, with a mixture 1 : 1 of activated magnesia (Micron brand No. 2642, Westvaco Chlorine Products Co., Newark, California) and Hyflo Super-Cel. No. 2641 magnesia is unsuitable, and if No. 2642 has absorbed moisture, a longer column should be used. Cover the mixture with 1 cm. of granular, anhydrous sodium sulphate. The carotenes pass rapidly through the column and are caught in a 25-ml. volumetric flask using a Fischer Filtrator. Wash the column with 10 per cent. acetone - light petroleum mixture until the volume of the eluate is nearly 25 ml. If the magnesia is freshly-prepared, use a greater concentration of acetone.

Photometry—Determine the optical density of the 25-ml. solution at 436 mμ. with a Coleman universal spectrophotometer, model 11, or a Klett photometer with a No. 44 filter. Convert to carotene concentration from a calibration curve. Density values are 0·15 to 0·95 for the Coleman, and 0·08 to 0·60 for the Klett photometer for samples containing 50 to 400 p.p.m. of carotene when 15 ml. are used for a chromatogram.

Carotene is extracted from fresh leaves more easily than from dry meals, fresh stem, or whole fresh plant material. Fresh leaves can be kept at room temperature for at least 30 min. without loss of carotene and, blanched and frozen, may be kept for at least 60 days. Field samples can be frozen in dry-ice, the carbon dioxide having little effect on the carotene content.

Extraction of leaves with 30 per cent. acetone (in light petroleum) is incomplete, but there is only a 1 to 2 per cent. loss with 40 per cent. acetone. The addition, during blending, of calcium cyanide or mercuric chloride to inactivate enzymes does not increase the yield of carotene.

The percentage losses are given for the extraction of carotene from dried alfalfa under various conditions. There was no loss when the alfalfa meal was heated for 18 hr. at 75° C. in a vacuum-oven, and it can be kept for 4 months at −18° C.

W. S. WISE

Colorimetric Evaluation of Derris Root. T. M. Meijer and A. Rachmad (*Rec. Trav. Chim.*, 1947, **66**, 312–316)—The authors describe a more precise modification of the method (Meijer, *Ibid.*, 1936, **55**, 954; Abstract, ANALYST, 1936, **61**, 858), for the estimation of rotenone and rotenone-like substances in Derris root, in which the colours obtained when the acetone-extracted matter is

treated with sulphuric acid containing a trace of sodium nitrite are measured and compared with those obtained from standard solutions of pure rotenone. Results agree closely with those obtained by the earlier, ether-extraction method, but this may be a coincidence, since the modified method is more accurate.

Procedure—Preparation of standards—Dissolve 50 mg. of pure rotenone in 10 ml. of acetone and dilute 0·5 ml. of this solution to 30 ml. with acetone. Transfer 0·1, 0·2, . . . 1 ml. of the diluted solution to glass-stoppered test tubes and evaporate to dryness in boiling water. Add to the residues 5 ml. of a reagent made by dissolving 0·1 g. of sodium nitrite in one litre of concentrated sulphuric acid, stopper the test tubes, shake well, and set aside in the dark for 30 min. Place the solutions in a 0·5-cm. cell and measure the colours photo-electrically, a solution of copper sulphate being interposed as a filter. Construct a curve in which the logarithms of the transmissions are plotted against the corresponding concentrations.

Evaluation of Derris root—Heat 1 g. of finely ground Derris root powder with 30 ml. of acetone for 2 hr. under reflux. Weigh the flask before and after refluxing, and make good any solvent lost. Filter, dilute 0·5 ml. of the filtrate to 30 ml. with acetone, and transfer 0·5 ml. of the diluted solution to a glass-stoppered test tube. Proceed as described above, and deduce the rotenone content from the curve already prepared.

A. H. A. ABBOTT

Separation and Purification of Some Constituents of Commercial Hexachlorocyclohexane. L. L. Ramsey and W. I. Patterson (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 337–346)—The separation of the isomers of hexachlorocyclohexane is difficult by conventional methods, such as fractionation crystallisation, and attempts were therefore made to use partition chromatography for this purpose. Hexachlorocyclohexane, however, is almost insoluble in water, so that another solvent had to be used as the stationary phase adsorbed on the silica gel; the best results were obtained with nitromethane. *n*-Hexane was used to develop the column. As hexachlorocyclohexane contains no ionic group, it was not possible to detect the position of the bands by means of an indicator, and therefore successive fractions of the filtrate were collected and evaporated to dryness. Satisfactory results were obtained using 10 g. of material with 200 g. of silica gel containing 100 ml. of nitromethane. Commercial hexachlorocyclohexane was first recrystallised to remove as much as possible of the α-isomer, which constitutes about 70 per cent. of the crude material. The remainder was then subjected to partition chromatography, giving the four known isomers of hexachlorocyclohexane in the pure state, together with two other compounds previously unknown; one appears to be octochlorocyclohexane and the other, heptachlorocyclohexane.

Procedure—To 30 g. of commercial hexachlorocyclohexane add 50 ml. of *n*-hexane saturated with nitromethane. Bring to the boiling point by heating in a steam-bath, and shake for about 5 min. Cool, filter, and wash the salt with 10 ml. of the solvent. Reserve the insoluble material for the preparation of the α- and β-isomers. Remove the solvent from the filtrate by evaporation under reduced pressure, weigh the residue, and re-dissolve in about 25 ml. of the hexane solvent.

To 200 g. of silica gel add sufficient nitromethane (95 to 115 ml.) to give a suitable rate of flow of solvent through the column as determined in a preliminary experiment. Then prepare a slurry of the silica gel with 600 ml. of the hexane solvent and transfer this to an adsorption tube, the bottom end of which is constricted and the constriction plugged with cotton wool. Connect the upper end of the tube to a compressed air supply and apply 3 to 7 lb. pressure. Release the pressure when the silica gel is so firm that it retains its shape on tipping. Pour on the hexane solution of hexachlorocyclohexane (about 10 g.) and apply sufficient pressure (not more than 7 lb.) to cause the solvent to percolate through the column at the rate of about 5 ml. per min. At the moment when all the solution has penetrated the gel, release the pressure, rinse out the flask with about 20 ml. of solvent, and transfer the rinsings to the column. Again apply pressure to the column, and when the solvent has again just penetrated the gel add another 20 ml. of solvent. After 175 ml. of filtrate have passed through the column, begin collecting 25-ml. fractions and continue to collect until no more solid comes through the column. Then transfer the silica gel from the column to a beaker, add 150 ml. of acetone, filter, and wash the gel with a further 200 ml. of acetone. The first four 25-ml. hexane fractions yield only an oil on evaporation, the 5th and the 7th to 9th fractions contain octo- and hepta-chlorocyclohexane respectively, and the 6th fraction contains a mixture of the two. The 10th to 12th fractions contain a mixture of heptachlorocyclohexane and the α-isomer of hexachlorocyclohexane, and fractions 13 to 17 contain the α-isomer and fractions 20 to 31 the γ-isomer of hexachlorocyclohexane. The intermediate fractions contain a mixture of the two. The acetone extract from the silica gel contains the δ-isomer together with small amounts of the β-isomer. Each of these fractions is recrystallised in order to obtain the pure isomer, whilst fractions containing mixtures are refractionated on a column of suitable size.

Results—The recovery of solid material from the column was almost quantitative (96 per cent.), but the separation of the constituents was not quite complete. Thus, when a mixture of 100 mg. each of the α- γ-, and δ-isomers was chromatographed, 93, 98 and 84 mg., respectively, were recovered, together with 5 mg. of intermediate fractions. The approximate composition of commercial hexachlorocyclohexane is: α-hexachlorocyclohexane, 65 to 70 per cent.; β-hexachlorocyclohexane, 5 to 6 per cent.; γ-hexachlorocyclohexane, 13 per cent.; δ-hexachlorocyclohexane, 6 per cent.; heptachlorocyclohexane, 4 per cent.; and octochlorocyclohexane, 0·6 per cent. F. A. ROBINSON

Gas Analysis

Determination of Benzene and Toluene Vapours in Air. M. S. Bikhovskaya (*Zavod. Lab.*, 1945, **11**, 537–541)—The method of Yant, Pearce, and Schrenk (*U.S. Bur. Mines*, 1936, *Rept. Invest.* 3323) was found to be unsatisfactory, and a modified method is described.

Toluene can be nitrated to trinitrotoluene in conditions under which benzene forms only dinitrobenzene, *e.g.*, by using Stepanov's nitrating mixture, 10 per cent. ammonium nitrate in concentrated sulphuric acid at 100° C. Hydrolysis of a portion of the mixed nitro-compounds by potassium hydroxide affects only the trinitrotoluene, giving salts of phenolic compounds which are not extractable by organic solvents; dinitrobenzene may, therefore, be separated by extraction, and then determined colorimetrically in acetone solution with potassium hydroxide. Trinitrotoluene, extracted from another portion of the product after nitration, gives, when shaken in 5 ml. of alcoholic solutions, with 0·05 ml. of 3 per cent. potassium hydroxide solution, a violet colour that reaches maximum intensity after 20 min., and then slowly decolorises, the test being sensitive to the equivalent of 0·005 mg. of toluene (*cf.* Rudolph, *Z. anal. Chem.*, 1926, **66**, 239). A similar colour is given in acetone or butanone solution with 25 per cent. aqueous ammonia solution. Either of these reactions may be used for determining toluene colorimetrically. Under similar conditions, dinitrobenzene gives a violet colour, but with only one-twentieth of the intensity. Quantitative determinations require the preparation of standard solutions simultaneously with the sample solutions.

