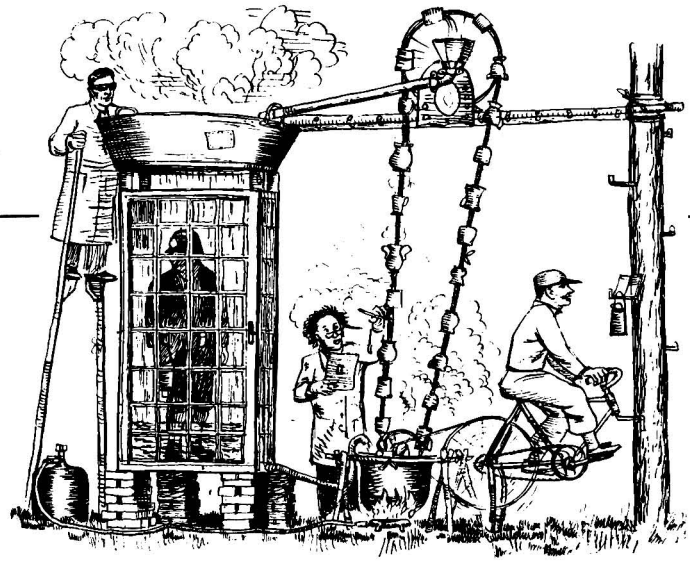
THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society for Analytical Chemistry

Editor: J. B. ATTRILL, M.A., F.R.I.C.
7-8 IDOL LANE, LONDON, E.C.3
Telephone: MANsion House 6608
Published for the Society by
W. HEFFER & SONS, LTD., CAMBRIDGE, ENGLAND

Volume 80 Price 6s. 6d.
Subscription Rate, inclusive of Analytical Abstracts, 80/- per annum, Post Free
No. 948, Pages 161-240 March, 1955

Our cartoonist's impression of Soxhlet extraction



THE ANALYST

New Analytical Laboratories

ANALYSTS in industry need to be versatile, and they are often called upon to help in designing new analytical laboratories. The advice the analyst gives will not always be taken, but that should not deter him. He should try to ensure that his new analytical laboratory will be much better than his old one, *i.e.*, such that the analytical work may be carried out more expeditiously and accurately. Moreover, he should be certain that the working conditions in his new laboratory are an improvement on those in the old one. His natural tendency will be to secure a laboratory that will enable him to cover the work previously carried out under the old conditions. In thinking only on these lines, however, he may make a mistake. Analytical chemistry is advancing rapidly, and more and more physical methods are being used. Consequently, it is wise to think ahead, and our analyst is advised to make his new laboratory as flexible as possible. By doing so he will be able to change his methods in the ensuing years with the minimum of disturbance to the general work of his laboratory.

We are all familiar with laboratories that were designed to carry out the general operations of gravimetric and volumetric analysis in the first place. Attempts are being made to adapt many of them to physical methods of analysis, but the task is often extremely difficult and, at times, impossible.

It is probable that the analyst designing a new analytical laboratory will not be given unlimited space. It is more likely that he will be given a certain area of floor space and asked to make the maximum use of it. He will do well to enlist the help of two or three senior members of his staff. Experienced analysts have spent a great deal of time in laboratories; their counsel is invaluable.

With this small team the analyst will be able to plan his new laboratory and, in doing so, he will take account of the views of the micro-analyst, spectroscopist and so on, not forgetting the junior members of his staff, who are often full of useful ideas.

As far as possible he will seek to make the various sections of his new laboratory "functional," *i.e.*, each one with a purpose. He will get away from the idea that every senior analyst must have an office; he should only have an office if that is essential to his work. He will avoid useless passages and corridors and, in doing so, give more bench space to each occupant of his laboratory. He will consider carefully what proportion of the bench space shall be allotted to fixed apparatus, such as titration benches, steam-distillation apparatus, saponification bench, gas analysis apparatus and centrifuge. He will be well advised to make sure that the benches used for general work are as free from fixed apparatus as possible. Analysts with plenty of room to work in will provide better results. There will be fewer breakages, fewer repeat experiments and, moreover, a reduction in the minor accident rate.

Fume chamber space should be adequate and really efficient. Analytical laboratories can be much healthier places than they used to be if operations that should be carried out in fume chambers are not performed in the open laboratory. Further, the fume chambers should no longer be regarded as repositories for dirty H_2S Kipps and bromine bottles. Tiled fume chambers can be washed down and kept scrupulously clean. A senior analyst should be in a position to ask a junior member of his staff to carry out an operation in a clean fume chamber and to leave the latter as clean as he found it. Further, the importance of adequate lighting, of plenty of sink accommodation and of ample supplies of hot water and distilled water cannot be over-emphasised.

There will always be controversy as to whether or not the best arrangement will be to provide a main laboratory and, round it, smaller laboratories or rooms designed for specific purposes. A sound decision will probably be made if some consideration is given to the work that may be performed in these smaller laboratories or rooms and how they fit into the general scheme of things. One of the most useful of these smaller laboratories will be the standardisation department, used for the standardisation of solutions, thermometers, viscometers, volumetric flasks, pipettes, burettes and so on. An efficient department of this kind will definitely increase the general accuracy of the whole department. Being so basic in character, the standardisation section will serve as a first-class initial training ground for the young analyst entering the profession.

Next we must think of the balance room. More and more free swinging balances are being replaced by aperiodic balances, preferably with rider weights. These balances require really efficient housing, and no chances must be taken, particularly if the balance room is situated on an upper floor. The micro-balance is a separate problem.

It is a good plan to set apart one room, adjacent to the main laboratory, as an analytical library. All the books should be readily available and none should be borrowed. The worst offenders, *i.e.*, in losing books by borrowing, are often the best analysts.

An analytical records office is a first-class investment; it should be possible to refer quickly to the results obtained in an analytical laboratory, and similar remarks apply to reports.

In these days most analysts are concerned with the determination of tr ce metals in some form or other. How many analysts are fighting a losing battle determining traces of iron in finished products under conditions in which rust from structures overhead is liable to vitiate the result! It is often desirable to set a laboratory apart for this purpose, *i.e.*, trace metal determination, with adequate precautions.

Good analytical laboratories often include separate furnace rooms containing furnaces and drying ovens, and rooms where solvent extractions are carried out.

Microchemical laboratories are probably best on ground floors, where attention can be paid to the really efficient housing of the balances.

Further, good analytical laboratories to-day often include special rooms designed for physico-chemical, spectrographic and spectrophotometric work. A safety room is a good investment; there is no need to alarm the whole laboratory when a Carius tube bursts.

There are probably no hard and fast rules about designing new analytical laboratories, but it may be useful to bear in mind some of the points made in this brief article.

J. H.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

SECRETARYSHIP OF THE SOCIETY

THE Council has accepted with regret the resignation of Mrs. D. V. Hicks from the Secretaryship of the Society and, for the purposes of the Companies Acts, this was resumed on January 1st, 1955, by the Honorary Secretary, Dr. K. A. Williams. Miss P. E. Hutchinson has been appointed Assistant Secretary.

SECRETARIAT OF THE ANALYTICAL METHODS COMMITTEE

THE Council of the Society has appointed Dr. Catherine H. Tinker to be the Secretary of the Analytical Methods Committee.

SPECIAL MEETING

A SPECIAL Meeting of the Society was held at 6 p.m. on Wednesday, February 2nd, 1955, in the Lecture Theatre of the Royal Institution, 21 Albemarle Street, London, W.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C. A lecture, accompanied by demonstrations, was given by Professor G. Schwarzenbach, Ph.D., on "The Complexones and their Analytical Application."

The lecture theatre was completely filled by 579 members and visitors, and over 30 people had to be turned away.

ORDINARY MEETING

AN Ordinary Meeting of the Society, organised by the Microchemistry Group, was held at 7.30 p.m. on Friday, January 28th, 1955, at the School of Pharmacy, Bloomsbury Square, London, W.C.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C.

The following papers were presented and discussed: "The Kjeldahl Method—Catalysts or Oxidants?" by A. E. Beet, B.Met., Ph.D. (see summary below); "Some Applications of Microchemical Analysis in Pharmaceutical Chemistry," by G. E. Foster, B.Sc., Ph.D., F.R.I.C. (see summary below); "The Composition of Tobacco Smoke: Some Minor Organic Constituents," by A. J. Lindsey, M.Sc., Ph.D., F.R.I.C., A.M.I.E.E. (see summary below).

THE KJELDAHL METHOD—CATALYSTS OR OXIDANTS?

DR. A. E. BEET said that for a wide range of substances, including cereals, feeding-stuffs, coals and alkaloids, it had been found that an oxidising agent, *viz.*, potassium permanganate, added gradually to the hot acid digest, had several marked advantages over the usual catalysts employed in the Kjeldahl method on both the semi-micro and micro scales. The conversion was very rapid and complete, the excess of oxidant served as an indication of complete conversion, and no destruction of ammonia occurred even on prolonged boiling.

When a 10-mg sample of tryptophan was used as the standard substance, the cause of the failure of the Badami - Whitaker method to yield reliable results was traced to a deficiency of about 50 mg of permanganate. This final addition of permanganate must be made *after* the attainment of the char-free stage in order to convert the colourless pyridine derivatives to ammonia.

Of other oxidants tested, only potassium dichromate was found to be satisfactory, but it gave no indication of end-point. Perhydrol (30 per cent. hydrogen peroxide) was not satisfactory with coals, undecomposed grey particles always remaining, but if excess of potassium permanganate was subsequently added, correct values were obtained.

There was some evidence that the nitrogenous constituents of all coals, high-rank and low-rank, were of the same type.

SOME APPLICATIONS OF MICROCHEMICAL ANALYSIS IN PHARMACEUTICAL CHEMISTRY

DR. G. E. FOSTER said that the increasing use of microchemical methods of analysis in the field of organic chemistry during the period following World War I had an important influence on the progress of medicine and pharmacy. During the period that elapsed before World War II a complete knowledge of the chemical structures of many hormones,

and vitamins was obtained and in some instances the products were synthesised. The use of these synthetic products in medicine to-day was undoubtedly due to microchemical analysis, which enabled their structures to be determined and their syntheses achieved.

The human doses of many of our most potent drugs were of the order of 1 to 5 milligrams, and single tablets and many ampoules of injections would contain no more material. In this field the scope for microchemical analysis needed no emphasis, but a few applications were discussed.

The British Pharmacopoeia and other books of standards described microchemical tests for limiting the amounts of undesirable impurities in pharmaceutical products, and sensitive colour reactions were extensively used for the determination of small quantities of some drugs, particularly alkaloids. The great sensitivity of the end-point of iodimetric titrations had led to the widespread use of such methods in microchemical work; some pharmaceutical applications were described.

Microchemistry was often of value in the solution of problems in the pharmaceutical industry. Reference was made to the study of the variation in the filling of 1-ml ampoules of injection and of vials of insulin zinc suspension. The replacement of the air in an ampoule of injection by nitrogen before sealing was sometimes important, and microchemistry had been used to study the efficiency of this operation.

Pharmacy advances on many fronts, engaging the attention of experts in diverse fields, amongst which microchemical analysis may justly claim a place.

The Composition of Tobacco Smoke: Some Minor Organic Constituents

Dr. A. J. Lindsey said that a review of the composition of tobacco smoke revealed that in the published literature there was much variation, both quantitative and qualitative, in the reports of its nature. Although over seventy compounds had been reported present, it was certain that many others were there.

It was desirable to standardise the conditions of smoking and to examine the mainstream smoke, produced artificially in a manner resembling as closely as possible normal human smoking. If this was done for hydrocarbons, it was found that, by chromatography followed by absorption spectrophotometry, a large number of polycyclic hydrocarbons were present. Acenaphthylene, anthracene, anthanthrene, azulene, pyrene, 1:12-benzperylene, 3:4-benzpyrene, fluoranthene and phenanthrene have all been detected and some have been determined.

The results have some significance when considered in the light of the statistical relationship between cigarette smoking and lung cancer.

Relevant publications by the author include "The Presence of Polycyclic Hydrocarbons in Cigarette Smoke," by R. L. Cooper and A. J. Lindsey (*Chem. & Ind.*, 1953, 1205), "Polycyclic Hydrocarbons in Cigarette Smoke," by B. T. Commins, R. L. Cooper and A. J. Lindsey (*Brit. J. Cancer*, 1954, **8**, 296), "The Presence of 3:4-Benzpyrene and Other Polycyclic Hydrocarbons in the Combustion Products of Cigarette Paper," by R. L. Cooper and A. J. Lindsey (*Chem. & Ind.*, 1954, 1260), "The Presence of 3:4-Benzpyrene in Cigarette Smoke," by R. L. Cooper, A. J. Lindsey and R. E. Waller (*Chem. & Ind.*, 1954, 1148) and "Carcinogens in Cigarette Smoke," by A. J. Lindsey (*Brit. Med. J.*, 1954, **ii**, 1352).

NEW MEMBERS

Ordinary Members

Leslie Alfred Allen, D.Sc., Ph.D. (Lond.), M.Sc. (Reading), D.I.C., F.R.I.C.; John Oliver Brice, A.R.I.C.; Robert Keith Chalmers, B.Sc. (Birm.), A.R.I.C.; Peter Craven, B.Sc. (Dunelm.); Glenn Harold Foxley, B.Sc. (Lond.), A.R.I.C.; Arnold Hamer, B.Sc. (Lond.), A.R.I.C.; William Horton Hardwick, B.A., B.Sc. (Oxon.); Frank Elias Harper, B.Sc. (Dunelm.), A.R.I.C.; Geoffrey William Higson, A.R.I.C.; Om Parkash Kapur, B.Sc. (Punjab); Souheil Laham, Phar.D. (Paris), Ph.G. (Haiti); Robert Henry Marriott, D.Sc. (Leeds), F.R.I.C.; Sidney Keenslyside Tweedy, B.Sc. (Lond.), A.R.I.C.; Amaresh Chandra Roy, M.Sc., D.Phil. (Allahabad), A.R.I.C.; Alfred Hubert Thorneloe, M.A. (Cantab.), B.Sc. (Lond.), A.R.I.C.; Mulk Raj Verma, M.Sc. (Punjab), M.S. (Akron).

JUNIOR MEMBERS

Peter Jackson Coope, B.Sc. (Lond.); John James Philip Knight; Alan Robert Oliver.

NORTH OF ENGLAND SECTION

THE Thirtieth Annual General Meeting of the Section was held at the Engineers' Club, Albert Square, Manchester, on Saturday, January 29th, 1955. The Chairman, Mr. T. W. Lovett, presided over an attendance of 51, including the President, the Honorary Secretary and the Honorary Assistant Secretary. The Annual Report of the Section and the Financial Statement for the year 1954 were approved. The following appointments were made for the ensuing year:—*Chairman*—Mr. J. R. Walmsley. *Vice-Chairman*—Mr. A. N. Leather. *Hon. Secretary and Treasurer*—Mr. A. Alcock, City Laboratory, 143 Regent Road, Salford, 5. *Members of Committee*—Messrs. E. G. Brown, K. A. Hyde, A. O. Jones, Arnold Lees, T. W. Lovett and R. Sinar. *Hon. Auditors*—Messrs. F. Dixon and C. J. House.

The President, Dr. D. W. Kent-Jones, F.R.I.C., on behalf of the members of the Section, presented Mr. Arnold Lees with a cheque in recognition of his services as Honorary Secretary during the past 15 years.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which Dr. J. Haslam, F.R.I.C., presented a paper entitled "The Importance of Analysis in Industry."

WESTERN SECTION

A JOINT Meeting of the Section with the local sections of the Royal Institute of Chemistry, the Society of Chemical Industry and the Chemical Society was held at 7 p.m. on Thursday, January 27th, 1955, at Bristol University, Woodland Road, Bristol.

A lecture on "Recent Advances in the Bacteriological Examination of Water" was given by E. Windle Taylor, M.A., M.D., D.P.H., M.R.C.S., L.R.C.P., Barrister-at-Law (see summary below).

RECENT ADVANCES IN THE BACTERIOLOGICAL EXAMINATION OF WATER

DR. E. WINDLE TAYLOR said that until about 75 years ago the assessment of purity of a water had depended upon chemical analysis, but with the discovery of bacteria and the association of some of them as being the causative agents of disease, a more delicate test for purity had become available.

The chief object of the bacteriological analysis of water was to ensure that the water was free from pathogenic bacteria by the time it passed into supply to consumers. The criterion of purity from the bacteriological standpoint was based on the isolation of normal intestinal organisms from water. If it could be shown that a sample was free from bacteria normally present in the human or the animal intestine, then it could reasonably be assumed that the water was free from any disease-producing bacteria.

Correct procedure in sampling was of absolute importance, otherwise the results of laboratory investigation were worthless. The bacteriological analysis was only a part of the investigation of a water supply, and the results must be interpreted in conjunction with the chemical analysis, inspection of the site and its history.

Improvements in the bacteriological examination of water had been concentrated in recent years on speeding-up the examination, and the present practice for the control of water supplies was to sample more frequently, but to limit the scope of the examination of each sample.

Dr. Taylor outlined new bacteriological procedures for the isolation of coli-aerogenes and other micro-organisms from water and discussed the significance of the results.

Finally he made a plea for uniformity in analytical procedure; Report No. 71, "The Bacteriological Examination of Water Supplies," published by the Ministry of Health, had done much towards this end. Uniformity of methods meant uniformity of interpretation and led to greater confidence in their value when the results and opinions therefrom were submitted and explained to lay persons.

A JOINT Meeting of the Section with the local sections of the Royal Institute of Chemistry, the Society of Chemical Industry and the Chemical Society was held at 7 p.m. on Friday, February 11th, 1955, at University College, Cardiff.

A lecture on "Some Applications of Modern Techniques in Analytical Chemistry" was given by J. R. Nicholls, C.B.E., D.Sc., F.R.I.C. (see summary below).

Some Applications of Modern Techniques in Analytical Chemistry

Dr. J. R. Nicholls said that analytical chemistry was concerned with the identification and determination of substances. For these purposes use was made of the chemical and physical properties of elements and radicles in such a way that specific characteristics were isolated and observed or measured. The isolation might be achieved by separating the entity possessing the desired characteristics or by removal of, or nullifying the effect of, other entities which exhibit interfering properties. The older methods had depended largely on one or more classical chemical reactions for separation or removal, although physical operations such as distillation and dialysis had also been employed. In modern times the properties of chelate and clathrate compounds had found analytical application, and use had been made more and more of physical phenomena for isolation of particular substances, including adsorption, electrophoresis, and emission and absorption of radiant energy in the visual, ultra-violet and infra-red regions and of X-rays.

He described some of these newer techniques and gave examples of their application, in particular to medical and clinical analysis, plastics analysis and forensic work.

INAUGURAL MEETING OF THE MIDLANDS SECTION

The Inaugural Meeting of the Midlands Section was held at 7.15 p.m. on Tuesday, February 22nd, 1955, at the Mason Theatre, The University, Edmund Street, Birmingham, 3. The President of the Society, Dr. D. W. Kent-Jones, was in the Chair, and was supported by the following members of the Council: Dr. K. A. Williams, Honorary Secretary, and Dr. D. C. Garratt.

The following Officers and Members of Committee were elected:—*Chairman*—J. R. Leech, J.P. *Vice-Chairman*—Dr. R. Belcher, F.R.I.C., F.Inst.F. *Hon. Secretary*—G. W. Cherry, M.A., B.Sc., 48 George Frederick Road, Sutton Coldfield, Warwickshire. *Hon. Treasurer*—F. C. J. Poulton, A.R.I.C. *Members of Committee*—Miss B. B. Bauminger, Messrs. H. J. G. Challis, F. P. Everett, S. H. Jenkins, C. A. Johnson, H. C. Smith, W. H. Stephenson and T. S. West and, *ex officio*, the President and Honorary Officers of the Society. Miss M. E. Tunnicliffe and H. J. Alcock were appointed as Honorary Auditors.

The Rules of the Section were then adopted. At the end of the business session Mr. J. R. Leech took the Chair, and the President addressed the meeting on the subject of "Developments in the Society for Analytical Chemistry."

MICROCHEMISTRY GROUP

The Eleventh Annual General Meeting of the Group was held at 7 p.m. on Friday, January 28th, 1955, at the School of Pharmacy, Bloomsbury Square, London, W.C.1. Dr. A. M. Ward, F.R.I.C., was Chairman. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Dr. G. F. Hodsman. *Vice-Chairman*—Mr. D. F. Phillips. *Hon. Secretary*—Mr. D. W. Wilson, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. *Hon. Treasurer*—Mr. G. Ingram. *Members of Committee*—Messrs. P. R. W. Baker, R. Belcher, A. Bennett, G. S. Crouch, M. A. Fill and F. Holmes. Dr. L. H. N. Cooper and Mr. H. Childs were reappointed as Hon. Auditors.

The Annual General Meeting was followed by the retiring Chairman's Address (see summary below).

Dr. A. M. Ward paid tribute to the work of the President during a period of important changes in the functions of the Society. He pointed out that the Group, by meeting outside the London area, could provide publicity which might effect a much-needed increase in membership of the Society in these areas, and emphasised that the progress of the Society depended on the quality and quantity of original work presented to it.

Dr. Ward referred to a new activity of the Microchemistry Group, namely their London Discussion Group, which would meet from time to time for informal discussion of topics of microchemical interest. He gave an outline of the first topic chosen for discussion, the direct determination of oxygen, and gave instances of its importance in inorganic analysis, as well as its more usual organic applications.

Determination of Anionic Detergents in Sewage, Sewage Effluents and River Waters

By J. LONGWELL and W. D. MANIECE

A method for the determination of anionic detergents in sewage and sewage effluents is put forward. Whilst this is based on the well-known principle of the formation, and extraction by chloroform, of a complex of the detergent and methylene blue, it differs from published methods in that the extraction is carried out in alkaline solution. Further, the chloroform extracts are washed with an acid solution of methylene blue. The method is applicable also to the determination of traces of anionic detergents in river water, drinking water and sea water.

The performance of the method is compared with that of Degens, Evans, Kommer and Winsor, which involves extraction in acid solution and omits washing of the solvent extracts. The new method is not subject to interference by inorganic ions, which give a positive reading in Degens's method. The new method gives also an improved recovery of sodium alkylbenzenesulphonate added to sewage and sewage effluents. (Sodium alkylbenzenesulphonate is the chief anionic detergent found in sewage.)

The method gives a better estimate of the detergent content of sewage, although the increase to be expected from an improved recovery over Degens's method is, in some cases, counterbalanced by the reduction of interferences. This reduction is most marked in comparing the estimates obtained by the two methods of the detergent content of effluents, river water and drinking water, when large quantities of sample are required and, consequently, the concentration of substances which interfere in Degens's method is increased.

The question of a reference standard in terms of which anionic detergent concentrations may universally be expressed is discussed. Sodium dioctylsulphosuccinate (Manoxol O.T.), which is readily available in a high state of purity, is suggested as the most suitable standard.

In 1953 the Minister of Housing and Local Government set up a Committee to examine and report on the effects of the increasing use of synthetic detergents. One of the first tasks of the Committee was to consider the effect of detergents on the functioning of sewage treatment plants and the possibility of detergents in sewage effluents affecting river-derived water supplies. Very little information was available on the amount of detergents in sewage and on the extent of their removal in sewage works. Any detergent not removed by biological purification of sewage would pass out into rivers and be diluted to low concentrations. It is important that these concentrations should be known.

It soon became obvious that a standard method to determine traces of anionic detergents in sewage and in river and estuary waters was essential and, moreover, that results should be expressed in terms of a standard reference substance which was readily available from suppliers of laboratory chemicals.

For this purpose a Sub-Committee on Methods of Analysis was set up, and its first consideration was the selection of the most promising method available for study and improvement so that a satisfactory procedure could be formulated in as short a time as possible.

Anionic detergents, mainly of the alkylarylsulphonate type, form the active ingredients of most household washing powders to-day and contribute the bulk of the synthetic detergents passing into town sewage. Various methods have been published for the determination of small quantities of anionic detergents in sewage, based either on titration with a standard cationic surface-active solution[1,2] or on the formation of a coloured methylene blue - anionic detergent complex, which can be extracted and measured colorimetrically.[3,4,5,6] The latter principle, in the opinion of the Sub-Committee, showed the most promise as the basis for a standard method and, accordingly, an investigation of this method was undertaken at the Government Laboratory. Difficulties immediately arose in applying the method to sewage and sewage effluents, because it was found that natural compounds in sewage as well as

detergent were determined and, in addition, it was subject to interferences from various inorganic ions. These defects have been critically discussed by Degens, Evans, Kommer and Winsor,[6] who modified the original method of Jones[3] to reduce the effect of these interferences. Nevertheless, it was not generally appreciated that Degens's method did not determine all the anionic detergent in the presence of organic material such as sewage solids. It was found that the recovery of alkylarylsulphonate added to sewage varied from about 80 to 96 per cent., depending on the type and strength of sewage present. The cause of this apparent loss is not fully understood, but it is believed that proteins present in sewage are largely responsible, and this is confirmed by some studies recently published by Edwards and Ginn,[2] in connection with the determination of anionic detergents by titration with quaternary ammonium compounds, who found that proteins interfered when the pH value of the solution was less than their iso-electric point, approximately 7. The method described in the present paper is put forward as a means of reducing this loss and, in addition, of eliminating interference due to thiocyanates and inorganic ions generally.

As a first step the absorption curves of methylene blue complexes with the anionic detergents sodium lauryl sulphate and Manoxol O.T. were determined with a Unicam SP600 spectrophotometer. All gave similar results with a maximum absorption at 650 mμ and all subsequent measurements were made at this wavelength.

Subsequently, the method was developed in three stages from that put forward by Degens *et al.* In the first stage the interference due to inorganic ions had to be removed, since it is necessary to determine traces of detergent in river and estuarine waters of a high salt content. Degens's method was found to give unreliable results when the sample extracted contained high concentrations of dissolved solids. This method consists in extraction by chloroform of an aqueous solution of the detergent in the presence of methylene blue and sulphuric acid. This was modified to include a washing of the chloroform extracts with a second aqueous acid solution of methylene blue, which effectively removed all traces of interference by salts and gave a reliable means of determining with ease traces of less than 1 p.p.m. of detergent in effluents, river water and sea water. This adapted method, however, when applied to sewage, did not give a materially improved recovery of added detergent (sodium alkylbenzenesulphonate) compared with Degens's procedure.

The second stage of development of the method was concerned with the removal of interference due to compounds naturally occurring in sewage. Proteins were thought to be a possible cause of this loss, and the behaviour of a 30 p.p.m. solution of gelatin was therefore examined. This was found to give emulsions similar to those given by sewage and not to affect the reagent blank, but it gave only about 90 per cent. recovery of added aklylbenzenesulphonate. The pH of the aqueous solution in the extraction was then varied from that of the Degens method (pH approximately 2) up to a high alkaline pH. Above pH 7 the chloroform extracts were coloured various shades of pink and blue, but the interfering pink colour was removed by the acid methylene blue washing procedure outlined above, although at a high pH (pH 11 and above) removal was not complete. At pH 10 interference was removed and it was found that there was complete recovery of added alkylbenzenesulphonate in the presence of gelatin.

The effect of addition of alkylbenzenesulphonate to sewages of different strengths was then investigated by the modified extraction procedure and, for comparison, similar experiments on the same sewage were also carried out by Degens's method.

Detergent content has hitherto been expressed as Teepol, as alkylarylsulphonate contained in one or other of the commercial products, or no reference at all has been used. Consequently, the third stage of the investigation was to select a standard which could be universally used. This standard should give a calibration curve similar to that given by sodium alkylbenzenesulphonate, it should be reasonably stable both as a solid and in solution, it should be chemically pure with a known molecular weight and it should be obtainable as a normal reagent from laboratory chemical manufacturers.

In the first instance attempts were made to obtain a chemically pure sodium lauryl sulphate. This was eventually obtained, but its keeping properties, particularly in solution, ruled it out from further consideration.

Manoxol O.T. (sodium dioctylsulphosuccinate) was found to give a calibration curve which was linear over the desired range. Its purity is of the order of 95 per cent. and a sample of the solid over 2 years old was found to be similar in calibration to three recent samples taken from three different production batches. Moreover, a solution of concentration

10 p.p.m. in distilled water was found to be stable for 3 months, the duration of the test. Lastly, Manoxol O.T. is readily procurable from chemical suppliers.

Experimental

1. The effect of some inorganic ions on both Degens's method and the modified version was investigated. Sulphides interfere in the alkali extraction by reduction of methylene blue and, if present, must be oxidised with hydrogen peroxide before carrying out the extraction.

Table I shows that, whilst by Degens's method a solution containing chloride, nitrate and thiocyanate has an apparent detergent content, it has no detectable blank when tested by the double-extraction method. Sulphide shows no blank by Degens's method and none by the alkaline method after oxidation with hydrogen peroxide.

Table I

Effect of some inorganic ions in (*a*) alkaline double extraction and (*b*) Degens's extraction

Ion	Quantity present in extraction: In μg	Quantity present in extraction: Equivalent to 10 ml of sample containing, p.p.m.	Apparent detergent content (as Manoxol O.T.): Alkali, μg	Apparent detergent content (as Manoxol O.T.): Degens, μg
Chloride	10,000	1000	<1	2
	2000	200	<1	<1
Nitrate nitrogen	1400	140	<1	24
	280	28	<1	6
Thiocyanate	300	30	<1	24
Sulphide	250 (not oxidised)	25	6	<1
	1300 (after oxidation)	130	<1	—

The cumulative effect of various inorganic ions is most noticeable when determining the detergent content of drinking water. Table II gives the results by both methods on three typical waters. Sea water is included as an extreme example. The source of the spring, well and sea water almost certainly precludes the presence of detergent, yet a definite amount is reported by Degens's method although not by the modified one. In the river-derived water, in which the presence of detergent may be expected, the figure given by the Degens's method is three times that found by the modified method.

Table II

Detergent content of different waters by (*a*) alkaline double extraction and (*b*) Degens's extraction

Type of water	Source	Detergent content in 100 ml of sample (as Manoxol O.T.): Alkali	Degens
London tap	Thames	6 μg ≡ 0·06 p.p.m.	20 μg ≡ 0·20 p.p.m.
Soft	Spring	less than 1 μg ≡ less than 0·01 p.p.m.	4 μg ≡ 0·04 p.p.m.
Hard	Well		9 μg ≡ 0·09 p.p.m.
Sea	Ocean		136 μg ≡ 1·36 p.p.m.

2. When Degens's method was carried out on a synthetic sewage free from detergent, an apparent detergent figure was obtained. This figure varied from 0·5 to 2·0 p.p.m., depending on the strength of the synthetic sewage. After considerable trouble, a rather dilute sewage free from detergents was found and a similar sewage blank was found. Furthermore, when known amounts of detergent were added to these detergent-free sewages, the recovery was incomplete, sometimes being as low as 80 per cent. Various experiments were carried out in attempts to eliminate this blank and increase the rate of recovery. These included purification of the methylene blue, the use of different solvents to extract the detergent - methylene blue complex and separate extraction in a Soxhlet apparatus of sewage solids. All these failed and no improvement was obtained until it was thought to

complex the detergent and methylene blue in alkaline solution and, after extraction in the usual way, to wash the extract with acid methylene blue as used by Degens. In addition, work was done on the elimination of the emulsion formed on the sewage - methylene blue in chloroform interface, as at one time it was thought that detergent might be secluded in this emulsion. This did not prove to be the case, but the emulsion was broken by the use of a glass rod as described in the method given in this paper.

The experiments on the addition of detergent to sewages and sewage effluent were carried out by mixing 10 ml of sample with 10 ml of approximately 10 p.p.m. alkylbenzenesulphonate solution and carrying out the extraction after 5 minutes. The detergent content of the sample before addition of the sulphonate was also determined.

Results are recorded in Table III for raw and settled sewages of various "strengths" measured by free and albuminoid ammonia and 4-hour oxygen absorption figures. They show that of the two methods compared, Degens's and the alkaline double extraction, the latter gives an improved recovery of added detergent in all cases. The estimates of the original detergent content of the samples by the two methods do not show as big a difference as might be expected, on the basis solely of improvement in recovery. This is probably due to a measure of compensation in the Degens method, when the value actually obtained is a balance between the percentage recovery of detergent originally present and the apparent detergent content of non-detergent compounds naturally present in sewage which react in the Degens method. Effluents, on the other hand, for which recovery by the two methods is high and the difference in recovery not marked, give lower results by the new method. The effluent sample 1 is probably an extreme case. Here the Degens figure is nearly twice that given by the new method.

TABLE III

RECOVERY OF DETERGENT (SODIUM ALKYLBENZENESULPHONATE) ADDED TO SEWAGES AND EFFLUENTS

Sample	Oxygen absorbed*	Detergent content†		Detergent content† after adding 10·7 p.p.m.		Recovery of added detergent	
		Degens's method, p.p.m.	Modified method, p.p.m.	Degens's method, p.p.m.	Modified method, p.p.m.	Degens's method, %	Modified method, %
Sewage 1	127	7·9	7·9	16·9	17·2	84	87
2	125	14·2	15·1	23·1	24·8	83	91
3	135	6·4	6·6	15·4	16·6	84	94
4	60	8·3	8·8	17·2	18·9	83	95
5	65	11·5	11·7	21·2	21·9	91	95
6	66	5·4	5·6	14·3	15·6	83	94
7	18·2	1·0	0·9	10·8	11·2	92	96
8	52	8·5	8·0	16·3	17·3	73	87
Effluent 1	10·7	5·7	3·2	15·9	13·6	95	97
2	14·5	6·8	6·6	16·7	16·9	93	96

* From *N*/80 acid potassium permanganate in 4 hours at 26·7° C. † As Manoxol O.T.

METHOD

REAGENTS—

Alkaline phosphate solution—Dissolve 10 g of analytical-reagent grade disodium hydrogen phosphate (anhydrous) in distilled water. Adjust the pH to 10 by addition of sodium hydroxide and make up to 1 litre with distilled water.

Neutral methylene blue solution—Dissolve 0·35 g of B.P. methylene blue in distilled water and make up to 1 litre.

Acid methylene blue solution—Dissolve 0·35 g of B.P. methylene blue in about 500 ml of distilled water, add 6·5 ml of analytical-reagent grade sulphuric acid and make up to 1 litre with distilled water.

Chloroform—Analytical-reagent grade. (It may be recovered after use by distillation over burnt lime.)

Manoxol O.T. solution—Dissolve 0·100 g of Manoxol O.T. (sodium dioctylsulphosuccinate) in distilled water and make up to 1 litre. Dilute 10 ml to 100 ml with distilled water to give a standard solution of 10 p.p.m.

PROCEDURE—

(*i*) The volume of sample taken should be chosen when possible to contain 20 to 150 μg of anion-active material. It is generally impracticable to take more than 10 ml of raw or settled sewage, owing to the degree of emulsion formation on shaking with chloroform, but it is possible to take up to 100 ml of good quality effluent, river and drinking water when the detergent content is very low.

(*ii*) Place the sample in a separating funnel and make up to 100 ml with distilled water. Add 10 ml of alkaline phosphate solution, 5 ml of neutral methylene blue solution and 15 ml of chloroform.

(*iii*) Shake gently and evenly twice a second for 1 minute. Allow to separate, breaking up any emulsion formed in the separator by gentle agitation with the flattened end of a glass rod. Run the clear chloroform layer into a second separating funnel containing 110 ml of distilled water and 5 ml of acid methylene blue solution. Rinse the first separating funnel with 2 ml of chloroform added from a burette and run this into the second separating funnel.

(*iv*) Shake the second separating funnel as in (*iii*) and allow the layers to separate. Run the chloroform layer through a small funnel plugged with cotton-wool moistened with chloroform into a 50-ml calibrated flask, rinsing with a further 2 ml of chloroform.

(*v*) Repeat from (*iii*) with two further 10-ml portions of chloroform and make up the combined extracts in the flask to the mark with chloroform.

(*vi*) Before carrying out a further determination, the separating funnels should be rinsed with dilute nitric acid to remove adsorbed methylene blue.

Sulphide interference—Sulphide, if present, should be oxidised before extraction. Place the required volume of sample in the first separating funnel, add 10 ml of alkaline phosphate solution and 2 ml of 20-volume hydrogen peroxide. Stand for 5 minutes and bulk to 110 ml with distilled water. Add 5 ml of neutral methylene blue solution, 15 ml of chloroform and proceed as in (*iii*) above.

Measurement—The optical density of the chloroform extract is measured in a suitable photo-electric absorptiometer either at 650 mμ or with an Ilford No. 607 orange filter.

Calibration—Suitable volumes (5, 10, 15 and 20 ml) of the dilute Manoxol O.T. solution are treated as above and the optical densities of the extracts determined. The optical density of a reagent-blank extract is also measured. This is constant for a given batch of reagents. In this sense, distilled water is a reagent and the same water and other reagents should, therefore, be used throughout one day, during which the reagent blank is determined.

The differences between the optical densities of the standards and the reagent blank are plotted against concentrations to give a calibration curve. The detergent concentration of a sample can then be determined from this curve. In practice, the calibration curve is a straight line and optical density can be converted to concentration by use of a factor based on the slope of the line.

This paper is published at the request of the Committee on Synthetic Detergents and by permission of the Government Chemist. Our thanks are due to Mr. H. J. Finch of Mogden Sewage Works and Mr. J. H. Simmons of this laboratory, who did much of the experimental work, and we acknowledge help and suggestions from various members of the Sub-Committee on Methods of Analysis. In addition, we thank The British Drug Houses Ltd. and Marchon Products Ltd. for samples of purified sodium lauryl sulphate and Messrs. Hardman and Holden Ltd. for samples of Manoxol O.T.

REFERENCES

1. Lewis, G. R., and Herndon, L. K., *Sewage Ind. Wastes*, **1952**, **24**, 1456.
2. Edwards, G. P., and Ginn, M. E., *Ibid.*, 1954, **26**, 945.
3. Jones, J. H., *J. Ass. Off. Agric. Chem.*, 1945, **28**, 398.
4. Evans, H. C., *J. Soc. Chem. Ind.*, 1950, **69**, s76.
5. Lester, W. F., and Raybould, R. D., *J. Inst. Sew. Purif.*, 1950, 393.
6. Degens, P. N., Evans, H. C., Kommer, J. D., and Winsor, P. A., *J. Appl. Chem.*, 1953, **3**, 54.

DEPARTMENT OF THE GOVERNMENT CHEMIST
GOVERNMENT LABORATORY
DUDLEY HOUSE
ENDELL STREET
LONDON, W.C.2

January 20th, 1955

Application of Paper-chromatographic Methods of Analysis to Geochemical Prospecting

By E. C. HUNT, A. A. NORTH and R. A. WELLS

Rapid and simple methods are described for the determination of copper, cobalt, nickel, niobium, tantalum, lead and uranium in soil samples. Following the breakdown of the samples and dissolution of the trace metals by methods applicable in the field, the trace metal or metals are separated chromatographically by upward diffusion of a solvent on slotted sheets of paper which enable ten separations to be performed simultaneously. The metals are detected by spraying the strips with suitable reagents and the amount present is determined by visual comparison with standards. Copper, cobalt and nickel are determined after a single separation. The same soil extract is used for the determination of both niobium and tantalum.

Prospecting by geochemical methods is based upon the observation that anomalous values for mineral concentration are often found in the rock, soil, waters or vegetation in the vicinity of an ore body. The various factors controlling the partial dispersion of metals from an ore body through the surrounding media have been described by Webb.[1] Two main types of geochemical dispersion are recognised: primary dispersions, which are formed during the period of mineralisation and which result in the distribution of trace metals in the wall rocks surrounding the ore body, and secondary dispersions. The second group are the most interesting from the geochemical prospector's point of view. They result from the weathering and breakdown of primary ore bodies which cause the metals constituting the mineralisation to be dispersed and re-distributed to an extent dependent upon local conditions and the properties of the metal concerned. Geochemical prospecting is particularly useful in detecting sub-outcropping ore bodies which are covered by a layer of residual soil. By analysis of the soil just below grass roots level it is often possible to detect an ore body covered by many feet of soil. This type of prospecting is rapidly growing in popularity and importance. It depends upon the systematic taking of samples on a grid pattern over the area to be investigated, and ultimately upon the analysis of large numbers of soil samples. A number of papers, particularly by the U.S. Geological Survey,[2] have described field methods for the speedy analysis of soils. Most of these methods have been based on conventional methods of separation and colorimetric estimation. A high order of accuracy is not required for geochemical work, but simplicity and cheapness are essential.

A number of papers describing separations of various metals with the aid of paper chromatography have originated from this laboratory. The present paper describes th application of a chromatographic technique to the analytical problems of geochemic prospecting. The method consists essentially in applying an aliquot of a suitable extrac of a soil to one end of a filter-paper strip and allowing an organic solvent mixture to diffuse up through the test spot. The solvent is chosen to produce a separation of the desired metal, which is then detected by spraying the strip with a suitable reagent. The quantity of metal present is estimated by comparison with standard strips. The apparatus usually employed for inorganic separations by paper chromatography is too bulky and fragile for use in the field and separations are not achieved in a sufficiently short time. For geochemical prospecting a specially cut sheet of paper has been designed to permit ten samples to be dealt with simultaneously. The time taken for the chromatographic separation varies from 10 to 40 minutes and the apparatus consists of a 600-ml glass or polythene beaker fitted with a cover. Consumption of reagents and operator's time are cut to a minimum, but the accuracy achieved is all that is required. Methods are provided for the estimation of copper, cobalt, nickel, niobium, tantalum, lead and uranium, and methods for a further series of metals are being investigated.

The paper sheet—

Although the procedures vary in detail and depend on the metal to be estimated, th chromatographic separation is carried out in each case on a specially designed sheet of p

(see Fig. 2). This consists of a rectangular sheet (21·3 cm × 11 cm) of Whatman's No. 1 filter-paper. Eleven slots (3 mm × 9 cm) are cut in the paper parallel to the short side so as to leave 12 strips 1·5 cm wide joined at top and bottom. These were originally cut by hand, but are now available commercially under reference number CRL/1. An aliquot of test solution is applied near to one end of each strip of paper so that, by leaving the two end strips vacant, ten sample solutions are placed on the sheet. The volume of test solution applied varies between 0·01 and 0·05 ml and it is applied with the aid of a capillary pipette so that it spreads right across the strip to form a rectangular patch. The sheet is bent round to form a cylinder and clipped at the top end with a paper clip. After suitable drying, the sheet is placed vertically in a covered beaker containing the solvent, the depth of which must not exceed 1 cm. The solvent is allowed to diffuse up the strip and reaches the level of the top of the slots in 10 to 40 minutes, the time depending upon the solvent. The sheet is removed just before the solvent reaches this level and the positions of the metals on the strip are located by various treatments of the sheet, which depend upon the metals to be estimated.

Copper, Cobalt and Nickel

Various solvent mixtures have been used for the chromatographic separation of copper, cobalt and nickel from each other and from other elements. Previous work in this laboratory indicated that mixtures of *iso*butyl methyl ketone or ethyl methyl ketone with hydrochloric acid should be suitable for the separation of these three metals if present in a soil extract. Since in either case it is the chlorides of metals which undergo separation, it is an advantage if the soil extract is in chloride solution. Direct extraction of the soil with hydrochloric acid was investigated, but was not found entirely satisfactory. Fusion of the soil with potassium bisulphate followed by solution of the melt in hydrochloric acid gave both a satisfactory extraction and a solution suitable for chromatographic separation. For geochemical work the *iso*butyl methyl ketone solvent was found to be satisfactory only if the copper content of the soil was less than 400 p.p.m. A mixture of ethyl methyl ketone (75 parts) with hydrochloric acid, sp.gr. 1·18 (15 parts) and water (10 parts) was found to be more universally applicable and is the recommended solvent. The ethyl methyl ketone solvent suffers from one disadvantage in that the commercial ketone usually requires some purification before it provides satisfactory results.[3] It is recommended that pure ethyl methyl ketone, now marketed specifically for chromatographic purposes, be used.

Experimental

Breakdown of sample—

A number of procedures including treatment with mineral acids and fusion with potassium bisulphate have been described by previous workers, by which copper, cobalt and nickel present in soil samples may be brought into solution. In most cases an attempt was made to obtain complete solution of the soil. In the present work this was not practicable, since complete solution of the soil leads to a large volume of sample solution. Since only a relatively small volume of solution can be used in chromatographic separation, an evaporation of the sample solution to low bulk would be necessary in order to avoid too large a dilution factor and a loss of sensitivity. Such an evaporation step is undesirable in a method designed as a quick field procedure. A method was sought therefore which would (*a*) give a solution of the main portion of copper in the sample, (*b*) result in a small volume of sample solution and (*c*) give a solution suitable for chromatographic separation. Treatment of the soil sample with a known volume of mineral acid appeared to offer a simple possible method and a number of mixtures was examined. One gram of soil was weighed into a 12-mm × 100-mm test tube and 1 ml of acid was added. The tube was heated for 1 hour in a boiling water-bath, with only the lower end of the tube immersed. The upper end of the tube acted as a condenser and after 1 hour's heating loss of acid was found to be less than 5 per cent. by weight. After cooling and allowing to settle, a portion of the supernatant liquid was removed by pipette for the chromatographic separation. The efficiency of extraction with various strengths and mixtures of hydrochloric, nitric and hydrofluoric acids was examined by this technique. Extraction with concentrated nitric acid or diluted hydrochloric acid (1 + 1) was found to give the most efficient extraction of copper, but very poor chromatographic separations resulted from the use of the concentrated nitric acid extract. Satisfactory chromatographic

results were obtained by using the diluted hydrochloric acid (1 + 1) extract, and a further study of the extraction efficiency obtained with this acid was made. On a series of ten soil samples tested, treatment with diluted hydrochloric acid (1 + 1) extracted about 45 per cent. of the total copper and about 80 per cent. of the total cobalt. The anomalous metal was more readily extracted in the case of cobalt and a 90 per cent. extraction efficiency of this portion of the cobalt was obtained. These extraction figures did permit the detection of anomalous copper and cobalt, but, since the extraction efficiency would probably vary with other soil samples, a more vigorous extraction procedure was looked for.

A number of field procedures which used a fusion with potassium bisulphate to break down the soil have been described, and the possibility of using this method in conjunction with chromatographic separation was investigated. To a 18-mm × 150-mm Pyrex-glass test tube was added 1 g of potassium bisulphate (80 mesh) and 0·5 g of the soil sample. The mixture was given a moderate fusion for 1 minute and then allowed to cool. Exactly 2 ml of diluted hydrochloric acid (1 + 1) containing 5 per cent. v/v of concentrated nitric acid were added to the cold melt. The reason for the addition of a small amount of nitric acid to the chloride solution is explained later, in the section on the chromatographic separation. The tube was then stood in the boiling water-bath for 10 minutes, which was sufficient time to obtain a solution of the melt. The solution was allowed to cool. The insoluble silica was deposited and potassium salts crystallised out to leave a layer of clear supernatant liquid, an aliquot of which was taken for the chromatographic separation.

The series of samples, which had already been examined by the direct acid extraction process, were tested by using the potassium bisulphate fusion. The results were satisfactory; the decrease in sensitivity brought about by the use of a larger volume of acid for the solution of the sample was compensated by the gain in sensitivity due to a more complete extraction. With this particular batch of soils, 100 per cent. extraction of copper and cobalt was obtained. The bisulphate fusion method of breakdown was adopted for future work and the results obtained on a series of soils were compared with those obtained by using dithizone for the determination of copper. When dealing with soils having a high copper content, satisfactory results were obtained on reducing the sample weight to 0·1 g and the weight of potassium bisulphate used to 0·5 g.

Chromatographic separation—

Various solvent mixtures have been used for the chromatographic separation of copper, cobalt and nickel from iron and other elements. Two solvent systems were investigated in the present work, *iso*butyl methyl ketone containing concentrated hydrochloric acid and ethyl methyl ketone mixtures with hydrochloric acid and water. The separation of cobalt, copper and nickel with ethyl methyl ketone - hydrochloric acid mixtures was reported in a previous paper,[4] but *iso*butyl methyl ketone was chosen for initial experiments in the present work. This solvent had the advantage over ethyl methyl ketone in being more stable and more readily obtainable in a pure form. It was known that successful separations of copper, cobalt and nickel could be obtained by using downward diffusion of a mixture of *iso*butyl methyl ketone (60 parts) and concentrated hydrochloric acid (40 parts). The length of solvent run when CRL/1 pattern sheets of filter-paper are used, however, is limited to 9 cm, which is insufficient to enable a separation to be achieved with the mixture of *iso*butyl methyl ketone and 40 per cent. of hydrochloric acid. On lowering the acid concentration in the solvent to 8 per cent. of hydrochloric acid, it was found possible to achieve a separation of copper and cobalt from certain soil extracts within the limit of solvent movement imposed by the strip. The separations were, however, sensitive to small variations of temperature, humidity and acid concentration and were satisfactory only if there were a considerable quantity of iron present in the test solution and if the soil sample contained less than 400 p.p.m. of copper. Whilst within these limits the *iso*butyl methyl ketone solvent gave satisfactory results on hydrochloric acid extracts of soil, results were less satisfactory when dealing with the acid extracts of potassium bisulphate fusions of soil samples. A large excess of potassium salts had little effect on the copper band, but the movement of cobalt was retarded and no clear-cut separation of cobalt from the original spot was obtained. In view of the limitations of *iso*butyl methyl ketone the alternative solvent, ethyl methyl ketone, was investigated.

In initial experiments with ethyl methyl ketone the effect of various additions of hydrochloric acid and water to the solvent on the separation was investigated. Optimum results

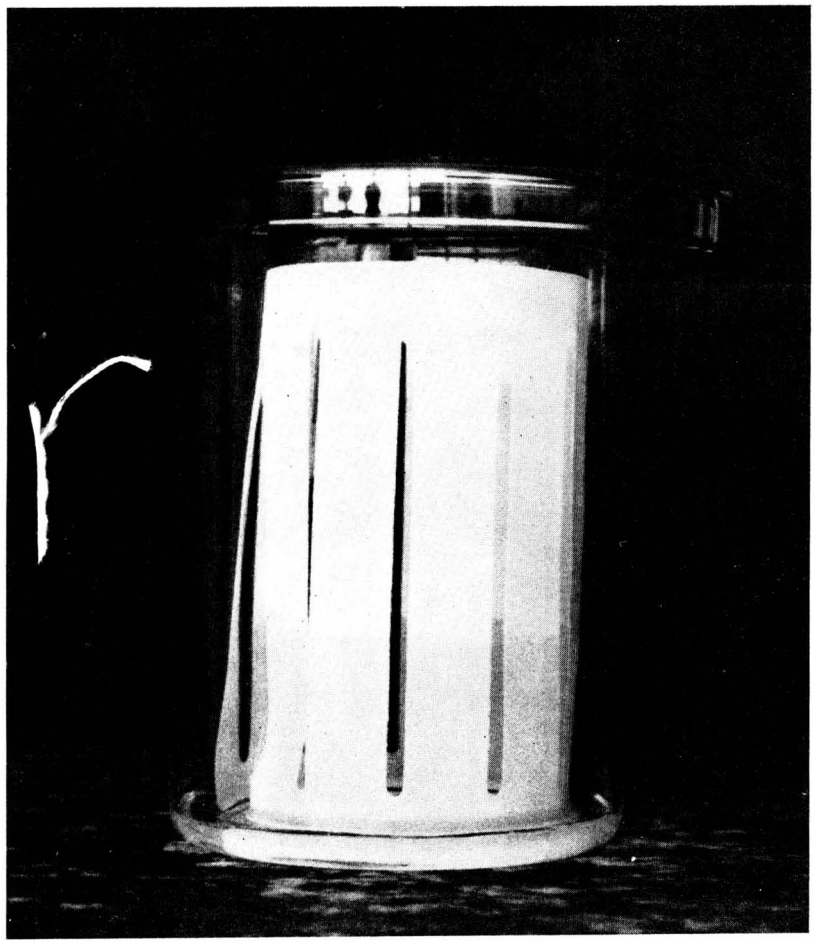


Fig. 1. Development of chromatogram

[To face p. 174

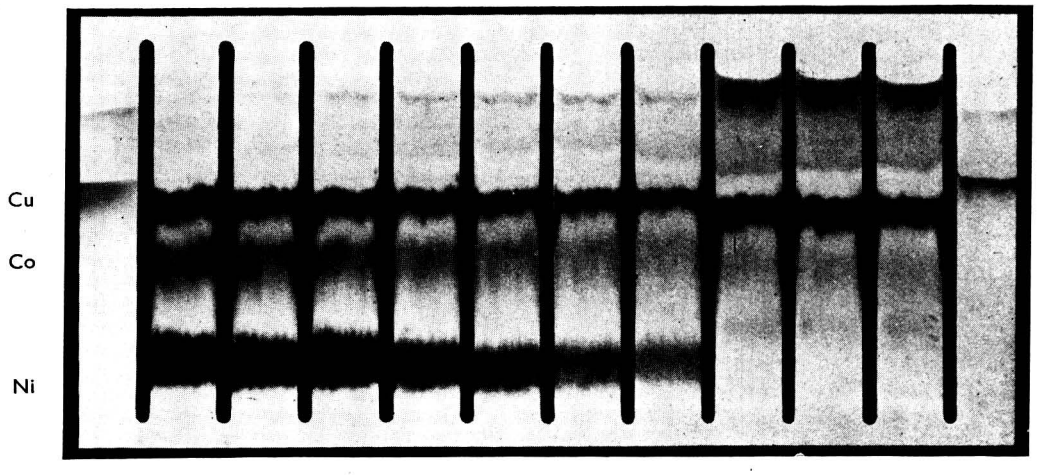


Fig. 2. The separation of nickel, cobalt and copper

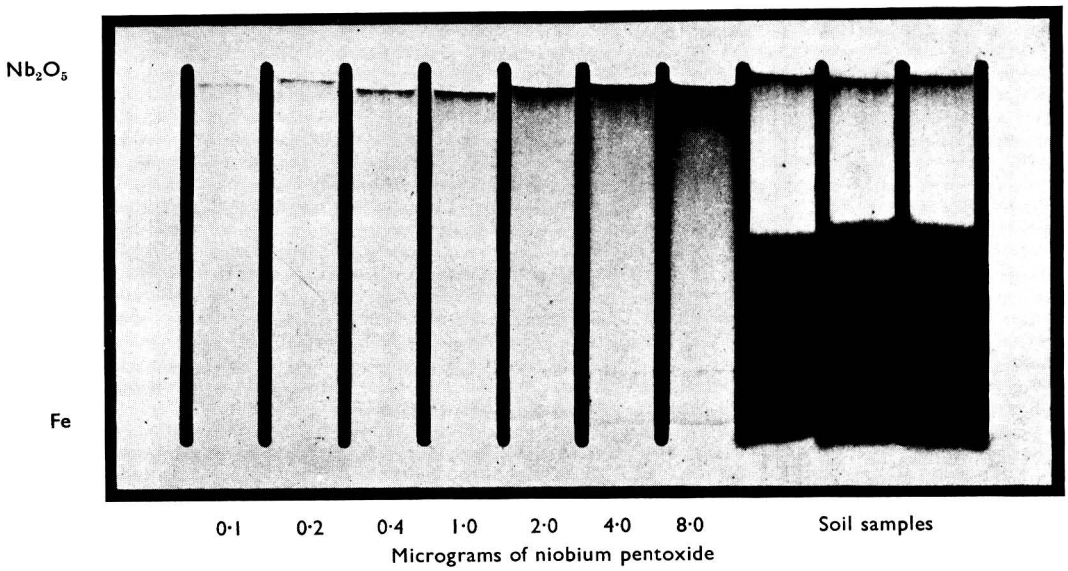


Fig. 3. The separation of niobium

were achieved by using as solvent ethyl methyl ketone containing 15 per cent. v/v of hydrochloric acid, sp.gr. 1·18, and 10 per cent. v/v of water. With this solvent the following approximate R_F values were obtained: copper, 0·65; cobalt, 0·45; nickel, 0·10. When the sheet was dried after application of the test solutions, three variables were found to affect the subsequent chromatographic separation. The humidity of the atmosphere controlled not only the drying time of the original spot, but also the moisture content of the whole sheet of paper. The temperature affected the rate of drying and also the rate of loss of hydrochloric acid from the test spot. Loss of hydrochloric acid from the test spot was extremely slow, and optimum results for the separation were achieved when the strip was dried in such a manner as to drive off most of the excess of hydrochloric acid. Time of drying was important in that a long drying time in an atmosphere of low humidity rendered part of the iron in the test spot immobile. This was particularly the case when the strip was dried in bright sunlight. This caused partial reduction of the iron to its ferrous condition, in which state it was not soluble in the ketonic solvent and remained in the original spot. All these variables were avoided by drying for a short period at a high temperature. This was achieved simply by floating a 600-ml beaker in a 2-litre beaker of boiling water. The sheet of paper bearing the test spots was rolled and clipped to form a cylinder in the normal manner and then stood, test spots lowermost, for 3 minutes inside the 600-ml beaker. Provided that the 600-ml beaker actually floated in the boiling water and was not merely suspended in the steam above it, the strip was maintained within a few degrees of 80° C. Optimum drying time at this temperature was 3 minutes. Longer drying time did not affect the chromatographic separation, but the paper was weakened by attack by traces of sulphuric acid present in the test solution after a bisulphate fusion. This often caused the strip to break away from the sheet in the neighbourhood of the test spot.

By using the optimum solvent and drying conditions, it was found that the copper tended to form two bands. These bands were close together, but their formation made comparison with standards difficult. This double-band formation was prevented by the addition of a small amount of nitric acid to the hydrochloric acid solution used in dissolving the bisulphate melt. The cause of the double band of copper and the action of nitric acid in preventing it is not clear. On investigation it did not appear that the double band of copper was due to the reduction of part of the copper salt to the cuprous condition. It may be due to the effect of the nitrate ion on the partition of copper into the solvent. The addition of 5 per cent. v/v of nitric acid, sp.gr. 1·42, to the diluted hydrochloric acid (1 + 1) solution had a second beneficial effect in that it speeded the solution of the bisulphate melt.

The optimum quantity of test solution to be applied to a strip was found to be 0·01 ml, *i.e.*, one-twohundredth of the total solution. When larger aliquots were used, incomplete extraction of copper from the test spot resulted. This appeared to be due to the large excess of potassium sulphate in the test solution.

After application of the test spots and drying at 80° C for 3 minutes, the cylinder of paper was stood with sample spots lowermost in a covered 600-ml beaker containing 20 ml of the ketonic solvent (see Fig. 1). The solvent diffused up the paper a sufficient distance to effect a separation in 30 to 40 minutes and satisfactory results were obtained over the temperature range 20° to 25° C. It was found that about five separate sheets could be run before loss by evaporation changed the constitution of the solvent sufficiently to make it necessary to use a fresh batch of solvent. Satisfactory chromatograms were obtained on standards prepared as described later and on hydrochloric extracts of soils or the hydrochloric acid solution of the melt from a bisulphate fusion of the soil sample.

Detection of metals—

When the solvent had diffused almost to the top of the strips, the sheet was removed from the beaker and allowed to dry in the air for 5 minutes. It was then placed in an atmosphere of ammonia vapour for 2 minutes. The atmosphere of ammonia vapour was achieved by placing a 50-ml beaker containing ammonium hydroxide, sp.gr. 0·880, in a 600-ml beaker and covering with a watch-glass. The neutralised sheet was then removed from the beaker and sprayed with a 0·1 per cent. solution of rubeanic acid. Nickel appeared as a blue-purple band just moving from the test spot, copper as an olive-green band, cobalt as a yellow-orange band and iron showed as the brown hydroxide in the solvent front (see Fig. 2). The lower limit of detection was 0·05 μg for each trace metal, which is equivalent to 20 p.p.m.

in the soil. The paper contained a small amount of copper, which was extracted and appeared in the normal position of the copper band. This copper impurity was not great but was sufficient to make difficult an exact estimation on samples at the 20 p.p.m. level. The colour obtained with nickel varied in shade with the quantity of ammonium chloride present on the paper, but reproducible results were obtained by adhering to the times and conditions given for detection above.

PREPARATION OF STANDARDS—

To prepare known solutions for the production of standard strips when the ethyl methyl ketone solvent was used, a solution of copper, cobalt and nickel chlorides containing 0·1 g of each metal in a 100 ml of solution was made. The salts of the metals were dissolved in 50 ml of hydrochloric acid, sp.gr. 1·18, and 5 ml of nitric acid, sp.gr. 1·42, and diluted to 100 ml with water. This gave a standard solution containing 10 μg of each metal per 0·01 ml. Further standards were made by diluting this solution with further mixed acids solution (50 ml of hydrochloric acid, sp.gr. 1·18, and 5 ml of nitric acid, sp.gr. 1·42, diluted to 100 ml). To prepare a standard chromatogram 0·5 g of potassium bisulphate was added to 2 ml of each standard solution in the test tube and the mixture was warmed, shaken and allowed to cool. The excess of bisulphate crystallised out and 0·01 ml of the supernatant liquid from each standard was applied to the paper sheet. The standards prepared depended upon the range of copper it was intended to cover, but a suitable range of standards covering 20 to 2000 p.p.m. of each metal in the soil was prepared by using a series of solutions containing 0·05, 0·10, 0·15, 0·20, 0·25, 0·30, 0·40, 0·50, 0·70, 1·0, 1·3, 1·6, 2·0, 2·5, 3·0, 4·0 and 5·0 μg per 0·01 ml of each metal. To cover the whole range two sheets of paper were required. The preparation of standards was a little time-consuming, but prepared sheets of standard chromatograms showed no signs of deterioration after keeping for 6 months.

RESULTS—

Initial experiments were carried out on ten soil samples from the African copper belt. These samples were taken in the region of a known copper anomaly. The total copper and cobalt in each sample was estimated accurately by classical procedures, and both metals

TABLE I

DETERMINATION OF COPPER AND COBALT AFTER CHROMATOGRAPHIC SEPARATION, WITH ETHYL METHYL KETONE AS SOLVENT

Sample number	Total copper, p.p.m.	Copper determined chromatographically after potassium bisulphate fusion and extraction with diluted hydrochloric acid (1 + 1), p.p.m.	Copper determined by dithizone after potassium bisulphate fusion and extraction with water, p.p.m.	Total cobalt, p.p.m.	Cobalt determined chromato-graphically, p.p.m.
1	26	20	—	5·0	<10
2	34	40	—	4·3	<10
3	135	80	—	8·7	<10
4	142	160	125	10·6	15
5	167	140	—	7·0	<10
6	168	140	125	14·7	10
7	292	260	225	31·7	24
8	296	280	220	25·3	20
9	387	320	275	30·0	30
10	395	360	375	27·6	20

were then estimated by using the chromatographic method. Results obtained for copper by both procedures are compared in Table I with results obtained by a colorimetric field procedure in which dithizone is used. The authors are indebted to Dr. J. S. Webb, of the Royal School of Mines, who supplied the results for the dithizone procedure and by whose permission these results and further results by the dithizone procedure recorded in later tables are quoted. It will be noticed that the percentage extraction recorded for the dithizone procedure is approximately 70 per cent. This low extraction results from the solution of copper being obtained by a bisulphate fusion followed by leaching of the melt with water.

In later experiments the melt was extracted with hydrochloric acid before the dithizone determination and the process became strictly comparable with the chromatographic procedure.

For some of the cobalt results in Table I a lower figure is recorded than 20 p.p.m., which was given previously as the limit of the method. These were obtained by carrying out repeat estimations on 0·02-ml aliquots of the test solution, which did not give a satisfactory chromatogram for determination of copper but did enable cobalt to be determined down to 10 p.p.m. From the results on this first batch of samples it appeared that fusion with potassium bisulphate followed by extraction with diluted hydrochloric acid (1 + 1) extracted virtually 100 per cent. of the copper and cobalt present in the soil. It appeared also that the copper and cobalt could be estimated with reasonable accuracy by visual comparison of the sample chromatograms with those of the standards.

No difficulty was experienced in using the ethyl methyl ketone solvent for determining copper and cobalt in a number of high-grade copper soils. This series of soils was prepared by mixing together a soil containing 48 p.p.m. of copper with a soil containing 3800 p.p.m. of copper in various proportions. The results obtained are compared (see Table II) with the results on the same samples obtained by the dithizone procedure and with calculated copper and cobalt values. The calculated values were obtained by carrying out a total estimation of copper and cobalt by normal analytical procedures on the lowest and highest bearing samples and calculating the remainder.

TABLE II

DETERMINATION OF COPPER AND COBALT IN HIGH-GRADE COPPER SOILS

Sample number	Copper determined chromatographically on 0·1-g sample, p.p.m.	Copper determined chromatographically on 0·5-g sample, p.p.m.	Copper calculated, p.p.m.	Copper determined by dithizone method, p.p.m.	Cobalt determined chromatographically on 0·1-g sample, p.p.m.	Cobalt determined chromatographically on 0·5-g sample, p.p.m.	Cobalt calculated, p.p.m.
11	—	20	48	20	—	<20	0
12	—	480	423	350	—	60	58
13	600	640	611	700	<100	80	87
14	900	1000	798	1000	150	120	116
15	1000	1200	1174	1150	100	160	174
16	1600	1600	1549	1650	250	180	232
17	1900	1800	1924	1950	250	220	290
18	2200	2400	2299	2550	360	320	348
19	2600	2800	2674	3650	450	360	406
20	3200	3200	3050	4250	500	400	464
21	3600	3400	3425	3750	500	400	522
22	4000	4000	3800	4650	550	480	580

The chromatographic results agreed reasonably well with the calculated results over the whole range, but figures obtained by the dithizone procedure were unreliable when the copper content approached 3000 p.p.m. Two series of chromatographic results are shown in Table II. One was obtained by using the normal 0·5-g sample weight and diluting to 4 ml instead of 2 ml when the copper content of the soil exceeded 2000 p.p.m. In the other series the sample weight was reduced to 0·1 g. It was felt that for higher concentrations of copper the comparison of chromatograms might be facilitated by reducing the amount of copper present in the test solution. However, no appreciable differences in the results for copper were obtained except on the soils containing little copper. In this case 0·1 g was too small a sample weight for the copper to be detected. The cobalt content in these soils was considerably less than the copper content and it was possible to make more accurate estimations of the cobalt when the larger sample weight was used.

Determinations of copper and cobalt were made on a further batch of twenty-four samples and compared with the results obtained by the dithizone procedure (Table III). To check reproducibility three separate determinations of copper were made on each soil by the chromatographic procedure in which ethyl methyl ketone was used as solvent. The dithizone results quoted are mean figures for a number of determinations on each sample. The chromatographic results on this series of samples tend to be a little lower than those obtained by the dithizone procedure, but the reason for this is not known. The mean of

the three results on each sample is given in the fifth column of Table III, whilst the percentage standard deviation from the mean is recorded in column 6. With the exception of the first five samples in which the copper concentration is low, the percentage standard deviation from the mean is less than 30 per cent., which is considered sufficiently accurate for geochemical prospecting purposes. Samples 23, 24 and 25 were identified subsequently as being portions of the same sample and the nine results on these samples can therefore be considered together and give a percentage deviation from the mean of 52·7 per cent. Samples 45 and 46 were also identified later as being portions of the same sample.

Table III

Reproducibility of chromatographic determinations of copper

Sample number	Copper determined chromatographically after potassium bisulphate fusion and hydrochloric acid extraction (3 separate determinations) A, p.p.m.	B, p.p.m.	C, p.p.m.	Mean	Standard Deviation × 100 / Mean	Copper by dithizone after potassium bisulphate fusion and hydrochloric acid extraction, p.p.m.	Cobalt determined chromatographically, p.p.m.
23	50	20	30	30	57·7	60	<10
24	40	20	20	27	42·7	45	<10
25	60	20	20	33	70·0	40	<10
26	100	40	60	67	45·6	80	10
27	120	60	80	87	35·1	120	10
28	80	100	100	93	12·4	120	10
29	100	120	100	107	10·8	120	10
30	100	120	120	113	10·2	130	16
31	100	100	140	113	20·4	140	14
32	160	100	100	120	28·9	160	15
33	140	120	180	147	20·8	170	15
34	160	140	140	147	7·8	170	16
35	160	160	140	153	7·5	210	16
36	200	140	160	167	18·3	180	24
37	200	200	160	187	12·3	200	24
38	180	180	200	187	6·2	240	26
39	180	200	200	193	6·0	—	26
40	220	160	200	193	15·8	250	20
41	160	200	240	200	20·0	250	18
42	240	200	240	227	10·2	270	20
43	240	280	240	253	9·1	270	30
44	280	280	320	293	7·9	280	28
45	240	280	360	293	20·8	280	30
46	240	360	320	307	19·9	290	24

In none of the samples so far recorded was nickel detected, but the method is applicable to the simultaneous detection of copper, cobalt and nickel. The available samples, known to contain nickel, were limited to three samples taken at various distances and depths from a mass of basic igneous rock. Results obtained for copper, cobalt and nickel on these three samples are shown in Table IV.

Table IV

Simultaneous detection of copper, cobalt and nickel

Sample number	Copper found chromatographically, p.p.m.	Cobalt found chromatographically, p.p.m.	Nickel found chromatographically, p.p.m.	Nickel found by classical procedure
47	140	140	1200	1850
48	60	100	400	410
49	20	<20	60	75

The total nickel in the samples was determined by complete solution followed by separation and colorimetric determination of the nickel. The chromatographic results appear to be in fair agreement with those obtained by classical procedures. The low result on sample 47 is probably explained by the fact that this sample was not a true soil sample

but a sample of igneous rock. The relatively mild fusion with potassium bisulphate would probably not result in sufficient breakdown of the sample for complete extraction of nickel to be obtained.

Method

Reagents—

Potassium bisulphate—Powdered to pass an 80-mesh sieve.

Nitric - hydrochloric acid mixture—Mix 50 ml of hydrochloric acid, sp.gr. 1·18, and 5 ml of nitric acid, sp.gr. 1·42, and dilute to 100 ml with water.

Ethyl methyl ketone—The pure dry solvent.

Hydrochloric acid, sp.gr. 1·18.

Paper—Sheets of Whatman's No. 1 filter-paper, reference number CRL/1.

Ammonium hydroxide, sp.gr. 0·880.

Rubeanic acid solution—Dissolve 0·1 g of rubeanic acid in 60 ml of ethanol and dilute to 100 ml with water.

Standard solutions—Prepare standard solutions of copper, cobalt and nickel in mixed acid solution (50 ml of hydrochloric acid, sp.gr. 1·18, and 5 ml of nitric acid, sp.gr. 1·42, diluted to 100 ml with water).

Procedure—

Weigh 0·5 g of the soil crushed to pass an 80-mesh sieve into a 18-mm × 150-mm Pyrex-glass test tube. Add 1 g of potassium bisulphate powdered to pass an 80-mesh sieve. Fuse gently for 1 minute. Allow the melt to cool and add 2 ml of mixed acid solution (50 ml of hydrochloric acid, sp.gr. 1·18, and 5 ml of nitric acid, sp.gr. 1·42, diluted to 100 ml with water). Stand the tube in a boiling water-bath for 10 minutes with only the bottom inch of the tube immersed. Remove the tube and allow to cool. Prepare the solvent by mixing 15 ml of hydrochloric acid, sp.gr. 1·18, and 10 ml of water with 75 ml of ethyl methyl ketone, then pour 25 ml of the mixture into a 600-ml beaker. Cover the beaker with an inverted petri dish. With the aid of a capillary pipette apply a 0·01-ml aliquot of the clear supernatant liquid from the settled sample solution to the end of one of the strips on a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. Aliquots of the solutions from ten samples can be applied to one sheet of filter-paper of this type. Bend the sheet of filter-paper to form a cylinder and clip with a paper clip. Place the cylinder of paper with sample spots lowermost in a 600-ml beaker floating in a boiling water-bath. After 3 minutes remove the paper cylinder and stand it in the solvent beaker. Allow the solvent to diffuse almost to the top of the strips, then remove the cylinder of paper and stand in the air for 5 minutes. Place the paper cylinder inside a second 600-ml beaker fitted with a cover and containing a 50-ml beaker in which has been placed a solution of ammonium hydroxide, sp.gr. 0·880, and leave for 2 minutes. Take the cylinder from the beaker, remove the paper clip, spread the sheet flat and spray both sides lightly with a 0·1 per cent. solution of rubeanic acid. Allow the sheet to dry and then compare the copper, cobalt and nickel bands with those produced on standard chromatograms. To prepare the solutions for standard chromatograms add 2-ml aliquots of the standard solutions containing copper, cobalt and nickel dissolved in mixed hydrochloric and nitric acids to 0·5 g of potassium bisulphate. Warm the solution to dissolve the potassium bisulphate and allow to cool. Use the supernatant liquid obtained in each case for preparing the standard chromatograms, using the same procedure as that employed for the soil-sample solutions.