Preparation of standards—In a 25-ml. graduated flask, fitted with a ground-glass stopper, place 5 ml. of a solution of 10 g. of ammonium nitrate, dried below 80° C., in 100 ml. of concentrated sulphuric acid ("nitrating mixture"), weigh the flask and contents, then add 0·05 ml. of benzene (and, correspondingly, of toluene in another flask), and weigh again. Place the flask in boiling water for 30 min., then cool, and make up to the mark with nitrating mixture. Dilute portions of the solution to suitable concentrations by adding diluted nitrating mixture (1 + 5). Treat a series of standards in parallel with the test samples.

Procedure—Pass a suitable volume of air, dependent upon the content of benzene and toluene, at the rate of 10 litres per hr., through two successive absorption vessels containing 2 ml. of the nitrating mixture in each. Place the absorption vessels in boiling water for 30 min., then cool, and transfer the solutions into a flask, washing with water. Divide the solution into two equal parts. *Determination of benzene*—To one portion add 40 per cent. sodium hydroxide solution to neutrality and 0·5 ml. in excess, leave for 30 min., and then transfer to a separating funnel and extract the solution twice with 10 ml. of ether. Run the aqueous solution through the tap, and pour the ether extract from the top of the funnel through a dry filter into a tube for which a ground-glass connection is available for connection to a condenser. Evaporate off the ether, removing residues in a current of air. Dissolve the dinitrobenzene in acetone (10 ml.), place 5 ml. in a colorimeter tube, add 0·05 ml. of 5 per cent. potassium hydroxide solution, and compare the colour intensity after 5 to 10 min. with that of standards, similarly prepared. The method is sensitive to 0·005 mg. of benzene. Alternatively, avoiding the need for evaporating the solvent, extract with butanone (at 30° to 35° C., otherwise salts crystallise out) instead of ether, separate the ketone layer, shake it energetically with 1 ml. of 40 per cent. sodium hydroxide, and carry out the colorimetric determination after 30 min., using standards prepared similarly at the same time. *Determination of toluene*—To the other portion, add 25 per cent. aqueous ammonia solution until it is just neutral (an excess must be avoided), transfer to a separating funnel, and extract twice with 10 ml. of ether. Separate the ether layer and remove the ether as previously described, dissolve the residue in 10 ml. of ethyl alcohol, take 5 ml. of the solution, add to it from a microburette 0·05 ml. of 3 per cent. potassium hydroxide, and compare the colour intensity after 5 min. with standards run at the same time. Alternatively, if the amount of benzene is not more than twenty times that of the toluene, extract with butanone and develop the colour in the butanone extract with 25 per cent. aqueous ammonia solution, or extract with ether as above, remove the ether, dissolve the residue in acetone, and develop the colour with 25 per cent. aqueous ammonia solution.

Some results obtained (in mg.) on known mixtures of benzene and toluene, respectively, in air, the amounts taken being given in parentheses, are: 0·015, 0·15 (0·018, 0·174); 0·13, 0·03 (0·14, 0·04); 0·030, 0·050 (0·034, 0·056); and 0·03, 0·008 (0·035, 0·01).

G. S. SMITH

Determination of Benzene in Gases Containing Butadiene and Higher Olefines. Behaviour of 1 : 3-Butadiene towards Ammoniacal Nickel Cyanide Solution. A. A. Balandin and M. N. Marushkin (*J. Anal. Chem. Russ.*, 1947, **2**, 3–6)—The use of an ammoniacal nickel cyanide solution for the determination, by means of an Orsat apparatus, of benzene vapour in air, etc. (Dennis and McCarty, *J. Amer. Chem. Soc.*, 1908, **30**, 233) is unsatisfactory when butadiene and butylenes are present, since gradual absorption of these gases occurs in the nickel cyanide solution; successive treatments of an initial 100 ml. of technically pure butadiene reduced the volume to less than 30 ml.; and the 5 per cent. sulphuric acid solution used for removing ammonia vapour absorbs butadiene slowly. Desorption from used solutions can also occur on admission of air.

G. S. SMITH

Water

Bromide Content of Underground Waters. Part I. Determination and Occurrence of Traces of Bromide in Water. Part II. Observations on the Chlorination of Water Containing free Ammonia and naturally

occurring Bromide. G. U. Houghton (*J. Soc. Chem. Ind.*, **1946, 65, 277–280, 324–328**)—In Part I, a colorimetric method and a volumetric method are described for the determination of bromide in waters of low organic content; the volumetric method gives the greater accuracy. By both methods, the sum of bromide plus iodide is determined, but iodide contents are usually negligible. A number of natural waters in South-East England have been examined, and bromide contents up to 2 p.p.m. of bromine are recorded.

(i) *Colorimetric method*—This is an adaptation of the phenol-red method of Stenger and Kolthoff (*J. Amer. Chem. Soc.*, 1935, **57**, 831), and depends on the conversion of phenol red by bromine to dyestuffs of the bromophenol blue type. Organic matter, free ammonia, and nitrite must be absent.

Reagents—(*A*) 0·05 g. of phenol red dissolved in 4·5 ml. of 0·01 *N* sodium hydroxide, and diluted to 150 ml. (*B*) 100 ml. of *N* sodium hydroxide + 130 ml. of *N* acetic acid. The buffered phenol-red solution is prepared by mixing 7·5 ml. of (*A*) and 92·5 ml. of (*B*).

Procedure—Evaporate 50 to 500 ml. of the sample to 25 ml. and add 0·5 *N* sulphuric acid to give a *p*H of 5·7. Filter, and dilute to 50 ml. with ammonia-free distilled water. Add 2 ml. of buffered phenol-red solution, 0·5 ml. of 0·35 per cent. aqueous chloramine-T solution, and mix rapidly in a Nessler cylinder. Allow to stand in the dark for 5 min., de-chlorinate with 0·2 ml. of 0·125 *N* sodium thiosulphate, and match against standards similarly prepared from dilute potassium bromide solution (1 ml. ≡ 5 μg. of bromine). The quantity of sample chosen should be such that the solution tested contains not more than 0·75 p.p.m. of bromine, and for this purpose it is advisable to carry out an approximate preliminary estimation of the bromide content, a large excess of the indicator solution being used.

(ii) *Volumetric method*—This is a modification of the method of D'Ans and Höfer (*Z. angew. Chem.*, 1934, **47**, 73), and consists in oxidising the bromide to bromate, which is titrated iodimetrically. Chlorine-water instead of hypochlorite is used for the oxidation. Nitrite and free ammonia are without effect on the determination, but iron must be removed. Bromate, which interferes, was not detected in any of the waters examined.

Reagents—Phosphate buffer solution: 22·5 g. of potassium dihydrogen phosphate + 90·0 ml. of *N* sodium hydroxide, diluted to 200 ml. *Chlorine water:* 1 ml. ≡ 2·0 mg. of available chlorine, freshly prepared by dilution with ammonia-free water from a concentrated solution standardised against 0·1 *N* sodium arsenite. 0·001 *N* *Potassium bromate solution:* prepare by the dilution of a 0·1 *N* solution obtained by dissolving 2·783 g. of potassium bromate in 1 litre of water. 0·001 *N* *Sodium thiosulphate solution:* standardise against 0·001 *N* potassium bromate as described below.

Procedure—Carry out preliminary determinations of chloride, permanent hardness, and iron, and choose a quantity of sample expected to contain 50 to 100 μg. of bromine, assuming the Cl : Br ratio to be 300 : 1. If iron is present, evaporate the sample to dryness, extract with successive portions of hot water, filter, and evaporate the filtrate to 2 to 3 ml. in a small flask. This method of removing iron is satisfactory for waters of appreciable bicarbonate content, but may require modification for acid waters. If iron is absent, omit the evaporation to dryness. Add 2 ml. of phosphate buffer, 4 ml. of 25 per cent. sodium chloride solution, and 10·0 ml. of the standard chlorine water. Heat on a water-bath for 15 min., the flask being fitted with a condenser. Wash down the condenser, add 2 ml. of 10 per cent. sodium formate solution, and heat for a further 5 min. to destroy the excess of chlorine. Cool rapidly to 25° C., add 3 drops of 10 per cent. ammonium molybdate solution, 1 ml. of 5 per cent. potassium iodide solution, and 5·0 ml. of diluted hydrochloric acid (1 + 3). Keep the solution in the dark for 5 min., and titrate the liberated iodine with 0·001 *N* thiosulphate, 1 ml. of 0·5 per cent. starch solution being added during the final stages of the titration. Carry out a blank determination on 2 ml. of distilled water.

Normally, 2 ml. of the buffer solution are adequate, but where waters of low bromide content and high permanent hardness are examined, *e.g.*, where more than 0·1 g. of permanent hardness salts can be expected in the concentrate, use proportionately more buffer solution to allow for the precipitation of phosphate by calcium and magnesium ions.

Standardisation of 0·001 *N* *thiosulphate*—Take 2 ml. of distilled water and add 2 ml. of phosphate buffer, 4 ml. of 25 per cent. sodium chloride solution, 10·0 ml. of 0·001 *N* potassium bromate, and 2 ml. of 10 per cent. sodium formate solution. Heat on the water-bath for 5 min., cool to 25° C., and proceed as in the determination. It is advisable to carry out a blank determination, water being used in place of the 0·001 *N* potassium bromate.

In Part II, the effect of bromide on the rate of sterilisation of waters with chlorine and on tests for residual chlorine is discussed.

A number of natural waters containing free ammonia were chlorinated and the speed of colour development was observed in the test for residual chlorine by the standard *o*-tolidine method. For waters containing 0·25 to 2·0 p.p.m. of bromine, the speed of colour development was considerably higher than for waters of negligible bromide content. Tests on distilled water containing sodium bicarbonate and free ammonia showed that the speed of colour development increases with increasing bromide content. For these reasons the presence of bromide invalidates the test laid down by the American Water Works Association for distinguishing between free chlorine and chlorine present as chloramine (*J. Amer. Water Works Assoc.*, 1943, **45**, 1315), since this test depends on the different speeds of colour development of chlorine and of chloramine with *o*-tolidine.

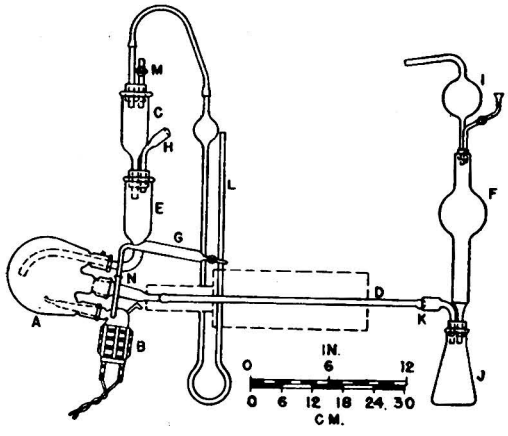
The effect of bromide content on the sterilisation with chlorine of natural waters containing free ammonia, but of low organic content, and of distilled water containing free ammonia and sodium bicarbonate was investigated. The test organism was *B. coli* (Type I), the time of chlorination was

varied, and the effect of bromide contents of 0 to 2 p.p.m. of bromine was observed. Bacterial counts indicated that the rate of kill increased rapidly with increasing bromide content. Further tests on a naturally polluted water gave similar results.

The effect of bromide on the rate of sterilisation and on the test for residual chlorine on waters containing free ammonia may be due to the formation of a bromine - ammonia compound or bromamine of lesser stability than chloramine. The reaction between bromine and ammonia at high dilution gave evidence of the formation of such a compound. H. J. CLULEY

Organic

Determination of Traces of Fluorine in Organic Compounds. W. B. Huckabay, R. H. Busey, and A. V. Metler (*Anal. Chem.*, 1947, **19**, 59–63)—In determining small amounts of halogen, direct vaporisation through a flame results in incomplete decomposition, lower values being obtained than by the recommended method. Final passage of the gases with oxygen over hot platinum ensures complete conversion of the fluorine to an absorbable form.



Apparatus—The general arrangement is shown in Fig. 1. In the 1-litre, Pyrex combustion flask, A, with standard ground-glass joints, methane and the sample vapour burn from the jet of the vaporiser, B. The vaporiser has a methane inlet, a ground-joint to accommodate the weight-pipette, G, and one that bears the outlet jet to fit into the combustion flask. The heating unit is of wound Nichrome wire taking about 500 watts. The weight-pipette has a stop-cock at one end, and a ground-joint at the other. The drying tube, C, contains a mixture of equal parts of 4-mesh calcium chloride and calcium sulphate, on a glass wool pad. Air enters the tube through the stopcock, M, and its flow is measured in L. Oxygen enters through H, passes through E, which is packed with sodium hydroxide pellets on a glass-wool pad, and joins with dry air to enter the combustion flask. A pre-heater rests on the adaptor, N, which joins the flask to the combustion tube, D, of Vycor or fused silica, containing three spirals of platinum wire, 6 cm. apart, a 5-cm. roll of platinum gauze, and lumps of 8-mesh silica, activated by impregnation with 1 per cent. platinum chloride solution and ignition at 250° C. The cold zone of the tube contains another platinum spiral to hold the silica in place. The combustion gases pass through another adaptor into the flask, J, through a fritted-glass scrubber, F, and spray trap, I, where a reliable suction is applied. The correct combustion temperatures are attained if the furnace and pre-heater have resistances of 28 and 14 ohms, respectively, and are placed in series across 110 volts A.C.

Procedure—Heat the furnace to between 600° and 800° C., and charge the absorber, J, with 25 ml. of water. Adjust the suction to give bubbles up to 1 in. of the trap tube, and then apply an oxygen flow just greater. Close the stopcock on the air inlet, and replace the vaporiser with a cork. Draw a liquid sample, of boiling point between 30° and 200° C., into the weight-pipette, weigh it to a milligram, and attach the pipette to the ground joint of the vaporiser, which is then not connected with the combustion flask. Pass methane through the vaporiser for a few min., then light the jet and adjust the flame to 4 cm. Attach the vaporiser to the combustion flask, reduce the oxygen flow to just less than the suction, and vaporise any water condensing in the flask or front part of the tube by applying a flame. Reduce the methane flow to give a 1-cm. flame, rotate the pipette through 180°, and allow the sample to flow into the vaporiser. Increase the heating to the vaporiser to give a 3-cm. flame and maintain between 2 and 4 cm. by regulating the temperature. When the heater has maintained maximum temperature for 5 min., stop the methane current, remove and weigh the pipette to obtain the true weight of the sample burnt. Purge the vaporiser with air for a few min., then apply a source of oxygen to the ground-joint, in the place of the pipette. Continue this oxidation for 5 min. after any residue in the vaporiser disappears. Stop the suction, and drain the absorbing solution into the flask, rinsing the walls of the absorber assembly with water, and combining the washings with the main solution.

If the sample contains more than 0·002 per cent. of fluorine, decolorise the solution by warming for 5 to 10 min. with 0·05 to 0·1 g. of activated charcoal, and filter the solution into a volumetric flask. When cool, dilute to the graduation, and treat 25-ml. portions with 25 ml. of 95 per cent. ethyl alcohol, 1 ml. of a buffer solution containing 9·4 g. of chloroacetic acid and 2·0 g. of sodium hydroxide in 100 ml. of water, and 3 drops of 0·05 per cent. sodium alizarin sulphonate solution. Titrate the fluorine present, which should not exceed 0·6 mg., with 0·01 or 0·003 *N* thorium nitrate solution until a colour similar to that of the permanent blank is obtained; the blank is composed of dilute cobaltous nitrate and potassium chromate (Eberz *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 259).

If the sample contains less than 0·002 per cent. of fluorine, add 0·1 *N* sodium hydroxide until the

solution is alkaline to phenolphthalein, and concentrate on a hot-plate to an appropriate volume; for 0·0002 to 0·0010 per cent. of fluorine, 15 ml. is a suitable volume. Neutralise the solution then with 0·1 *N* hydrochloric acid and titrate as above.

In treating gaseous samples, the sample container must have valves at both ends. Connect it vertically with the vaporiser through a Hoke needle valve in a ground-joint fitting into the pipette joint. The link between container and valve should be of copper tubing, and that between the valve and the vaporiser, a short length of neoprene tubing. Weigh the sample by weighing the container before and after the combustion, and regulate the flow by adjusting the needle valve.

Heavy liquids may be weighed in a weight-pipette or drawn directly into the vaporiser and their weight determined by weighing this before and after the combustion. Solids should be dissolved in a known amount of an organic solvent containing neither fluorine nor sulphur. The heavy residue left is destroyed by passage of oxygen through the methane inlet as before while maintaining a temperature approaching red heat.

The determination takes 8 hr. on 0·0001 per cent. contents, but only 2 hr. on relatively high contents.

Results—The standard materials used were fluorohydrocarbons dissolved in acetone and *iso*-octane. The maximum error incurred on samples containing 0·01 to 0·26 per cent. of fluorine was 46 parts per thousand, with an average of 22 parts per thousand for the 8 samples analysed. In the range 0·005 to 0·001 per cent. of fluorine, the maximum error was 69 parts per thousand. Greater errors were found on samples with less than 0·001 per cent. of fluorine.

Organic bromides may be analysed by the same procedure, and the absorbate treated for the gravimetric determination of silver bromide. The maximum error on contents greater than 0·01 per cent. was 77 parts per thousand, and below 0·01 per cent. the accuracy was within 0·0003 per cent.

The titration error is 0·05 ml., which represents a significant variation on small titres (one-tenth of the titre of 0·01 *N* thorium nitrate when a 25-g. sample is burned and its fluorine content is 0·0005 per cent.), but this can be compensated for by increasing the sample size and concentrating the absorbate. Water is adequate as an absorbing solution for fluorine, though 2 *N* sodium hydroxide should be used for the other halogens, any oxyhalogens so formed being reduced by adding sodium nitrite. Traces of sulphate in the titrating solution give high results, and should be removed by distillation or the titre corrected by determining the sulphur content. M. E. DALZIEL

Use of Emodin as an Indicator. Z. M. Umanski (*Zavod. Lab.*, 1945, **11**, 404–405)—Emodin (1 : 6 : 8-trihydroxy-3-methylanthraquinone), found in nature in the form of anthraglucosides in the bark of alder buckthorn, in the roots of rhubarb, and in certain kinds of sorrel, may be used as an acidimetric indicator in the *p*H range 6·8 to 7·6. The colour change is sharp (lemon-yellow at *p*H 6·8, yellow at 7, orange-yellow at 7·2, orange at 7·4, and reddish-orange at 7·6) and the indicator may be used satisfactorily both for titration of acid with alkali and *vice versa*, but in the latter titration the transition is sharpest in hot solutions. Neutral salts have scarcely any effect on the sensitivity. The indicator is used as a 0·1 per cent. alcoholic solution or as emodin paper, and is superior to litmus and neutral red.

Preparation of emodin paper—(i) *Yellow paper.* Soak filter paper in a 1 per cent. alcoholic emodin solution, and dry in the absence of ammonia fumes. The yellow colour is turned to red by one drop of 0·005 *N* sodium hydroxide. (ii) *Red paper.* Soak filter paper in 0·005 *N* sodium hydroxide, dry, dip into a 0·1 per cent. solution of emodin in 0·005 *N* sodium hydroxide, and again dry. The red colour is turned to yellow by one drop of 0·005 *N* hydrochloric acid.