Niobium

A rapid chromatographic method for the determination of niobium in low-grade ores developed at this laboratory has already been published.[3] An obvious extension of this technique was the development of a field method for the determination of niobium in soils for geochemical prospecting purposes.

For the determination of niobium in low-grade ores a fluoride solution of the sample was applied to a paper strip and the niobium was separated chromatographically. Under the conditions of the experiment the niobium was detected as a diffuse orange-yellow band after spraying with tannic acid solution and was estimated by comparison with a series of

standard chromatograms. The limit of niobium detection by this method was of the order of 0·01 per cent. of Nb_2O_5. Modification of the procedure chiefly by the use of a solvent to separate niobium in a narrow band allows the detection of as little as 0·0004 per cent. of Nb_2O_5. Whilst it is possible to separate the fluorides of tantalum and niobium chromatographically on cellulose columns and paper strips by using ethyl methyl ketone with hydrofluoric acid mixtures as solvent,[5,6,7,8] the proposed method relies upon the separation of niobium together with tantalum, if present, from iron and other metals in the soil extract. The use of tannic acid as a detecting reagent sensitive only towards niobium renders interference from tantalum negligible. The procedure is simple and sufficiently speedy to allow approximately 60 determinations per analyst per day to be carried out with an accuracy of ± 50 per cent. or better.

Experimental

Breakdown of sample—

It was found that niobium could not be extracted completely from soils by digesting them with dilute or concentrated hydrofluoric acid in the cold, nor was complete extraction achieved on leaching the melt obtained by fusion of the sample with potassium bisulphate with a small volume of hot 2 per cent. oxalic acid solution. For a large number of samples a satisfactory procedure was to evaporate the soils to dryness with concentrated hydrofluoric acid and to treat the residue with a small volume of dilute hydrofluoric acid to obtain the niobium in solution. It was found that 1 g of soil treated with 5 ml of acid in a small platinum dish could be evaporated to dryness on a steam-bath in ½ to 1 hour. More rapid evaporation on the hot-plate led to the formation of a hard cake, which was difficult to break up. Small polythene beakers of about 10 ml capacity could be used in place of the platinum dishes. No trouble due to distortion of the polythene was encountered, but the evaporation of 5 ml of acid, even when the beaker was almost completely immersed in a steam-bath, took 2 to 3 hours. To the dry residue 2 ml of dilute hydrofluoric acid (25 ml of 40 per cent. w/w hydrofluoric acid and 75 ml of water) was added with the aid of a polythene pipette fitted with a rubber bulb. The mixture was stirred with a polythene rod to break up the cake and the dish was then covered and left to stand for 30 minutes. The 2 ml of acid used was the minimum volume that gave a portion of clear supernatant liquid on standing for a short time. An aliquot of this liquid was used for the chromatographic separation of the niobium, described in the next section.

Part of the organic matter present in some soils interfered with the detection and determination of niobium, since it was not destroyed during the hydrofluoric acid breakdown and moved with the niobium during the chromatographic separation. In such cases the organic matter was destroyed by pre-heating the soil to a dull red heat for a few minutes. Prolonged heating at high temperatures was avoided, since it tended to make the niobium resistant to hydrofluoric acid attack.

Most of the niobium-bearing soils available during the course of this work contained niobium as pyrochlore. This was extracted completely by the hydrofluoric acid treatment. In a few cases the niobium was present as ilmenorutile and similar refractory materials, which were resistant to attack by hydrofluoric acid. In these cases fusion with potassium bisulphate was employed to break down the sample. The soil was fused with four times its weight of finely ground potassium bisulphate in a platinum dish. The melt was warmed and stirred with 5 ml of concentrated hydrofluoric acid solution and then evaporated to dryness. To the residue were added 2 ml of dilute hydrofluoric acid solution. The mixture was stirred and allowed to stand. In some cases it was necessary to add more than 2 ml of dilute hydrofluoric acid solution in order to obtain a clear supernatant liquid after stirring the mixture and allowing to stand. An aliquot of the clear supernatant liquid was taken for the chromatographic separation.

It is recommended that the air-dried soil should be crushed with a pestle in a mortar and the fraction passing through an 80-mesh sieve used for the analysis. Experiments with several soils showed that this fraction and the fraction retained on the sieve did not differ in their niobium contents, which were identical with that of the fraction passing a 240-mesh sieve. Grinding the soil to pass completely through a 240-mesh sieve gave the same result, but the hydrofluoric acid insoluble residue did not settle easily and difficulty was experienced in obtaining a clear supernatant liquid.

CHROMATOGRAPHIC SEPARATION—

By means of a capillary pipette, 0·05-ml aliquots of the soil extracts in dilute hydrofluoric acid were applied to the centre ten strips of a paper sheet, reference number CRL/1. The tip of the pipette was drawn across the strip from side to side commencing about 1 cm from the lower end and working up the strip to empty the pipette. The rectangular spots formed then occupied the lower 4 to 5 cm of the strips. Suitable pipettes were prepared from thick-walled capillary tubing of Pyrex glass of internal diameter about 1·2 mm, by drawing out one end to provide a tip and then calibrating them by weighing the water they delivered. The pipettes were fitted with small rubber bulbs to facilitate the withdrawal of supernatant liquid from the sample beaker without disturbing the solid matter and transfer to the paper strip. The hydrofluoric acid attacked the glass and after a period of use each pipette was discarded.

The spots were allowed to dry for 1 hour. When the atmosphere had a relative humidity greater than 66 per cent., the niobium bands obtained after the chromatographic separation were diffuse instead of narrow and loss of sensitivity resulted. When this occurred, the spots were dried in a closed vessel over fused calcium chloride for 1 hour. After drying, the sheet, rolled and clipped to form a cylinder, was placed, sample spots lowermost, into a 600-ml polythene beaker containing approximately 20 ml of solvent and the beaker was covered with a 12-cm diameter petri dish. The solvent, consisting of pure ethyl methyl ketone containing 15 per cent. v/v of 40 per cent. w/w hydrofluoric acid, effected a separation of niobium and tantalum from other metals present. The sheet was removed from the beaker when the solvent had diffused almost to the top of the strips. This took about 20 minutes. Provided that the separation was carried out at a temperature of 20° to 25° C, niobium, if present, was found in a narrow band in the solvent front. At temperatures below 20° C niobium tended to form a diffuse band and it was necessary to increase the acid content of the solvent to 20 per cent. v/v. With this solvent the time required for the chromatographic separation was slightly longer.

When fusion of the soil with potassium bisulphate was employed for its breakdown, the high concentration of potassium salts in the final extract with dilute hydrofluoric acid interfered with the chromatographic separation of niobium. A satisfactory separation could, however, be achieved by reducing the sample aliquot taken to 0·02 ml and drying the spots for from 1 to 2 hours. The solvent mixtures used for the separation were stored in polythene bottles and were stable for several days. It was found necessary to replace the solvent in the beaker after it had been used for five or six separations or after a period of 2 to 3 hours owing to change in its composition due to evaporation.

DETECTION OF BANDS—

After removal of the developed sheet from the solvent-containing beaker, it was allowed to stand for a few seconds in air to permit the solvent to evaporate and was then placed in an atmosphere of ammonia. Three minutes' exposure to ammonia vapour was found sufficient to completely neutralise the free acid on the sheet, which was then spread flat and sprayed on both sides with a 2 per cent. aqueous solution of tannic acid. It was found desirable to prepare fresh reagent every few days. If the sheets were sprayed before exposure to ammonia or re-exposed after spraying, a dark, uneven and undesirable background was formed.

Niobium was detected in a narrow orange-yellow band in the solvent front (Fig. 3). As little as 0·1 μg of Nb_2O_5 was visible. Larger amounts gave more intensely coloured wider bands and part of the niobium trailed back from the front of the bands. These variations assisted in the comparison of bands with standards. After keeping overnight, the orange-yellow colour of the niobium - tannic acid complex was stable, and sheets of standards could be kept for several weeks. A slight increase in the intensity of the niobium bands was noted on keeping overnight, and, unless standards were prepared daily, the sample chromatograms were best left overnight before a visual comparison of the band intensities was made.

If present, tantalum also moved in the solvent front but gave only a pale buff-coloured band with tannic acid under the conditions of the test. Approximately 25 μg of tantalum could be mistaken for 0·1 μg of niobium, since the orange-yellow colour of the niobium complex was only just evident at this low level, but greater errors were unlikely.

On spraying the lower half of the strips, the original sample spots turned black owing to the presence of iron. Immediately above and still in contact with the iron band a yellow band was found when the sample contained titanium. Molybdenum and vanadium, if present, gave brown and grey bands, respectively, just overlapping the yellow band due to titanium.

PREPARATION OF STANDARDS—

Standard chromatograms were prepared by applying suitable aliquots of standard solutions of niobium to paper strips, drying the spots for 1 hour and then proceeding as for a sample solution. A standard niobium solution was prepared from Specpure niobium pentoxide which had been heated to 500° to 600° C for 30 minutes. Then 0·400 g of this oxide was weighed into a platinum dish and warmed with hydrofluoric acid and a little nitric acid until solution was complete. The solution was evaporated to dryness on a water-bath and the evaporation repeated twice with hydrofluoric acid alone. The residue was dissolved in the minimum volume of warm hydrofluoric acid and allowed to cool. After dilution, the solution was transferred to a 100-ml calibrated flask, diluted to the mark and transferred as quickly as possible to a polythene bottle. Suitable aliquots of this solution were diluted to give a series of standards. The dilution technique was to put, by pipette, a suitable volume of the standard solution into a polythene bottle, to add 1 ml of 40 per cent. w/w hydrofluoric acid and to dilute to 50 ml with water from a burette. The standard solutions were stable if stored in polythene bottles having screw caps. It was usually necessary in preparing standard chromatograms to prepare a separate solution for each standard band required, but in the case of niobium all the metal applied to the paper strip was carried forward in the solvent front and it was possible to use aliquots of 0·01 to 0·05 ml of the same standard solution in order to give several standard bands. The series 0·1, 0·2, 0·4, 1·0, 2·0, 4·0 and 8·0 μg of Nb_2O_5 was found to constitute a satisfactory range of standards. The lowest corresponded to 4 p.p.m. of Nb_2O_5 and the highest to 320 p.p.m. of Nb_2O_5 in a soil if a 0·05-ml aliquot was taken from 2 ml of solution obtained from 1 g of soil. The use of smaller aliquots extended the upper range of the method, but for higher-grade materials the method already published[3] for the determination of niobium in low-grade ores was preferable.

RESULTS—

Tests on soils from the Sukulu area of Africa, which contain a relatively large amount of niobium (0·1 to 0·6 per cent.), have already been reported.[3] Few other soils of known niobium content were available for testing the method. Fourteen soil samples, which had been collected in the vicinity of a sub-outcropping tungsten ore body for the purpose of carrying out tungsten determinations, were available. Scheelite often contains traces of niobium, and wolframite usually contains traces of both niobium and tantalum.[9] This series of samples was therefore examined for niobium by the field procedure. Thirteen of the samples were found to contain 60 p.p.m. of Nb_2O_5 and the remaining sample 40 p.p.m. of Nb_2O_5. The accuracy of these figures was checked in two ways. To each of two of the soil extracts were added 60 μg of Nb_2O_5 and the chromatographic separation was repeated. Values of 120 p.p.m. in the soil were obtained on both samples, so indicating that all the niobium in solution was extracted in the solvent front. Two of the samples containing 60 p.p.m. of Nb_2O_5 were bulked together and a 10-g sample of the mixture was taken for accurate analysis. The samples were broken down by attack with hydrochloric acid and hydrogen peroxide, and an acid hydrolysis was carried out to separate the niobium in a fraction of the original bulk of the sample. This fraction was dissolved in dilute hydrofluoric acid and the niobium separated chromatographically by using a cellulose-column procedure.[10] The niobium in the eluate was determined colorimetrically[11] and the 10-g sample of soil was found to contain 0·6 mg of Nb_2O_5, equivalent to 60 p.p.m. of Nb_2O_5 in the soil.

A further fourteen samples collected in the region of pegmatite veins and suspected of containing niobium were subjected to the field procedure. Values ranging from 24 to 50 p.p.m. of Nb_2O_5 were found for these samples, which was the order expected, but no attempts have been made to confirm the figures by other methods.

Method

Reagents—

Hydrofluoric acid, 40 per cent. w/w.

Hydrofluoric acid, diluted—Mix 1 volume of 40 per cent. w/w hydrofluoric acid and 3 volumes of water.

Paper—Sheets of Whatman's No. 1 filter-paper, reference number CRL/1.

Ethyl methyl ketone—The pure dry solvent.

Ammonium hydroxide, sp.gr. 0·880.

Tannic acid solution—Dissolve 2 g of tannic acid in 100 ml of water.

Potassium bisulphate—Powdered to pass an 80-mesh sieve.

Standard solutions—Prepare standard solutions of niobium in hydrofluoric acid.

Procedure involving hydrofluoric acid extraction—

Weigh 1 g of the soil, powdered to pass an 80-mesh sieve, into a 10-ml polythene beaker, add 5 ml of 40 per cent. w/w hydrofluoric acid and evaporate to dryness on a water-bath. To the residue add 2 ml of diluted hydrofluoric acid (1 volume of 40 per cent. w/w hydrofluoric acid and 3 volumes of water) by means of a polythene pipette fitted with a rubber bulb. Stir the mixture with a polythene rod and stand for 30 minutes. Extract 0·05 ml of the supernatant liquid with a glass pipette fitted with a rubber bulb and transfer to one end of a paper strip on a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. Allow the sheet to dry in the atmosphere for 1 hour. If the relative humidity of the atmosphere is greater than 66 per cent., dry the sheet for 1 hour in a calcium chloride desiccator. Prepare the solvent by mixing 85 ml of ethyl methyl ketone with 15 ml of 40 per cent. w/w hydrofluoric acid, and pour about 20 ml into a 600-ml polythene beaker 30 minutes before the drying of the paper strip is complete. Cover the beaker with a 12-cm diameter petri dish. Place the sheet, rolled and clipped to form a cylinder, in the beaker. Allow the solvent to diffuse upwards from the sample spots almost to the top of the strips. Remove the sheet and allow the solvent to evaporate and expose the strips to ammonia vapour for 3 minutes. Spread the sheet flat and spray it on both sides with a 2 per cent. aqueous solution of tannic acid. Allow the sheet to dry before making a comparison of the niobium band colour intensities with the standards. Standard chromatograms must be prepared daily if an immediate comparison of the sample chromatograms is to be made; otherwise the sample sheet should be kept overnight before comparison with old standards.

Procedure involving potassium bisulphate fusion—

Weigh 1 g of the soil, powdered to pass an 80-mesh sieve, into a small platinum dish. Add 4 g of powdered potassium bisulphate and fuse for 1 to 2 minutes. To the cool melt add 5 ml of 40 per cent. w/w hydrofluoric acid and evaporate to dryness on a water-bath. Add 2 ml of dilute hydrofluoric acid (1 volume of 40 per cent. w/w hydrofluoric acid and 3 volumes of water) by means of a polythene pipette fitted with a rubber bulb. Stir the mixture with a polythene rod and stand for 30 minutes. Extract 0·02 ml of the supernatant sample liquid with the aid of a glass pipette fitted with a rubber bulb and transfer to one end of a paper strip on a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. Allow the sheets to dry in the atmosphere for from 1 to 2 hours and then proceed with the chromatographic separation as given above for the hydrofluoric acid extraction.

Tantalum

In the method for the determination of niobium in soils it was stated that both niobium and tantalum are extracted together. A satisfactory test was available for the detection of niobium in the presence of tantalum, but, in the absence of a specific test for tantalum, it was necessary to separate tantalum from niobium chromatographically in order to determine tantalum. The process finally adopted was a modification of the technique already described for the determination of niobium. The separation of niobium from tantalum on both columns of cellulose and strips of paper has already been reported.[5,6,7,8] In both cases separation of the fluorides of the metals was effected with the aid of ethyl methyl ketone as solvent. For geochemical prospecting purposes the problem was to obtain a separation of tantalum from niobium and other metals present in a soil sample on the limited length of

a paper strip (9 cm) provided by a sheet of filter-paper of CRL/1 pattern. It was found that ethyl methyl ketone containing 2 per cent. v/v of 40 per cent. w/w hydrofluoric acid and 8 per cent. v/v of water was the most suitable solvent for the separation, but results were sensitive to changes in temperature and to the method used for drying the test spots on the paper. With appropriate attention to these variables, it was found possible to separate tantalum and to determine as little as 4 p.p.m. of Ta_2O_5 in a soil with the aid of quinalizarin as detecting reagent.

It was somewhat more difficult to obtain a complete solution of the tantalum present in a soil than was the case for niobium. Methods investigated included fusion with potassium bisulphate, attack by hydrofluoric acid and attack by mixtures of hydrofluoric and nitric acids. Although direct attack with hydrofluoric acid did not give complete solution of tantalum in all cases, solutions obtained by this method were more amenable to chromatographic treatment than were others and this is the procedure recommended for geochemical prospecting work.

Experimental

Breakdown of samples—

Most tantalum minerals are completely decomposed by fusion with potassium bisulphate, and this method of attack was investigated. One-gram samples of soil were fused with 4 g of potassium bisulphate. Melts were evaporated to dryness with 40 per cent. w/w hydrofluoric acid and the residues were treated with a measured small volume of hydrofluoric acid of various concentrations. Very poor recoveries of tantalum were obtained. For example, a soil sample was "spiked" with 1000 p.p.m. of Ta_2O_5 and, after fusion with potassium bisulphate and evaporation with hydrofluoric acid, was treated with 2 ml of 25 per cent. hydrofluoric acid (1 part of 40 per cent. w/w hydrofluoric acid and 3 parts of water). Only 15 per cent. of the tantalum was found after chromatographic separation. This was probably due to the presence of a large excess of potassium salts, so permitting the formation of insoluble potassium fluorotantalate. The slightly better recovery achieved on increasing the volume of dilute hydrofluoric acid used to leach the bisulphate melt supported this view. Thus, on using 4 ml instead of 2 ml of dilute hydrofluoric acid, a 20 per cent. recovery was obtained.

In view of the poor results obtained in the presence of large amounts of potassium salts, direct attack with hydrofluoric acid and also with hydrofluoric and nitric acid mixtures was investigated. Various 0·1-g samples of tantalum minerals were weighed into platinum dishes and treated with 5 ml of 40 per cent. w/w hydrofluoric acid. After evaporation to dryness on a steam-bath, various volumes of dilute hydrofluoric acid were added to the residues. Aliquots of the extracts were applied to paper strips and the tantalum was separated and estimated chromatographically. This series of experiments was then repeated, 5 ml of 40 per cent. w/w hydrofluoric acid and 1 ml of nitric acid, sp.gr. 1·42, being used for the initial treatment. On samples of tantalite, 85 per cent. of the tantalum was extracted by using the mixed acids compared with 55 per cent. by using hydrofluoric acid alone. For a tantalite - columlite, hydrofluoric acid extracted 80 per cent. of the tantalum, whilst a hydrofluoric - nitric acid mixture extracted 100 per cent. of the tantalum. On both euxenite and samarskite samples extraction was more than 90 per cent. with both the mixed acids and hydrofluoric acid alone. Extraction of tantalum from samarskite was slightly more efficient when the mixed acids were used, but no difference was detectable in the extraction of the euxenite. Both methods failed to extract more than 20 per cent. of the tantalum from simpsonite. No microlite was available for testing. Results on pyrochlore indicated that complete extraction of tantalum was obtained by treatment with hydrofluoric acid. From these results it appeared that direct attack on soil samples with hydrofluoric acid would be satisfactory for geochemical purposes but that extraction with mixed nitric and hydrofluoric acids would be preferable.

The presence of organic matter in some soil samples interfered with the chromatographic separation. It resulted in the formation of a brown band in the solvent front, which made the comparison of tantalum bands extremely difficult. Removal of this interference was obtained by ignition of the soils to a dull red heat before treatment with hydrofluoric acid. When mixed hydrofluoric and nitric acids were used for extracting the soil, interference from organic matter was again experienced. This occurred even after ignition of the soil and was probably due to decomposition products resulting from the attack by traces of nitric acid on the paper strip. For this reason use of nitric acid for the extraction of soil samples was

avoided, and with all the soil samples so far examined satisfactory results have been obtained by using direct attack with hydrofluoric acid.

CHROMATOGRAPHIC SEPARATION—

Several solvent mixtures of ethyl methyl ketone with 1 to 2 per cent. v/v of 40 per cent. w/w hydrofluoric acid and 8 to 12 per cent. v/v of water were found to be capable of separating tantalum from niobium on strips of paper of 9 cm length. The conditions of drying the sample spots and in some cases the temperature at which the separations were performed, however, had to be controlled within narrow limits. The extent to which niobium moved during chromatographic separation was dependent on the amount of hydrofluoric acid in the system. The higher the hydrofluoric acid content of the solvent the greater was the movement of niobium. The acid content of the solvent could not, however, be reduced below 2 per cent. v/v if reasonable amounts of tantalum were to move in narrow bands in the solvent front. When the separation was carried out at 20° C, 8 per cent. by volume was found to be the optimum amount of water to add to the ethyl methyl ketone solvent. With this solvent mixture of ethyl methyl ketone containing 2 per cent. v/v of 40 per cent. w/w hydrofluoric acid and 8 per cent. v/v of water, a separation of tantalum from niobium was obtained, provided that the amount of hydrofluoric acid in the test spot was reduced to a low level. In order to obtain complete extraction of tantalum from the soil it was necessary to take up the residue from the initial hydrofluoric acid attack in 25 per cent. hydrofluoric acid (1 part of 40 per cent. w/w hydrofluoric acid and 3 parts of water). That is, the amount of acid in the soil extract applied to the paper could not be reduced for a given aliquot other than by a prolonged drying of the spot. It was necessary to dry the spots formed by the application of 0·05 ml of a soil solution to paper strips for from 2 to 3 hours in order to obtain a separation of tantalum from niobium. The tantalum was then obtained as a band moving in the solvent front with the niobium a short distance behind, not always completely separated from the titanium, which moved partially from iron and other immobile metals in the original test spot. It was found possible to reduce the drying time to 1½ hours by developing the chromatogram at 25° C or by reducing the size of sample aliquots applied to the strip. By using 0·02 ml of sample solution, the effective length of the strip was increased, and it was possible to achieve a separation at 20° or 25° C after drying for 1 hour.

Attempts were made to reduce the time required for drying the spots by drying at 80° C as described earlier in the section on copper, cobalt and nickel separations. Drying at an elevated temperature reduced the water content of the whole sheet of paper and resulted in niobium moving freely in the solvent front. If, after drying at a high temperature, the paper was allowed to stand at room temperature for some time in order to regain moisture, the separation of tantalum from niobium was possible, but results were not reproducible.

The chromatographic separation was carried out in a similar manner to that described for the determination of niobium in soils, a 600-ml covered polythene beaker containing 20 ml of the solvent mixture being employed. Each 20-ml portion of the solvent could be used for 2 to 3 hours or for the running of five or six sheets, whichever involved the shorter time.

During the development of the procedure for the determination of tantalum, the only soil samples available were those in which niobium was present in considerably greater concentration than the tantalum. In normal geochemical prospecting practice, attempts to locate areas of interest relating to such samples would be based on a determination of the niobium. In looking for areas of major tantalum interest, tantalum would be the predominant element. This would simplify the method in that the quantity of niobium present on a strip would be small and the time taken for drying the test spots on the strip could be reduced to 1 to 1½ hours, even on using 0·05-ml aliquots of soil extracts and carrying out the chromatographic separation at 20° C.

DETECTION OF BANDS—

Detailed attention was given to the use of alizarin S and quinalizarin as reagents for the detection of tantalum. The second of these was finally adopted as giving the most sensitive test. By using quinalizarin it was possible to detect 0·1 μg of Ta_2O_5 on a paper strip. This was equivalent to 4 p.p.m. of Ta_2O_5 in the soil when a 2-ml extract of 1 g of soil was prepared and a 0·05-ml aliquot of the extract was applied to the paper strip. To achieve this sensitivity it was necessary to spray the developed chromatogram immediately after its removal from

the polythene beaker containing the solvent. When the paper sheet was allowed to dry before spraying, maximum sensitivity was not achieved. The spray reagent was prepared by dissolving 0·05 g of quinalizarin in 10 ml of pyridine and diluting to 100 ml with acetone. After spraying with the quinalizarin reagent, the sheet was exposed to ammonia vapour, when the alizarin formed a coloured lake with the tantalum. The background colour due to unreacted reagent was then reduced to a pale pink by exposure to acetic acid vapour. This was carried out by placing the sheet around a 50-ml beaker containing glacial acetic acid inside a larger beaker fitted with a cover. Tantalum was detected as a narrow mauve-pink band in the solvent front. If present, niobium was detected as a similarly coloured band between the original spot and the tantalum. The width of the niobium band depended upon its concentration. It was always behind and separated from the tantalum band, but sometimes overlapped a mauve-pink band due to titanium, which just began to move from the original spot. Iron and other metals, which did not move from the original test spot, resulted in the formation of a dark blue colour. All the colours formed were stable and underwent no change in intensity on keeping for long periods.

PREPARATION OF STANDARDS—

Standard chromatograms were prepared by applying aliquots of standard tantalum solutions to paper strips, drying the spots, developing the sheet chromatographically, spraying with quinalizarin reagent, exposing to ammonia vapour and then acetic acid vapour. By using a solvent mixture containing 2 per cent. v/v of 40 per cent. w/w hydrofluoric acid and 8 per cent. v/v of water, the tantalum bands were diffuse unless the standard solutions contained free hydrofluoric acid. Satisfactory bands were obtained by preparing standard solutions in 5 per cent. hydrofluoric acid and allowing the test spots to dry for ½ to 1 hour before developing the sheets chromatographically. Longer drying times caused the tantalum bands to be diffuse.

A standard tantalum solution was prepared by weighing 0·0819 g of tantalum metal into a platinum dish and warming on a steam-bath with hydrofluoric acid and a little nitric acid until solution was complete. The solution was evaporated to dryness and the residue again evaporated twice with further hydrofluoric acid to remove nitric acid. The residue was dissolved in 5 ml of warm hydrofluoric acid and the solution was diluted to 100 ml with water. This solution contained the equivalent of 10 μg of tantalum oxide per 0·01 ml and further standard solutions were prepared by dilution of this solution with 5 per cent. hydrofluoric acid (5 ml of 40 per cent. w/w hydrofluoric acid and 95 ml of water). A suitable series of standard solutions contained 0·1, 0·2, 0·5, 1·0, 2·0, 3·0, 5·0, 7·0 and 10·0 μg of tantalum oxide per 0·01 ml.

RESULTS—

Tantalum was estimated in three soil samples known to contain pyrochlore. The soils contained 1600, 1800 and 5900 p.p.m. of Nb_2O_5 and the corresponding tantalum results obtained were 100, 100 and 300 p.p.m. Thus the niobium to tantalum ratio for these soils appeared to be between 16 and 20 to 1. In check experiments the mixed oxides were extracted quantitatively from the soil samples and the tantalum was estimated colorimetrically. In these experiments a ratio of 17 to 1 was obtained for the niobium and tantalum concentrations, in good agreement with the results obtained by the field procedure.

A sample of Nigerian granite, also known to contain pyrochlore, was analysed for niobium and tantalum. The sample was found to contain 2500 p.p.m. of Nb_2O_5 and 160 p.p.m. of Ta_2O_5. Here again the ratio of niobium to tantalum was approximately 16 to 1.

Ten samples collected in the region of pegmatite veins were also analysed for niobium and tantalum. The niobium content varied between 24 and 50 p.p.m. of Nb_2O_5. No tantalum could be detected. In view of the low niobium content this result could perhaps be expected, since the limit of detection of Ta_2O_5 by the method is 4 p.p.m.

METHOD

REAGENTS—

Hydrofluoric acid, 40 per cent. w/w.

Hydrofluoric acid, diluted—Mix 1 volume of 40 per cent. w/w hydrofluoric acid and 3 volumes of water.

Paper—Sheets of Whatman's No. 1 filter-paper, reference number CRL/1.

Ethyl methyl ketone—The pure dry solvent.

Quinalizarin solution—Dissolve 0·05 g of quinalizarin in 10 ml of pyridine and dilute to 100 ml with water.

Ammonium hydroxide, sp.gr. 0·880.

Acetic acid, glacial.

Standard solutions—Prepare standard solutions of tantalum in 5 per cent. hydrofluoric acid.

PROCEDURE—

Weigh 1 g of the soil, powdered to pass an 80-mesh sieve, into a 10-ml polythene beaker. Add 5 ml of 40 per cent. w/w hydrofluoric acid and evaporate to dryness. Add 2 ml of dilute hydrofluoric acid to the residue by means of a polythene pipette fitted with a rubber bulb. Stir the mixture with a polythene rod and allow to stand for 30 minutes. Extract 0·05 ml of the supernatant liquid with a capillary pipette and transfer it to one end of a paper strip on a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. Aliquots from ten soil solutions may be applied to one sheet. Allow the sheet to dry in the atmosphere for at least 1 hour. If niobium is present, dry for 1½ hours if temperature is about 25° C. If the temperature is 20° C or less, increase the drying time to 2 hours. Prepare the solvent mixture by mixing 2 ml of 40 per cent. w/w of hydrofluoric acid and 8 ml of water with 90 ml of pure ethyl methyl ketone and pour about 20 ml of the mixture into a 600-ml polythene beaker 30 minutes before the strips are dried completely. Cover with a petri dish. Place the sheet, clipped to form a cylinder, in the beaker with sample spots lowermost and allow the solvent to diffuse upwards until it almost reaches the top of the strips. Remove the sheet from the solvent beaker and spray immediately with a solution of quinalizarin. Expose the sheet to ammonia vapour for a few minutes, then expose to acetic acid vapour for a further few minutes and compare the tantalum bands with those of standard chromatograms.

Lead

In earlier work[4] the chromatographic separation of a number of metals from lead was achieved by using diffusion of methanol containing 5 per cent. v/v hydrochloric acid, sp.gr. 1·18, down a paper strip to which had been applied a chloride solution of the metals. This separation was repeated by using upward diffusion of the solvent. Separation from lead was achieved on the 9-cm long strips available on a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. The separation was unusual in that the desired metal was the least mobile of the metals present in the test solution and moved only a short distance from the position of the test spot. Other metals, including copper, bismuth, cadmium, mercury, iron, aluminium, magnesium, zinc, cobalt, nickel and tin, moved in a mixed band which trailed back from the solvent front. It was found that the separation could be carried out by using either a nitric acid or hydrochloric acid extract of a soil. With the chloride solution, the movement of metals other than lead was slightly greater than with nitrate solutions.

Detection of lead was achieved by spraying the strip with a solution of dithizone. This reagent gave an extremely sensitive test for lead, but the sensitivity was considerably reduced by the presence of small amounts of ammonia, and the pink dithizonate colour faded very rapidly in sunlight. By control of conditions and the use of a buffered solution of dithizone, it was possible to detect as little as 0·1 μg of lead. Under the conditions used in the method this was equivalent to the detection of 10 p.p.m. of lead in the soil. Because of the instability of the dithizone it was necessary to prepare a fresh set of standards each day.

Several methods of extracting lead from soil samples were investigated. Other workers[2] used digestion of the soil with diluted nitric acid (1 + 3) for the extraction of lead in geochemical work and this was the procedure ultimately adopted in the present method. Experiments in which the lead was extracted by digestion with diluted hydrochloric acid (1 + 1) gave satisfactory results on all the samples so far examined, but in view of the general use of nitric acid for lead extraction use of hydrochloric acid has not been proposed in the final procedure.

Experimental

Breakdown of sample—

Other workers who used the dithizone mono-colour method for the colorimetric determination of lead have reported a satisfactory extraction of lead from soils and rocks by digestion with diluted nitric acid (1 + 3). In the present work satisfactory extraction of lead was obtained by heating 1 g of sample in a test tube for 1 hour in a boiling water-bath with 1 ml of diluted nitric acid (1 volume of nitric acid, sp.gr. 1·42, and 3 volumes of water). One gram of the air-dried soil sample, crushed to pass an 80-mesh sieve, was weighed into a 12-mm × 100-mm test tube. One millilitre of diluted nitric acid solution was added and the tube was warmed gently until any effervescence due to the decomposition of carbonates ceased. The tube was then heated on a boiling water-bath for 1 hour. Provided that only the lower end of the tube was immersed in the bath, loss of acid solution by evaporation was negligible. The tube was set aside to cool and to allow solid matter to settle. The clear supernatant liquid was used for the chromatographic separation.

For soils containing a large proportion of carbonate it was necessary to increase the strength of the nitric acid solution used in order to maintain a sufficiently high concentration of free acid to complete the extraction of lead. For samples containing large quantities of lead it was necessary to decrease the sample weight taken or to use a larger volume of nitric acid in the extraction process.

Both hydrochloric acid extraction and potassium bisulphate fusion of the sample have been used in the extraction of metals other than lead from soils, and these methods of attack were investigated for lead with a view to being able to carry out the determination of several metals on different aliquots of the same extract. Digestion of 1 g of soil for 1 hour with 1 ml of diluted hydrochloric acid (1 + 1) in a test tube in a boiling water-bath resulted in a satisfactory extraction of lead, and the chromatographic separation was readily carried out on the chloride extract. In further experiments 0·5-g samples of soil were fused with potassium bisulphate and the melts were dissolved in hydrochloric or nitric acid. Chromatographic separation of lead was not satisfactory when carried out on the resulting solutions, since a great deal of the iron present remained behind with the lead in the test spot. Melts from further bisulphate fusions were treated with ammonium acetate solution. Chromatographic separation of lead carried out on the resulting acetate solution was satisfactory, but only 10 per cent. of the lead was found in the ammonium acetate solution.

Nitric acid digestion of the soil was adopted in the final procedure in view of the satisfactory results obtained by a number of workers who used this method.

Chromatographic separation—

The optimum chromatographic separation of lead on a 1·5-cm × 9-cm paper strip cut from a sheet of Whatman's No. 1 filter-paper, reference No. CRL/1, was obtained by using 0·01 ml of a nitric acid extract of a soil. A separation was just possible on applying 0·02 ml of the extract to the paper strip, but 0·05 ml covered more than half the strip and so reduced its effective length that a separation could not be achieved. After application of 0·01 or 0·02 ml of sample extract to the paper, the strip was allowed to dry in air for 30 minutes and was then transferred to the solvent beaker. As in previous work, the sheet of paper holding the sample strips was rolled to form a cylinder, clipped at one end and stood vertically in a 600-ml covered beaker containing 20 ml of solvent mixture. The solvent was allowed to diffuse almost to the top of the strips (30 minutes), the sheet of paper was then removed and the lead detected by the method given in the following section.

The solvent used was methanol containing 5 per cent v/v of hydrochloric acid, sp.gr. 1·18. Pure dry methanol had to be used in preparing the solvent mixture in order to obtain exactly reproducible lead bands.

Detection of bands—

The lead present on the paper strips was detected by neutralising the free acid and spraying with a freshly prepared aqueous solution of dithizone. The lead was detected as a pink band in the region of the original test spot, well separated from a mixed coloured band due to other metals, which trailed back from the solvent front. In order to obtain reproducible lead bands an exact procedure had to be followed. After removal from the solvent beaker the sheet was dried in air for at least ½ hour. Thorough drying was necessary in order to remove

a large proportion of the hydrochloric acid. The sheets were then exposed for 2 minutes to an atmosphere of ammonia and then again stood in air for 15 minutes. This procedure ensured that the concentration of ammonium chloride and ammonia on the sheet was low. The sheet was then sprayed with a brown solution of dithizone prepared by dissolving a few milligrams of the solid reagent in 20 ml of acetone containing 1 drop of 2 *N* ammonium hydroxide solution and diluting to 100 ml with a 5 per cent. aqueous solution of ammonium acetate.

The strength of the dithizone solution used depended upon the quantity of lead present. By using a 0·002 per cent. solution of dithizone it was possible to detect 0·1 μg of lead. When several micrograms of lead were present on each strip, however, spraying with such a dilute dithizone solution did not provide sufficient reagent for complete reaction with the lead. In this case the strength of the dithizone solution was increased to 0·02 per cent. By using this solution the lower limit of detection was 0·2 μg. The decrease in sensitivity with the stronger dithizone solution was due to background colour of unreacted reagent on the strip, which prevented the detection of the pink band due to 0·1 μg of lead. By using the more dilute dithizone solution it was thus possible to detect as little as 10 p.p.m. of lead in a soil, whilst use of the more concentrated solution limited the detection to 20 p.p.m. of lead. Ammonium acetate was added to the spray reagent solution in order to give reproducible sensitivity and to improve the stability of the pink lead dithizonate colour.