Preparation of emodin from the bark of alder buckthorn—Methods described previously (*cf.* Klein, *Handbuch der Pflanzenanalyse*, 1932, III, 1027) are cumbersome and do not always yield a product sufficiently pure for use as an indicator. A convenient and economical method, devised by the author and T. K. Griunberg, is as follows. Treat 50 g. of the finely-divided bark in a 1-litre flask with a mixture of 150 g. of 33 per cent. sulphuric acid and 300 g. of chloroform, and boil under reflux for 1 hr. Filter the hot solution through a Buchner funnel and leave the filtrate to cool in a separating funnel for 18 to 20 hr. As the mixture cools, the chloroform layer deposits an orange precipitate and the acid layer a precipitate of sulphates containing some of the emodin. Filter the chloroform layer, wash the insoluble matter two or three times with 2- to 3-ml. volumes of chloroform, dry it at 50° to 60° C., and re-crystallise from hot toluene, alcohol, or ether, to give orange needles, m.p. 255° to 256° C. The yield may be increased by collecting the insoluble matter from the acid layer, washing with chloroform, and re-crystallising. Normally the total yield of pure emodin is about 0·6 g. G. S. SMITH

Polarographic Reduction of Aliphatic Aldehydes. I. Polarographic Properties of Formaldehyde. R. Bieber and G. Trümpler (*Helv. Chim. Acta.*, 1947, **30**, 706–733)—The polarographic reduction of formaldehyde has been investigated; and the theory of the reaction and the characteristics of experimental polarographic curves are described in this paper.

Formaldehyde gives rise to a polarographic reduction wave that increases in height with an increase in the *p*H value of the solution. At low *p*H values, the curves have definite maxima, and the half-wave potential is near to the discharge potential of the supporting electrolyte, so that no well-defined limiting current is obtained, but in alkaline solutions, well-defined curves showing no maxima and suitable for analytical purposes are produced. For quantitative measurements, solutions should be well buffered at *p*H 9·0 owing to the variation of diffusion current with *p*H value. The wave-height is proportional to formaldehyde concentration when the latter is in the range 0·02 to 0·10 *M*. J. G. WALLER

Inorganic

Recommended Specifications for Analytical Reagent Chemicals. American Chemical Society Committee on Analytical Reagents (*Anal. Chem.*, 1947, **19**, 210–215)—The limits and tests included are based on published work, on the experience of the Committee in examining reagent chemicals as sold, and on studies of the tests described.

Requirements and tests are given for the following reagents: benzine (petroleum benzine, light petroleum), red mercuric oxide, red mercuric iodide, methyl orange, methyl red, fuming nitric acid, sodium cobaltinitrite, and soluble starch for iodimetry.

Corrections for specifications previously published are also included. These are for the iron content of hydrochloric acid; the ammonia-insoluble fraction of molybdic anhydride; the alkalinity of 95 per cent. and absolute ethyl alcohol; the chloride content of ammonium thiocyanate; the substances in amyl alcohol darkened by sulphuric acid; the calcium and strontium salts in barium acetate, barium carbonate, barium chloride, and barium nitrate; the iron and hydrogen-sulphide-soluble contents of cadmium chloride and cadmium sulphate; the assays of cuprous chloride and potassium biphthalate; the nitrogen content of potassium bromide; the silica and ammonium hydroxide precipitate in anhydrous potassium carbonate, potassium hydroxide, sodium carbonate and sodium hydroxide; the nitrogen content of potassium sulphate; the free acid in silver nitrate; the neutrality of sodium oxalate; the chloride in sodium tungstate; the iron and lead in zinc chloride; the iron in zinc oxide; and the arsenic and iron in zinc sulphate.

M. E. DALZIEL

Colorimetric Determination of Small Quantities of Aluminium in Steel. I. K. Kuskova (*J. Anal. Chem. Russ.*, 1947, **2**, 7–16)—Aluminium 8-hydroxquinolinate may be extracted by *iso*-amyl alcohol from a neutral, aqueous solution containing an excess of oxine (*cf.*, extraction by chloroform, Moeller, *Ind. Eng. Chem., Anal. Ed.*, **1943, 15**, 346; Gentry and Sherrington, ANALYST, **1946, 71**, 432). The colour of the extract, greenish-yellow, is due entirely to the aluminium complex, and comparison with standards, or a colorimetric titration, allows the aluminium content to be determined. The method is direct, and larger amounts can be dealt with than in the other colorimetric methods for aluminium, including Teitelbaum's oxine method (*Z. anal. Chem.*, 1930, **82**, 366), that have been proposed. It is applied to the determination of aluminium in steel.

Extraction and colorimetric determination of aluminium—To the aluminium solution in an Eggertz tube, add aqueous ammonia solution until reaction is faintly alkaline, acidify with one drop of sulphuric acid (the total volume should be 50 ml.), add 1 g. of ammonium acetate, and either 0·5 to 2 g. of ammonium chloride or 1 to 2·5 g. of ammonium sulphate, followed by the oxine solution (the optimum quantity corresponding to 0·5 mg. of aluminium is 2 ml. of a 0·5 per cent. solution of 8-hydroxyquinoline in 4 per cent. acetic acid), mix, then add 10 ml. of *iso*-amyl alcohol, and shake well several times. Compare the colour intensity of the alcoholic layer with that of suitable standards prepared at the same time under the same conditions. The colour is stable for 30 min., after which time the intensity slowly increases. Potassium and sodium chlorides, up to 1 g., and sulphates, up to 1·5 g., have no effect on the colour intensity. Between 2 and 250 μg. of aluminium can be determined.

Determination of aluminium in steel—Dissolve 2 g. of steel in 24 ml. of diluted sulphuric acid (1 + 9) by gentle heating. Filter off the carbides, wash them with hot distilled water, ignite in platinum, fuse with 3 g. of potassium pyrosulphate, extract the melt with water, and add the solution to the main filtrate. Evaporate to 80 ml., cool, add 1 g. of ammonium chloride, and then add aqueous ammonia solution (1 + 1 or 12·5 per cent.) until the precipitate produced by a single drop re-dissolves with difficulty. Add 1 ml. of glacial acetic acid, 2 g. of ammonium acetate, and then, dropwise, 20 ml. of 10 per cent. ammonium benzoate solution (*cf.* Kolthoff, Stenger, and Moscovitz, *J. Amer. Chem. Soc.*, 1934, **56**, 812; Stenger, Kramer, and Beshgetoor, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 797; Smales, ANALYST, **1947, 72**, 14). If less than 0·01 per cent. of aluminium is present, precipitate in a volume of 50 ml. and halve the quantities of acetic acid and ammonium acetate. Boil the solution gently for 5 min., but no longer, stirring it by means of a glass rod, filter, and wash the precipitate 8 to 10 times with an ammonium benzoate wash solution, 100 ml. of 10 per cent. ammonium benzoate solution and 20 ml. of glacial acetic acid diluted to 1 litre with water. If the chromium content of the steel is 0·2 per cent. or over, dissolve the precipitate in concentrated hydrochloric acid and re-precipitate as described above. Ignite the paper and precipitate in platinum, remove silica by means of hydrofluoric acid, fuse the residue with 2 to 3 g. of potassium pyrosulphate, extract the melt with 40 to 60 ml. of water, dilute the solution to 100 ml., add 2 ml. of concentrated sulphuric acid, pass hydrogen sulphide to precipitate platinum, and filter. To the filtrate add 3 ml. of concentrated sulphuric acid, evaporate somewhat, add a few drops of perhydrol to oxidise iron, and evaporate just to fuming. Add 45 ml. of distilled water, boil to dissolve the salts, cool the solution to 6° C., wash down the sides of the beaker with water, and precipitate iron and titanium with 6 per cent. aqueous cupferron solution, adding an excess of about 5 ml. and maintaining the temperature at 6° C. Filter, wash the insoluble matter several times with diluted sulphuric acid (1 in 10) containing 2·5 g. of cupferron per litre, and evaporate the filtrate to fuming. Destroy cupferron by addition of nitric acid and some water followed by evaporation to fuming, repeating this procedure as necessary, but avoiding prolonged fuming. If chromium is present, add, before the evaporation, several props of perhydrol. Transfer the sulphuric acid solution (volume about 8 to

12 ml.) to a 100-ml. measuring flask, dilute to the mark with water, and pipette 10 ml. of the solution into an Eggertz cylinder. Make just alkaline to litmus paper with dilute ammonia solution (1 + 1), and then just acid with diluted sulphuric acid (1 + 4). In presence of chromium, add an extra quantity of acid (4 drops for 0·2 per cent. of chromium and 12 drops for 2 per cent.). Cool the solution, add by means of a pipette 2 ml. of 50 per cent. ammonium acetate solution, mix, add from a pipette 2 ml. of 0·5 per cent. oxine solution in 4 per cent. acetic acid solution, again mix, then add 10 ml. of *iso*-amyl alcohol, and shake the contents of the cylinder vigorously. Compare the colour intensity of the alcoholic layer with that of a standard by means of colorimetric titration.

Preparation and use of standard—Place 5 ml. of 20 per cent. ammonium sulphate solution in an Eggertz cylinder, run in from a graduated pipette a standard solution of potassium aluminium sulphate (1·7588 g. of the recrystallised salt are dissolved in water containing 1·5 ml. of concentrated sulphuric acid, and made up to 1 litre with water; the aluminium content is determined gravimetrically after precipitation with aqueous ammonia solution; for use it is diluted four-fold so that 1 ml. is equivalent to 0·025 mg. of aluminium) in quantity equivalent to less than the expected aluminium content of the aliquot part of the solution to be tested, neutralise to litmus with aqueous ammonia, and proceed as described above, correcting the volume by addition of distilled water. After the addition of *iso*-amyl alcohol, run into the standard, portions of the standard aluminium solution, shaking well after each addition, until the colours of the alcoholic layers match.

If the solution during precipitation with cupferron has not been sufficiently cool, small amounts of iron, which would interfere with the determination of aluminium, may remain in solution. To 10 ml. of the test solution must then be added 1 ml. of 25 per cent. sulphosalicylic acid solution and aqueous ammonia solution until it is definitely alkaline. At the same time, treat 10 ml. of water with 1 ml. of sulphosalicylic acid solution, make alkaline with aqueous ammonia solution, and add 0·01 per cent. ferric sulphate solution until the colour matches that of the test solution. Note the number of millilitres required and add this quantity to the standard when determining the aluminium.

The method as described is unsuitable for steels containing more than 2 per cent. of chromium. Most of the chromium should then be removed as chromyl chloride.

All the reagents must be chemically pure and those used after the cupferron precipitation should contain no iron. In blank experiments, it is recommended that 0·2 mg. of iron be added as ferric sulphate to give a complete separation of traces of aluminium by benzoate. Glass-ware should be made of Pyrex. The Eggertz tubes should be of the same size, preferably 15 mm. in diameter.

An accuracy of the order of one part in ten is attainable over the range 0·01 to 0·1 per cent. of aluminium in steel. G. S. SMITH

Precipitation of Beryllium Hydroxide by Means of α-Picoline. E. A. Ostroumov and B. N. Ivanov-Emin (*Zavod. Lab.*, **1945**, **11**, 386–391)—The precipitation of beryllium hydroxide by means of aqueous ammonia solution is incomplete, and the precipitate is contaminated by certain metallic hydroxides that are not precipitated by aqueous ammonia solution in absence of beryllium. Pyridine is also unsuitable as a precipitating agent since, although its aqueous solution has a *p*H of about 6·5 and beryllium hydroxide precipitates at *p*H 5·7, the high degree of dissociation of the pyridine salt that is formed during the hydrolysis of the beryllium salt so lowers the dissociation of the weakly-dissociated pyridine base that the hydroxyl concentration becomes insufficient for quantitative precipitation of beryllium hydroxide. It is now shown that α-picoline (2-methyl pyridine) precipitates beryllium hydroxide quantitatively, the *p*H of the final solution being about 7. The precipitate is dense, adsorption is low, the *p*H is insufficiently high for precipitation of magnesium, calcium, strontium, barium, and manganese hydroxides, and the reagent forms fairly stable complexes with manganese, cobalt, nickel, and zinc, so that quantitative separation of beryllium from these elements is possible. Precipitation is carried out in the nearly boiling solution and ammonium chloride is added to give a more compact precipitate. The presence of sulphates tends to give a finely-dispersed basic beryllium salt that is not completely converted into the hydroxide by boiling with the α-picoline solution, but the addition, before precipitation, of ammonium chloride in an amount equivalent to or up to double the sulphate content gives a dense, easily-coagulated hydroxide precipitate.

General procedure—To 100 ml. of the beryllium chloride solution add 5 per cent. aqueous ammonia solution until a cloudiness appears, and then clear the solution by adding two or three drops of 10 per cent. hydrochloric acid solution. Add 5 g. of ammonium chloride, heat to 100° C., add a few drops of methyl red indicator and then 20 per cent. α-picoline solution, with vigorous stirring, until the solution becomes yellow, followed by a further 10 to 15 ml. of the reagent. Bring the solution again to the boiling point, and maintain at 100° C. for 20 min., stirring occasionally. Filter the solution while hot, wash the precipitate with 3 per cent. ammonium nitrate solution containing a few drops of α-picoline, dry, and ignite it in platinum at 1000° to 1100° C., cool, and weigh under cover. Results are accurate to 2 parts in 500.

Experiments carried out in this way, but in presence of 6 to 7 g. of ammonium chloride, on solutions containing the equivalent of 0·05 g. of the oxides of manganese, cobalt, or nickel, in presence of 0·05 g. of beryllium oxide, indicate that only 0·02 to 0·04 mg. of the foreign oxide contaminates the beryllium oxide. In absence of ammonium salts, zinc salts give zinc hydroxide (incompletely), but in their presence there is formed a crystalline precipitate (*cf.*, the similar compound with pyridine, Kolthoff and Hamer, *Pharm. Weekblad*, 1924, **61**, 1222), moderately soluble at room temperature, but readily soluble on heating.

Separation of beryllium from zinc—Add aqueous ammonia solution until a precipitate appears, clear the solution with one or two drops of 10 per cent. hydrochloric acid solution, dilute to 150 to 200 ml., and add ammonium chloride in the proportion of 0·15 g. per ml. Boil and treat with 20 per cent. α-picoline solution as described above, but keep the solution hot for 30 to 40 min. Filter hot over boiling water (to avoid cooling of the solution), wash with hot, 3 per cent. ammonium nitrate solution containing α-picoline, etc. If the zinc content greatly exceeds that of the beryllium, dissolve the precipitate in 10 per cent. hydrochloric acid solution, and re-precipitate.

With solutions containing the equivalent of 0·05 g. each of beryllium and zinc oxides the weight of zinc oxide found in the first precipitate is 0·06 to 0·07 mg.

Determinations of 0·05-g. quantities of the oxides of barium (determined as sulphate), strontium (as sulphate in presence of alcohol), calcium (as oxalate), and magnesium (as pyrophosphate) in the filtrates after precipitation of beryllium hydroxide equivalent to 0·05 g. of the oxide showed an error of one or two parts in 500. G. S. SMITH

Spectrographic Analysis of Zinc-Base Alloys. L. Larrieu (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 403–407)—A rapid routine method for the quantitative analysis of alloy constituents and certain impurities, a spark discharge and grating spectrograph being used, is described.

Attention has been directed to the analysis of No. 2 Zamak alloy, which contains aluminium 3·5 to 4·6 per cent., copper 2·5 to 3·5 per cent., magnesium 0·02 to 0·10 per cent., iron 0·10 per cent. maximum, cadmium 0·005 per cent. maximum, tin 0·005 per cent. maximum, and lead 0·007 per cent. maximum. A smooth disc of the alloy forms the upper electrode, and a hollow-tipped rod, machined from National Carbon Company "spectroscopic carbon," packed with ammonium chloride, is the lower electrode in a Petrey spark stand. Two 30-second exposures, with no pre-spark, from different regions of the disc are superimposed on one spectrogram recorded on Eastman Spectrum No. 2 film, a fresh lower electrode being used for each exposure.

Two A.R.L. - Dietert comparator densitometers are used to measure transmission values for line plus background and adjacent background of a selected line of each element to be determined. Using zinc as the internal standard, the following lines have been found to be satisfactory for the analysis of the copper-bearing zinc-base alloys:—

Element	Wavelength
Zinc (internal standard)	2670 A.
Cadmium	2288 A.
Lead	2833 A.
Magnesium	2928·7 A.
Iron	2967 A.
Aluminium	3060 A.
Tin	3175 A.
Copper	3194 A.

Calculations are made on calculating boards to determine the line intensity ratios corrected for background. The working curves for the analysis were prepared from data obtained from the spectrographic and chemical analysis of three standard samples of this alloy.

The analysis of certain lots of this alloy has been repeated several times over a period of many months with the following precision:—

	Average content %	Standard deviation %
Lead	0·01066	0·000639
Aluminium ..	4·09	0·13

D. A. POYNTER

Spectrographic Analysis of Zinc-Base Alloys. R. W. Smith and J. E. Hoagbin (*Anal. Chem.*, 1947, **19**, 86–92)—A condensed spark method for the control of zinc-base die-casting alloys has been developed. The method permits the determination of aluminium, magnesium, and copper as alloying constituents, lead, tin, and cadmium as undesirable impurities, and occasional other impurities such as iron.

Investigation of the optimum excitation conditions for these determinations showed that a combination of high capacity and low added inductance is most favourable for the determination of aluminium and magnesium, but a moderate capacity and a very high added inductance are necessary for the accurate determination of the other elements previously mentioned. These features are incorporated in the circuit.

Chill-cast electrodes of the alloy under examination, turned down to 3/16 in. diameter with a 45° chamfer, are inserted into the electrode holders by means of a special loading jig to give an analysis gap of 2·9 mm. Two separate exposures of each sample are made after a 20-seconds pre-spark, one with little added inductance, and the other with the 7 *m*H added inductance for reasons already stated. The operations involved in recording the spectra of a sample are automatically controlled by a Multiflex seven-circuit timer which is arranged to move the shutter, move the lens, rack the plate, and switch in the high added inductance, all in the proper sequence for a complete exposure. The spectra are recorded by a Bausch and Lomb large Littrow instrument on an Eastman process plate.

Working curves have been prepared for aluminium 3·0 to 6·0 per cent., copper 0·75 to 1·60 per cent., magnesium 0·03 to 0·11 per cent., cadmium 0·003 to 0·06 per cent., lead 0·004 to 0·016 per cent., and tin 0·003 to 0·016 per cent., chemically analysed standards being used. Standard samples of zinc containing magnesium, tin, lead, and cadmium were prepared by making master alloys containing known amounts of these elements (about 0·1 per cent.) and carefully diluting these with calculated quantities of high-purity zinc. A calculating board is used to interpret the densitometer readings obtained from the plates; this incorporates a device that allows adjustment to

be made for day to day shift in the working curves. Various experiments have been carried out, without success, to try to account for this shift.

The line pairs used to construct the curves are:—

Constituent line, A.		Comparison line, A.	
Al	2367	Zn	2712
Al	2816	Zn	3018
Al	2660	Zn	2712
Cu	2961	Zn	2682
Cu	2369	Zn	2712
Mg	2792	Zn	3018
Fe	2967	Zn	2682
Pb	2833	Zn	2670
Sn	2839	Zn	2670
Cd	2288	Zn	2670
Cd	3403	Zn	2670

Some figures are given to demonstrate the precision and accuracy of the method.