In the determination of other metals, described in earlier sections, comparison of chromatograms was made by comparing the colour intensity of particular bands. Comparison of the intensity of the pink coloured bands due to lead was not readily made, but determinations could be made by comparing band widths. The widths of the lead bands were found to be related to the concentration of lead. Thus, for separations performed at 25° C, a band width of 1·5 cm was obtained for 2·0 μg of lead, 2·5 cm for 10 μg of lead and 3·5 cm for 25 μg of lead, the band width obtained for 0·2 μg of lead being approximately the width of the original test spot. For separations carried out at 20° C, band widths were still related to concentration but approximately 4 mm wider than those obtained at 25° C.

Preparation of standards—

For the preparation of standard chromatograms a stock solution containing 0·4 g of AnalaR lead nitrate in 100 ml of diluted nitric acid (1 + 3) was prepared. This solution contained 25 μg of lead per 0·01 ml, and portions of this solution were diluted with further diluted nitric acid (1 + 3) to give a series of solutions such that each differed in lead content from its neighbour by 30 to 40 per cent. Then 0·01-ml aliquots of these solutions were applied to paper strips and treated in a similar manner to that already described for sample solutions. The standard chromatograms obtained were found to be stable for a few days if kept in the dark, but it was found preferable to prepare fresh standards daily.

Results—

Determination of lead was carried out on a number of lateritic soils and weathered shales. Results obtained chromatographically were compared with those obtained spectrographically (see Table V).

TABLE V

DETERMINATION OF LEAD IN SOILS

Sample number	Lead found spectrographically, p.p.m.	Lead found after extraction with diluted nitric acid (1 + 3), p.p.m.	Lead found after extraction with diluted hydrochloric acid, p.p.m.
1	90	60	40
2	175	50	40
3	180	300	240
4	400	470	300
5	2500	2500	3000
6	4000	3700	4000
7	6000	8300	10,000
8	10,000	10,400	12,500

Chromatographic separations were carried out on both nitric and hydrochlorié acid extracts of the soil samples. The results given in column 3 of Table V are the mean for three determinations carried out after nitric acid extraction. The results in column 4 are single chromatographic results after a hydrochloric acid extraction.

Method

Reagents—

Nitric acid, diluted—Mix 1 volume of nitric acid, sp.gr. 1·42 and 3 volumes of water.
Paper—Sheets of Whatman's No. 1 filter-paper, reference number CRL/1.
Methanol—The pure dry solvent.
Hydrochloric acid, sp.gr. 1·18.
Ammonium hydroxide, sp.gr. 0·880.
Dithizone solution—Dissolve 20 mg of dithizone in 20 ml of acetone plus 1 drop of 2 *N* ammonium hydroxide solution and dilute to 100 ml with a 5 per cent. w/v aqueous solution of ammonium acetate.
Standard solutions—Prepare standard solutions of lead in diluted nitric acid (1 +3).

Procedure—

Weigh 1 g of soil, ground to pass an 80-mesh sieve, into a 12-mm × 100-mm rimless test tube. Add 1 ml of diluted nitric acid (1 + 3) and digest on a water-bath for 1 hour with only the bottom inch of the tube immersed. Remove the tube, cool and allow to settle. Apply 0·01 ml of the clear supernatant sample liquid to the end of a paper strip cut from a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. Allow the spots to dry in the atmosphere for 30 minutes. Prepare the solvent by adding 1 ml of hydrochloric acid, sp.gr. 1·18, to 19 ml of re-distilled methanol and pour into a 600-ml beaker fitted with a petri-dish cover. Place the sheet of paper, rolled and clipped to form a cylinder, with sample spots lowermost in the beaker and allow the solvent to diffuse to the top of the strips. Remove the sheet of paper and allow it to dry in the atmosphere for at least ½ hour. Expose the sheet to ammonia vapour for 2 minutes and then stand again in air for a further 15 minutes. Spread the slotted sheet of paper flat and spray both sides lightly with a freshly prepared solution of dithizone. Compare the lead bands obtained with those of standard chromatograms.

Uranium

The high solubility of uranyl nitrate in many organic solvents has been utilised in chromatographic procedures for the determination of uranium in minerals and ores. In earlier work[12] in which strips of paper were used it was found that a variety of solvents containing nitric acid could be employed to separate uranium from other less mobile metals. The chromatographic separation of uranium by employing cellulose columns, and its subsequent determination by conventional techniques, has become a well established analytical procedure.

It has been found possible to modify the paper-strip separation of uranium described by earlier workers and to apply it to the determination of uranium in soils and rocks for geochemical prospecting purposes. In the modified procedure the 1-inch wide strips of paper normally used have been replaced by 1·5-cm × 9-cm strips available on sheets of Whatman's No. 1 filter-paper, reference number CRL/1. Satisfactory solution of uranium was obtained on most samples by treatment with a mixture of hydrofluoric and nitric acids. The solvent used was ethyl acetate containing 10 per cent. v/v of nitric acid, sp.gr. 1·42, and 5 per cent. v/v of water. With this solvent uranium was extracted in the solvent front, where it was detected by spraying the sheet with potassium ferrocyanide solution. By comparison of the brown coloured uranium ferrocyanide bands obtained with those of standard chromatograms it was possible to determine the uranium content of soils and rocks down to 2 p.p.m.

Experimental

Breakdown of sample—

Several procedures were investigated for the extraction of uranium from soil samples. These included fusion with potassium bisulphate followed by extraction with nitric acid,

fusion with potassium hydroxide followed by extraction with nitric acid, direct extraction with diluted nitric acid (1 + 3) and extraction with a mixture of hydrofluoric and nitric acids. Neither of the fusion procedures was satisfactory. A chromatographic separation was attempted on the solution obtained by digesting the melt from a fusion of the soil with potassium bisulphate with nitric acid. The extremely high concentration of sulphate prevented the extraction of uranium. In experiments with potassium hydroxide fusions the melt from the alkali fusion was acidified with nitric acid and a chromatographic separation was carried out on the resulting solution. The acidification of the melt required so much acid that dilution of the uranium became excessive and the method lost sensitivity. Difficulty was also experienced in dealing with samples high in silica, owing to hydrated silica separating out both in the test solution and on the filter-paper.

With a number of soils, extraction with diluted nitric acid (1 volume of nitric acid, sp.gr. 1·42, and 3 volumes of water) was satisfactory. For this type of sample 1 g of the soil was weighed into a test tube and heated with 1 ml of diluted nitric acid (1 + 3) in a boiling water-bath for ½ hour. With only the lower part of the tube immersed, losses by evaporation were negligible. After standing in order to allow solids to settle and the solution to cool, an aliquot of the supernatant liquid was taken for the chromatographic separation. In order to achieve the maximum sensitivity, the minimum volume of acid (1 ml) was used to digest the sample. For materials with a high carbonate content it might be necessary to use concentrated acid and in some cases to use more than 1 ml per gram of sample in order to ensure complete extraction. In more refractory samples, such as granite, digestion of the sample with nitric acid failed to extract all the uranium. In these cases treatment with a mixture of hydrofluoric and nitric acids was employed. One gram of sample was weighed into a small platinum dish and 2 to 3 ml each of concentrated nitric acid and hydrofluoric acid were added. The dishes were placed on a steam-bath and the solutions evaporated to dryness. To the residue was added 1 ml of diluted nitric acid (1 + 3) and the mixture was stirred and then allowed to stand until a clear supernatant liquid was obtained. In a few cases it was necessary to add a further 1 ml of acid and to re-stir and settle before a clear supernatant liquid, suitable for the chromatographic procedure, was obtained. This breakdown procedure was more lengthy than direct attack with dilute nitric acid, but was preferred since it was more generally applicable.

Chromatographic separation—

Previous work[12] indicated that ethyl acetate containing 10 per cent. v/v of nitric acid, sp.gr. 1·42, would be a suitable solvent for the chromatographic separation of uranium. This was confirmed in experiments on samples of soil extracts. When the chromatographic separation of gold on paper strips was studied,[13] the addition of 5 per cent. by volume of water to the ethyl acetate - nitric acid mixture was found to improve the extraction of gold. This modified solvent was also found to improve the chromatographic separation of uranium in that narrower bands of uranium were obtained. AnalaR ethyl acetate was used and was found to be satisfactory. With ethyl acetate containing traces of ethanol it was necessary to remove the alcohol if an optimum separation was to be obtained. This was performed by washing the ester with an aqueous solution of calcium chloride. The alcohol-free ester was then dried over anhydrous sodium sulphate and finally re-distilled.

It was found that 0·01 to 0·05-ml aliquots of the soil extracts in dilute nitric acid could be used for the chromatographic separation. They were applied to the ten centre strips of sheets of Whatman's No. 1 filter-paper, reference number CRL/1, and the spots formed were allowed to dry in the atmosphere for 1 hour. In conditions of high relative humidity a longer drying time was advantageous. The sheet was rolled and clipped to form a cylinder and then placed, sample spots lowermost, in a 600-ml beaker containing 20 ml of the solvent mixture. The beaker was covered with a petri dish and the solvent was allowed to diffuse almost to the top of the strips. The time required for the solvent diffusion was approximately 15 minutes. Twenty millilitres of solvent mixture could be used for five or six sheets or for 2 to 3 hours before being replaced.

Detection of bands—

After chromatographic development of the paper strips the organic solvent was allowed to evaporate before the sheet was sprayed lightly on both sides with a 5 per cent. aqueous solution of potassium ferrocyanide. Uranium appeared as a brown band in the solvent

front, whilst iron, copper, molybdenum and other cations giving a colour with ferrocyanide moved only slightly, if at all, from the original test spot. As little as 0·1 μg of uranium gave a detectable brown band. On keeping, the sprayed sheets became discoloured owing to the oxidation of excess of potassium ferrocyanide with subsequent production of Prussian blue. It was found impossible to wash out excess of potassium ferrocyanide from the paper without partial removal of the uranyl ferrocyanide. It was thus necessary to prepare fresh standards daily. After allowing the sprayed sheet to dry, visual comparison of the bands with standards gave an estimate of the uranium content of the sample accurate to ± 30 per cent., provided that not more than 20 μg of uranium were present on the strip.

PREPARATION OF STANDARDS—

For the preparation of standard chromatograms a stock solution of uranyl nitrate was prepared by dissolving 0·12 g of U_3O_8 in 25 ml of concentrated nitric acid and diluting to 100 ml with water. This solution, containing 10 μg of uranium per 0·01 ml, was diluted further with diluted nitric acid (1 + 3) for the production of further standard solutions. Standard chromatograms were prepared by applying 0·02-ml aliquots of the standard solutions to strips on sheets of Whatman's No. 1 filter-paper, reference number CRL/1, and carrying out the chromatographic separation as already described for sample extracts. A suitable range of standards was found to be 0·1, 0·2, 0·3, 0·5, 0·7, 1·0, 1·5, 2·0, 3·0, 5·0, 7·0, 10·0, 15·0 and 20·0 μg of uranium. If 1 g of sample was extracted with 1 ml of acid solution, and a 0·05-ml aliquot of the extract was used, this series of standards corresponded to 2 to 400 p.p.m. of uranium in the soil. If the sheets of standard chromatograms were kept in the dark when not in use, they were satisfactory for a few days, but it was preferred to prepare standards daily.

RESULTS—

The procedure was tried out on a number of uraniferous soils and granites. The samples were treated with a mixture of nitric and hydrofluoric acids and the residues from evaporation were re-dissolved in dilute nitric acid. The results obtained (Table VI) were compared with those obtained by fluorimetric estimation of the uranium after separation by the cellulose-column procedure.

TABLE VI

DETERMINATION OF URANIUM

Sample	Uranium as U_3O_8 determined by visual comparison of ferrocyanide bands, %	Uranium as U_3O_8 determined fluorimetrically, %
soil	0·001	0·002
soil	0·002	0·002
soil	0·0007	0·001
soil	0·0007	0·0015
granite	0·003	0·003
granite	0·0007	0·001
granite	0·001	0·001
granite	0·038	0·045*

* Uranium determined colorimetrically.

METHOD

REAGENTS—

Nitric acid, sp.gr. 1·42.
Hydrofluoric acid, 40 *per cent. w/w.*
Nitric acid, diluted—Mix 1 volume of nitric acid, sp.gr. 1·42, with 3 volumes of water.
Paper—Sheets of Whatman's No. 1 filter-paper, reference number CRL/1.
Ethyl acetate—The pure dry solvent.
Potassium ferrocyanide, 5 *per cent. aqueous solution.*
Standard solutions—Prepare standard solutions of uranium in diluted nitric acid (1 + 3).

PROCEDURE—

Weigh 1 g of the sample, powdered to pass an 80-mesh sieve, into a small platinum dish and add 2 ml of nitric acid, sp.gr. 1·42, and 2 ml of 40 per cent. w/w hydrofluoric acid. Place the dish on a water-bath and evaporate to dryness. Add 1 ml of diluted nitric acid (1 + 3) and stir with a glass rod. Leave to cool and settle. With a capillary pipette apply 0·05 ml of the clear supernatant sample liquid to the end of a paper strip cut from a sheet of Whatman's No. 1 filter-paper, reference number CRL/1. Allow the sample spot to dry for 1 hour in air. Prepare 20 ml of the solvent mixture by shaking 2 ml of nitric acid, sp.gr. 1·42, and 1 ml of water with 17 ml of AnalaR ethyl acetate and pour the mixture into a 600-ml beaker covered with a petri dish. Roll and clip the paper sheet to form a cylinder and place in the solvent beaker with sample spots lowermost. Allow the solvent to diffuse upwards almost to the top of the strips, then remove the sheet from the beaker and allow the solvent to evaporate in air. Spread the sheet flat and spray lightly on both sides with a 5 per cent. aqueous solution of potassium ferrocyanide. Allow the sheet to dry and compare with standard uranium chromatograms.

CONCLUSIONS

The procedures described provide speedy and simple methods for the determination of copper, cobalt, nickel, niobium, tantalum, uranium and lead in soil samples. By using the methods as given, the lower limit of detection of each metal is: copper, cobalt and nickel, 20 p.p.m.; niobium and tantalum, 4 p.p.m.; lead, 10 p.p.m.; and uranium, 2 p.p.m. in the sample. By suitable modifications, which would tend to lengthen the procedures, it would be possible to extend these limits to detect even smaller quantities of the metals. The method can obviously be applied to the determination of a number of other metals and details of procedure for the determination of several metals at present under investigation will form the subject of further papers.

The apparatus is sufficiently simple and the chemicals required sufficiently few for the methods to be used in the field. The procedures for copper, cobalt, nickel and niobium have in fact already been used successfully in field investigations.

The determination of each of the metals studied has been carried out on a portion of a soil sample crushed to pass an 80-mesh sieve. This was obtained by roughly crushing the sample and sieving out the portion which passed an 80-mesh sieve. In all the cases dealt with in the present paper this procedure gave satisfactory results, but in other cases it might prove necessary to crush the whole sample to pass an 80-mesh sieve in order to obtain a representative sample. Good liaison between the chemist and the geologist can help in deciding points of this type and is in fact absolutely necessary for the successful application of the geochemical prospecting method. Information regarding the type of dispersion and the mineral form of the dispersed metal can usually be supplied by the geologist, whilst the frequency and pattern of sampling, the depth at which samples are taken and the interpretation of the results are all problems which are the responsibility of the geologist and which require for their solution a knowledge of the soil profile and local topography.

Whilst the chromatographic separation technique has been applied in the present paper to the analysis of soil samples, its use could be extended to cover other analytical problems. Thus it might be used in the analysis of plant ashes obtained in biogeochemical prospecting work. The method has been used for the analysis of precipitates and of various solutions for trace metals in the laboratory and also for the determination of lead in zinc metal. This use of the method for the determination of trace impurities in a metal might be extended to deal with a number of problems of interest to the metallurgical analyst.

The authors wish to express their thanks to Dr. J. S. Webb of the Royal School of Mines for his generosity in supplying samples and for permission to quote results supplied by him.

This paper is published by permission of the Director of the Chemical Research Laboratory.

REFERENCES

1. Webb, J. S., *Trans. Inst. Min. & Met.*, 1952–53, **62**, 321.
2. Lakin, H. W., Almond, H., and Ward, F. N., U.S. Geological Survey Circular **161**.
3. Hunt, E. C., and Wells, R. A., *Analyst*, 1954, **79**, 351.
4. Burstall, F. H., Davies, G. R., Linstead, R. P., and Wells, R. A., *J. Chem. Soc.*, 1950, 516.

5. Burstall, F. H., Swain, Patricia, Williams, A. F., and Wood, G. A., *Ibid.*, 1952, 1497.
6. Williams, A. F., *Ibid.*, 1952, 3155.
7. Mercer, R. A., and Williams, A. F., *Ibid.*, 1952, 3399.
8. Burstall, F. H., and Williams, A. F., *Analyst*, 1952, **77**, 983.
9. Rankama, K., and Sahama, T. G., "Geochemistry," The University of Chicago Press, 1950, p. 608.
10. Mercer, R. A., and Wells, R. A., *Analyst*, 1954, **79**, 339.
11. Hunt, E. C., and Wells, R. A., *Ibid.*, 1954, **79**, 345.
12. Arden, T. V., Burstall, F. H., and Linstead, R. P., *J. Chem. Soc.*, 1950, S311.
13. Kember, N. F., and Wells, R. A., *Analyst*, 1951, **76**, 579.

CHEMICAL RESEARCH LABORATORY
TEDDINGTON
MIDDLESEX

October 15th, 1954

Simultaneous Absorptiometric Determination of Tantalum and Niobium in Ores

BY A. E. O. MARZYS

The thiocyanate - acetone method for niobium is combined with a modified procedure for determining tantalum with pyrogallol, so making it possible to carry out both determinations on one sample solution. The titanium content of the solution is determined on another aliquot for use in applying a correction and, except in the case of low-grade soils, it represents the total titanium content figure of the sample. Tartaric acid is used for the first time as extracting medium for the pyrogallol method, in which the use of 4 *N* hydrochloric acid and of a suitable combination of ammonium oxalate added to tartaric acid gives a maximum absorption peak at 350 mμ, considerably reducing titanium interference and eliminating the interference due to niobium. The procedures for niobium and titanium are based on those described in a previous paper. The method is adapted for use with a spectrophotometer or a filter-type absorptiometer. No chemical separations are necessary for elements commonly occurring in earth-acid ores. The procedure is suitable for both low and high-grade ores.

THE analytical chemistry of niobium and tantalum has been enriched in recent years by a considerable amount of published work, which has kept pace with the intensified exploitation and exploration of the proved and potential sources of supply of the earth-acid metals. Most of these are situated in the outlying underdeveloped parts of the world, such as tropical Africa, and the chemists there, required often to produce hundreds of results within a short space of time in more or less primitive laboratories, find several shortcomings in the recent methods. The interference of titanium invariably associated with niobium and tantalum is often inadequately treated, and expensive instruments such as spectrophotometers are involved. Techniques involving the use of radioactive tracers or irradiation in an atomic pile are obviously out of the question, and the necessity with most methods of preparing separate solutions for niobium and for tantalum determinations greatly lengthens the time of analysis.

Attempts made in the past to obviate this last difficulty included the use of pyrogallol by Russian workers between 1936 and 1948,[1] applications of this reagent for simultaneous determination of niobium and tantalum still appearing in literature,[2,3] and the use of hydrogen peroxide after discovering absorption peaks of the two metals at two different wavelengths in the ultra-violet region.[4,5] Both approaches have sometimes been combined,[6,7] whilst pyrogallol for tantalum was also combined with hydroquinone for niobium in steel analysis.[8]

The recent development of spectrographic and allied as well as chromatographic techniques has provided perhaps the most rapid ways of estimating niobium and tantalum in such methods as fluorescent X-ray spectroscopy[9] or paper-strip chromatography.[10] These, however, cannot equal in accuracy and precision the photometric methods lately made available.

The object of the present work was to select the most sensitive and accurate photometric methods for the determination of niobium and tantalum, substantially free from titanium

interference, and to attempt to combine them into a procedure for the simultaneous determination of both metals in one sample solution with the use of inexpensive apparatus such as a filter-type absorptiometer.

Recent work[11,12,13] has shown the thiocyanate method to be capable of fulfilling these requirements as far as niobium is concerned, its sensitivity, flexibility and essential freedom from titanium interference having been lately proved by its application to low-grade ores,[14,15] tantalum metal[16] and titanium alloys.[17] In these respects the thiocyanate method is superior to the perniobic acid method recently greatly improved by the study of its ultra-violet spectrum by Telep and Boltz,[18] although greater precision is claimed for the latter. Other reagents such as hydroquinone,[8,19] phosphomolybdate[20] and hydrochloric acid[21] can be used only after a preliminary separation of titanium or iron or both.

As for tantalum, pyrogallol has been for a long time the only colorimetric reagent utilised to any extent for quantitative work, although rhodamine B appears to offer some

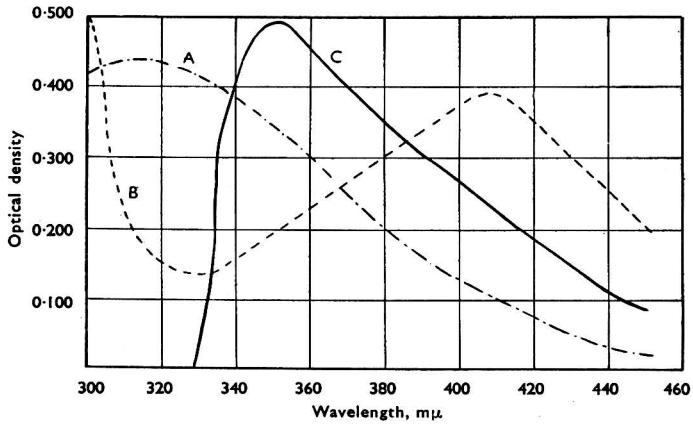


Fig. 1. Absorption spectra of the tantalum - pyrogallol complex (20 μg of Ta_2O_5 per ml): curve A, under conditions described by Dinnin[24]; curve B, under conditions described by Hunt and Wells[3]; curve C, under conditions of present method

promise in this direction.[22,23] Even pyrogallol, however, as employed until recently with sulphuric acid, giving an absorption peak at around 400 mμ, is subject to serious interference by titanium. The same criticism as mentioned previously applies to the pertantalic acid absorption peak at 285 mμ.

The first positive step towards obviating this difficulty was made last year by Dinnin.[24] He found that by replacing sulphuric acid by hydrochloric acid in an appreciably increased concentration, the tantalum - pyrogallol absorption peak appeared at 325 mμ and the interferences by titanium and niobium were reduced to negligible proportions.

It was therefore desirable to see whether the thiocyanate method for niobium and the pyrogallol - hydrochloric acid method for tantalum could be combined in a procedure of simultaneous determination of both in one sample solution on a filter-type absorptiometer. The time-honoured peroxide method for titanium, whose determination was necessary for applying a correction, could be adapted for any medium, and there was little need to look for other procedures except to test the chromotropic acid as a reagent which is said to afford better sensitivity and selectivity than hydrogen peroxide.

Experimental

Tantalum—

The considerable difference in the wavelength of the absorption peak obtained under the conditions described by Dinnin[24] in comparison with those given by Ikenberry, Martin and Boyer[8] and Hunt and Wells[3] was confirmed and can be seen from Fig. 1, curves A and B, determined with a Uvispek spectrophotometer. By using the Spekker absorptiometer at 365 mμ the method was rather less sensitive, the interference due to titanium increased

slightly, whilst that of niobium decreased. Under the conditions described by all previous authors, the titanium interference was considerable at any wavelength.

These findings pointed to the possibility of using the 365-mμ wavelength available with the Spekker absorptiometer for the determination of tantalum in the presence of considerable amounts of titanium and niobium, with the use of a very small correction for titanium.

All the pyrogallol methods so far published involved the extraction of the bisulphate melt with ammonium oxalate. The thiocyanate method for niobium involves extraction with tartaric acid. In order to make possible the use of one sample solution for both, it was necessary to test (*a*) whether niobium could be determined with thiocyanate in an oxalate extract, or (*b*) if tantalum could be determined with pyrogallol in a tartaric acid extract.

The first alternative was easily eliminated when it was found that the smallest quantity of oxalate bleached completely the niobium - thiocyanate complex. With tartaric acid, the absorption of the tantalum - pyrogallol complex was somewhat reduced at 365 mμ, but it was still quite adequate; those due to titanium and niobium were increased; the absorption of the niobium complex in particular was too great at this wavelength for practical use. The comparative examples of the optical densities of 2 mg of each metal in 50 ml of 0·25 per cent. ammonium oxalate and 1·5 per cent. of tartaric acid (the latter concentration being used in the niobium - thiocyanate method) are shown in Table I.

TABLE I

OPTICAL DENSITIES OF 2 mg OF TANTALUM, NIOBIUM AND TITANIUM WITH PYROGALLOL AT 365 mμ IN 50 ml OF (*a*) 0·25 PER CENT. AMMONIUM OXALATE AND (*b*) 1·5 PER CENT. TARTARIC ACID

Element as	Optical density	
	in ammonium oxalate	in tartaric acid
Ta_2O_5	0·972	0·824
Nb_2O_5	0·142	0·190
TiO_2	0·034	0·080

The idea was then conceived of adding progressive amounts of ammonium oxalate in hydrochloric acid to the tartaric acid extracts of the three metals and measuring their optical densities produced with pyrogallol. The remarkable difference in their behaviour under this treatment can be seen from Fig. 2. Whilst little effect was produced on tantalum beyond a

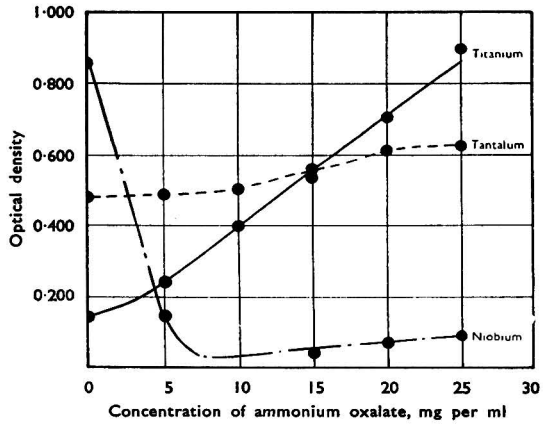


Fig. 2. Effect of ammonium oxalate on the optical density of the pyrogallol complex of tantalum (20 μg per ml), niobium (200 μg per ml) and titanium (40 μg per ml) in 7·5 per cent. tartaric acid and 4 *N* hydrochloric acid

slight increase, titanium absorption increased steadily, whilst niobium showed a sharp fall to a minimum at about 0·375 g of ammonium oxalate per 50 ml, whereafter its optical density crept up very slowly. With this mixture of tartaric acid and ammonium oxalate, the optical density due to 2 mg of niobium in 50 ml was only 0·006, as compared with 0·142 in ammonium

oxalate alone and 0·190 in tartaric acid alone. This suggested that if ammonium oxalate was added to the tartaric acid in this proportion, the correction for niobium obviously necessary in any medium at 365 mμ, as can be seen from the figures in Table I, could be eliminated in practice. This still left the necessity of applying a correction for titanium, which was somewhat increased but was still considerably below that in all other pyrogallol methods except Dinnin's. The proportion of oxalate was fixed at 0·375 g in 50 ml, which corresponded with the minimum absorption of the niobium complex. Its addition was conveniently combined with the addition of 8 *N* hydrochloric acid, in which it was dissolved, to make the final concentration 4 *N*, the optimum found by Dinnin.[24] Also the method of preparing pyrogallol and stabilising it with stannous chloride employed by this author was found to be satisfactory. Analytical-reagent grade pyrogallol gave almost transparent solutions with tantalum. The curves obtained under these conditions with tantalum, niobium and titanium are shown in Fig. 3.

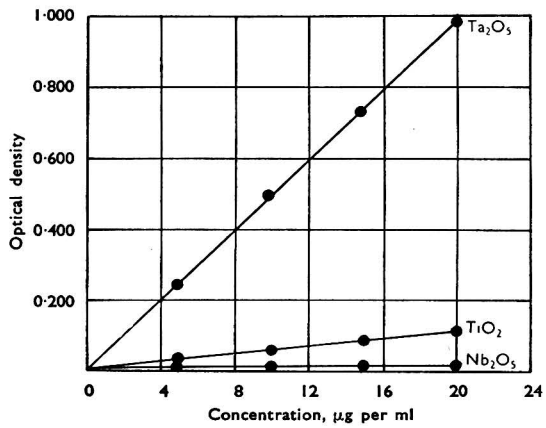


Fig. 3. Standard curves of the pyrogallol complexes of the three oxides measured at 365 mμ in 4-cm cells under the conditions of the present method

The emphasis laid by Dinnin[24] on the difficulties connected with the preparation of true and stable tantalum solutions was found to be fully justified, but contrary to his claims the extraction of the bisulphate melt with tartaric acid, necessary for the present method, was found to be easier than with ammonium oxalate provided that (*a*) the extracting solution was used boiling hot, (*b*) continuous stirring was maintained during extraction and (*c*) the solution was cooled rapidly.

The complex solutions were found to reach maximum optical density in about a quarter of an hour and they remained stable for several hours. The absorption curve obtained on the Uvispek spectrophotometer (curve C in Fig. 1) showed a peak at about 350 mμ which was sharper than in Dinnin's method. The shift of the peak towards the higher wavelength was responsible for the fact that very little sensitivity was sacrificed by the use of the Spekker absorptiometer at 365 mμ in comparison with the spectrophotometer at 350 mμ.

INTERFERENCES WITH THE TANTALUM DETERMINATION—

Interferences were checked in a manner analogous to that described in the previous paper on niobium.[15] No serious interferences were found to be caused by niobium, iron, aluminium, copper, antimony, zirconium, manganese, cobalt, phosphate, fluoride, alkaline earths and alkali metals. Those due to other elements are shown in Table II.

The possible methods of dealing with these interferences are mentioned in the method; they are essentially identical with those described in the previous paper on niobium.

The transmissions at 365 mμ with pure pyrogallol (ordinary grade gives dark-coloured solutions) are so excellent that 4-cm cells are best used, so giving improved sensitivity.

NIOBIUM—

The procedure for the determination of niobium was fully described in the previous paper.[15] It has now been in use in the author's laboratory for about 2 years and has been also adopted by a number of other laboratories. The only modification made since its introduction was the increase in the amount of bisulphate used for fusion and the use of 15 per cent. tartaric acid for extraction, subsequently diluted to 7½ per cent. in making up the solution, instead of 10 per cent. throughout. This amendment facilitates fusion and extraction and it has been incorporated in the sample-solution part of the description of the general method given below.

TABLE II

EFFECT OF INTERFERING ELEMENTS ON THE DETERMINATION OF TANTALUM

Interference expressed as	Percentage error when weight ratio of interference to Ta_2O_5 is		
	1 to 1	10 to 1	100 to 1
TiO_2	+16	+185	+242
V_2O_5	+11	+11	+100
MoO_3	+6	+242	—
WO_3	+22	+157	—
U_3O_8	−11	+2	+96
ThO_2	−3	+15	Precipitation
NiO	−14	−7	+22
Cr_2O_3	+30	+204	—
PtO_2	Precipitation	Precipitation	Precipitation
SnO	+2	+36	+38
MgO	+22	Precipitation	Precipitation
Bi_2O_3	−2	+4	+58

TITANIUM—

The hydrogen peroxide method has been used successfully for a long time now in conjunction with niobium analyses with the thiocyanate for the purpose of evaluating a correction. In the course of the present investigation the use of the chromotropic acid was tried out, but with the tartaric extract of the melt the solutions were very dark coloured and the blank had a very low transmission. The stability was poor, as well as the reproducibility of the results, and the sensitivity under best conditions just equalled that with hydrogen peroxide at 405 mμ. For that reason the latter was retained. This method is so well known and generally used that little else need be said regarding its use. The results are utilised in applying a correction to both niobium and tantalum readings, and in this connection it should be noted that in the sample solution procedure for soils described in the method, a considerable part of their titanium content may be soluble in hydrochloric acid. If therefore the total titanium content of the soil is required, the part dissolved in hydrochloric acid should be determined separately, which presents no difficulties; otherwise total titanium must be determined on a separate sample, without preliminary leaching.

METHOD FOR SOLUTION OF THE SAMPLE

REAGENT—

Tartaric acid, 15 per cent.—Dissolve 150 g of the acid in water and dilute to 1 litre.

PROCEDURE FOR HIGH-GRADE ORES—

Fuse 0·1000 g of finely ground material with exactly 12·5 g of fused sodium bisulphate in a silica crucible of 40 ml capacity. Begin the fusion over a very small flame until the bisulphate liquefies, then gradually increase the flame; swirl the contents of the crucible while holding it with the tongs, and maintain the crucible at a dull-red heat for about 10 minutes. Avoid frothing and excessive loss of sulphur trioxide.

Allow the crucible to cool down and in the meantime heat 125 ml of tartaric acid solution to boiling. Extract the melt by filling the crucible two-thirds full with boiling tartaric acid solution, boil it with stirring continuously until the melt disintegrates and rinse out the contents into a 250-ml beaker with distilled water. Repeat the extraction with further quantities of tartaric acid, and add the extracts to the beaker together with the lid and crucible. Boil the contents of the beaker with stirring until a clear solution is obtained, then

remove and rinse the crucible and lid. Filter, if necessary, cool rapidly in iced water to room temperature and make up to 250 ml in a calibrated flask; alternatively, cool down, make up to 250 ml and centrifuge portions of the solution as needed.

PROCEDURE FOR LOW-GRADE ORES—

Soils—Leach and ignite a 1·0000-g sample in a silica crucible as described in the previous paper[15] (top of p. 335). After ignition, fuse and extract the residue as described for high-grade ores, but use 5 g of sodium bisulphate and 50 ml of tartaric acid, and make up the extract to 100 ml with water.

Granites and other siliceous rocks—Weigh out 1·0000 g into a platinum crucible. Add 3 drops of sulphuric acid, fill one-third full with 40 per cent. hydrofluoric acid and evaporate to dryness, then expel sulphur trioxide by heating. Repeat treatment at least three times. Thereafter proceed as with soils.

When a platinum crucible was used for fusion, any platinum introduced into the sample solution should be removed with hydrogen sulphide before diluting the 15 per cent. tartaric acid extract in a manner described in the previous paper[15] (Modification 1, p. 336).

Other materials—With some intractable materials it may be necessary to carry out the preliminary separation of mixed oxides by the Schoeller tannic acid method[25]; thereafter treat the residue in the manner described for high-grade ores. The modification of the Schoeller tannic acid method by Milner and Smales[14] can also be used; however, these authors state specifically that their precipitation procedure is not quantitative and employ a radiometric correction for losses; this paper has been devised to serve in the circumstances where such techniques are not available, as stated in the introduction.

METHOD FOR DETERMINATION OF TANTALUM

REAGENTS—

Standard tantalum solution—Fuse 0·0500 g of Specpure tantalum pentoxide (J.M.620) with 12·5 g of fused sodium bisulphate. Carry out the fusion and extraction with 125 ml of 15 per cent. tartaric acid exactly as described for the sample and make up the solution to 250 ml. Then 1 ml = 0·2 mg of Ta_2O_5.

Bisulphate - tartaric acid blank solution—Fuse 12·5 g of sodium bisulphate, extract with 125 ml of 15 per cent. tartaric acid and make up to 250 ml as described for the sample solution.

Ammonium oxalate - hydrochloric acid solution—Dissolve 15 g of ammonium oxalate in about 150 ml of water. With stirring, add 760 ml of concentrated hydrochloric acid. Cool and make up to 1 litre.

Pyrogallol solution—Dissolve in water 50 g of pyrogallol with 25 ml of concentrated hydrochloric acid and 10 ml of 2 *M* stannous chloride and make up the volume to 250 ml. The 2 *M* stannous chloride is made up by dissolving 113 g of stannous chloride, $SnCl_2.2H_2O$, in concentrated hydrochloric acid and making it up to 250 ml with the same acid. It is essential that the pyrogallol should be of the highest possible analytical-reagent grade, freshly re-sublimed.