	Precision (Average deviation %)	Accuracy (Average deviation %)
Aluminium ..	1·2	3·0
Copper	2·4	4·8

Precision is the average deviation per cent. from the mean value of many determinations of the same sample, and accuracy is the average deviation per cent. from the chemically determined value. It will be noted that both the precision and the accuracy of the aluminium determinations are better than those achieved with copper. This is because the electrical conditions and circuit constants were chosen to give the best possible aluminium determination, since this was regarded as the most critical factor, and the one for which an accuracy comparable with that obtained by chemical methods was desired.

As readings of line intensity were not corrected for background, relatively poor precision in the determination of elements present in low concentration has been attained. This is shown by the following table:—

Determination	Precision (Average deviation %)
Mg (0·03% standard) ..	2·1
Pb (0·007% standard)	8·6
Sn (0·006% standard)	9·3
Cd (0·005% standard)	6·8

D. A. POYNTER

Spectrophotometric Determination of Fluorine in Glass. M. C. Parrish, J. H. Widmyer, A. J. Brunner, and F. R. Matson (*Anal. Chem.*, 1947, **19**, 156–157)—By the application of an absorptiometric technique, with control of the temperature and the volume of solution, increased precision is obtained using Steiger's procedure for determination of fluorine (*J. Amer. Chem. Soc.*, 1908, **30**, 219), which depends on the bleaching action of fluorides on the yellow titanium - hydrogen peroxide colour. The method is suitable for glasses with fluorine contents up to 2 per cent., but higher contents may be determined by dilution of the sample with fluorine-free and boron-free glass. If more than 2·5 per cent. of boron and 0·5 per cent. of fluorine occur together in a glass, low results are obtained.

Procedure—Fuse 1 g. of glass with 3·5 ± 0·1 g. of sodium carbonate, extract with water, filter under suction, and wash with hot water. To complete precipitation of silica, ferric oxide, and alumina, add 20 ml. of zinc nitrate reagent (10 g. of zinc oxide and 20 ml. of concentrated nitric acid diluted to 200 ml.). Filter under suction, and wash with hot water. Cool, and add accurately 4 ml. of 6 per cent. hydrogen peroxide, 10 ml. of titanium sulphate solution (1 ml. ≡ 0·001 g. of TiO_2) and, slowly from a burette, 3 ml. of sulphuric acid. Adjust the volume to 100 ml. and the temperature to 30° C. Prepare a blank solution by the same method using a fluorine-free glass. Using 1-cm. cells and incident light of wavelength 440 mμ., measure the absorption of the blank solution, the test solution being used as reference. Read off the fluorine content from a calibration curve prepared by carrying out the method on samples obtained by mixing a glass of known fluorine content and a fluorine-free glass to give a range of 0 to 2 per cent. of fluorine. H. J. CLULEY

Physical Methods, Apparatus, etc.

Constant Temperature Cell Bath for Use with the Spekker Photo-electric Absorptiometer. R. B. Ingle Finch and C. A. Parker (*Chem. and Ind.*, 1947, 257)—When fitted with a constant temperature bath the "Spekker" photo-electric absorptiometer may be used for the determination of velocity constants for any reaction involving a colour change. A U-shaped bath has been constructed from ¼-in. Perspex sheet and is mounted on a brass plate which replaces the normal cell carrier. Accommodation has been allowed for three, 1-cm. "tall - form" absorption cells each fitted with a loosely fitting stopper moulded from polythene. The sides of the bath are polished and do not absorb appreciably in the visible region. No difference in transmission was detected for the three positions of the stage. Water from a thermostat pump is circulated through a lagged copper tube cemented to the bottom of the bath. Drainage back to the thermostat takes place through two, ¼-in. bore tubes cemented ⅜ in. from the top of the bath. A felt filter is incorporated in the pump circuit. The observed variation of the cell contents was ±0·03° and ±0·05° at 20° and 60° C., respectively. Random errors of single Spekker measurements may be averaged by taking readings at sufficiently small time intervals. Four values of the velocity constant for the same first order reaction, obtained under identical conditions, showed good agreement. G. A. BASSETT

Separation of Anions by Adsorption on Alumina. H. Kubli (*Helv. Chim. Acta*, 1947, **30**, 453–463)—Commercial aluminas, and pure alumina treated with sodium carbonate solution adsorb cations, but after being treated with acid the aluminas adsorb anions. When a solution of the sodium salts of a mixture of inorganic acids is passed through a column of acid-treated alumina the anions, having different affinities for the alumina, are adsorbed on different zones (Schwab

and Dattler, *Z. angew. Chem.*, 1937, **50**, 691). The three experimental sections of this paper report the order in which the common anions are adsorbed on a column of acid-treated alumina, the limit of detection of certain anions by adsorption on alumina, and examples of quantitative separations.

Order of adsorption of anions—Preparation of the alumina—Shake 20 g. of basic alumina ("Neuhausen") with 100 ml. of diluted perchloric acid (1 + 1) and allow to stand for 2 hr. Filter off the alumina, wash with 50 ml. of water, and dry for 1 hr. at 120° C.

Columns—Fit a series of glass tubes or acetyl cellulose straws about 7 cm. long and 2·3 mm. in diameter into a rubber plate resting on a suction tube. In each tube place a wad of cotton wool and press down over the wad 100 to 200 mg. of the alumina. Wash with 0·1 to 0·2 ml. of re-distilled water. Add the test solution from a micro-pipette with a fine capillary jet, using pressure to force the liquid out. Suck the air-free liquid through the column at a controlled rate and wash with 0·1 to 0·2 ml. of water added in portions from the micro-pipette.

Comparison of two anions—Exactly 0·5 ml. of a 10^{-3} *N* solution of the sodium or potassium salts of two of the anions concerned was passed through the column, using the technique described. A similar quantity of a suitable reagent was added in the same way and run through the column to produce a coloured compound of one or both of the anions on the alumina. For some of the anion mixtures, two reagents were used. The region in which each anion had been adsorbed was apparent from the position of the coloured bands.

Schwab and Dattler have listed the order of adsorption of some of the common anions. The purpose of the present work was to fit other anions into that list. The extended list is: OH'–PO_4'''–C_2O_4''–F'–SO_3'', $[Fe(CN)_6]''''$, CrO_4''–S_2O_3''–SO_4''–$[Fe(CN)_6]'''$, Cr_2O_7''–NO_3', CNS'–I'–Br'–Cl'–NO_3'–MnO_4'–ClO_4'–CH_3COO'–S''.

Limit of detection of anions—The technique used was similar to that already described, the column being treated with a solution of the sodium or potassium salt of the anion and then, if necessary, with a suitable reagent. The limits of detection of several anions are given in the table.

Anion	Reagent	Limit of detection μg.
MnO_4'	—	0·1
Cr_2O_7''	—	1
	$AgNO_3$	0·5
S_2O_3''	$AgNO_3$	0·12
$[Fe(CN)_6]'''$	$FeSO_4$	0·3
$[Fe(CN)_6]''''$	$FeCl_3$	0·5
CNS'	$FeCl_3$	0·2
Cl'	$AgNO_3$, light	0·25
	" " I	0·05
	" " II	0·01
Br'	$AgNO_3$, light	0·2
	" " I	0·01
	" " II	0·005
I'	$AgNO_3$, light, I	0·04
	" " II	0·01
PO_4'''	$(NH_4)_2MoO_4$, benzidine, NH_3 vapour	0·01

In the test for chloride, bromide, and iodide the columns were treated with silver nitrate and then exposed to light. The two reagents used to increase the sensitivity of the tests were a metol-hydroquinone photographic developer (I), and a solution of 1 g. of metol and 5 g. of citric acid in 50 ml. of water, the solution containing one drop of 2 *N* silver nitrate per ml., added immediately before use (II). The latter reagent deposits silver on any silver nuclei in the column.

For most of the anions the height of the adsorption zone is roughly proportional to the concentration of anion in the solution added to the column.

Quantitative separations—Preparation of the column—Place about 40 g. of basic alumina in a filter crucible, wash slowly with 100 ml. of 0·5 *N* hydrochloric acid, followed by 100 ml. of water, drain the alumina by suction, and dry at 120° C. for 4 hr. Run 4 g. of the alumina into a 9-mm. diameter glass tube filled with water.

Procedure—From 5 to 10 ml. of a 0·025 to 0·05 *M* solution of the compound concerned were passed through the column at a rate of 30 drops per min., and the column was washed with 10 to 15 ml. of water.

Under conditions described, sulphate from potassium sulphate is quantitatively adsorbed but the potassium ion is not. The sulphate can be recovered by washing the column with 20 ml. of 0·05 *N* sodium hydroxide. The ions of sodium hydrogen phosphate and of potassium dichromate are quantitatively separated.

The difficulties met with in the determination of magnesium and sulphate in the presence of iron and phosphate (Samuelson, *Z. anal. Chem.*, 1939, **116**, 328) can be overcome by using the adsorption technique. Ten ml. of a solution containing ferric iron, magnesium, phosphate, and sulphate ions were passed through a column containing 10 g. of hydrochloric-acid-treated alumina, the column was washed with 15 ml. of 0·5 *N* hydrochloric acid, and the effluent analysed for iron and magnesium. The column was washed with 50 ml. of diluted 0·880 ammonia (1 + 1) and the solution of the anions so obtained diluted to 100 ml. Analysis of the cation solution gave: iron 11·1 mg., theory 11·17 mg.; and magnesium 10·1 mg., theory 10·00 mg. Analysis of 40-ml. portions of the anion solution gave: phosphate 2·0 mg., theory 2·07 mg.; sulphate 27·4 mg., theory 27·35 mg.

B. ATKINSON

Dielectric Identity Test for Plasticisers. Polyvinyl Chloride Plastics Type. M. A. Elliott, A. R. Jones, and L. B. Lockhart (*Anal. Chem.*, 1947, **19**, 10–15)—A method has been worked out to establish the identity of a plasticiser from a given polyvinyl chloride product with that from previously manufactured batches of the same product. The method compares the unknown with the standard, and will detect contaminants present in sufficient quantity to affect the electrical or mechanical properties of the finished plastic. It should form a useful control test in the manufacture of chlorinated or other polar organic liquids. Under high-radio-frequency conditions, each

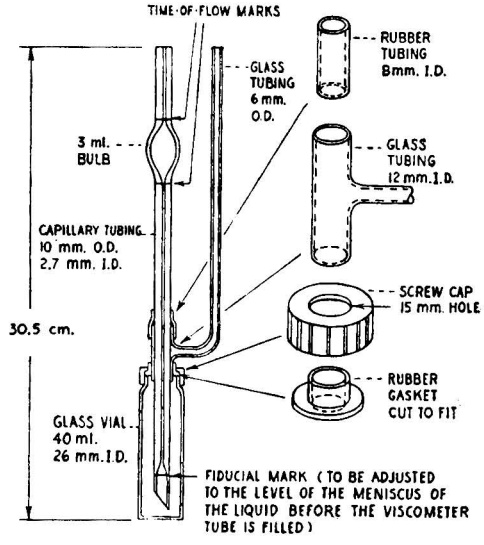
plasticiser shows a characteristic minimum value of dielectric constant and loss factor (anomalous dispersion). As this value is a function of viscosity of the liquid and therefore of temperature, it is simpler, experimentally, to keep the frequency constant and vary the temperature.

Extraction of plasticiser—Place 6 g. of sample (about 20 mesh) in a Wiley - Richardson type of extraction apparatus and add 50 ml. of a mixture of (3 : 2, by volume) benzene and methyl alcohol. Extract for 20 hr. and allow to cool. Filter, if necessary, and evaporate solvent on a steam-bath. Add 40 ml. of ethyl alcohol, filter off any precipitate formed, and again evaporate the solvent. The addition of alcohol and the second evaporation may be omitted if it is desired to retain alcohol-insoluble materials in the extract. Place the plasticiser in a vacuum oven at 110° C. and atmospheric pressure for 0·75 hr.; then evacuate to less than 3 mm. of mercury for another 0·75 hr. Store the plasticiser in a desiccator until the electrical measurements are made. Oven treatment control is important.

Electrical measurements—The design of the test cell, now being manufactured commercially, permits rapid cleaning and refilling, and requires only 0·3 ml. of sample. The capacitance and conductance of the filled cell are measured and the temperature is noted at intervals as it rises slowly after cooling by "dry ice" to a temperature 10° C. below the peak value of the loss factor curve. In routine measurements, it is necessary to cover only a range of about 10° C. around this peak. The electrical equipment used comprised a signal generator, a Twin-T impedance measuring circuit, and a well-shielded, high-frequency receiver. All measurements were made at a frequency of 10 megacycles per sec. in a shielded room. The most satisfactory method found to detect the bridge balance was to modulate the 10 megacycle current with an audio-frequency of 800 cycles, with the receiver set for normal operation. The vertical plates of an oscillograph were connected to the phone jacks of the receiver and the horizontal plates directly to the audio-oscillator. At balance, a crescent-shaped pattern appears on the oscillograph screen and variations of capacitance and conductance can be detected independently. Leads between the Twin-T circuit and the cell are of "silver-plated phenolic tubes"; a method of plating these tubes is given. Stray capacitance of the cell is determined with a liquid of known dielectric constant. Curves are reproducible to within 2 per cent. in height and 0·5° C. in temperature. Other analytical procedures involve determination of chlorine, phosphorus, and refractive index. G. A. BASSETT

Capillary-Type Viscometer: for Use with Solutions Containing Volatile Solvents, with Application to Measurements of Viscosities of Nitrocelluloses. D. P. Shoemaker, E. Hoerger, R. M. Noyes, and R. H. Blaker (*Anal. Chem.*, 1947, **19**, 131–132)—A diagram of the viscometer is given in the figure. Solutions to be examined are prepared in a glass vial which has a threaded mouth and screw cap. The viscometer assembly is then screwed on to the vial in place of the cap. An advantage of the instrument is the small volume, about 10 ml., of liquid required.

The capillary tube of the viscometer passes through a short slightly broader tube bearing a side arm, and is held in position by the short piece of rubber tubing. At its lower end the broader tube is held in the rubber gasket which fits inside the perforated screw cap. The gasket is pressed



on to the lip of the vial when this cap is screwed on. Fit the apparatus with a capillary such that the time of flow is in the range 100 to 500 sec.

Procedure—The special features of the procedure are as follows. After screwing on the assembly adjust the mark at the base of the capillary to the level of the liquid meniscus. Place the apparatus in a thermostat. Connect the side arm to a source of compressed air to raise the liquid up the viscometer, release the pressure, and measure the time for the liquid to fall between the two marks above and below the bulb. Calibrate the viscometer with liquids of known viscosity.

The viscometer is shown to give satisfactory results for the viscosities of 10 per cent. solutions of several nitrocelluloses. Determinations of viscosity on seven samples of Hercules nitrocellulose No. 6278, dissolved in a mixture of ethyl alcohol and acetone in the ratio 10 to 90 by volume, gave an average viscosity of 46·02 stokes with a total spread of 3·3 per cent. and a mean deviation of 0·7 per cent. If representative samples have to be taken from a large amount of material, quicker results will be obtained by using a larger vial and an increased weight of sample. B. ATKINSON

Apparatus for Rapid Conductometric Titrations. Determination of Sulphate. L. J. Anderson and R. R. Revelle (*Anal. Chem.*, 1947, **19**, 264–268)—The apparatus consists essentially of a power unit supplying 20 volts at 1000 cycles,

a Wheatstone bridge of the continuous deflection type, and a vacuum tube [valve] voltmeter which is used to indicate conductance changes. The titration cells, of 500-ml. capacity for macro-titrations and of 100-ml. capacity for micro-titrations, are fitted with pure silver electrodes, which are superior to platinum electrodes for avoiding electrolysis of solutions. Two cells containing similar volumes of test solution are included in the circuit, the second cell acting as a compensator to balance out changes of conductance due to variation of temperature and other effects; this device renders unnecessary rigid control of the temperature of the test solution. Changes of conductance of 0·02 per cent. can be detected with the apparatus.

For the determination of sulphate, the solution is "seeded" with crystals of barium sulphate. Precipitates of larger crystal size are thus obtained, and result in the following advantages: (i) precipitation is more rapid, and the time required for titration is considerably reduced, (ii) errors due to adsorption are minimised, and (iii) the uniform nature of the precipitates obtained from one titration to the next permits precision of determination. To obtain sharp end-points concentrated solutions of barium nitrate (0·2 *M* for macro- and 0·35 *M* for micro-titrations) should be used.

Quantities of sulphate down to 1 mg. have been determined with an accuracy of ±1 per cent. Titrations are successful in the presence of 50 times the quantity of chloride. Calcium interferes, but this may be overcome by the addition of oxalate provided that the sulphate to calcium ratio is at least 6 : 1. The method has been applied to the determination of sulphate in sea-water.

For details of the apparatus and of the titration technique the original paper must be consulted.

H. J. CLULEY

Calcium Chloride as a Polarographic Supporting-Electrolyte. N. Ya. Khlopin (*J. Anal. Chem. Russ.*, 1947, **2**, 55–59)—Polarography in a calcium chloride solution simplifies the determination of individual cations in a mixture (*cf.* Heyrovsky). At *p*H 4·5 to 6·0, the deposition potentials of various metals now studied are: bismuth −0·16 v., copper −0·28 v., tin −0·42 v., lead −0·48 v., cadmium −0·66 v., nickel −0·86 v., zinc −1·04 v., ferrous iron −1·3 v., manganese −1·44 v., and aluminium −1·66 v., with respect to the saturated calomel electrode. A concentration of calcium chloride of 30 per cent. (density 1·28271 at 17·9° C.) is recommended. Removal of oxygen is unnecessary. The bismuth, lead, cadmium, zinc, and manganese waves are well-defined; that of copper has sometimes a slight maximum, but since the value drops to that of the diffusion current the height is easily measured. If the preliminary treatment of a sample is such that the dry residue may be extracted with the minimum volume of 30 per cent. calcium chloride solution, then very small concentrations of the whole series of cations named above may be determined without previously separating them. This property of calcium chloride solution distinguishes it from other supporting electrolytes. Under the conditions used, *viz.*, temperature 20° C., height of mercury column 36 cm., and $m^{2/3}t^{1/6}$ 2·22 to 2·25, the diffusion currents, I (milliamps) and cation concentrations C (mg.-mol. per litre) are accurately expressed by $I = bC$, where $b = 3{\cdot}016$ for bismuth, 4·335 for copper, 3·129 for lead, 4·98 for cadmium, 4·454 for zinc, and 5·903 for manganese. The diffusion coefficients, D, calculated from the Ilkovic equation are then: $0{\cdot}13 \times 10^{-5}$ for bismuth and lead, $0{\cdot}26 \times 10^{-5}$ for copper and zinc, $0{\cdot}33 \times 10^{-5}$ for cadmium, and $0{\cdot}48 \times 10^{-5}$ cm.^{2}sec.$^{-1}$ for manganese.

Nitrates and free hydrochloric acid must be removed. Treatment with hydroxylamine hydrochloride in the presence of hydrochloric acid is recommended to prevent the formation, during evaporation, of basic salts of bismuth and copper. When tin is present the *p*H should be 7, but at this value the height of the lead wave is less than that at *p*H 1·5 to 6·5; hence calibration curves should be obtained under the same conditions. For the determination of bismuth, copper, and lead in tin for tinning, the bulk of the tin should be separated first. Manganese gives a good wave at *p*H 6·5 to 7·0, but traces of undecomposed hydroxylamine interfere and must be carefully removed, since otherwise the manganese and hydrogen waves become indistinguishable. With cadmium, the half-wave potential in 30 per cent. calcium chloride is quite independent of *p*H, and serves usefully in the analysis of limestone materials for defining the reduction potentials of the various cations present.