PROCEDURE—

With a pipette, place a 20-ml aliquot of the sample solution in a 100-ml calibrated flask. Add 50 ml of ammonium oxalate solution in hydrochloric acid and 20 ml of pyrogallol solution, both from burettes. Make up to the mark and allow the mixture to stand for half an hour. Measure the absorption at 365 mμ with a Spekker type H760 absorptiometer with a mercury-vapour lamp and Wood's glass filters, or with a spectrophotometer (Hilger Uvispek or Unicam SP600) at 350 mμ. Use 4-cm cells and a blank containing 20 ml of tartaric acid - bisulphate solution and the other reagents. At the same time read the optical density values for solutions containing 5, 10, 15 and 20 ml of standard tantalum solution, made up to 20 ml with the tartaric acid - bisulphate solution and the appropriate amounts of the other reagents as used for the sample solution. The four points should give a straight line on a graph, from which the tantalum content can be obtained by using the optical density readings of the sample solution corrected for titanium. The titanium correction is obtained from the graph prepared as described on p. 200 in a manner analogous to that in the case of niobium[15] (see Fig. 4).

Method for determination of niobium

Proceed as described in the previous paper on low-grade ores,[15] but use 12·5 g of sodium bisulphate for fusion of standard niobium pentoxide and 125 ml of 15 per cent. tartaric acid for extraction. With high-grade ores, use a smaller aliquot of the sample solution made up to 10 ml with the bisulphate - tartaric acid blank solution, or preferably dilute 20 ml of sample solution to 100 ml with the blank solution and use a 10-ml aliquot. Note that more sodium bisulphate and 15 per cent. tartaric acid subsequently diluted with water on making

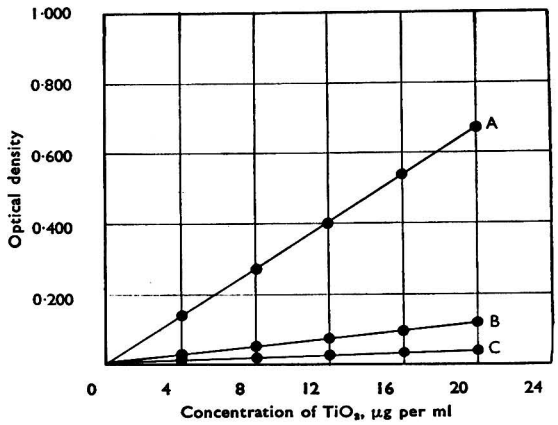


Fig. 4. Correction graph for titanium in the determination of tantalum and niobium: A, titanium with hydrogen peroxide at 405 mµ in 4-cm cells; B, titanium with pyrogallol at 365 mµ in 4-cm cells (under the conditions of the tantalum procedure); C, titanium with thiocyanate and acetone at 405 mµ in 1-cm cells (under the conditions of the niobium procedure)

up the sample solution to 7·5 per cent. has been used, and therefore the blank solution to be used is also the same as for tantalum and titanium determinations. The other reagents are the same as described in the previous paper.[15]

Method for determination of titanium (for the evaluation of corrections)

Reagents—

Standard titanium solution—Fuse 0·0500 g of Specpure titanium dioxide (J.M.435) with 12·5 g of fused sodium bisulphate. Carry out the fusion and extraction with 125 ml of 15 per cent. tartaric acid exactly as described for the sample and make the solution up to 250 ml. Then 1 ml = 0·2 mg of TiO_2.

Bisulphate - tartaric acid blank solution—As for tantalum and niobium determinations.

Sulphuric acid, diluted (1 + 1, *v/v*).

Hydrogen peroxide, 20-volume.

Procedure—

Follow the procedure described in the previous paper[15] (p. 335), under titanium correction, but use 100-ml flasks, doubling all the volumes indicated, and use 4-cm instead of 2-cm cells. This procedure was found to lead to somewhat increased accuracy.

Calibration graphs for titanium—

Use 4, 8, 12 and 16-ml aliquots of the standard titanium solution made up to 20 ml with the bisulphate - tartaric acid solution and develop the colour (*a*) with hydrogen peroxide, phosphoric and sulphuric acids as described above, (*b*) with pyrogallol, ammonium oxalate and hydrochloric acid as described for tantalum and (*c*) with stannous chloride - thiocyanate and acetone as described for niobium. Plot the readings to obtain the three curves of a correction graph (Fig. 4).

Modifications in the presence of interfering elements

The removal of copper, mercury, platinum, tin, bismuth and molybdenum when present was described in the previous paper[15] and will apply to all the three determinations. The correction for tungsten in the case of tantalum can be carried out in a manner analogous to that described for niobium. The interference by large quantities of tantalum is reduced in the niobium determination by resorting to the ether-extraction procedure described previously[15] (Modification 2). For greatest accuracy in the presence of considerable tantalum, determine tantalum first and add an appropriate approximate amount of standard tantalum solution to the aliquots of standard niobium solution used for the calibration graph. Vanadium interference is much less serious in the case of tantalum than it is in the case of niobium, but when present, a preliminary separation of niobium and tantalum by Schoeller's tannic acid procedure will be necessary.[25] Other metals which must be chemically separated by the usual procedure, owing to their interference in the tantalum determination, are chromium, nickel, uranium and thorium, although their interference in the niobium determination is eliminated by ether-extraction. In practice, however, these metals rarely or never occur together with tantalum in quantities likely to introduce an appreciable error and the chemical separations will be seldom necessary.

Results

The method was first tested on a range of synthetic mixtures, prepared by mixing known weights of pure oxides in varying proportions. The results are shown in Table III.

Table III

Analyses of synthetic mixtures of oxides of tantalum, niobium and titanium

Nb_2O_5		Ta_2O_5		TiO_2	
Taken, mg	Found, mg	Taken, mg	Found, mg	Taken, mg	Found, mg
20·2	20·7	5·2	5·4	15·8	15·8
5·6	5·6	13·0	12·7	23·2	23·0
10·1	9·9	45·3	45·5	4·8	5·0
20·0	20·1	14·7	15·0	5·0	5·1
18·8	19·7	19·8	20·1	20·9	20·8
4·4	4·4	14·3	14·6	21·3	20·8

Subsequently, the method was applied to a range of both high-grade and low-grade ores of niobium and tantalum. The results were compared with those obtained by gravimetric or combinations of gravimetric, colorimetric and chromatographic methods, and the analyses were carried out either in the author's or some other laboratories as indicated. Several examples of comparative figures are shown in Table IV.

The reproducibility of the tantalum procedure was tested in a manner analogous to that previously reported for niobium.[15] Thirty-two determinations, starting with a separate 0·1-g sample of columbite - tantalite (J.129), were made in groups of eight by four different operators. Two determinations were spoilt by accidents and the experimental error was calculated on the remaining 30; it included the cumulative errors of sampling, weighing, taking aliquots, making up solutions, etc., as well as individual variations from operator to operator. The mean value of optical density for the 30 samples was 0·270, corresponding with 24·83 per cent. of Ta_2O_5. The standard deviation was 0·007 (2·7 per cent.). The standard error of the mean for 30 determinations was 0·50 per cent. The statistical analysis of the niobium procedure was given in the previous paper.[15]

Conclusions

The described method provides a convenient combination of the most sensitive photometric methods of determining tantalum and niobium on one sample solution. When no preliminary leach is employed, the total titanium content is determined simultaneously. The methods selected are substantially free from interference of the three metals in question among themselves, and from that caused by other metals commonly associated with them. Chemical separations are necessary only in the case of elements very rarely occurring in the

ores of the earth acids, either high or low grade; normally the determinations can be carried out without any separations at all.

This leaves the solution of the sample as the longest part of the procedure. The necessity for preparing separate melts, hitherto necessary if the thiocyanate and pyrogallol methods were to be used for niobium and tantalum, respectively, has been eliminated, and the aliquots of the same extract are used for three determinations. Incidental to that, small interferences of niobium in the tantalum - pyrogallol procedure was virtually eliminated by a suitable combination of tartaric acid and ammonium oxalate.

TABLE IV

ANALYSES OF SOME HIGH-GRADE AND LOW-GRADE ORES OF NIOBIUM AND TANTALUM

Sample No.	Description of material	Nb_2O_5 Present method, %	Nb_2O_5 Other methods, %	Ta_2O_5 Present method, %	Ta_2O_5 Other methods, %	Remarks
B142	Sukulu soil, Uganda	0·19	0·20	0·009	0·01	Used for analysis by 12 laboratories in a collaborative scheme by Rio Tinto, Ltd.
L41	Sukulu soil, Uganda	0·17	0·17	0·013	0·01	Analysed by Mineral Resources Division, Colonial Geological Surveys and by Uganda Development Corporation Limited (U.D.C.)
L42	Sukulu soil, Uganda	0·60	0·61	0·023	0·02	Analysed by Mineral Resources Division, Colonial Geological Surveys and by Uganda Development Corporation Limited (U.D.C.)
L44	Nkumbwa soil, N. Rhodesia	1·10	1·08	0·001	0·00	Analysed by Mineral Resources Division, Colonial Geological Surveys and by Uganda Development Corporation Limited (U.D.C.)
L45	Coarse pyrochlore fraction from Sukulu soil, Uganda	25·5	25·7	0·56	0·53	Analysed by U.D.C. Ltd.
J130	Columbite, origin unknown	64·1	~63·2	6·5	~7·0	Analysed by U.D.C. Ltd.
J129	Columbite - tantalite, origin unknown	38·8	38·2	28·05	28·0	Analysed sample furnished by Mr. E. A. W. Hebdon, London University
J131	Nigerian granite	0·20	0·21	0·01	trace	Analysed samples supplied by C.R.L., Teddington
J132	Nigerian granite	0·24	0·24	0·02	trace	Analysed samples supplied by C.R.L., Teddington
K7	Columbite - tantalite, Uganda	29·1	29·2	39·6	39·3	Analysed by the Geological Survey of Uganda
K8	Columbite - tantalite, Uganda	39·4	39·8	36·0	36·5	Analysed by the Geological Survey of Uganda
K9	Columbite - tantalite, Uganda	26·7	26·3	42·3	42·4	Analysed by the Geological Survey of Uganda

In most results described as obtained by "other methods" at least the mixed oxide content was obtained gravimetrically by Schoeller's tannic acid procedure.

The method, although eminently suitable for a spectrophotometer such as the Unicam SP600, Uvispek or Beckmann DU was adapted for use with the Hilger Spekker absorptiometer, as was its initial purpose. It is thought that apart from the spectrographic procedures, it provides a means of the most rapid and accurate determination of earth acids in a given sample of ore of any grade between 0·01 and 100 per cent. yet published.

The success of the method depends greatly on the skill acquired in carrying out the fusions in sodium bisulphate, to ensure on one hand complete solution of the earth-acid content and on the other to avoid the loss of sulphur trioxide, which causes errors in the niobium determination. The preparation of a true solution of tantalum pentoxide is always difficult, the more so if the standard oxide is really pure, as is the case with the Specpure material. In certain cases the expedient of adding some niobium oxide to the weighed amount of the tantalum standard was resorted to, the presence of niobium facilitating the solution whilst causing no interference in the optical-density measurements of the standard solution.

Another case of interaction between the three metals is the rapid reduction in the optical density of the niobium - thiocyanate complex with certain samples comparatively high in tantalum. This effect, caused probably by hydrolysis, has been reported by other writers on the thiocyanate method and can be obviated by taking the measurements as soon as possible after dissolving the sample, and by using the ether-extraction modification described in the previous paper.[15] In the extreme cases, tantalum should be determined first and

standard tantalum solution added to the aliquots of niobium solution used for the calibration graph in the same proportion as found in the sample.

The author wishes to express his thanks to the Manager, Technical Development Division, Uganda Development Corporation, Limited, for permission to publish this paper, and to the staff of the Chemical Section for their assistance in the experimental work; in particular to Mr. G. W. K. Ssali who carried out its greater part under the author's supervision.

Thanks are due also to Mr. J. B. Pollock of the Geological Survey of Uganda, Mr. P. F. Shilton and Mrs. J. M. Brown for their help at various stages of the present investigation.

References

1. See, for example, Krivoshlykov, N. F., *T.L.K.Kh.T.I.*, 1939, No. 7, 103; *Khim. Referat. Zhur.*, 1939, **2**, No. 5, 59.
2. Gôto, H., and Kakita, Y., *Sci. Rep. Res. Inst. Tôhoku Univ.*, 1950, **2**, 249.
3. Hunt, E. C., and Wells, R. A., *Analyst*, 1954, **79**, 345.
4. Palilla, F. C., Adler, N., and Hiskey, C. F., *Anal. Chem.*, 1953, **25**, 926.
5. Langmyhr, F. J., *Tidsskr. Kjemi. Bergves.*, 1953, **13**, No. 7, 164.
6. Thanheiser, G., *Mitt. K.-Wilh.-Inst. Eisenforsch.*, 1940, **22**, 255.
7. Boyd, T. F., and Galan, M., *Anal. Chem.*, 1953, **25**, 1568.
8. Ikenberry, L., Martin, J. L., and Boyer, W. J., *Ibid.*, 1953, **25**, 1340.
9. Campbell, W. J., and Carl, H. F., *Ibid.*, 1954, **26**, 800.
10. Hunt, E. C., and Wells, R. A., *Analyst*, 1954, **79**, 351.
11. Alimarin, I. P., and Podvalnaya, R. L., *J. Anal. Chem., U.S.S.R.*, 1946, **1**, 30.
12. Freund, H., and Levitt, A. E., *Anal. Chem.*, 1951, **23**, 1813.
13. Lauw-Zecha, A. B. H., Lord, S. S., jun., and Hume, D. N., *Ibid.*, 1952, **24**, 1169.
14. Milner, G. W. C., and Smales, A. A., *Analyst*, 1954, **79**, 315.
15. Marzys, A. E. O., *Ibid.*, 1954, **79**, 327.
16. Hastings, J., and McClarity, T. A., *Anal. Chem.*, 1954, **26**, 683.
17. Norwitz, G., Codell, M., and Verderame, F. D., *Anal. Chim. Acta*, 1953, **9**, 561.
18. Telep, G., and Boltz, D. F., *Ibid.*, 1952, **24**, 163.
19. Johnson, C. M., *Iron Age*, 1946, **157**, No. 14, 66.
20. Davydov, A. L., Vaĭsberg, Z. M., and Burkser, L. E., *Zavod. Lab.*, 1947, **13**, 1038.
21. Kanzelmeyer, J. H., and Freund, H., *Anal. Chem.*, 1953, **25**, 1807.
22. Shapiro, M. Ya., *J. Appl. Chem., U.S.S.R.*, 1938, **11**, 1028.
23. Gillis, J., *Mikrochemie*, 1944, **31**, 273.
24. Dinnin, J. I., *Anal. Chem.*, 1953, **25**, 1803.
25. Schoeller, W. R., "The Analytical Chemistry of Tantalum and Niobium," Chapman & Hall, London, 1937.

Uganda Development Corporation Ltd.
Technical Development Division
Entebbe, Uganda

September 9th, 1954

Separation of Rhodium and Iridium by Ion Exchange

By M. L. CLUETT, S. S. BERMAN and W. A. E. McBRYDE

Rhodium and iridium can be separated as chloro salts by passing a solution containing both metals through a column of anion-exchange resin and eluting the rhodium from the column with 2 per cent. sodium chloride solution. Iridium is quantitatively retained on the column provided that it is in the quadrivalent state and this is ensured by including bromine water in the sample and eluting solutions. Iridium can be subsequently eluted from the column by successive treatment with 5 *M* ammonium hydroxide and 6 *M* hydrochloric acid.

Attempts to use ion-exchange resins for the purification or separation of platinum metals have revealed the lack of suitable elutriants for the chloro salts of these metals when they are absorbed on anion-exchange resins. This led to the proposal[1] to ash the resin containing absorbed precious-metal salts to recover the metal. Recently, MacNevin and Crummett have given an account of the behaviour of platinum-metal salts toward ion-exchange resins[2] and of certain separations of these metals by ion exchange. Kraus, Nelson and Smith[3] have studied the ion exchange of palladium, platinum and iridium in hydrochloric acid solutions of various concentrations; they demonstrated the separation of iridium from platinum or palladium. A brief outline of a separation of four of these metals in which a cation-exchange resin was used has been given by Stevenson, Franke, Borg and Nervick.[4] The following is a description of the separation and recovery of rhodium and iridium by anion exchange.

This particular separation is commonly made by selective reduction of rhodium to the metal[5]; the procedure is undoubtedly time-consuming and may not be quantitative, especially when small amounts of metal are to be separated.[6]

Experimental

Simple batch and column experiments showed that iridium as chloro-iridate was strongly absorbed by anion-exchange resins, while rhodium as chlororhodite was only moderately absorbed. In the column all the added iridium appeared as a narrow, almost black, band at the top of the resin, and this band seemed stubbornly to resist elution by other ions. However, a certain amount of this iridium leaked away from the column or was removed by shaking batches of resin with various aqueous solutions. The iridium removed in this manner was colourless in solution and in all respects behaved as chloro-iridite; for instance, the brown colour of chloro-iridate could be regenerated by warming the solution with a small quantity of nitric acid. Although all the added iridium had been quadrivalent, the inference was drawn that a small amount of reduction accompanied by desorption occurred on continued contact with the resins or with the aqueous solutions used as elutriants. No appreciable desorption of iridium occurred, however, when the feed solution and solutions used for elution contained a small amount of dissolved bromine. As long as the iridium remained in the quadrivalent state, it was firmly retained on the resin. The difference in ion exchange between iridium^{III} and iridium^{IV} in chloride solutions has been indicated recently by Kraus *et al.*[3]

Although rhodium as chlororhodite was absorbed on the resins, it could be removed quantitatively by a 2 per cent. solution of sodium chloride or ammonium chloride. When rhodium was fed on to a column in a 2 per cent. salt solution, some of the metal passed through in the sample solution. The behaviour in ion exchange depended somewhat on the previous history of the rhodium solution. Freshly prepared solutions of sodium chlororhodite in water or 0·05 *M* hydrochloric acid are pink, but on standing they change to a brown colour. The pink colour can be regenerated from the brown by the addition of an excess of a soluble chloride and then warming the solution. The change of colour seems to be associated with hydrolysis of the $RhCl_6'''$ ion to ions such as $RhCl_5OH'''$ or $RhCl_5H_2O''$. From pink rhodium solutions the metal was more strongly absorbed and less easily eluted than from the brown. This difference is clearly shown by the results in Fig. 1; this represents the course of elution

of rhodium added to the resin as a brown solution (curve A), and as a pink solution (curve B). Hence the complete removal of rhodium from columns required a larger volume of elutriant when the metal was added as the pink chlororhodite ion. When bromine was used in the sample and eluting solutions, the colour of the rhodium passing down the resin bed was largely masked owing to the brown colour of the resin and the strong red-brown adsorption band due to the bromine.

So the separation of rhodium from iridium involved addition of the two metals as chloro salts to a column of resin and subsequent elution of the rhodium with 2 per cent. sodium chloride solution. The presence of bromine added to these solutions to keep the iridium

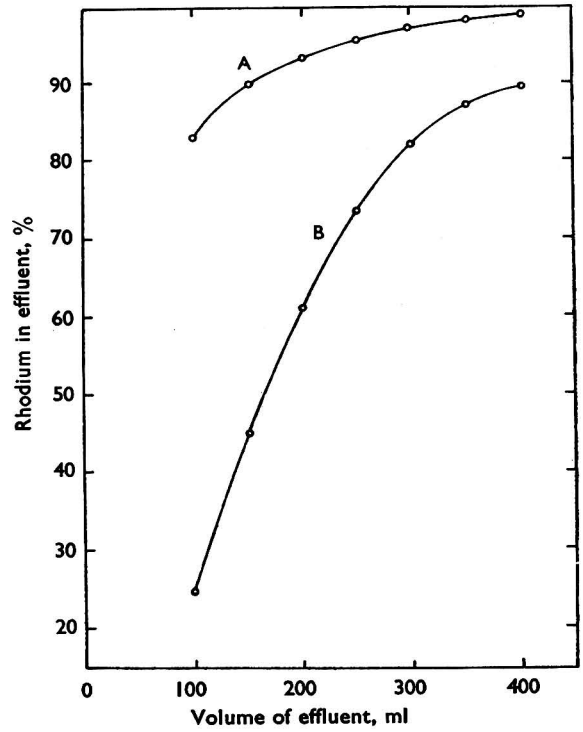


Fig. 1. Integral elution curves for rhodium. Curve A, rhodium added to the resin as a brown solution; curve B, rhodium added to the resin as a pink solution

in the quadrivalent state precluded the use of ammonium chloride solutions for elution because of the production of gas, which ruptured the resin bed.

The elution of iridium from the column was more difficult than expected. Of many solutions tried as elutriants only ammonium hydroxide containing ammonium chloride removed a useful proportion of the metal. However, all the absorbed iridium was rarely removed with this solution. A small amount, about 5 to 10 per cent., of the iridium still remained. This residue could be completely removed by a small volume of 6 *M* hydrochloric acid or of 8 *M* nitric acid. Originally this elution of iridium by ammonium hydroxide was thought to be due to the formation of a cationic ammine. But it was observed that chloro-iridate was reduced by the addition of 5 *M* ammonium hydroxide to a pale green solution from which the iridium was completely removed by absorption on Amberlite IRA-400 resin. When the pale green solution was acidified with hydrochloric acid, it acquired a slightly more yellow colour and appeared to contain chloro-iridite ion. Owing to the presence of ammonium chloride in this second solution, the iridium was scarcely absorbed at all from it by the resin. The exact constitution of the iridium ions in the ammonium hydroxide and

ammonium chloride solutions produced in this manner has not been established, but undoubtedly the iridium is tervalent and in the form of anions that are not appreciably absorbed on Amberlite IRA-400 in the presence of moderate concentrations of chloride ion. The elution of quadrivalent iridium involves reduction to chloro-iridite by ammonia, and the function of the ammonium chloride and the hydrochloric acid is to displace the reduced iridium from the resin.

Method

Stock solutions of rhodium and iridium were prepared by dissolving highly refined chloro salts, which had been obtained from the International Nickel Company of Canada, in 0·05 *M* hydrochloric acid. These solutions were both standardised by gravimetric methods[7,8] and the iridium solution was also checked by a volumetric procedure.[9]

The sample solution consisted of a 2 per cent. solution of sodium chloride in 0·1 *M* hydrochloric acid containing 5 per cent. by volume of saturated bromine water. To this solution were added known amounts of the rhodium or iridium stock solutions. The same solution served for the elution of rhodium. Elution of iridium was done with a solution 5 *M* in ammonium hydroxide and *M* in ammonium chloride, followed by either 6 *M* hydrochloric acid or 8 *M* nitric acid.

The columns were conventional in design, 12 mm in internal diameter and about 30 cm high. A dropping funnel served as reservoir for the sample solution, and the flow-rate of the liquid was controlled by regulating the influx of air into the reservoir through a fine capillary tube. Sufficient resin was taken for most of the experiments to give a bed about 7 cm deep, although deeper beds were prepared when 100 mg of the metals were separated. Sample solutions were run through the columns at a flow-rate of about 25 ml per hour; the elutriants were passed at about 40 ml per hour.

The resin used in these experiments was Amberlite IRA-400 of an analytical grade. Some qualitatively similar observations were recorded with some other commercially available resins, but these were not investigated in detail. In the course of the work several lots of the resin were used and small differences between lots were observed in the amount of solution required for elution of rhodium, and at least one lot imparted yellow colour to eluting solutions containing ammonium hydroxide. The resin was pre-treated by stirring it in a beaker with a large excess of *M* sodium chloride solution to ensure complete conversion to the chloride form. This step was found necessary even with the analytical grade of resin, whose specification indicated 100 per cent. in the chloride form, and at the same time it made possible the removal of fine particles and small pieces of foreign matter. The prepared resin was stored in *M* sodium chloride solution.

Procedure—

Determinations of rhodium were made absorptiometrically on aliquot portions of effluents by the stannous chloride procedure of Sandell.[10] Dilutions were made with 2 *M* hydrochloric acid. Optical densities were measured with a Beckman model DU spectrophotometer at 470 mμ.

Determinations of small amounts of iridium (less than 1 mg) were made absorptiometrically after oxidation with ceric sulphate.[11] Larger amounts were determined by potentiometric titration.[9] Attempts to use the photometric procedure for larger amounts of iridium eluted from columns were not always successful; several such analyses gave high results, sometimes by as much as 7 per cent. The cause of this discrepancy was probably colour-throwing by the resin into the ammoniacal eluting solution.

Several residues were examined by means of a Hilger Medium Quartz spectrograph with d.c. arc source.

Absorption and recovery of iridium—

One to 5 mg of iridium were fed into a column in 200 ml of solution. A further 300 ml of the sample solution were then passed through the resin bed. From the evaporated effluent solutions any iridium was precipitated as hydrated dioxide on a carrier of titanium dioxide. The precipitate was digested overnight, separated by filtration and washed free of salts. The filter-paper was destroyed and the precipitate was dissolved in mixed acid; the resulting solution was evaporated to dryness. The residue was suspended in 1 per cent. hydrochloric

acid, transferred to a carbon spectrographic electrode and the solution evaporated to dryness under an infra-red lamp. An arc was struck between the electrodes and the iridium lines were compared with those from standards. The results, which are summarised in Table I, show that iridium is nearly quantitatively absorbed and not eluted by the sample solution.

TABLE I

ELUTION OF IRIDIUM^{IV} BY SOLUTION USED FOR DISSOLVING SAMPLE

Iridium in sample solution, mg	Iridium found in direct effluent from sample solution, μg	Iridium eluted with 300 ml of solution, μg	Iridium found in total effluent, μg
1	~10	<5	
5			~10
5	~10	<5	
5	~10	<10	

The recovery of iridium from the resin bed was accomplished by elution with (*i*) 500 ml of 5 *M* ammonium hydroxide in *M* ammonium chloride solution, followed by (*ii*) 300 ml of 6 *M* hydrochloric acid or 8 *M* nitric acid. Representative recoveries are shown in Table II.

TABLE II

DOUBLE ELUTION OF IRIDIUM

Iridium in feed solution, mg	Iridium found in effluent (*i*), mg	Iridium found in effluent (*ii*), mg	Total iridium recovered, mg
8·90	8·58	0·35	8·93
8·90	8·53	0·35	8·88
8·90	8·66	0·22*	8·88
8·90	8·71	0·24	8·95
8·90	8·58	0·31	8·89

* 8 *M* nitric acid was used as elutriant (*ii*).

ABSORPTION AND RECOVERY OF RHODIUM—

Approximately 10 mg of rhodium were fed into the resin column in 200 ml of solution. The resin bed was eluted with 300 to 500 ml of this solution, the volume required depending on the previous history of the rhodium solution as previously recorded and also on the particular batch of resin used. Representative recoveries are shown in Table III.

TABLE III

ELUTION OF RHODIUM BY SOLUTION USED FOR DISSOLVING SAMPLE

Rhodium in sample solution, mg	Rhodium found in direct effluent from sample solution, mg	Rhodium eluted with solution, mg	Total rhodium recovered, mg
10·21	7·68	2·60	10·28
10·21	8·76	1·44	10·20
10·05	4·57*	5·48	10·05

* 100 ml of feed solution used.

This method of eluting rhodium leaves the metal in a solution containing large amounts of sodium chloride, which was removed from several solutions by passing the entire effluent, diluted fivefold, through a bed (2·5 cm × 30 cm) of Amberlite IR-120 cation-exchange resin previously treated with 10 per cent. hydrochloric acid. The resin removed nearly all the sodium ions. In future, hydrochloric acid will be used for this elution, but sufficient results are not available to be included in this paper.

SEPARATION OF RHODIUM AND IRIDIUM—

Known amounts of rhodium and iridium were fed into the resin column in 200 ml of solution. The rhodium was quantitatively eluted with the further addition of 500 ml of the solution used for the sample. The iridium was eluted as described above with 500 ml of ammonium hydroxide and 300 ml of hydrochloric acid. Results of several such separations are shown in Table IV.

TABLE IV

SEPARATION OF RHODIUM AND IRIDIUM

Metal in sample solution		Rhodium recovered, mg	Iridium recovered, mg
Rhodium, mg	Iridium, mg		
10·21	8·90	10·27	8·96
10·21	8·90	10·20	8·91
10·12	9·48	10·20	9·49
10·12	9·48	10·02	9·43
51·32	47·45	50·9*	47·2†
51·32	94·9	51·1*	91·0†

* Eluted with 950 ml of solution used to dissolve sample.
† Eluted with 950 ml of ammonium hydroxide and 300 ml of hydrochloric acid. This volume is probably insufficient for the last sample.

The last two separations in Table IV involved larger amounts of the metals and required larger volumes of the eluting solutions. For these samples analyses were made only on portions of the effluents containing a known fraction of the whole; hence, about 10 mg of iridium and 1 mg of rhodium were actually determined. One sample of rhodium separated in this manner and freed from sodium ions by a cation-exchange resin was converted to the metal by ignition and reduction and examined spectrographically for the presence of iridium. Similar samples for comparison were prepared from the stock solutions of the metals. The d.c. arc spectrum revealed less than 15 μg of iridium in 10 mg of reduced metal.

DISCUSSION OF RESULTS

The procedure described in this paper might be of use in the later stages of the systematic separation of the platinum group metals as currently practised. It possesses the advantage of simplicity of manipulation by comparison with procedures in which rhodium is selectively reduced from solution. Furthermore, it is expected that extensions of this work will shortly be available and separation of the four commonest platinum metals by ion exchange of their chloro salts will be possible.

The strong absorption of chloro-iridate and chloroplatinate by these anion-exchange resins as compared with many common ions and with chloro-iridite and chlororhodite in these experiments might be explained if the formation of insoluble "compounds" such as $(R_4N)_2IrCl_6$ or $(R_4N)_2PtCl_6$ with the resin is postulated. These would be analogous to the chloroplatinate and chloro-iridate salts used for the characterisation of organic amines, and their formation is consistent with the fact that the anion-exchange resins are polymerised organic amines. It is not intended to suggest that the mechanism of the binding of chloro-iridate to a resin is any different from that by which groups such as nitrate or sulphate are bound, but whatever causes ammonium chloro-iridate to be insoluble in water might also ensure a strong resin - chloro-iridate attachment.

We thank Miss Alice Fischel and Mrs. Norah Wilson for many experiments performed by them that assisted in the development of this procedure. This and related work was aided by grants from the University's Advisory Committee on Scientific Research and from the Research Council of Ontario, for which grateful acknowledgment is made.

REFERENCES

1. Nachod, F. C., U.S. Patent 2,371,119, March 6th, 1945.
2. MacNevin, W. M., and Crummett, W. B., *Anal. Chem.*, 1953, **25**, 1628.
3. Kraus, K. A., Nelson, F., and Smith, G. W., *J. Phys. Chem.*, 1954, **58**, 11.

4. Stevenson, P. C., Franke, A. A., Borg, R., and Nervick, W., *J. Amer. Chem. Soc.*, 1953, **75**, 4876.
5. Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffman, J. I., "Applied Inorganic Analysis," Second Edition, John Wiley and Sons, Inc., New York, 1953, p. 380.
6. Barefoot, R. R., and Beamish, F. E., *Anal. Chim. Acta*, 1953, **9**, 47.
7. Currah, J. E., McBryde, W. A. E., Cruikshank, A. J., and Beamish, F. E., *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 120.
8. Hill, Martha A., and Beamish, F. E., *Anal. Chem.*, 1950, **22**, 590.
9. McBryde, W. A. E., and Cluett, M. L., *Can. J. Res., B*, 1950, **28**, 788.
10. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Second Edition, Interscience Publishers, Inc., New York and London, 1950, p. 523.
11. Maynes, A. D., and McBryde, W. A. E., *Analyst*, 1954, **79**, 230.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TORONTO
TORONTO 5, ONTARIO, CANADA

July 16th, 1954

The Determination of Amino-acids with Ninhydrin

BY E. W. YEMM AND E. C. COCKING

The colorimetric method for the determination of amino-acids based on the reaction with ninhydrin has been systematically examined. It has been found possible to obtain a stoicheiometric reaction for most amino-acids with a quantitative yield of diketohydrindylidenediketohydrindamine, the probable end-product of the reaction, by a simple procedure suitable for routine analysis.

THE purple colour formed by the reaction of amino-acids in acid solution with ninhydrin (triketohydrindene hydrate) is attributed to the anion of diketohydrindylidenediketohydrindamine (DYDA).[1,2] It seems that the first step in the reaction is the oxidative de-amination of the amino-acid with the formation of ammonia and the reduction of ninhydrin to hydrindantin. The ammonia then condenses with the hydrindantin to form DYDA.[3,4,5] As recently pointed out by Troll and Cannan,[6] this scheme is consistent with the fact that ammonia forms DYDA with ninhydrin only in the presence of a reducing agent capable of producing hydrindantin. It is, however, inconsistent with the fact that ammonia reacts more slowly and less completely than do amino-acids under the same conditions. It is therefore concluded that the de-amination and condensation are coupled in some way.

Moore and Stein[7] developed the reaction as a convenient photometric method for the determination of amino-acids. They obtained consistent and reproducible results by the introduction of a reducing agent into the system to prevent the oxidation of the hydrindantin formed as a result of oxidative de-amination. The method was further simplified by Smith and Agiza[8]; but in both methods the reaction of different amino-acids with ninhydrin did not give a quantitative yield of DYDA, so that standardisation was required for individual amino-acids. Seeking an explanation for the apparent lack of stoicheiometry of the reaction, Troll and Cannan[6] sought conditions under which the rate of formation of DYDA was increased relative to its rate of destruction. Organic solvents were found to accelerate the development of colour to varying degrees, and ultimately an 80 per cent. solution of phenol in water, buffered at pH 5 with pyridine, was adopted as the most effective solvent. A solution of potassium cyanide was employed as the reducing agent rather than the less stable stannous chloride. The conclusion of Moore and Stein[7] that pH 5 was optimal for reaction was confirmed. All amino-acids tested, except tryptophan and lysine, gave colours equivalent to 97 to 102 per cent. of that of pure DYDA in the same solvent. Troll and Cannan found, furthermore, that the absorption spectra of the colours obtained from amino-acids in their procedure were identical with that of DYDA.

For routine analysis this modified procedure has several disadvantages: it is difficult to control the pH accurately, high water blanks may be obtained and it involves the extensive use of a pyridine - phenol solvent. It has been found possible to retain the advantage of the quantitative reaction of Troll and Cannan[6] by a simple procedure suitable for routine analysis.

METHOD

MATERIALS—

Methyl Cellosolve, $CH_3OCH_2{\cdot}CH_2OH$—Samples gave a clear solution when mixed with an equal volume of water and a faint or negative peroxide test with 10 per cent. potassium iodide.

Ninhydrin—To ensure a low blank reading in the photometric procedure the ninhydrin should be pale yellow with a greenish tint and completely odourless. It should dissolve in methyl Cellosolve to give a clear light yellow solution: if it does not meet these specifications, it may be recrystallised according to the method of Hamilton and Ortiz.[9]

Potassium cyanide—AnalaR reagent as supplied by The British Drug Houses Ltd.

Distilled water—Treat all distilled water with Permutit to remove traces of ammonia.

Amino-acids—For standardisation use synthetic products as far as possible; recrystallise all amino-acids at least once and dry over concentrated sulphuric acid *in vacuo* for 48 hours before use. Prepare stock solutions (2×10^{-3} *M*) in 0·1 *N* hydrochloric acid and store in the cold with a little thymol to prevent fungal growth; prepare standard solutions (2×10^{-4} *M*) by dilution with citrate buffer, pH 5 (0·2 *M*).

REAGENTS—

Citrate buffer, pH 5 (0·2 M)—Dissolve 21·008 g of citric acid, $C_6H_8O_7{\cdot}H_2O$, in 200 ml of distilled water, add 200 ml of *N* sodium hydroxide and dilute to 500 ml; store in the cold with a little thymol.

Potassium cyanide, 0·01 M—Dissolve 0·1628 g of potassium cyanide in distilled water and dilute to 250 ml. This solution is stable for at least 3 months at room temperature.

Ethanol in water, 60 per cent. by volume.

Potassium cyanide - methyl Cellosolve solution—Dilute 5 ml of 0·01 *M* potassium cyanide to 250 ml with methyl Cellosolve. This solution is stable for at least 1 month at room temperature.

Methyl Cellosolve - ninhydrin solution—Prepare a 5 per cent. w/v solution of ninhydrin in methyl Cellosolve. This solution is stable for at least 6 months at room temperature.

Potassium cyanide - methyl Cellosolve - ninhydrin solution—Mix 50 ml of the methyl Cellosolve - ninhydrin solution with 250 ml of the potassium cyanide - methyl Cellosolve solution. The resulting solution is at first red, but soon becomes yellow. It should be stored overnight before use to ensure low blank readings and is stable for at least 1 week when kept in a stoppered flask at room temperature.

BLANK DETERMINATION—

Preliminary experiments indicated that to ensure low blank readings it was necessary to take precautions against contamination of all solutions by ammonia, especially the pH 5 buffer solution. The blank value at a volume of 5·7 ml, read against water, should not be greater than an optical density of 0·12, if reliable results are to be obtained.

PROCEDURE FOR DETERMINING AMINO-ACIDS—

Mix 0·5 ml of citrate buffer, pH 5 (0·2 *M*), with 1 ml of an amino-acid solution containing 0·05 to 5·6 μg of amino nitrogen. Add to this either 0·2 ml of the methyl Cellosolve - ninhydrin solution and 1 ml of the potassium cyanide - methyl Cellosolve solution or 1·2 ml of the potassium cyanide - methyl Cellosolve - ninhydrin solution. Heat the well mixed solution for 15 minutes at 100° C, and cool for 5 minutes in running tap water. Make up the solution to a convenient volume with ethanol, shake well, and determine the optical density, using a 1-cm glass cell, on a Unicam spectrophotometer SP500 at 570 mμ for all amino-acids. Determine proline and hydroxyproline at 440 mμ. An E.E.L. portable colorimeter (Evans Electroselenium Ltd.) with No. L200 tubes and yellow filter No. 626 (maximum transmission at a wavelength of 570 mμ) is equally satisfactory and much more suitable for routine work involving measurements on a large number of tubes. Proline and hydroxyproline are determined with violet filter No. 621 (maximum transmission at 460 mμ). Read all tubes against blanks containing 1·5 ml of citrate buffer, pH 5 (0·2 *M*), and 1·2 ml of the potassium cyanide - methyl Cellosolve - ninhydrin solution subjected to the same procedure as above. Preliminary experiments indicated that the heating time

was not critical and that most amino-acids had reacted fully after heating at 100° C for 10 minutes. Furthermore, the boiling point of the water - methyl Cellosolve mixture is greater than 100° C; using tubes stoppered with a glass marble, evaporation gave losses during the heating period that were negligible. The colour was stable at room temperature for at least 3 hours before ethanol was added and for at least 1 hour after its addition.

Isolation of pure DYDA

DYDA was isolated, as its sodium salt, from aqueous solution by the method of Moore and Stein.[7] For analysis 50 mg of the sodium salt was recrystallised from 15 ml of a water - *n*-propanol mixture (1 + 1). The crystals were dried over concentrated sulphuric acid *in vacuo* for 48 hours, and the millimolar extinction coefficient at 570 mμ was determined in the same solvent mixture as employed in the procedure described above. It was found to be 21·6 and is in agreement with that found by Troll and Cannan[6] for the sodium salt of DYDA in a solution of pyridine, phenol and ethanol; and, as was also found by Troll and Cannan, the absorption spectrum of the sodium salt was identical with that of the unfractionated reaction mixture obtained from amino-acids in the ninhydrin analysis.

Results

For each of the amino-acids various amounts containing from about 0·1 to 6·0 μg of amino nitrogen were examined by the above procedure. The standard curves determined by using an E.E.L. colorimeter for amino-acids in the concentration range 0·05 to 2·8 μg of amino nitrogen are shown in Fig. 1. The individual readings were reproducible to within 1 per cent. of the mean of four determinations for all amino-acids studied. From optical density measurements on the spectrophotometer the millimolar extinction coefficient of the reaction mixture, at 570 mμ, was calculated in each case, and since that of the sodium salt of DYDA was known the percentage yield of DYDA could be determined. The results are summarised in Table I.

Table I

Extinction coefficients and estimated yields of DYDA

Amino-acid	Millimolar extinction coefficient at 570 mμ	Yield of DYDA, %
Alanine	21·6	100
Arginine	21·6	100
Aspartic acid	21·6	100
Asparagine	9·7	45
Cysteic acid	21·4	99
Cystine	21·6	100
Glutamic acid	21·6	100
Glutamine	21·6	100
Glycine	21·6	100
Histidine	21·6	100
Leucine	21·6	100
*iso*Leucine	21·6	100
Lysine	23·4	108
Methionine	21·6	100
Serine	21·4	99
Threonine	21·6	100
Tryptophan	18·0	83
Valine	21·4	99
Phenylalanine	19·1	89
Tyrosine	19·1	89
Ammonia	7·1	33

It was found that all the amino-acids studied gave colours equivalent to 100 ± 1 per cent. of that of pure DYDA except tyrosine and phenylalanine (89 per cent.), tryptophan (83 per cent.) and lysine (108 per cent.). Ammonia reacted to yield a colour equivalent to only 33 per cent. of that of pure DYDA. This value for ammonia is, however, approximate and varies somewhat from one batch of reagent to another. Proline and hydroxyproline react with ninhydrin in an entirely different way; de-amination does not occur, no DYDA is produced, reducing agents are without influence and the imino-acid residue that results

from decarboxylation condenses directly with the ninhydrin to form a yellow end-product.[10] Nevertheless, the same method can be used for their estimation, although the optical density readings are only about one-fourth of those obtained with equimolar solutions of amino-acids.

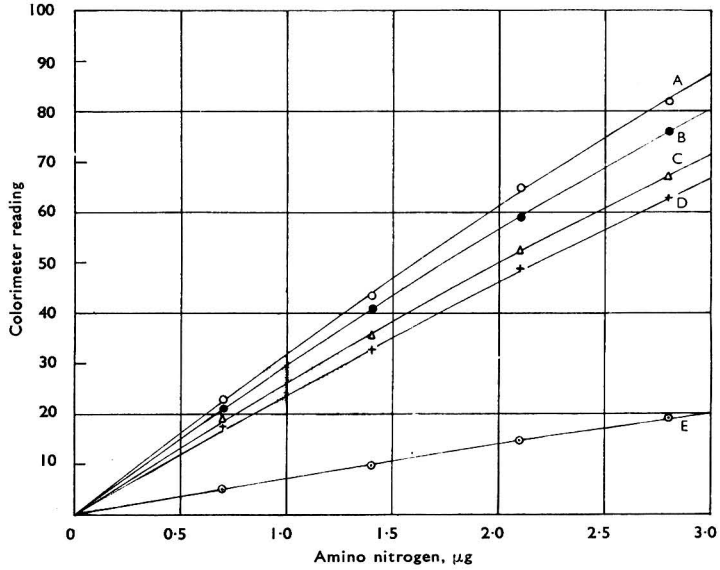


Fig. 1. Standard graphs determined on colorimeter with reaction mixture adjusted to 5·7 ml—
A, lysine;
B, alanine, arginine, aspartic acid, cysteic acid, cystine, glutamic acid, glycine, histidine, leucine, *iso*leucine, methionine, serine, threonine and valine;
C, phenylalanine and tyrosine;
D, tryptophan;
E, proline

Discussion of results

It is clear from the results shown that a stoicheiometric reaction occurs for most of the amino-acids studied, with a quantitative yield of DYDA. The low yield with tryptophan is to be expected, since the optimum pH for reaction is in this case 6, and the high yield for lysine is probably brought about by the partial reaction of the terminal amino group. The yield of DYDA from tyrosine and phenylalanine is largely dependent on the relative proportion of water to methyl Cellosolve, for it is found that the reaction is almost complete when the relative proportion of water to methyl Cellosolve is increased from 1:0·8 to 1:0·4. The yields with the procedure described are however quite reproducible. The results for cystine indicate that only one-half of the amino groups react; it seems probable that one reacts and that the other is protected by cyclisation. Cysteine is known to be the only amino-acid that does not form DYDA; from it a yellow product similar to that given by proline is obtained.[7,8] Cysteic acid on the other hand reacts normally.

The procedure described above has several advantages over that of Moore and Stein.[7] The more complete reaction gives greater sensitivity and highly reproducible results, whilst the lower sensitivity to ammonia leads to smaller and more consistent blanks. Moreover a shorter and less critical heating period is required. There is also a considerable economy of ninhydrin, for only about one-half of the quantity is required compared with that used in the method of Moore and Stein, and furthermore the method avoids the complication of having to store reduced ninhydrin under nitrogen.

Application to protein hydrolysates

The procedure described was developed for the estimation of the amino-acids of protein hydrolysates separated chromatographically on ion-exchange resin columns by elution with buffers of progressively increasing pH, as in the method of Moore and Stein.[11] One-millilitre

fractions of effluent were obtained in optically matched tubes by using a capacity-change drop counter. Each fraction was treated with 0·5 ml of citrate buffer, pH 5 (0·2 *M*), to which *N* sodium hydroxide had been added so that the final pH of the treated fraction was 5. Then 1·2 ml of the potassium cyanide - methyl Cellosolve - ninhydrin solution were added as in the standard procedure. The tubes were well shaken and heated in racks of twenty-five at 100° C for 15 minutes. They were cooled in running tap water for 5 minutes, 3 ml of ethanol were added, the tubes were shaken and the colour intensity was read directly in an E.E.L. colorimeter, a No. 626 filter being used. In view of the low absorption of DYDA at 440 mμ it has been found possible to estimate proline when mixed with small quantities of other amino-acids. This is readily achieved on an E.E.L. colorimeter, No. 621 and 626 filters being used. No interference was noted from special chemicals such as benzyl alcohol, thiodiglycol and ethylenediaminetetra-acetic acid added to the columns to improve their performance. The complete reaction makes possible the more accurate calculation of the amount of an amino-acid present when one peak is incompletely separated from another; moreover, the low yield from ammonia ensures lower and more consistent blank readings, thus making possible the more exact determination of the amount of an amino-acid present under any particular peak. With the above procedure it has been found possible by using semi-automatic pipettes to analyse 100 samples in about 3 hours.

One of us (E. C. C.) is indebted to the Agricultural Research Council for a Research Studentship.

References

1. MacFadyen, D. A., *J. Biol. Chem.*, 1950, **186**, 1.
2. MacFadyen, D. A., and Fowler, N., *Ibid.*, 1950, **186**, 13.
3. Ruhemann, S., *J. Chem. Soc.*, 1910, **97**, 1438.
4. ——, *Ibid.*, 1911, **99**, 792.
5. ——, *Ibid.*, 1911, **99**, 1486.
6. Troll, W., and Cannan, R. K., *J. Biol. Chem.*, 1953, **200**, 803.
7. Moore, S., and Stein, W. H., *Ibid.*, 1948, **176**, 367.
8. Smith, A. M., and Agiza, A. H., *Analyst*, 1951, **76**, 623.
9. Hamilton, P. B., and Ortiz, P. J., *Anal. Chem.*, 1950, **22**, 948.
10. Grassman, W., and von Arnim, K., *Annalen*, 1934, **509**, 288.
11. Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1951, **192**, 663.

Department of Botany
University of Bristol

August 3rd, 1954

Appendix

A CAPACITY-CHANGE DROP COUNTER

By R. E. Ricketts

A simply constructed and reliable capacity-change drop counter is described. Fractions of 1 to 5 ml may be automatically collected, and measurements made over a period of several months indicate an accuracy of delivery better than ±1 per cent.

It was found necessary to collect a large number of fractions of eluate from resin columns employed for the fractionation of protein hydrolysates. The equipment used was designed to operate in conjunction with a chromatographic table,[1] a set number of drops being counted into each test tube.

In view of the possibility of breakdown of unstable solutions owing to electrical contact, it was decided to use a capacity-operated switch. To guard against failure of valves or other components, two channels operating simultaneously were used, with "test channel" buttons to enable the operator to check, periodically, each channel separately.

The circuit diagram of the apparatus is shown in Fig. 2. Valves V_1 and V_2 are connected as a Hartley oscillator whose feedback depends on the ratio of C_1 and C_2. Radio-frequency voltage from the oscillator is applied through C_3 to the thyratrons, V_3 and V_4. The supply voltage to the oscillator is arranged to be 180° out of phase with that applied to the thyratrons, consequently rectification occurs at the grids of the thyratrons so charging C_3 negatively, thus biasing the

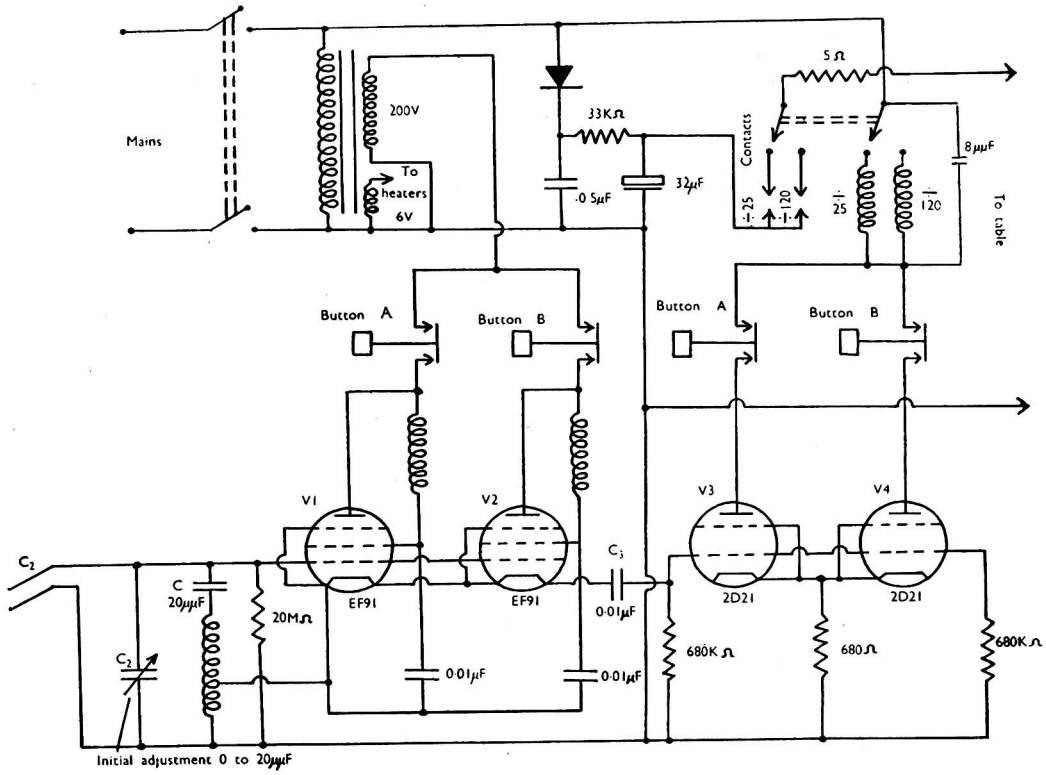


Fig. 2. Circuit diagram of drop counter

thyratrons and ensuring that no current passes through the counting relay. As a drop approaches the platinum wires which form C_2, the critical value is upset, oscillation ceases, the bias is removed and the counting relay is energised. At some pre-determined number of drops the counting relay in turn energises the table relay by discharging a 32-μF condenser, and the next tube moves into place.

Reference

1. Hough, L., Jones, J. K. N., and Wadman, W. H., *J. Chem. Soc.*, 1949, 2511.

Chemistry Department
University of Bristol

August 3rd, 1954

The Fractionation and Determination of Corticosteroids in Urine

By E. R. COOK, BARBARA DELL* and D. J. WAREHAM

Corticosteroids may be fractionated by a simple partition technique employing silicic acid columns impregnated with a controlled amount of water and using benzene - ether mixtures as the eluting phase. The method has been applied to the fractionation of extracts of (*a*) adrenal tissue and (*b*) urine hydrolysed with acid and also with β-glucuronidase preparations.

Total 17-hydroxycorticosteroids may be determined in urine extracts by a modified procedure employing very much smaller silicic acid columns. The average excretion of total 17-hydroxycorticosteroids liberated by crude β-glucuronidase preparations is 3·5 mg per 24 hours (males).

Romanoff, Wolfe and Pincus[1,2] have described the separation of urinary corticosteroids into three fractions of increasing polarity on silica gel columns, followed by further detailed fractionation by the paper systems of Zaffaroni, Burton and Keutmann[3,4,5,6]. Silica gel columns were also used by Katzenellenbogen, Kritchevsky and Dobriner[7] to separate the acetates of adrenal steroids, aqueous methanol or ethanol being employed as stationary phase and mixtures of dichloromethane and light petroleum as mobile phase. Haines[8] has separated adrenocortical hormones on silica gel columns impregnated with ethylene glycol by using mixtures of dichloromethane in *cyclo*hexane.

Schneider[9,10] described the chromatographic isolation of certain corticosteroids from chloroform extracts of pooled male urine. Magnesium silicate - Celite columns were employed, and the acetylated steroids were eluted with mixtures of benzene, benzene - ether and ethyl acetate - ether. Similar columns were used by Haines, Johnson, Goodwin and Kuizenga[11] to demonstrate the presence of pregnenolone in hog testes.

The phenylhydrazine - sulphuric acid reaction of Porter and Silber[12] is considered to be specific for the 17:21-dihydroxy-20-ketosteroids and has been applied to the determination of 17-hydroxycorticosteroids in blood by Nelson and Samuels.[13] Chloroform extracts of blood or plasma were adsorbed on Florosil or magnesium silicate - Celite columns, which were then eluted with methanol - chloroform and the extracted 17-hydroxycorticosteroids estimated by a micro modification of the Porter and Silber reaction. Bayliss and Steinbeck[14] emphasise that it is essential to carry out sulphuric acid blanks in order to eliminate the errors due to non-specific chromogens. A similar method has been used by Glenn and Nelson[15] to determine the 17-hydroxycorticosteroids in urine.

Morris and Williams[16] were able to determine the individual adrenocortical hormones in peripheral blood by the application of partition chromatography followed by the polarographic estimation of Δ^4-3-ketosteroids, previously developed by Butt, Morris, Morris and Williams.[17] The column employed consisted of Hyflo Supercel, with either (*a*) 25 per cent. aqueous ethanol as the stationary phase and toluene as the mobile phase or (*b*) ethylene glycol as the stationary phase and 20 per cent. light petroleum in toluene as the mobile phase.

Heftmann and Johnson[18] were able to separate individual corticosteroids with a column of 30 g of silicic acid impregnated with water and a mobile phase consisting of light petroleum and dichloromethane of continuously increasing polarity. This communication describes the separation of corticosteroids on a column of 5·5 g of silicic acid, by using water as the stationary phase and benzene followed by mixtures of benzene and ether as the mobile phase. The method has also been adapted to the determination of total 17-hydroxycorticosteroids in urine.

Method

Reagents—

Benzene and ethyl acetate—Fractionally distil analytical-reagent grade material through a 6-pear column. Discard the first and last 10 per cent.

Ethanol and methanol—Heat the B.P. grade of absolute alcohol under reflux over freshly ignited calcium oxide (sulphur free) for 3 to 4 hours, then fractionate through a 6-pear column.

* Present address: Department of Pathology, Sunnybrook Hospital, Toronto, Ontario, Canada.

The distillate is occasionally faintly turbid with calcium oxide owing to "creep," and must be re-fractionated. Add 16 g of anhydrous analytical-reagent grade sodium acetate and 24 g of analytical-reagent grade semicarbazide hydrochloride to 100 ml of the distillate, heat under reflux for 2 hours, allow the precipitate of sodium chloride to settle, and add the clear supernatant liquid to 1 litre of the dried fractionated alcohol. Heat under reflux for 2 hours, and then distil through a 6-pear column.

Ether—Fractionally distil reagent-grade material from ferrous sulphate every 2 days.

Silicic acid—Spread out moist silicic acid (supplied by British Drug Houses Ltd.) in a thin layer, preferably on Pyrex-glass plates, and dry for 3 to 4 days in an oven at 45° to 50° C. Roughly crush the dry powder and extract for 6 hours in a Soxhlet apparatus with ether boiling over ferrous sulphate. Dry the extracted silicic acid for 48 hours at 30° to 35° C and sieve through a brass screen of 100 mesh, aperture 0·006 inches. Store in an air-tight bottle. Determine the moisture content of the fine powder by igniting at 600° C for 2 hours. Silicic acid prepared in this way usually contains from 20 to 25 per cent. of water.

Sulphuric acid reagent—Mix 310 ml of concentrated analytical-reagent grade acid with 190 ml of water.

Phenylhydrazine hydrochloride—Crystallise three times from purified ethanol.

Propylene glycol—Distil under reduced pressure.

Toluene—Analytical-reagent grade, sulphur free.

β-Glucuronidase—An extract of the common limpet (*Patella vulgata*) assayed by the technique of Henry and Thevenet.[19]

CHROMATOGRAPHY—

Weigh into a small beaker a quantity of the prepared silicic acid, calculated to give 5·5 g on a dry-weight basis. Add distilled water dropwise to bring the total weight to 11·5 g, *i.e.*, the final mixture contains 5·5 g of dry SiO_2 and 6·0 g of water (including the water held in the ether-washed silicic acid). Well stir the mixture with a thick glass rod, and add benzene saturated with water. Stir once more, break up all the agglomerates present, and then pour the fine suspension on to a small glass-wool plug in the bottom of a Pyrex-glass column, 1·8 cm in internal diameter, 25 cm long and fitted with a 250-ml bulb reservoir. Allow the column to drain until the level of the solvent approaches the top of the silicic acid, then carefully add 2 to 5 ml of wet benzene containing 1·0 to 4·0 mg of mixed corticosteroids and allow the liquid to drain into the surface of the solid. Elute the column with 30 ml of benzene followed by 120 ml of benzene - ether (13 + 2), 120 ml of benzene - ether (9 + 2), 120 ml of benzene - ether (6 + 2), 120 ml of benzene - ether (1 + 1) and 100 ml of ether. The solvents are saturated with water before use. Collect the eluate in 10-ml aliquots by using an automatic fraction cutter,[20] evaporate to dryness in an oil-bath at 90° C followed by suction from a water-pump, and determine the corticosteroid in the residue.

DETERMINATION OF CORTICOSTEROIDS—

Method I—The colorimetric modification of the Hagedorn and Jensen blood sugar technique devised by Gordon and Pelly[21] was used to determine the corticosteroids in the eluates from the preliminary chromatographic experiments. The method does not give equal extinctions per molecule for different corticosteroids and was used only for qualitative purposes.

Method II—This technique is based on the procedure of Saffran, Grad and Bayliss,[22] who utilised the characteristic absorption at 240 mμ to determine corticosteroids with a Δ^4-3-keto grouping. Dissolve the dry residue in 5 ml of methanol and determine the absorption at 220, 240 and 260 mμ in the Unicam ultra-violet spectrophotometer, using the 1-cm quartz cells. The true absorption at 240 mμ is calculated by the method of Allen.[23] Corrected absorption at 240 mμ is—

$$CA_{240} = A_{240} - \frac{A_{220} + A_{260}}{2}.$$

The corticosteroid value is read from a graph constructed by plotting the corrected reading at 240 mμ against quantities of cortisone from 10 to 200 μg. In later experiments ethanol was used to avoid the tendency of methanol to creep over the lip of the cuvette.

The method was not entirely satisfactory when applied to other than pure corticosteroids and frequently gave low results due to over-correction.

Method III—Dissolve the residue in 5·0 ml of methanol or ethanol, determine the ultraviolet absorption as in method II, and then evaporate 2·0-ml duplicates to dryness in an oil-bath at 90° to 100° C followed by suction from a water-pump. The 17-hydroxycorticosteroids present are determined by a slight modification of the colour reaction described by Porter and Silber.[12]

Dissolve the duplicates in 0·5 ml of ethanol, and to one tube add 4·0 ml of phenylhydrazine - sulphuric acid reagent (65 mg of recrystallised phenylhydrazine hydrochloride + 100 ml of sulphuric acid reagent), and 4·0 ml of sulphuric acid reagent to the other. Stopper the tubes and incubate in the dark at 60° C for 20 minutes together with similar reagent blanks.

Transfer the coloured solutions to 0·5-cm cells and determine the optical density at 405 mμ in a Hilger photo-electric absorptiometer, using the mercury-vapour lamp.

Any non-specific sulphuric acid chromogens present are subtracted from the phenylhydrazine - sulphuric acid reading, and the corticosteroid value is calculated from a standard graph calibrated with 10 to 200 μg of cortisone. Thus curve must be checked at frequent intervals.

Results

The order in which the corticosteroids are eluted and the separations possible are shown in Fig. 1. The rate of movement of the corticosteroid through the column is largely deter-

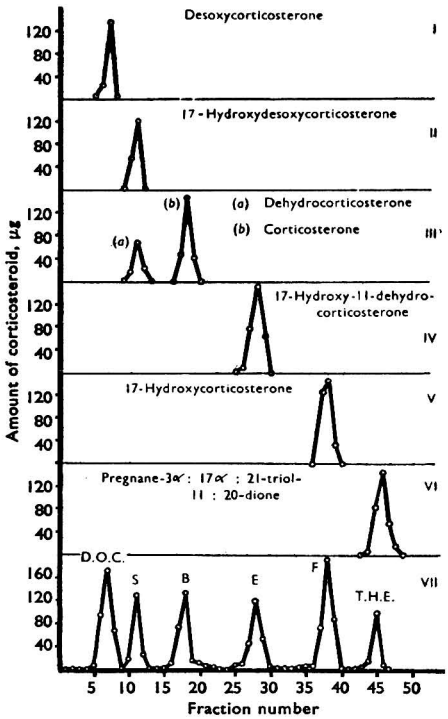


Fig. 1. (*i*) to (*vi*) The elution sequence of individual corticosteroids from silicic acid columns impregnated with water; (*vii*) the separation of a mixture of desoxycorticosterone (D.O.C.), 17-hydroxydesoxycorticosterone (S), corticosterone (B), 17-hydroxy-11-dehydrocorticosterone (E), 17-hydroxycorticosterone (F) and pregnane-3α:17α:21-triol-11:20-dione (T.H.E.)

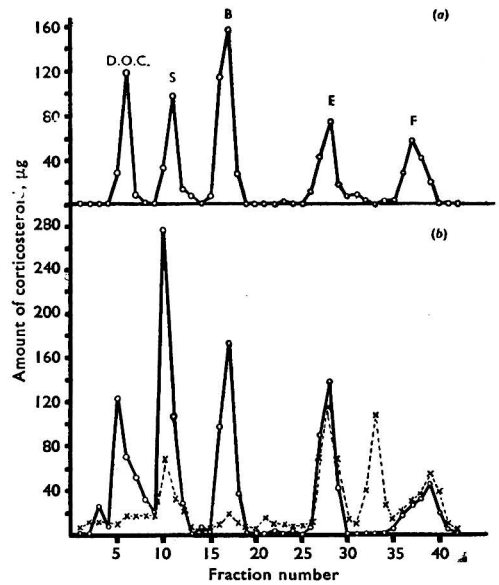


Fig. 2. (*a*) The partition chromatography of a mixture of desoxycorticosterone (D.O.C.), 17-hydroxydesoxycorticosterone (S), corticosterone (B), 17-hydroxy-11-dehydrocorticosterone (E) and 17-hydroxycorticosterone (F): the evaporated fractions were estimated by the absorption at 240 mμ; (*b*) the fractionation of an adrenal extract: the dry fractions were estimated by the absorption at 240 mμ (full line, method II) and by the Porter and Silber reaction (broken line, method III)

mined by the volume of water held by the inert phase, a decrease in water level resulting in a greater separation of the corticosteroid peaks. Reproducible chromatograms can be obtained with different batches of silicic acid only if the final total water content is strictly controlled. The technique does not separate 17-hydroxy-11-desoxycorticosterone from dehydrocorticosterone, but a mixture may easily be quantitatively differentiated by determining the absorption at 240 mμ followed by the Porter and Silber reaction.

Recoveries of added steroids are given in Table IA and average 97·6 ± 8·5 per cent. It was considered possible that the method of evaporating the eluates to dryness might be a contributory factor to the occasional low recoveries, but investigations with standard solutions of 17-hydroxy-11-dehydrocorticosterone and 17-hydroxycorticosterone showed that little loss occurred this way, measured by either the Porter and Silber reaction or by ultra-violet absorption.

FRACTIONATION OF ADRENAL AND URINE EXTRACTS—

An ethyl acetate extract of a commercial adrenal preparation (Eucortone, by Allen and Hanbury's Ltd.) was evaporated to dryness at 50° C under reduced pressure and chromatographed. The eluates were evaporated to dryness, the residues dissolved in 5 ml of methanol or ethanol, and the ultra-violet absorption at 220, 240 and 260 mμ was determined. Two-millilitre duplicates were evaporated to dryness, and the Porter and Silber reaction carried out (method III). Little correction was needed for non-specific material in both methods. Fig. 2 shows the result of a typical experiment.

Fractions 30 to 35 contain a material which gives a positive Porter and Silber reaction but does not absorb at 240 mμ. The elution of this substance between the positions occupied by 17-hydroxy-11-dehydrocorticosterone and 17-hydroxycorticosterone suggests that it may be a reduction compound of 17-hydroxy-11-dehydrocorticosterone, but insufficient material has been isolated for identification purposes.

The steroid conjugates contained in 6 litres of fresh, pooled, male urine were extracted by the method of Edwards, Kellie and Wade.[24] Three kilograms of analytical-reagent grade ammonium sulphate were added, and the urine was extracted four times with 2 litres of a mixture of ether and ethanol (3 + 1). The mixed extracts were evaporated to dryness at 45° C under reduced pressure and the residue was dissolved in 150 ml of distilled water.

The aqueous solution was adjusted to pH 1·0 with hydrochloric acid, and extracted three times with 100 ml of chloroform which had been previously washed with 0·5 *N* sodium hydroxide and distilled water. The mixed, yellow, chloroform extracts were washed four times with 30 ml of 0·1 *N* sodium hydroxide and three times with 50 ml of water. The chloroform solution was dried over anhydrous analytical-reagent grade sodium sulphate for 2 hours, and filtered through a glass sinter. The filtrate was evaporated to dryness at 45° C under reduced pressure, the residue dissolved in benzene, and an aliquot, equivalent to 3·7 litres of urine, was chromatographed as detailed above.

After extraction at pH 1·0 with chloroform, the aqueous solution was adjusted to pH 4·6 with sodium hydroxide, and 2×10^6 units of β-glucuronidase (standardised by the method of Henry and Thevenet[19]) were added. After incubation at 37° C for 72 hours, the solution was extracted three times with 150 ml of ethyl acetate. The combined extracts were washed three times with 150 ml of 0·1 *N* sodium hydroxide, three times with 100 ml of water, dried over anhydrous analytical-reagent grade sodium sulphate, filtered, and evaporated to dryness at 45° C under a vacuum. The red oily residue was dissolved in benzene, and an aliquot, equivalent to 4·3 litres of urine, was chromatographed.

Fig. 3*a* shows the chromatographic pattern obtained by estimating the dried fractions from the pH 1·0 extract by the Porter and Silber reaction and by the method of ultra-violet absorption. Fig 3*b* shows that a different pattern was obtained from the extract hydrolysed by β-glucuronidase. Very small amounts of non-specific sulphuric acid chromogens appeared to be present in either extract, but considerable correction had to be made for material interfering with the ultra-violet absorption. The very large initial peak in Fig. 3*b*, measured by ultra-violet absorption, is due to the early elution of 17-ketosteroids.

Tubes 40 to 44 contained 1·1 mg of a material which had no absorption at 240 mμ but was positive in the Porter and Silber reaction. This substance was chromatographed with parallel standard corticosteroids by the paper-partition method of Zaffaroni, Burton and Keutmann,[3,4,5,6] by using propylene glycol as the stationary phase, and toluene saturated with propylene glycol as the mobile phase. The paper strips were dried in a gentle stream

TABLE I

RECOVERY OF CORTICOSTEROIDS FROM COLUMNS OF SILICIC ACID, EMPLOYING WATER AS STATIONARY PHASE AND BENZENE - ETHER MIXTURES AS MOBILE PHASE

Column	Corticosteroid															
	Desoxy-corticosterone		17-Hydroxy-desoxy-corticosterone		Dehydro-corticosterone		Corticosterone		17-Hydroxy-11-dehydro-corticosterone		17-Hydroxy-corticosterone		Pregnane-3α:17α:21-triol-11:20-dione	
	Added, μg	Recovery, %	Added, μg	Recovery, %	Added, μg	Recovery, %	Added, μg	Recovery, %	Added, μg	Recovery, %	Added, μg	Recovery, %	Added, μg	Recovery, %
A. 5·5 g of silicic acid (calculated on dry-weight basis) + 6·0 g of water. Mean recovery = 97·6 ± 8·5 per cent.	66	97	107	121	70	100	93	107	54	82	74	96	150	107
	99	100	172	104	100	93	104	94	60	100	160	93	347	98
	125	99	198	88			160	91	147	95	160	104		
	138	85	216	93			204	102	212	92				
	167	98					265	88						
	194	111												
B. 0·55 g of silicic acid (calculated on dry-weight basis) + 0·6 g of water. Mean recovery of 1 to 15 μg of steroid = 91 ± 10·0 per cent. 15 to 960 μg of steroid = 94·8 ± 4·9 per cent.									1	71	1	76	3	92
									2	85	2	89	6	95
									3	97	3	88	7	94
									4	100	5	94	57	97
									13	92	15	103	103	95
									15	98	34	94	115	99
									31	98	49	94		
									77	91	98	82	230	96
									212	100	108	90		
									564	99				
									960	98				

of warm air, and the compounds were located by spraying with a freshly prepared alkaline solution of triphenyltetrazolium chloride (2 parts of aqueous 0·2 per cent. triphenyltetrazolium chloride + 1 part of 10 per cent. sodium hydroxide). The urine component had split into two compounds, one running parallel with authentic tetrahydrocortisone (pregnane-3α:17α:21-triol-11:20-dione), the other more polar.

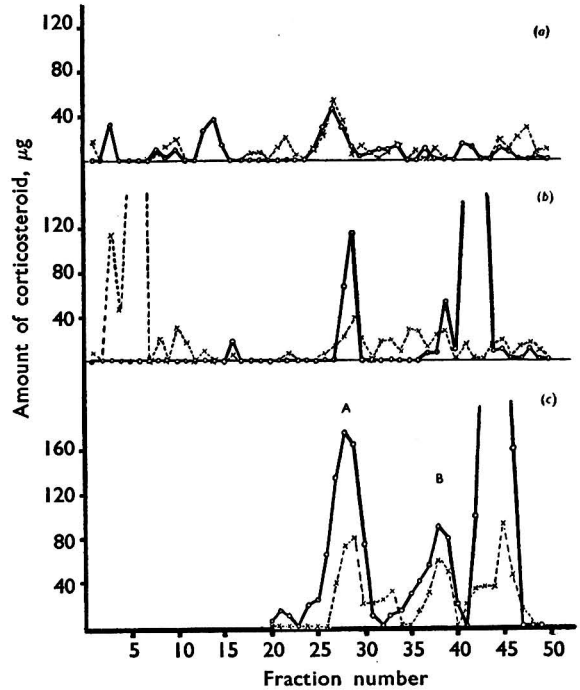


Fig. 3. The fractionation of urine extracts: (*a*) chloroform extract of urinary steroid conjugates adjusted to pH 1·0; (*b*) ethyl acetate extract of urinary steroid conjugates that have been treated with β-glucuronidase; (*c*) ethyl acetate extract of urine after hydrolysis with β-glucuronidase. Full line, corticosteroid values estimated by the Porter and Silber reaction; broken line, corticosteroid values determined by the absorption at 240 mμ

In order to study these compounds in more detail a large-scale extraction of urine was carried out. Nineteen litres of pooled male urine were brought to pH 4·6 with hydrochloric acid, $3{\cdot}5 \times 10^6$ units of β-glucuronidase added, and incubated for 96 hours. The cooled urine was extracted with ethyl acetate, the combined extracts were washed with 0·1 *N* sodium hydroxide and water, and distilled to dryness at 45° C under reduced pressure. The yellow residue was dissolved in 30 ml of water, washed with 30 ml of benzene, and the benzene extracted four times with 30 ml of water. The five water extracts were combined and shaken three times with 150 ml of ethyl acetate, which was then evaporated to dryness under reduced pressure. The residue was dissolved with warming in 10 ml of benzene and a 5-ml aliquot was chromatographed (see Fig. 3*c*).

The substances in the smaller peaks, A and B, were tentatively recognised as 17-hydroxy-11-dehydrocorticosterone (peak A) and 17-hydroxycorticosterone (peak B), on the following evidence—

(*i*) Both possess the Δ^4-3-keto grouping, as shown by ultra-violet absorption at 240 mμ and by the soda fluorescence test of Bush.[25]

(*ii*) The substances are positive in the Porter and Silber reaction, specific for a 17:21-dihydroxy-20-ketosteroid.

(*iii*) They react with triphenyltetrazolium chloride in alkali, indicative of an α-ketol group.

(*iv*) They occur in the positions occupied by 17-hydroxy-11-dehydrocorticosterone and 17-hydroxycorticosterone in the silicic acid, water and benzene - ether system, and run parallel to authentic specimens in the paper and propylene glycol - toluene system.