To obtain the solution for polarography, one of the following methods is used: (i) when the concentrations are not too small, mix the solution with sufficient more concentrated calcium chloride solution to give a final solution which is 30 per cent. in calcium chloride, (ii) when the material available is small in amount and the cation concentrations are very low, normally evaporate to dryness, and extract the residue with an accurately measured volume, 0·5 to 2 ml., of 30 per cent. calcium chloride solution, or (iii) add a known quantity of calcium chloride, evaporate to dryness and then extract with the correct amount of water.

Some examples, but without specified details, of the use of the method are given: (i) analysis of foundry smokes, *e.g.*, the determination of 0·4 mg. of lead and 0·1 mg. of copper per cu. metre, (ii) the determination of tetra-ethyl lead in air, absorption being carried out in castor oil containing iodine, *e.g.*, determination of 0·0005 mg. of lead per litre of air, using 600 litres, (iii) the determination of zinc and copper in tinned fish, and (iv) the determination of 0·17 per cent. of lead in tin for tinning.

G. S. SMITH

Improvements in Polarographic Instrumentation. [A Polarographic Cell for Routine Use.] G. E. Philbrook and H. M. Grubb (*Anal. Chem.*, 1947, **19**, 7–10)—The cell consists of a weighing bottle (25 × 60 mm.) fitted through a rubber bung into a 150-ml., lipless beaker that serves as a thermostat. Through a stopper in the

weighing bottle pass the dropping mercury cathode capillary, an inlet for the anode pool mercury, and three stainless steel hypodermic needles through which nitrogen can be passed to remove dissolved oxygen from the solution, or over the liquid while the polarogram is recorded, and vented to the atmosphere. A fourth needle packed with beeswax admits a connection to the anode mercury pool.

The cell, designed for routine use, is composed of equipment that is readily replaced.

J. G. WALLER

Reviews

PLANT PHYSIOLOGY. By M. THOMAS, M.A., F.R.S.E. Third Edition. Pp. xi + 504. London: J. & A. Churchill, Ltd. 1947. Price 28s.

At first sight a book dealing with plant physiology might be regarded as somewhat outside the province of a chemist. The definition of physiology is given elsewhere as the science of organic functions and vital phenomena of animals and plants. Professor Thomas interprets the meaning of vital phenomena so broadly and with such a well-defined appreciation of the chemical and physical integrations that his work has a special appeal to the chemist, and particularly to the biochemist and soil chemist. In the First Edition (1935) of his work he writes: "It cannot be too strongly emphasised that there is an immense scope for pioneering investigation for those who can apply in the field or in the laboratory modern knowledge of physics and chemistry to plant physiology." Attention is confined to the analysis of the principal physiological processes that occur in green plants, having in view the formulation in terms of physico-chemical concepts of problems for further consideration.

The present edition includes new matter treating of the oxidative enzymes, zymase and phosphorylases, cells as osmotic systems, nitrogen metabolism, photosynthesis, and plant growth substances. It is divided into four parts: protoplasm; the functional purpose of water, solutes and gases; nutrition and metabolism; and growth and movement of plants. The first part comprises chapters on the biological characteristics, the physico-chemical properties and the active enzymic system of protoplasm. The second part elaborates the complex inter-relation between the cell, the soil and the aqueous and gaseous phases, against the background of their relation to the living plant. The third part includes chapters on photo-synthesis of carbohydrates, respiration, and the metabolism of di- and tri-carboxylic acids. The final part is divided into growth, plant growth substances, and plant movements. Then follows an appendix of 39 pages, dealing with the chemistry of plant metabolism products, and an appendix of 22 pages devoted to a brief résumé of the physical chemistry of aqueous disperse solutes, surface phenomena, hydrogen-ion concentration and osmotic pressure. Finally, a bibliography extends over 7 pages.

Of special interest is the chapter on protoplasm as a chemically active system. This covers 52 pages and presents a conspectus of the factual knowledge of, and diverse hypotheses in connection with, enzymes and their fundamental functions in the life of the cell.

The classical view that the aqueous conditions of a cell are dependent on osmotic pressure is reviewed in the light of the theory that metabolic processes exert a significant effect—a viewpoint of interest to the chemist. It is suggested that metabolic activity may exercise a predominant influence on the rate of absorption of solutes and the final amounts absorbed, although comparison of plant sap with that of a solution outside the plant indicates that it is rare for a diffusible substance to exist at the same concentration inside and outside living cells.

Micro-chemistry now enters increasingly into the elucidation of problems concerned with plant life. By its means methods for estimating carbohydrates and nitrogenous substances have been greatly improved. It might seem, however, that microscopy is being stretched to its limit when bacteria are brought into service as indicators. *B. Termo* enclosed with green tissue under a cover-slip change from a condition of quiescence to one of mobility when the merest trace of oxygen is liberated from the green tissue; other bacteria become luminescent under similar conditions.

The discussion of plant growth substances extends over only 22 pages and includes an account of auxins. Advances in knowledge of this subject have been rapid in recent years and are having many unexpected commercial applications. It seems probable that it will be found necessary to give more space to this subject in the next edition.

Professor Thomas' book may not be indispensible to the chemist, but it may well be said that its value—and interest—is as great for the chemist as for the plant physiologist.

GEORGE TAYLOR

PHYSICAL METHODS GROUP

A MEETING of the Physical Methods Group organised by the Polarographic Discussion Panel will be held in Leeds on Friday, April 9th, 1948. In the afternoon visits will be paid to the Brotherton Library, University of Leeds, to the Research Laboratories of the Physical Chemistry Department of the University and to the Central Research Department of Brotherton & Co., Ltd.

At 5.30 p.m. a meeting will be held in the Chemistry Department of the University. The following papers will be read and discussed:—

"Polarography in Germany," by G. W. C. Milner, M.Sc., A.R.I.C.

"The Polarographic Analysis of Light Alloys and Metals," by W. Stross, M.D.

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

MICROCHEMISTRY GROUP

JOINT MEETING AT ABERDEEN

A JOINT Meeting of the Microchemistry Group with the local sections of the Chemical Society, the Society of Chemical Industry and the Royal Institute of Chemistry will be held at Aberdeen on May 4th and 5th, 1948. The programme will be as follows:—

Tuesday, May 4th. Choice of visits at 10 a.m. to—

The Research Laboratories, Chemistry Department, University of Aberdeen.
The Macaulay Institute for Soil Research.
The Rowett Institute for Research in Animal Nutrition.
The Torry Research Station (D.S.I.R.) for Research on Fish Products.

Luncheon at the kind invitation of the University of Aberdeen.

Meeting at 2.15 p.m.—Symposium on "The Analysis of Traces and Ultra-micro Quantities," at which the following papers will be read.

"Ultra-Microchemical Methods," by Cecil L. Wilson.

"The Simultaneous Concentration of Trace Elements with Organic Precipitants," by R. L. Mitchell.

"Trace Determination by means of the Polarographic Method of Analysis," by G. W. C. Milner.

And after tea—

"Micro-Diffusion Analysis," by T. G. Brady.

Wednesday, May 5th.

At 9.45 a.m. there will be a paper read by Cecil L. Wilson:

"The Microscope as a Chemical Tool,"

and later a coach trip through Deeside.

Further details will be circulated to members of the Group later.

THE BIOMETRIC SOCIETY—BRITISH REGION

THE Biometric Society, a new international organisation, whose aim is the furtherance of quantitative biology in all its aspects, came into being at a conference held in Woods Hole, Massachusetts, during September, 1947. Its President is Professor R. A. Fisher, F.R.S., and its Secretary, Dr. C. I. Bliss, of New Haven, Connecticut. It is hoped to hold international meetings every few years, and all members will receive the journal *Biometrics*.

For the purpose of more local activities, the Society is organised into regions, of which it is proposed that a British Region shall form one. A provisional committee, under the chairmanship of Dr. J. W. Trevan, Vice-President for the Region, is now engaged in drafting proposals for the regional organisation and activities, which it is intended will primarily provide a means of bringing together all those biologists and biochemists who are interested in the application of quantitative methods with the statisticians and mathematicians who can co-operate in developing these methods. It is hoped shortly to present these proposals to an inaugural meeting of the Region. Further information can be obtained from the Regional Secretary, Dr. K. Mather, of the John Innes Horticultural Institution, London, S.W.19.

APPLICATIONS are invited for the post of administrative officer to the Pneumokoniosis Research Unit of the Medical Research Council at Cardiff. Duties would include the organisation and supervision of supply arrangements, accounts, filing, and junior staff appointments for the various departments of the Unit. Applicants should have had administrative experience, and if possible, knowledge of medical and laboratory equipment. Initial salary of £450–£600 according to age and experience. Applications, stating age, qualifications and experience, accompanied by the names of two referees, one professional and one personal, should be sent to the Director, Pneumokoniosis Research Unit, Llandough Hospital, Nr. Cardiff, immediately.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
SOUTH KENSINGTON, S.W.7

PHYSICS DEPARTMENT
(Technical Optics Section)

A COURSE OF EIGHT LECTURES
By
B. K. JOHNSON, D.I.C.,
On
MICROSCOPY
will be given on
Tuesdays and Thursdays, at 4 p.m., commencing on Tuesday, 27th April, 1948.

The lectures, which will be accompanied by practical demonstrations, will include modern advanced methods in microscopy. They will be suitable for those having to use the microscope in technical practice.

Application for admission should be made to the Registrar of the Imperial College, Prince Consort Road, S.W.7. The fee is £2 2s. 0d. for the Lectures. Students of the College and Inter-Collegiate students will be admitted free (on production of an Inter-Collegiate ticket).

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