(*v*) The absorption maxima shown by substance A in concentrated sulphuric acid are identical with those shown by 17-hydroxy-11-dehydrocorticosterone, *i.e.*, 280, 340 and 415 mμ (Zaffaroni[26]). The absorption curve of peak B in concentrated sulphuric acid gave inconclusive results.

The two compounds are present in quantities equivalent to 71 μg of 17-hydroxy-11-dehydrocorticosterone and 35 μg of 17-hydroxycorticosterone in 1 litre of pooled male urine. These figures agree with the levels determined by Cope and Hurlock,[27] who found that extracts of patients' urines hydrolysed by β-glucuronidase contained the equivalent of 20 to 185 μg of 17-hydroxy-11-dehydrocorticosterone and 41 to 102 μg of 17-hydroxycorticosterone in 24 hours. Gray and Lunnon[28] report similar values.

The combined material from tubes 43 to 46 totalled 3·1 mg as measured by the Porter and Silber reaction and was fractionated by a large-scale application of the paper-partition technique of Zaffaroni, Burton and Keutmann. Whatman 3-mm chromatography paper was extracted for 6 hours in a Soxhlet apparatus with ether boiling over ferrous sulphate, dried in an oven at 37° C for 2 days, and then soaked in 25 per cent. propylene glycol in chloroform. The excess of solvent was blotted off, and the paper dried in a stream of warm air. The extract was dissolved in 1·0 ml of methanol and loaded stripwise across the paper, which was then eluted for 9 days with toluene saturated with propylene glycol and then dried in a stream of warm air. The two previously observed compounds were located by spraying narrow strips of the paper with alkaline triphenyltetrazolium chloride, the unused parallel areas eluted with methanol, and the Porter and Silber reaction was applied to aliquots of the two extracts. The recovery of the less polar substance was 2·5 mg, equivalent to 277 μg per litre of urine, and was provisionally identified as pregnane-3α:17α:21-triol-11:20-dione on the basis of: (*a*) the absence of a Δ^4-3-keto grouping as shown by the lack of absorption at 240 mμ and a negative soda fluorescence reaction, (*b*) the positive reaction with the Porter and Silber reagent and with triphenyltetrazolium chloride, (*c*) examination of the sulphuric acid chromogen reveals maxima at 330 and 410 mμ (Zaffaroni[26]) and (*d*) when mixed with authentic pregnane-3α:17α:21-triol-11:20-dione and chromatographed by the paper-partition method of Zaffaroni *et al.* there is no separation.

The more polar material was present in an amount corresponding to 27 μg per litre of urine, and appeared to be contaminated, giving a brown solution on the addition of the sulphuric acid reagent in the Porter and Silber reaction. It has not yet been identified.

The level of pregnane-3α:17α:21-triol-11:20-dione reported here, *i.e.*, 277 μg per litre of urine, agrees with the figure of 214 μg per litre determined by Schneider,[10] but it is highly probable that both values are low owing to the use of insufficient enzyme. The beef spleen β-glucuronidase preparation employed by Schneider was not assayed, while the 184 units per ml of urine used in this experiment is considered to be below the concentration required for maximal yields. Cope and Hurlock,[27] employing 1000 units (Fishman) of β-glucuronidase per ml of urine, reported that the excretion of pregnane-3α:17α:21-triol-11:20-dione varied from 840 to 2650 μg per 24 hours. These workers also showed that increasing the amounts of enzyme above 50 units per ml of urine yielded greatly increased quantities of steroid giving the Porter and Silber reaction, although no comparable increase in biological activity, attributed to 17-hydroxy-11-dehydrocorticosterone and 17-hydroxycorticosterone, took place. It was necessary to use 500 units per ml of urine to obtain maximum yields of steroids. It will be shown later that the hydrolysis of urine with 1000 units (Henry and Thevenet) per ml will give yields of total 17-hydroxycorticosteroids comparable with those reported by Cope and Hurlock,[27] and by Gray and Lunnon.[28]

The fractionation technique described here permits the satisfactory separation of small amounts of corticosteroids in pure mixtures and in extracts of adrenal tissue. However, the disproportionate occurrence of 17-hydroxy-11-dehydrocorticosterone, 17-hydroxycorticosterone and pregnane-3α:17α:21-triol-11:20-dione in urine extracts necessitates the hydrolysis of large volumes of urine in order to obtain sufficient material for adequate chromatographic analysis.

DETERMINATION OF TOTAL 17-HYDROXYCORTICOSTEROIDS IN URINE FOLLOWING HYDROLYSIS WITH β-GLUCURONIDASE—

It has been shown that the amount of corticosteroids which can be extracted from urine following hydrolysis with β-glucuronidase may be 10 to 20 times greater than following hydrolysis at pH 1·0[29,30,31,32,33] and that the corticosteroids so obtained consist principally of the 17-hydroxy compounds, particularly pregnane-3α:17α:21-triol-11:20-dione.[10,15,27,28,34] The excretion of these compounds depends upon the functional ability of the adrenal cortex,[27,28,34] and their estimation in urine may prove to be of clinical value as an index of adrenal activity. We have accordingly considerably modified the previously described chromatographic technique in order that 17-hydroxy-11-dehydrocorticosterone, 17-hydroxycorticosterone and pregnane-3α:17α:21-triol-11:20-dione can be eluted as one fraction. Pregnane-3α:11β:17α:21-tetrol-20-one may also be included, but we have not had the necessary pilot substance for confirmation. The modified procedure has been used to determine the 17-hydroxycorticosteroids liberated by β-glucuronidase, but there is evidence that the values so obtained do not entirely represent the total quantity of 17-hydroxycorticosteroids present in the urine specimen.[35,36,37]

SMALL-COLUMN METHOD

Weigh out 0·55 g of prepared silicic acid (calculated on a dry-weight basis) in a 2-inch × 1-inch sample tube, and add water dropwise to bring the total weight to 1·15 g (*i.e.*, the final preparation contains 0·55 g of dry SiO_2 and 0·6 g of water, including that held initially in the silicic acid).

Stir the mixture with a thick glass rod, add 8 to 12 ml of benzene, previously equilibrated with water, and break up all the lumps. Pour the fine suspension on to a glass-wool plug in the bottom of a Pyrex-glass column 20 cm long, 6 mm in internal diameter, with a constriction of approximately 1 mm internal diameter near the lower end and a 100-ml bulb reservoir at the upper end.

Wash the column with a further 8 to 12 ml of benzene saturated with water, and allow to drain until the solvent level approaches that of the silicic acid. Dissolve the corticosteroids in 2 ml of wet benzene, with warming if necessary, and load carefully on the column with a capillary pipette. Elute the column with 15 ml of benzene - ether (13 + 2) and 20 ml of benzene - ether (1 + 2). Both solutions are previously equilibrated with water.

Reject the first eluate, and adjust the second to 20 ml with benzene in a calibrated flask. With a pipette put 10 ml into a Pyrex-glass test tube, and transfer the remaining solution to a similar test tube, using 1 ml of benzene to wash out the calibrated flask. Add a small chip of boiling-stone, and evaporate the duplicates almost to dryness in an oil-bath at 50° C rising to 100° C as the ether boils off. Remove the last traces of solvent in a vacuum-desiccator, and estimate the 17-hydroxycorticosteroids in the dry residue by the Porter and Silber reaction described in method III.

The ether used must be fractionally distilled from ferrous sulphate just before use, but the silicic acid is stable for at least 4 months. The water content increases slightly and should be determined every 2 to 3 weeks.

Table IB shows that the recovery of pure 17-hydroxycorticosteroids from the column is 91 ± 10 per cent. for 1 to 15 μg and 94·8 ± 4·9 per cent. for 15 to 960 μg of steroid.

HYDROLYSIS AND EXTRACTION OF URINE—

Adjust 25 to 100 ml of urine, depending upon the level of corticosteroid expected, to pH 4·6 with hydrochloric acid, and hydrolyse with 1000 units of β-glucuronidase per ml at 37° C for 24 hours. No buffer solution is added, as there is no appreciable change in pH under these conditions.

Cool the urine, and extract three times with equal volumes of ethyl acetate. Wash the combined extracts once with one-fifth of the volume of 0·5 *N* sodium hydroxide, twice with one-fifth of the volume of water, and then evaporate to dryness at 45° to 50° C under reduced pressure from a water-pump. Dissolve the residue in 10 ml of benzene and then extract five times with 10 ml of water. Combine the aqueous extracts, and extract three times with 50 ml of ethyl acetate. Discard the aqueous layer and evaporate the ethyl acetate to dryness at 45° to 50° C under reduced pressure.

Dissolve the dry residue in 2·0 ml of wet benzene and chromatograph on the small column detailed previously. Discard the first eluate, divide the second into halves, and evaporate each half to dryness in an oil-bath and then under reduced pressure. Dissolve the residue in 0·5 ml of ethanol and determine the 17-hydroxycorticosteroid value by method III. Reagent blanks should be carried through the complete process, but generally contain very little interfering matter. We have observed that in extracts of urines from untreated subjects, the Porter and Silber reaction is enhanced by sulphuric acid reacting non-specific chromogens which generally account for approximately one-third of the final colour. The nature of this interfering material is not known.

The benzene - water partition is somewhat tedious, but is necessary to remove excessive non-specific chromogens, which would otherwise give deep brown solutions in the colorimetric reaction. Known quantities of 17-hydroxy-11-dehydrocorticosterone and 17-hydroxycorticosterone were taken through this purification technique with an average recovery of 96 per cent. (range 93 to 102 per cent.).

Fig. 4 shows some typical absorption curves obtained by applying the Porter and Silber reaction to chromatographed urine extracts. They show the characteristic peak at 405 to 410 mμ.

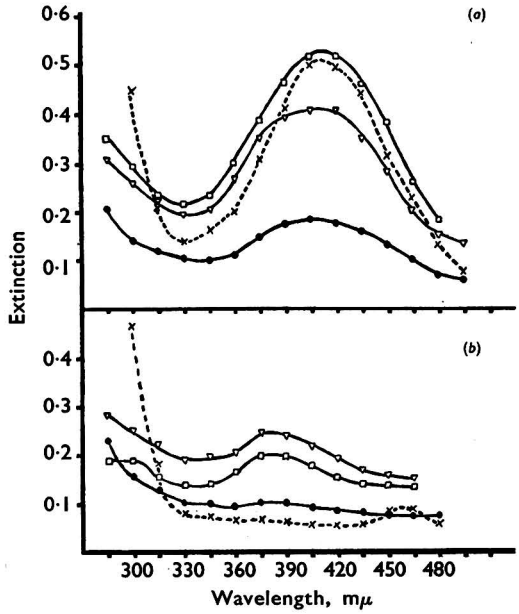


Fig. 4. The absorption curves of the colour produced by applying the Porter and Silber reaction to purified urine extracts and to 17-hydroxycorticosterone: (*a*) reaction with phenylhydrazine - sulphuric acid reagent; (*b*) reaction with sulphuric acid reagent. Broken line, 17-hydroxycorticosterone. The absorption curves were determined with the Hilger ultra-violet spectrophotometer

Results

Table II illustrates the recovery of pure 17-hydroxycorticosteroids carried through the complete technique, and also of pure 17-hydroxycorticosteroids added to hydrolysed urines.

The daily 17-hydroxycorticosteroid excretion varies considerably between persons, but remains reasonably constant for the individual. A small number of male subjects, aged 18 to 36 years, consisting of fourteen normal healthy males and fourteen mental patients with no gross endocrinological disorder, gave a mean excretion level of 3·5 mg per 24 hours (range 1·8 to 6·9). This figure agrees approximately with the mean level of 3·6 mg per 24 hours reported by Baggett, Kinsella and Doisy[34] for five males, but is lower than the values given by Reddy, Jenkins and Thorn,[37] who found that a series of fifteen men and fifteen women excreted an average of 5·8 mg per 24 hours and 3·8 mg per 24 hours, respectively.

The latter workers claim that their method estimates not only 17-hydroxycorticosteroid glucuronides but also other 17-hydroxycorticosteroid conjugates believed to be present in urine.[35,36,37,38] These conjugates have not yet been isolated, but are thought to be sulphates.

The enzyme preparations used in the work reported here were extracted from the visceral hump of the common limpet (*Patella vulgata*) by the method of Stitch and Halkerston[39,40]

TABLE II

RECOVERY OF PURE 17-HYDROXYCORTICOSTEROIDS BY THE PROPOSED PROCEDURE

Experiment	17-Hydroxycorticosteroid			
	Urine specimen	Added, μg	Found, μg	Recovery, %
17-Hydroxy-11-dehydrocorticosterone		20	19·3	96·5
		40	37·1	92·7
		100	89·0	89·0
17-Hydroxycorticosterone		13	11·3	87·0
		26	26·5	102·0
		52	49·6	95·4
Pregnane-3α:17α:21-triol-11:20-dione		15	13·4	89·4
		30	29·5	98·3
		60	57·9	96·5
Urine A	45·3			
Urine A + 17-hydroxycorticosterone	45·3	38	82·0	96·6
	45·3	38	80·0	91·3
Urine B	31·5			
Urine B + 17-hydroxycorticosterone	31·5	52	77·8	89·0
Urine C	56·0			
Urine C + pregnane-3α:17α:21-triol-11:20-dione	56·0	60	113·0	95·0
Urine D	7·0			
Urine D + pregnane-3α:17α:21-triol-11:20-dione	7·0	50	57·0	100·0
Urine E	42·0			
Urine E + pregnane-3α:17α:21-triol-11:20-dione	42·0	50	92·0	100·0

and contained 1 to 1·5 × 10^6 units (Henry and Thevenet[19]) of β-glucuronidase and 8 to 16,000 units of sulphatase (measured by the hydrolysis of dehydro*iso*androsterone sulphate[39,40]) per gram of dried powder. It is not yet known if the steroid alcohol sulphatase plays any part in the hydrolysis of 17-hydroxycorticosteroid conjugates.

The authors are indebted to Dr. de Monchy of Organon, Oss, Holland, for invaluable advice, and wish to acknowledge the generous gift of tetrahydrocortisone from Dr. Dorfman of the Worcester Foundation for Experimental Biology, Shrewsbury. We are grateful to Dr. Reiss for his constant interest and encouragement during the course of the work.

REFERENCES

1. Romanoff, L. P., Wolf, R. S., and Pincus, G., *Fed. Proc.*, 1952, **11**, 131.
2. Romanoff, L. P., Wolf, R. S., Constandse, M., and Pincus, G., *J. Clin. Endocrinol. and Metab.*, 1953, **13**, 928.
3. Zaffaroni, A., Burton, R. B., and Keutmann, E. H., *Science*, 1950, **111**, 6.
4. Burton, R. B., Zaffaroni, A., and Keutmann, E. H., *J. Biol. Chem.*, 1951, **188**, 763.
5. ——, ——, ——, *Ibid.*, 1951, **193**, 769.
6. Zaffaroni, A., and Burton, R. B., *Ibid.*, 1951, **193**, 749.
7. Katzenellenbogen, E. R., Kritchevsky, T. H., and Dobriner, K., *Fed. Proc.*, 1952, **11**, 238.
8. Haines, W. J., "Recent Progress in Hormone Research," Academic Press, New York, 1952, Volume 7, p. 255.
9. Schneider, J. J., *J. Biol. Chem.*, 1950, **183**, 365.
10. ——, *Ibid.*, 1952, **194**, 337.
11. Haines, W. J., Johnson, R. H., Goodwin, M. P., and Kuizenga, M. H., *Ibid.*, 1948, **174**, 925.
12. Porter, C. C., and Silber, R. H., *Ibid.*, 1950, **185**, 201.
13. Nelson, D. H., and Samuels, L. T., *J. Clin. Endocrinol. and Metab.*, 1952, **12**, 519.
14. Bayliss, R. I. S., and Steinbeck, A. W., *Biochem. J.*, 1953, **54**, 523.
15. Glenn, M. E., and Nelson, D. H., *J. Clin. Endocrinol. and Metab.*, 1953, **13**, 911.
16. Morris, C. J. O. R., and Williams, D. C., *Biochem. J.*, 1953, **54**, 470.
17. Butt, W. R., Morris, P., Morris, C. J. O. R., and Williams, D. C., *Ibid.*, 1951, **49**, 434.
18. Heftmann, E., and Johnson, D. F., *Anal. Chem.*, 1954, **26**, 519.
19. Henry, R., and Thevenet, M., *Bull. Soc. Chim. Biol.*, 1952, **34**, 886.

20. Grant, R. A., and Stitch, S. R., *Chem. & Ind.*, March 24th, 1951, No. 12, 230.
21. Gordon, J. J., and Pelly, J. E., *Nature*, 1951, **168**, 603.
22. Saffran, M., Grad, B., and Bayliss, M. J., *Endocrinology*, 1952, **50**, 639.
23. Allen, W. M., *J. Clin. Endocrinol.*, 1950, **10**, 71.
24. Edwards, R. W. H., Kellie, A. E., and Wade, A. P., *in* Eckstein, P., and Zukerman, S., *Editors*, "The Determination of Adrenocortical Steroids and their Metabolites," Dennis Dobson Ltd., London, 1953, p. 53.
25. Bush, I. E., *Biochem. J.*, 1952, **50**, 370.
26. Zaffaroni, A., *J. Amer. Chem. Soc.*, 1950, **72**, 3828.
27. Cope, C. L., and Hurlock, B., *in* Eckstein, P., and Zukerman, S., *Editors, op. cit.*, p. 25.
28. Gray, C. H., and Lunnon, J. B., *in* Eckstein, P., and Zukerman, S., *Editors, op. cit*, p.64.
29. Cox, R. I., and Marrian, G. F., *Biochem. J.*, 1951, **48**, xxxiii.
30. Cohen, S. L., *Fed. Proc.*, 1951, **10**, 173.
31. Venning, E. H., *J. Clin. Endocrinol.*, 1951, **11**, 769.
32. Daughaday, W. H., Farr, A. L., and Houghton, E., *Endocrinology*, 1951, **49**, 146.
33. Kinsella, R. A., jun., and Glick, J. H., jun., *J. Biol. Chem.*, 1953, **203**, 1003.
34. Baggett, B., Kinsella, R. A., jun., and Doisy, E. A., *Ibid.*, 1953, **203**, 1013.
35. Marrian, G. F., *in* Eckstein, P., and Zukerman, S., *Editors, op. cit.*, p. 89.
36. Norymberski, J. K., *in* Eckstein, P., and Zukerman, S., *Editors, op. cit.*, p. 50.
37. Reddy, W. J., Jenkins, D., and Thorn, G. W., *Metabolism*, 1952, **1**, 511.
38. Smith, R. W., Mellinger, R. C., and Patti, A. A., *J. Clin. Endocrinol. and Metab.*, 1954, **14**, 336.
39. Stitch, S. R., and Halkerston, I. D. K., *J. Endocrinology*, 1953, **9**, xxxvi.
40. ——, ——, *Nature*, 1953, **172**, 398.

THE BIOCHEMICAL AND ENDOCRINOLOGICAL RESEARCH DEPARTMENT
BRISTOL MENTAL HOSPITALS
BARROW HOSPITAL
BARROW GURNEY, NR. BRISTOL

September 9th, 1954

An Improved Apparatus for the Determination of Gaseous Elements in Metals by Vacuum Fusion on a Micro Scale

BY J. N. GREGORY AND D. MAPPER

This paper describes some improvements to a vacuum fusion apparatus previously described by the same authors in this journal.

The improvements relate mainly to more efficient pumping and recovery of the evolved gases, and the introduction of a low-pressure method of gas analysis.

The latter has enabled the complete results on a series of samples to be obtained in about half a day, compared with 2 or 3 days by the previous method.

IN a recent paper,[1] we described an apparatus which has been used for the determination of gaseous elements in uranium and steel on a micro scale, at the 10 to 100 p.p.m. level. In an apparatus of this type it is essential to pump the evolved gases away from the high-temperature region as rapidly as possible. It is also necessary to confine the metal and its vapour as much as possible to the region of maximum temperature in the crucible. If these two requirements are not adequately met, losses will occur owing to reaction of the evolved gases with metal deposited on the lower-temperature regions of the crucible and heat shield.

It was found that the pumping rate and the method of confinement of the metal sample to the crucible in the apparatus previously described were not adequate in the case of beryllium, which has a very high vapour pressure at the temperatures required to decompose the oxide. Very high pumping rates are needed with careful confinement of the metal to the crucible to avoid large recombination losses and massive evaporation of the metal to the walls of the silica jacket.

The modifications described in this paper were specifically designed to make the apparatus more efficient for the determination of gases in beryllium, but they will also give more efficient determination of gases in other metals.

The publications by Madley and Strickland-Constable[2] and Ransley[3] drew our attention to the possibility of using low-pressure methods for rapid analysis of the gas, without removal from the apparatus. The method by which this was achieved is described in this paper.

Apart from the modifications described here the operation of the apparatus is exactly as previously given in detail.[1]

Description of new apparatus

Furnace design—

The mechanism for adding samples and the method of measuring temperatures and observing the crucible are exactly as described previously.[1] However, it has been found that the silica funnel should not be brought into direct contact with the graphite crucible

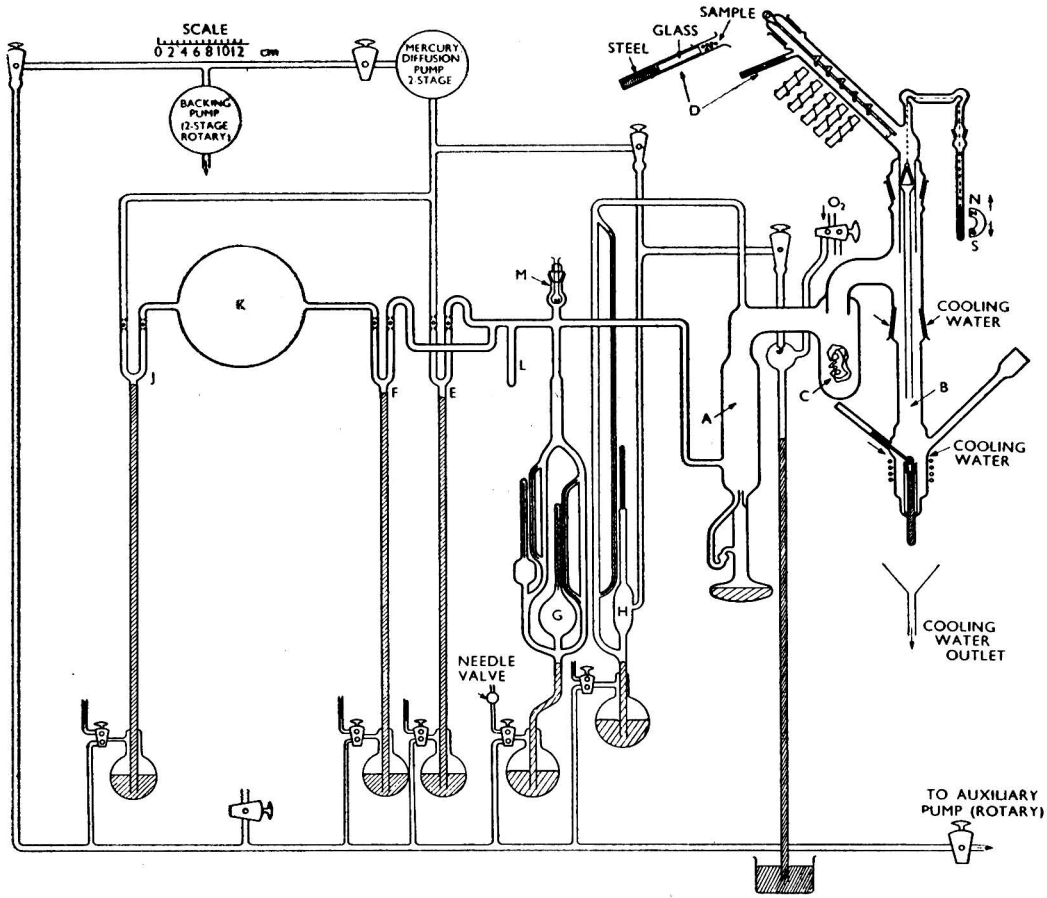


Fig. 1. Micro vacuum fusion apparatus

at any temperature above 1000° C. If the contact is prolonged, there is a risk of formation of carbon monoxide by reduction of the silica. The sample can be readily introduced by having the end of the funnel about 5 mm from the mouth of the crucible at quite high temperatures, without incurring this risk.

An additional sample carrier has been added (D, Fig. 1). This is of considerably greater capacity than the bucket and is used for holding sufficient material for a metal bath (1 to 3 g). It consists of an inclined tube, at the closed end of which is a cylinder of soft iron followed by a cylinder of glass. Both these cylinders are just slightly smaller in diameter than the internal dimension of the glass tube and can slide freely. The pieces of metal which are to constitute the bath are placed in the tube and rest on the upper end of the glass cylinder. The metal is ejected by raising the iron cylinder with a magnet. The glass cylinder

is interposed to permit the addition of magnetic metals by this device. The glass is of such a length that it cannot be ejected completely from the side tube.

The oblique entry crucible described in the previous paper has been replaced by an open crucible fitted with a removable lid. This is shown in Fig. 2. The essential dimensions of the crucible, heat shield and jacket are unchanged except for the top of the crucible. The lid consists of a graphite sphere ($\frac{7}{16}$ inch diameter) and rod ($\frac{1}{4}$ inch diameter) in one piece ($2\frac{1}{2}$ inches in length). A piece of soft iron is fitted to the end of the stem, which can slide in the side tube. The sphere normally seals the top of the crucible, but can be moved away for introducing the sample or temperature measurement by lifting the soft iron in the sleeve with a magnet. The fit between the sleeve and the stem should be such that the lid falls

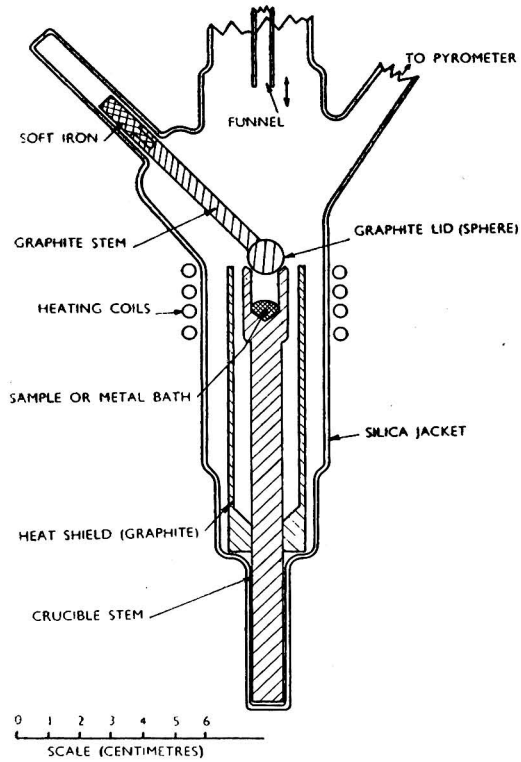


Fig. 2. Graphite crucible, heat shield and silica furnace assembly showing modifications

back on to the crucible when released. To facilitate release of the evolved gases a very small groove is filed in the crucible at one point on and normal to the circle of contact with the lid. During the outgassing process the lid is left on the crucible. It is considered that this design gives a much better control of volatile metals than the previous oblique entry crucible.

PUMPING SYSTEM—

The modifications are shown on Fig. 1, in which the path of the evolved gases is confined to the heavily outlined sections. The main vacuum pump, A, has been moved much closer to the furnace and the pumping path made as clear as possible. The liquid-air trap, the only function of which was to entrap water in the event of the collapse of the silica furnace jacket, B, has been replaced by a modified trap, C, with a similar function, but with less pumping resistance. This trap contains gold foil to prevent mercury from entering the furnace. The mercury cut-off between the pump and the furnace has also been removed. This limits slightly the flexibility of the apparatus, but the loss is justified in terms of increased pumping speed.

GAS COLLECTION AND MEASUREMENT—

The gas is collected, as before, in the volume cut off by the pump, A, and the cut-offs, E and F, and measured by the pressure rise indicated by the McLeod gauge, G.

To accommodate larger gas volumes within the range of the McLeod gauge and to prevent excessive backing pressures on the pump, A, a larger collecting volume may be used. This is achieved by opening F and closing J, by which the volume is increased by about 1 litre. Calibration of the system is carried out by the introduction of metered volumes of gas via H as before.

GAS ANALYSIS—

The most marked improvement in the apparatus is in the method of gas analysis. This modification has removed the necessity of collecting the gas at atmospheric pressure or in a breaker-seal tube and thereby eliminates the Toepler pump and one diffusion pump.

The gas is now analysed within the smaller collection volume by a fractional-condensation method combined with oxidation on a platinum filament. This method operates more efficiently at relatively low pressures (within the range of 0 to 0·10 mm on the McLeod gauge) and the optimum sample for analysis is that which gives a pressure of 0·025 to 0·05 mm in the smaller collecting volume, *i.e.*, 10 to 20 μl. However, gas samples as small as 3 to 5 μl can be analysed in this system. If the gas collected is larger in amount than the optimum, the excess can be removed by expansion into the evacuated bulb, K, and then re-closing F, or by trapping some of the gas in the bulbs of the McLeod gauge and pumping the rest of the sample away through E. It is obvious that many combinations of these manipulations can be used to reduce the volume by various fractions and they can be used successively when large amounts are involved. It can usually be arranged to reserve some of the excess of gas in K for a subsequent duplicate analysis, if necessary, for this gas may be diffused back through F after evacuation of the analysis volume via E. When a sample of the gas giving a pressure not more than 0·05 mm in the smaller collecting volume has been isolated, the analysis is commenced. Except on very rare occasions the gas contains only carbon monoxide, hydrogen, nitrogen and occasionally carbon dioxide. The latter is seldom present in more than a few per cent.

The first operation in the analysis is to measure accurately the initial pressure, a. A liquid-nitrogen bath is then placed on the cold finger (L, Fig. 1) and the pressure, b, again measured, the reduction being due to carbon dioxide (water if present is also condensed, but may be differentiated by a solid carbon dioxide - acetone bath). With the liquid nitrogen still on the cold finger, a measured excess of pure oxygen is added via H. A volume equal to the original sample gives a satisfactory excess. The total pressure, c, is then measured. The platinum filament is now heated until it just glows visibly in the dark. Within about 30 seconds the hydrogen and carbon monoxide are oxidised and the products condensed in the cold finger. The new pressure, d, is read on the McLeod gauge. The liquid nitrogen is then replaced by a solid carbon dioxide - acetone bath (—78° C), which condenses the water alone. A further pressure, e, is measured. Each pressure is measured twice with about a 1-minute interval to check completion of the reaction or absorption.

The insertion of the cold traps causes reduction in pressure due to cooling of the uncondensed gas in the finger, and all pressures measured with the traps in position must be corrected accordingly. It was found that a correction factor of +10 per cent. was necessary with the liquid-nitrogen trap and +3 per cent. with the carbon dioxide trap in position. This correction factor can be easily determined by measuring the pressure of a permanent gas with and without the cold trap. No correction for ambient temperatures is necessary unless there is a variation of more than 2° C during the 10 to 15 minutes required for the analysis.

It can be shown very easily that the five pressure measurements, a, b, c, d and e, are sufficient to give a complete analysis of the gas. That is—

$$\begin{aligned}
&\text{Carbon dioxide, per cent. v/v} &&= 100\,(a-b)/a\\
&\text{Carbon monoxide, per cent. v/v} &&= 100\,(e-d)/a\\
&\text{Hydrogen, per cent. v/v} &&= 100\,(\tfrac{2}{3}c+\tfrac{1}{3}d-e)/a\\
&\text{Nitrogen, per cent. v/v} &&= 100\,(\tfrac{2}{3}d+b-\tfrac{2}{3}c)/a.
\end{aligned}$$

The nitrogen determination is by difference, but many analyses by mass spectrograph have confirmed that other unreactive gases are extremely unlikely to occur in the nitrogen fraction.

This method of analysis has very greatly improved the speed and simplicity of the micro vacuum fusion method. Previously, the gases were analysed by mass spectrograph or the rather tedious Blacet - Leighton method. Both methods involved a delay of at least a day between evolution and analysis, and the external handling of the gas incurred a considerable risk of loss or error due to accident. The gas from one sample is now analysed and the estimation carried to completion before the next sample is added to the crucible. The furnace is usually shut down or run at a low temperature during the analysis to avoid errors due to accumulation of blank gases.

The low-pressure method of analysis is particularly suited for the micro vacuum fusion system as it requires only one stage in the compression of the evolved gas and this compression arises automatically from the necessity of maintaining a high vacuum in the furnace system. Also, the nature of the gases produced enables very simple condensation absorptions to be used and no chemical absorbents are required. The latter can produce outgassing problems and are more difficult to manipulate.

As the low-pressure methods are quite standard practice, it was only necessary to do a few tests on gases of known composition to ascertain that the method was functioning satisfactorily in this apparatus. Analyses were carried out on pure hydrogen, pure carbon monoxide and a mixture of both. The hydrogen was commercial cylinder gas, passed through a red-hot silica tube to convert oxygen to water, and then through Carbosorb and Anhydrone. The carbon monoxide was produced from sodium formate and sulphuric acid and was passed through Carbosorb and Anhydrone.

The results of these tests are given in Table I. The samples were also analysed for carbon dioxide, water and nitrogen and the result was zero in every case. Our experience

TABLE I

ANALYSIS OF GASES WITH MODIFIED APPARATUS

Gas	Volume taken for analysis, μl (S.T.P.)	Volume recovered by analysis, μl (S.T.P.)	Recovery, %
Hydrogen	15·0	15·0 $\pm$ 0·4*	100 $\pm$ 3
Carbon monoxide	17·2	17·2 $\pm$ 0·4*	100 $\pm$ 2
Carbon monoxide and hydrogen mixture	16·0 (CO_2)	16·0 $\pm$ 0·4*	100 $\pm$ 2·5
	5·8 (H)	5·8 $\pm$ 0·4*	100 $\pm$ 7

* Estimated probable experimental error.

with a large number of analyses shows that the accuracy of the method depends more on the sensitivity of the McLeod gauge than on any other factor. Over the range 10 to 25 μl it is estimated that the volume can be measured to approximately $\pm 0{\cdot}4$ μl, so that the percentage error depends on the amount of each gas present. Owing to the increased sensitivity of the McLeod gauge at lower pressures, the volumetric accuracy is probably better than $\pm 0{\cdot}1$ μl when quantities of sample of the order of 1 to 5 μl are being handled.

The precision of this method is definitely superior to mass-spectrograph analysis as applied in the previous paper[1] and is at least equal to that of the Blacet - Leighton method, with considerably increased speed, easier manipulation and less risk of extraneous errors.

CONCLUSIONS

The modifications described in this apparatus, particularly the analysis method, have given very considerable advantages in speed and efficiency. With the older methods of analysis,[1] the lag between the collection of the gas and the reporting of the analysis was often 3 to 4 days or more and further estimations had to be commenced before the previous results were known. This made development work particularly difficult and interrupted the continuity of use of the apparatus. With the new system the analysis is only a small interruption (about 10 to 15 minutes) in the sequence of operations, and the complete analysis for the gaseous elements in a series of five metal samples is carried through in the one apparatus by one operator in about 3 hours (excluding the graphite outgassing, which takes up to 6 to 8 hours).

The authors wish to thank Mr. A. A. Smales for his interest and encouragement in this project. They also wish to thank the Director, A.E.R.E., Harwell, for permission to publish this paper.

REFERENCES

1. Gregory, J. N., Mapper, D., and Woodward, J. A., *Analyst*, 1953, **78**, 414.
2. Madley, D. G., and Strickland-Constable, R. F., *Ibid.*, 1953, **78**, 122.
3. Ransley, C. E., *Ibid.*, 1947, **72**, 504.

ANALYTICAL CHEMISTRY GROUP
ATOMIC ENERGY RESEARCH ESTABLISHMENT
HARWELL, NR. DIDCOT, BERKS.

August 23rd, 1954

The Determination of Oxygen in Beryllium by the Vacuum Fusion Method on a Micro Scale

With a Note on the Determination of Oxygen in Zirconium

BY J. N. GREGORY AND D. MAPPER

It is shown that the oxygen present as impurity in beryllium metal can be determined by means of the micro-scale vacuum fusion apparatus, by using samples of 2 to 10 mg containing as little as 0·2 per cent. of oxygen, provided that a rigorous experimental procedure is followed. This consists essentially of exact temperature control and the use of a molten-platinum bath in the graphite crucible.

The factors determining the optimum conditions are discussed fully and the difficulties involved in working with beryllium are stressed. The results obtained for different types of beryllium and beryllium "spiked" with beryllium oxide are given, and thus the degree of accuracy possible with such very small samples is shown.

Hydrogen can also be determined by this method.

THE possible use of beryllium as a reactor structural material has been reviewed by Udy, Shaw and Boulger.[1] Since investigations have shown that its ductility[2] and other properties may be adversely affected by the presence of oxygen, a suitable analytical method for the determination of 1 per cent. or less of oxygen in beryllium metal was required. Numerous methods of attacking this problem are being investigated, but, with the exception of one specialised approach, these have not yet led to any routine method. The specialised technique of radioactivation has been described by Osmond and Smales,[3] who have also listed the various other methods being applied and tentatively compared the results so far obtained. The present authors, having designed a micro vacuum fusion apparatus for the determination of trace gases in steel and uranium,[4] were led to consider the application of this method to the problem of oxygen in beryllium.

In principle, a micro-scale vacuum fusion method for oxygen in beryllium should not differ essentially from that previously described.[4] However, certain properties of beryllium, such as the high volatility of the metal and the very refractory nature of the oxide, made necessary a number of major modifications before the apparatus was suitable for this metal. These modifications, together with changes in the method of analysis of the evolved gases, have considerably improved the apparatus as a whole and have been fully described in another paper.[5] The basic principles involved in micro-scale vacuum fusion methods have been considered by the authors in their earlier paper,[4] and their application to the determination of the oxygen content of beryllium will be dealt with below.

CONDITIONS FOR THE DETERMINATION OF OXYGEN IN BERYLLIUM—

It has been established that at a temperature of 1860° C the oxygen present, as beryllium oxide or otherwise, in beryllium metal can be evolved quantitatively as carbon monoxide, provided that the graphite crucible is covered by a lid and contains a molten-platinum bath in which the ratio of platinum to beryllium is not less than 50 to 1 by weight and that

the total amount of beryllium present is not allowed to exceed 70 to 80 mg. The platinum can be readily recovered by chemical separation. The experimental work and theoretical considerations which led finally to these optimum conditions will be treated later, but a general survey here of the factors determining these optimum conditions will illustrate the nature of the difficulties which had to be overcome. The most recently reported figure for the melting point of beryllium is 1315° ± 20° C, its boiling point (extrapolated) is 2970° C and the vapour pressure at 1950° C is as high as 10^{-2} atmospheres. On the other hand, in the graphite reduction of refractory beryllium oxide a very high temperature is required, and we have shown experimentally that such a reaction takes place only slowly at 2000° C. It is evident that extensive evaporation of beryllium will also occur at this temperature, and since in all vacuum fusion methods it is essential to keep volatilisation of the metal to a minimum, some means must be found of reconciling these conflicting requirements. A platinum bath has been found satisfactory for lowering the temperature required for rapid reduction of oxide and minimising the evaporation of beryllium at the operating temperature. More conventional metals, such as iron, normally used as metal baths, are too volatile at the high temperatures required.

Experimental and Results

The development of a vacuum fusion method for the determination of oxygen in beryllium suffers a great disadvantage in that no reliable standard or accurately analysed metal is available. Accordingly, the problem was tackled in several stages—

(*a*) By the use of accurately weighed micro-samples of beryllium oxide the conditions under which oxygen could be quantitatively freed from beryllium were obtained.

(*b*) A large number of analyses were carried out on samples of metal, the oxygen content of which had been determined by other methods. The figures given by these methods, although not consistent, indicated the approximate oxygen content. Two samples were used in this work, a powder containing about 0·38 per cent. of oxygen and a flake beryllium containing about 1·2 per cent. of oxygen.

(*c*) When a large number of analyses had shown reasonably consistent results for the powder sample, it was "spiked" with known quantities of beryllium oxide incorporated by careful grinding, and a number of analyses were carried out on the mixture.

Attempts to prepare standards by introducing known quantities of oxygen into beryllium by oxidation at elevated temperatures, as was done previously with uranium,[4] proved unsuccessful. At low temperatures, less than 400° C, no appreciable oxidation occurred, and at higher temperatures no container material could be found which did not also absorb large quantities of oxygen.

Beryllium oxide experiments—

Very small pieces of hard-fired beryllium oxide were weighed accurately on a microbalance and loaded in the apparatus in the normal way with a 2·5-g bath of platinum. The carbon monoxide evolved was determined by the low-pressure combustion method, already described in detail.[5] Although the experiments were conducted at a temperature of 1900° or 1950° C, it was confirmed later that the reduction of beryllium oxide is also quantitative

Table I

Recovery of carbon monoxide from beryllium oxide

Weight, mg	Temperature, °C	Reaction time, minutes	Carbon monoxide evolved, μl at S.T.P.	Carbon monoxide expected, μl at S.T.P.	Recovery, %
0·596	1950	9	556	533	104
0·468	1900	4	415	419	99
0·456	1900	6·5	415	409	101
0·487	1900	7	477	436	109
0·860	1900	5	791	771	103
0·713	1900	4	670	640	105

Average = 103 ± 5 per cent.

and sufficiently rapid at 1860° C. The quantities of gas evolved with the smallest weighable beryllium oxide samples are, of course, far in excess of those normally dealt with on the micro-apparatus, but the apparatus is constructed to cope with such large quantities, should they arise. The results obtained are given in Table I.

As one of the most serious errors in this method is caused by chemisorption of carbon monoxide on evaporated films of metal, this experiment was repeated with 40 mg of beryllium previously added to the crucible. The beryllium was heated for 20 minutes at 1900° C before addition of the oxide samples. The results are given in Table II.

TABLE II

RECOVERY OF CARBON MONOXIDE FROM BERYLLIUM OXIDE IN PRESENCE OF EVAPORATED BERYLLIUM

Weight, mg	Temperature, ° C	Time, minutes	Carbon monoxide evolved, μl at S.T.P.	Carbon monoxide expected, μl at S.T.P.	Recovery, %
0·612	1950	7	530	548	97
0·703	1950	6	587	630	93
0·711	1950	6	619	637	97
0·566	1950	9	475	507	94

Average = 95 ± 5 per cent.

These recoveries are obtained under conditions of beryllium evaporation considerably more severe than those experienced in an actual determination. Even under these conditions the decrease in recovery when excess of beryllium is present is only just significant.

ANALYSES OF POWDERED AND FLAKE BERYLLIUM—

Small single pieces are the best type of sample for vacuum fusion analysis, but powdered samples can be analysed after pelleting to a suitable size in a cold press. The pellets are sufficiently stable for handling and weighing, but occasionally the large samples disintegrate before melting when added to the hot crucible. The samples were analysed under the conditions described above, and Table III shows the results for the powdered beryllium. Series A, B and C are separate "runs."

TABLE III

OXYGEN CONTENT OF POWDERED BERYLLIUM SAMPLES

Series	Sample weight, mg	Oxygen found, %
A	10·2	0·51
	8·8	0·39
	10·3	0·43
	11·5	0·22
	10·9	0·25
B	8·2	0·50
	6·9	0·21
	6·2	0·19
	8·3	0·27
	11·8	0·26
C	5·3	0·49
	5·4	0·48
	7·9	0·22
	3·3	0·43
	3·6	0·42

Average = 0·35 per cent.; σ = 0·12

Some results for flake beryllium are given in Table IV. Series F and G refer to the samples obtained from a piece of solid beryllium which has been sintered from the powder at a high temperature. Consequently, the possibility of further oxidation of the metal

taking place had to be considered. This is seen to be the case, when these results are compared with series H, which were obtained on the untreated flake beryllium.

TABLE IV

OXYGEN CONTENT OF FLAKE BERYLLIUM

Series	Sample weight, mg	Oxygen found, %
F	4·6	1·57
	2·9	1·61
	5·1	1·50
	3·6	1·52
G	2·5	1·72
	2·4	1·89
	2·3	1·90
	1·6	1·61
		Average = 1·67 per cent.; σ = 0·15
H	11·2	1·19
	10·6	0·91
	3·4	1·11
		Average = 1·07 per cent.

POWDERED BERYLLIUM SPIKED WITH BERYLLIUM OXIDE—

Mixtures of powdered beryllium (oxygen, 0·35 per cent.; see Table III) and beryllium oxide were prepared by intimate mixing and grinding so that the oxygen content was increased by exactly 1·5 per cent.

Samples of this mixture were pelleted and analysed for oxygen and the results are given in Table V.

TABLE V

OXYGEN CONTENT OF POWDERED BERYLLIUM SPIKED WITH 1·5 PER CENT. OF OXYGEN

Series	Sample weight, mg	Spike oxygen, %	Total oxygen found, %	Spike oxygen found, %
D	13·3	0	0·34	−0·01
	10·5	1·5	1·89	1·54
	10·9	1·5	1·84	1·49
E	5·9	1·5	1·92	1·57
	8·6	1·5	2·14	1·79
	6·5	1·5	1·65	1·30
	5·6	1·5	1·66	1·31

The last column is obtained by deducting the average oxygen content shown in Table III from the total found. The average of this column is 1·50 per cent. (σ = 0·17).

By using the experimental technique described in this paper and provided the sample weights are not too large (for discussion of this point, see later), we have obtained no evidence that the scatter of results shown in Tables III, IV and V is due to any other factor than inhomogeneity of the beryllium samples. This is borne out, too, by the results in Table V for mixtures of powdered beryllium and beryllium oxide. On the scale on which these experiments were conducted, this scatter is not surprising; it could only be overcome by taking large samples, which is quite impossible with beryllium, the volatility of the metal being too great for reliable results.

An outstanding feature of the micro-scale experiments was the rapid evolution of gas at 1860° C, such that the actual heating time at the operating temperature was only 3 to 4 minutes, with practically all the gas evolved in the first minute. Blanks performed before and after the addition of each beryllium sample showed that all the gas was evolved quantitatively, and the results of each series show that there was no progressive fall of oxygen content with each added sample, so that at the beryllium to platinum ratios used in these

experiments, losses due to chemisorption were negligible. It must be emphasised that this ratio is the determining factor in the number of samples that can be analysed. Should this become too great the experiment must be stopped, the furnace tube cleaned out, and the apparatus re-assembled with a new crucible and fresh platinum. Platinum is easily outgassed at the operating temperature (1860° C) prior to the addition of the beryllium samples. At the end of the experiment it is readily recoverable with little loss.

The temperature control is important and should be maintained at 1860° $\pm$ 20° C during the whole experiment. Once beryllium has been added to the crucible, the temperature must on no account be allowed to rise above the operating value.

The present apparatus is designed to hold five samples, and with an oxygen content of 0·2 to 1·0 per cent., sample weights of 2 to 10 mg can be used and the samples analysed consecutively. The total time required for the whole experiment after the outgassing has been completed, including the gas analysis, is 2 to 3 hours. It is obviously desirable to work with samples as small as possible and it is in this respect that the micro-scale vacuum fusion apparatus is eminently suitable, as it is particularly designed to deal with volumes of gas down to 5 to 10 μl.

The metal bath—

Attempts were made initially to eliminate chemisorption losses by first distilling the beryllium from the crucible at a moderately high temperature on to a graphite or silica condenser, which could then be withdrawn from the region of the heated crucible, thus permitting the oxide to be later reduced in a "beryllium-free atmosphere." However, no simple method could be found of doing this satisfactorily. Attention was then directed to the use of a molten-metal bath, which in our earlier work with uranium[4] had proved unnecessary, and platinum proved most satisfactory in this respect. Its inert nature and low vapour pressure at the operating temperature of 1860° C were important factors.

Some thermodynamic considerations will prove instructive here in illustrating the advantages of the platinum bath. Following the procedure of Sloman and Harvey,[6] only the simplest mechanism will be considered here, namely—

$$BeO_{solid} + C_{solid} = CO_{gas} + Be_{liquid}$$

but it must be emphasised that carbide formation should also be taken into consideration. By ignoring the presence of a molten-metal bath and by using the free-energy equations for this reaction as tabulated by Kubaschewski and Evans,[7] who give full details of the calculations, it is easily found that the pressure of carbon monoxide in equilibrium with beryllium oxide and graphite at 1860° C is $3{\cdot}2 \times 10^{-3}$ mm of mercury. This is quite small and, whilst purely thermodynamic considerations can give no information concerning the rate of the reaction, the pressure is very much less than that required for satisfactory vacuum fusion analysis, and it has been confirmed experimentally that the rate of reduction of beryllium oxide by carbon alone is indeed too slow for the vacuum fusion method. The use of a molten-platinum bath, as has been stated, enormously speeds up this reaction, and it is obvious that further large favourable free-energy changes must be involved in the solution of the beryllium in the molten platinum, which most probably functions here also as a medium or solvent for bringing the oxide and graphite into intimate contact. The lowering of the activity of the metallic beryllium by its solution in platinum greatly reduces its volatility and a marked reduction of evaporation is noticed. Provided, therefore, a large excess of platinum is present, the reaction proceeds smoothly and quickly.

Presence of other gases

Hydrogen has been consistently found in all samples of beryllium analysed. The determination of hydrogen presents no difficulties, as it is evolved at a very much lower temperature. Some carbon monoxide was also present with the hydrogen evolved at these lower temperatures. This probably arises from adsorbed water or hydrated oxide.

In the powdered beryllium the average hydrogen content for 34 samples was 0·015 per cent. ($\sigma = 0{\cdot}006$); in one type of flake beryllium the average was 0·022 per cent. ($\sigma = 0{\cdot}010$) for 18 samples, and in another type 0·042 per cent. ($\sigma = 0{\cdot}020$) for 8 samples.

Nitrogen was not found to be present in any of the beryllium samples analysed.

Determination of oxygen in zirconium

It was reported in our previous paper[4] that attempts to analyse for oxygen and nitrogen in zirconium were unsuccessful, and it seemed desirable to repeat this investigation with the modified apparatus and similar experimental conditions which had proved satisfactory in the case of beryllium. By adopting a precisely similar technique to that described for the experiments with beryllium oxide, it was shown that zirconium oxide could be rapidly decomposed (comparable times to those for beryllium oxide) by using a platinum bath at a temperature of 1860° C, and the carbon monoxide was evolved quantitatively, so giving an average recovery of 103 $\pm$ 5 per cent. It seems that oxygen could therefore be readily determined in zirconium under these experimental conditions.

Conclusions

The results have shown that the analysis of small quantities of oxygen and hydrogen in beryllium can be carried out satisfactorily on the micro scale, provided rigorous control is made of the experimental conditions. These conditions have been specified exactly and present no difficulty. The accuracy of the method is difficult to assess owing to the absence of any satisfactory standards, but there is no reason to think it is inferior to other methods of analysis.

The micro vacuum fusion method has the following particular advantages—

(*i*) Small samples only are required. This is convenient, among other aspects, for safety and health precautions.

(*ii*) A positive approach is adopted, *i.e.*, the oxygen is being directly determined, unlike the other methods (except radioactivation) where it is estimated after everything else has been removed.

(*iii*) It is suitable for both powders and solids.

(*iv*) The analysis is very rapid. Five samples can be analysed completely in about two working days.

APPENDIX

Experimental procedure

Since detailed instructions of operation have already been given,[4,5] it will only be necessary here to outline the experimental procedure and emphasise the salient features. The weighed samples are loaded into the buckets and 2·5 g of platinum cut into small pieces from suitable thick rod are placed in the side-arm. After the graphite crucible and heat shield have been outgassed, the crucible lid is raised, the funnel lowered and the platinum added carefully. A blank at 1860° C is carried out, after about 30 minutes outgassing at 2000° C, and if it is less than 0·5 μl per minute the beryllium is ready to be added. The induction heater is turned off, the lid removed from the crucible, the funnel lowered into position and the first sample added to the cooled crucible. The funnel is then raised, the lid replaced and heater turned to the pre-determined setting so that the temperature is 1860° C. The evolved gases are collected, and after about 4 minutes, when gas evolution has ceased, the heater is turned off and the gases are analysed. A similar procedure is followed for the next four samples. A Pirani gauge has been fitted into the gas-collecting volume so that the point when gas evolution has ceased is indicated immediately, without the necessity of frequent manipulation of the McLeod gauge, which is only used for the final absolute pressure measurement.

The authors wish to thank Mr. A. A. Smales for his interest and valued advice in this project, and Dr. T. L. Johnston of Metallurgy Division for his assistance in the preparation of the beryllium samples. Thanks are also due to the Director, A.E.R.E., for permission to publish this paper.

References

1. Udy, M. C., Shaw, H. L., and Boulger, F. W., *Nucleonics*, **1953**, **11**, 1125.
2. Pearsall, C. S., MIT, 1104, U.S.A.E.C., 1952.
3. Osmond, R. G., and Smales, A. A., *Anal. Chim. Acta*, **1954**, **10**, 117.

4. Gregory, J. N., Mapper, D., and Woodward, J. A., *Analyst*, **1953**, **78**, 414.
5. Gregory, J. N., and Mapper, D., *Ibid.*, 1955, **80**, 225.
6. Sloman, H. A., and Harvey, C. A., *J. Inst. Metals*, 1952, **80**, 391.
7. Kubaschewski, O., and Evans, E. Ll., "Metallurgical Thermochemistry," Butterworth - Springer Ltd., London, 1951, p. 305.

ANALYTICAL CHEMISTRY GROUP
ATOMIC ENERGY RESEARCH ESTABLISHMENT
HARWELL, NR. DIDCOT, BERKS.

August 23rd, 1954

Notes

POLAROGRAPHIC DETERMINATION OF MAGNESIUM IN COAL ASH AND COKE ASH

THE methods described in Fuel Research Survey Paper No. 50[1] for the determination of the major constituents of an ash are time-consuming and require relatively large amounts of sample. The advent of modern apparatus, such as the flame photometer and polarograph, has led to the development of new methods designed to shorten the time and reduce the amount of sample necessary for an analysis. Thus sodium and potassium[2] and calcium[3] can be determined with the flame photometer by using samples of only 0·1 g.

Methods for the indirect polarographic determination of magnesium have been described[4,5,6] and that of Stone and Furman[5] has been applied by the present authors to the determination of magnesium in coal ash and coke ash.

METHOD

APPARATUS—

A Cambridge pen-recording polarograph with a cell bath thermostatically controlled at 25° ± 0·1° C. The capillary drop time was 2·6 seconds in 0·1 *M* potassium chloride solution.

REAGENTS—

Sulphuric acid, 20 per cent. v/v.

Hydrofluoric acid, 40 per cent. w/v—AnalaR.

Ammonium hydroxide, sp.gr. 0·880—Laboratory-reagent grade.

Ammonium hydroxide - ammonium chloride buffer solution—Adjust the pH of a 0·036 *M* solution of ammonium chloride to 10 by the addition of ammonium hydroxide, sp.gr. 0·880, using a pH meter. The final solution is approximately 0·25 *M* in ammonium hydroxide.

Standard oxine solution—Dissolve 0·5 g of AnalaR oxine (recrystallised from 40 per cent. ethanol) in 1 litre of 5 per cent. ethanol.

Standard magnesium solution, approximately 100 μg of MgO per ml—Dissolve the calculated weight of magnesium in the minimum of dilute hydrochloric acid and dilute with water to the required value. Determine the actual concentration of magnesium by precipitation as magnesium ammonium phosphate with subsequent ignition to magnesium pyrophosphate.

PROCEDURE—

Solution of ash and removal of group 3A metals—Follow the procedure given by Edgcombe and Hewett,[3] finishing with 50 ml of solution.

Removal of manganese and platinum—Measure, by pipette, 10 ml of the above solution into a small silica crucible, add a small crystal (about 2 mg) of hydroxylamine hydrochloride and evaporate to dryness. Heat to 450° to 500° C during 30 minutes and maintain at this temperature for 1 hour. Cool, extract by boiling with 5 ml of water for a few minutes and cool. Filtration is not necessary.

Determination of magnesium—Wash the aqueous extract above into a 25-ml calibrated flask containing 10 ml of the buffer solution and 5 ml of the standard oxine solution and make up to the mark with distilled water. Mix thoroughly and allow to stand with frequent shaking for 2 hours. At the end of this period transfer a suitable quantity of the suspension to the polarographic cell, place the cell in the bath, maintained at 25° C, and pass nitrogen (to remove oxygen) for 15 minutes. Make the polarogram at sensitivity 1/15, damping position 3, from −1·0 volt to −1·6 volts against a mercury-pool anode. Measure the wave height recorded. Prepare a calibration curve

by making polarograms in the manner described above, using the requisite amounts of the standard magnesium solution. Carry out periodically blank determinations on the standard oxine solution.

Calculation of results—If x = μg of MgO in the solution, then—

$$\text{Magnesium oxide content, per cent.} = \frac{x \times 2{\cdot}5}{\text{sample weight in mg}}.$$

Results

Seven ashes of known composition were examined by this procedure and the results are shown in Table I. Confirmation of the non-interference of calcium was obtained by determining the magnesium polarographically in the filtrates obtained after precipitation of calcium as oxalate by the normal method.

Table I

Determination of magnesium in coal ash and coke ash

	MgO found by		
CaO present, %	Normal gravimetric method, %	Polarographic method, %	Polarographic method on filtrate from calcium oxalate precipitation, %
11·1	0·8	0·8	0·8
7·8	1·2	1·4	1·4
3·1	1·8	1·8	1·9
6·0	3·2	3·2	3·4
12·1	3·4	3·5	3·5
—	4·1	4·1	4·1
14·9	5·7	5·9	5·5

The results show that the polarographic method is as accurate as the normal gravimetric method for amounts of magnesium oxide up to 5 per cent. and that the presence of up to 15 per cent. of calcium as oxide is without effect on the result.

This work forms part of the programme of the Fuel Research Board of the Department of Scientific and Industrial Research and is published by permission of the Director of Fuel Research.

References

1. "Improved Methods for the Quantitative Analysis of Coal Ash and Coke Ash," Fuel Research Survey Paper No. 50, H.M. Stationery Office, London, 1949.
2. Collins, G. C., and Polkinhorne, H., *Analyst*, 1952, **77**, 430.
3. Edgcombe, L. J., and Hewett, D. R., *Ibid.*, 1954, **79**, 755.
4. Carruthers, C., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 412.
5. Stone, K. G., and Furman, N. H., *Ibid.*, 1944, **16**, 596.
6. Kolthoff, I. M., and Lingane, J. J., "Polarography," Interscience Publishers Inc., New York and London, 1952, Volume II, p. 430.

L. J. Edgcombe
G. R. E. C. Gregory

Fuel Research Station
Blackwall Lane
Greenwich, London, S.E.10

First submitted, *May 13th*, 1954
Amended, *October 22nd*, 1954

DETERMINATION OF DIBUTYL PHTHALATE IN PROPELLANTS

The direct determination of dibutyl phthalate in propellants containing both nitrocellulose and nitroglycerine was previously carried out in this laboratory by using the method developed by Lamond.[1] In this method dibutyl phthalate is separated from nitroglycerine by decomposing the latter with ammonium sulphide. The dibutyl phthalate is then extracted with light petroleum and determined by saponification.

Two disadvantages arise from the use of ammonium sulphide as the reagent for destroying nitroglycerine. In the first place it is unpleasant to handle. Secondly, sulphur is dissolved in the light petroleum and has to be removed before saponifying the dibutyl phthalate. This involves its precipitation and separation by filtration as mercuric sulphide, and considerably prolongs the time required to complete the determination.

If the ammonium sulphide could be replaced by an alternative reagent, these disadvantages might be overcome, and the method made more suitable for routine production control. It was with this in view that the work described in this paper was undertaken.

Experimental

Dibutyl phthalate and nitroglycerine could be readily extracted from nitrocellulose with ethyl ether. The only other ether-soluble ingredient common in propellants of the type dealt with in this report was carbamite (diethyldiphenylurea). As this compound did not interfere with the saponification of dibutyl phthalate, it was not necessary to remove it. The problem was to find a suitable reagent that would remove nitroglycerine and leave the dibutyl phthalate in a condition in which it could be readily determined by saponification.

Dickson and Easterbrook[2] have described a method for destroying nitroglycerine in the presence of nitro compounds by treatment with ferrous chloride in methanolic solution. Experiments were carried out to see if this reagent could be used instead of ammonium sulphide to destroy nitroglycerine in the presence of dibutyl phthalate.

The composition of the particular propellant concerned was such that a convenient sample contained 1 g of nitroglycerine. According to Dickson and Easterbrook, 1 g of nitroglycerine required 25 ml of saturated ferrous chloride solution for its destruction in 40 ml of methanol and 25 ml of concentrated hydrochloric acid. The reaction was to proceed at room temperature for 24 hours.

Synthetic mixtures were prepared containing nitroglycerine and dibutyl phthalate in the proportions encountered in the course of a determination. These mixtures were treated with the reagents prescribed above. As the reaction time of 24 hours would be far too long for the purpose of production control, the mixtures were heated under reflux on a hot-plate for 1 hour instead.

The dibutyl phthalate was extracted with ethyl ether from the ferrous chloride solution. The ethyl ether solution was washed with water to remove traces of iron and the solvent removed in a current of air. The residual ester was saponified with ethanolic potassium hydroxide.

The results obtained were generally low, which suggested that some dibutyl phthalate was undergoing acid hydrolysis in the ferrous chloride solution. A small loss of dibutyl phthalate was also suspected in the water washings of the ether extract. A few results were too high, and it was noticed that these high results coincided with the formation of a red colour in the ether extract of the ferrous chloride solution. This red colour was attributed to the formation of an ether-soluble iron complex compound with the decomposition products of nitroglycerine. Iron was thus introduced into the saponification stage and precipitated as hydroxide, using up some of the alcoholic potassium hydroxide and giving a saponification figure which was too high.

Further experiments showed that by reducing the acidity of the ferrous chloride solution from 2·8 *N* to 1·5 *N*, the loss of dibutyl phthalate through acid hydrolysis was eliminated. By using a 5 per cent. solution of potassium sulphate instead of water as the wash liquid, good recovery of dibutyl phthalate was achieved. It was also found that an increased methanol to water ratio prevented the occasional formation of the red colour in the ether extract, provided that the period of heating under reflux was kept to a minimum. This period varied with the temperature of the hot-plate, but ¾ hour was usually sufficient.

Method

On the basis of the experiments outlined above, the following method was developed for determining dibutyl phthalate in the ethyl ether extract of propellants containing both nitrocellulose and nitroglycerine.

Reagents—

Ferrous chloride, anhydrous.

Potassium hydroxide, 0·4 N ethanolic solution—This should contain about 10 per cent. of water, which will prevent precipitation of the potassium phthalate formed in the reaction.

Hydrochloric acid, 0·1 N.

Potassium sulphate, 5 per cent. w/v aqueous solution.

Procedure—

Weigh out sufficient of the finely ground cordite sample to contain 1 g of nitroglycerine into a sintered-glass crucible of grade 3 porosity, and extract for 4 hours with pure ethyl ether. In current compositions this will provide an extract containing about 1 g of nitroglycerine, 0·2 g of

dibutyl phthalate and 0·3 g of carbamite. Transfer the extract to a 250-ml conical flask, remove the ethyl ether in a current of dry air and add 10 g of anhydrous ferrous chloride, 60 ml of methanol, 15 ml of concentrated hydrochloric acid and 15 ml of distilled water. Heat under reflux on the sand-bath for ¾ hour, cool and dilute with 100 ml of distilled water. Extract the dibutyl phthalate by shaking for 3 minutes with 150 ml of ethyl ether in a separating funnel. Wash the ethereal layer first with 100 ml then with 50 ml of 5 per cent. potassium sulphate solution. Transfer the extract to a 250-ml conical flask and remove the solvent in a current of dry air. Add exactly 10 ml of 0·4 *N* ethanolic potassium hydroxide from a burette and heat under reflux for ¾ hour on the sand-bath. Dilute with 50 ml of distilled water and titrate with 0·1 *N* hydrochloric acid, using thymol blue as indicator. The end-point occurs when the blue colour has just given place to yellow.

Carry out a blank determination on a synthetic mixture of similar composition to the ethyl ether extract of the cordite, but without the dibutyl phthalate. From the difference of the titres calculate the amount of dibutyl phthalate present in the sample. The equivalent weight of dibutyl phthalate is 139·2.

Results

The recoveries of samples of dibutyl phthalate in the presence of 1 g of nitroglycerine and 0·3 g of carbamite were as follows—

Dibutyl phthalate taken, g ..	0·159	0·165	0·177	0·186	0·198	0·204
Dibutyl phthalate found, g ..	0·158	0·166	0·176	0·187	0·196	0·202
Recovery, per cent.	99	101	99	101	99	99

Discussion

The method described is designed to meet a particular need, namely a routine composition control to a specification range of propellants containing dibutyl phthalate, and seeks to combine speed with a moderate degree of accuracy. Thus, although a more efficient extraction of dibutyl phthalate from the ferrous chloride solution would be achieved by several successive shakings with ether, the time involved would be prohibitive. A single shaking, as described, has been found to be a satisfactory compromise.

The method has been used in this laboratory for some time and has proved satisfactory over several hundred determinations. The over-all time for a set of six determinations is 10 hours, of which only 3 hours are operator's working time.

Summary

A routine method for the determination of dibutyl phthalate in the ether extract of propellants containing both nitrocellulose and nitroglycerine is described. The method entails the reduction of nitroglycerine by ferrous chloride in the presence of methanol, followed by the extraction and saponification of the dibutyl phthalate. The method has been found satisfactory for routine control purposes.

Grateful acknowledgment is made to Miss S. N. McGeoch and to the various members of the laboratory staff who assisted in this work, in particular Mr. A. O'Hare and Mr. R. Sinnott. Acknowledgment is also made to the Chief Scientist, Ministry of Supply, for permission to publish this Note.

References

1. Lamond, J., *Analyst*, 1948, **73**, 674.
2. Dickson, W., and Easterbrook, W. C., *Ibid.*, 1922, **47**, 112.

Royal Ordnance Factory
Bishopton
Renfrewshire

M. A. C. Mullaly
June 24th, 1954

Ministry of Food

STATUTORY INSTRUMENT*

1955—No. 221. The Bread (Amendment) Order, 1955. Price 2d.

This Order, which came into operation on February 20th, 1955, amends The Bread Order, 1953 (S.I., 1953, No. 1283; Analyst, 1953, 78, 566) by providing for the production of national milk bread containing a prescribed minimum of skim milk powder. The following definition is given—

"National milk bread" means national bread or national brown bread except that in its production not less than six parts by weight of skim milk powder have been added per one hundred parts by weight of national flour or national brown flour used.

CIRCULAR MF 21/54*

Food and Drugs Amendment Act, 1954.

This circular (price 2d.), dated November 26th, 1954, draws attention to the Food and Drugs Amendment Act, 1954, which received the Royal Assent on November 25th, 1954.

Apart from section 28 (which deals with the restriction of private slaughterhouses and the provisions of which came into effect on November 25th, 1954), the Food and Drugs Amendment Act, 1954, will not come into operation immediately but on a date to be appointed by order of the Minister of Food. Time will be given to consolidate the Food and Drugs Amendment Act, 1954, with the Food and Drugs Act, 1938, the Food and Drugs (Milk, Dairies and Artificial Cream) Act, 1950, and the Slaughterhouses Act, 1954, and to make Food Hygiene Regulations under the consolidation measure to replace section 13 of the Food and Drugs Act, 1938, which the Food and Drugs Amendment Act, 1954, repeals. It is proposed to bring the Food and Drugs Amendment Act, 1954, the consolidation measure and the Food Hygiene Regulations into force simultaneously, in the early months of 1955.

British Standards Institution

NEW SPECIFICATIONS†

B.S. 2540:1954. Silica Gel for Use as Desiccant for Packages. Price 2s. 6d.
B.S. 2541:1954. Activated Alumina for Use as Desiccant for Packages. Price 2s. 6d.

Publications Received

OXINE AND ITS DERIVATIVES. Volume I. OXINE—PART 1. By R. G. W. HOLLINGSHEAD, M.A. Pp. xx + 322. London: Butterworth's Scientific Publications. 1954. Price 42s.

OXINE AND ITS DERIVATIVES. Volume II. OXINE—PART 2. By R. G. W. HOLLINGSHEAD, M.A. Pp. xxiv + 323–616. London: Butterworth's Scientific Publications. 1954. Price 42s.

NON-AQUEOUS TITRATION. A monograph on acid-base titrations in organic solvents. By SANTI R. PALIT, D.Sc., F.R.I.C., F.N.I., MIHIR NATH DAS, D.Phil., and G. R. SOMAYAJULU, M.Sc. Pp. viii + 122. Calcutta: Indian Association for the Cultivation of Science. 1954. Price Rs.3; $1.00; 5s.

GUIDE FOR SAFETY IN THE CHEMICAL LABORATORY. Prepared by and published for the General Safety Committee of the Manufacturing Chemists' Association Inc. Second Printing. Pp. x + 234. New York: D. Van Nostrand Co. Inc.; London: Macmillan & Co. Ltd. 1954. Price 32s.

THE NATIONAL FORMULARY 1955. Pp. 210. London: The British Medical Association *and* The Pharmaceutical Press. 1955. Price 5s. (interleaved copies, 8s.).

INDIAN FOOD LAWS. Compiled by Major N. V. R. IYENGAR, M.Sc., A.I.I.Sc., F.R.I.C., B. K. SUR, M.Sc., Ph.D., and G. T. KALE, D.Sc. Pp. viii + 221. Mysore, India: Central Food Technological Research Institute. 1954. Price Rs.4/8.

HANDBUCH DER MIKROCHEMISCHEN METHODEN, in 5 volumes. Edited by F. HECHT and M. K. ZACHERL. Volume I, Part 1, ORGANISCHPRÄPARATIVE UND MIKROSKOPISCHE METHODEN: PREPÄRATIVE MIKROMETHODEN IN DER ORGANISCHEN CHEMIE, by H. LIEB and W. SCHÖNIGER; MIKROSKOPISCHE METHODEN, by L. KOFLER and A. KOFLER. Pp. vi + 236. Vienna: Springer-Verlag. 1954. Price 81s. (To subscribers to the whole handbook, 64s. 6d.)

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.
† Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

ANALYTICAL CHEMIST. The British Drug Houses Ltd., Graham Street, London, N.1, require an Analytical Chemist for work on the analysis of organic pharmaceutical compounds. Candidates should be of B.Sc. standard. Previous experience desirable but not essential. Salary according to qualifications and experience. Five-day week, Canteen, Sports Club, Pension and Profit-Sharing Schemes. Write, stating age, experience, if any, to The Staff Manager.

AN ANALYTICAL CHEMIST is required by the British Thomson-Houston Co. Ltd., for work in the Research Laboratory on non-routine inorganic analysis covering a wide field, including the development of physico-chemical methods and microchemistry. Qualifications should include B.Sc. or A.R.I.C.; industrial experience in inorganic analysis is not essential. The group working on analytical problems is now expanding and there are good prospects for a candidate who has the necessary skill and initiative. Apply to the Director of Research, The British Thomson-Houston Co. Ltd., Rugby, giving age, qualifications, industrial experience (if any) and quoting reference AC.

ASSISTANT EDITOR required by the Society for Analytical Chemistry for *Analytical Abstracts*. Applicants should possess a degree or chemical qualification. Some experience of analysis is desirable. Apply, stating age and details of training and experience, to The Editor, *Analytical Abstracts*, Peek House, 20, Eastcheap, London, E.C.3.

ABSTRACTORS are required for "Analytical Abstracts," especially those who can abstract German, Dutch or Scandinavian papers in the field of Foods, Drugs, Agriculture and Organic Chemistry. Apply for further particulars to The Editor, "Analytical Abstracts," 20, Eastcheap, London, E.C.3.

DURHAM COUNTY COUNCIL—LABORATORY STAFF

APPLICATIONS are invited for the following appointments in the new County Laboratory. All the appointments are subject to the provisions of the Local Government Superannuation Acts and to the passing of a medical examination.

Applications stating age, qualifications and full details of experience and appointments held, together with names and address of three referees should be sent to the County Analyst, Shire Hall, Durham, not later than Saturday, 26th March, 1955.

Applicants must state whether they are related to any member or senior officer of the Council. Canvassing, directly or indirectly, will disqualify.

DEPUTY COUNTY ANALYST—Salary scale: £1,000 × £50–£1,250 per annum. Candidates must be qualified in accordance with the Public Analysts' Regulations, 1939, and the Fertilisers and Feeding Stuffs Act, 1926. This appointment is subject to the approval of the Minister of Food and the Minister of Agriculture and Fisheries and is terminable by three months' notice in writing on either side.

FIRST ASSISTANT ANALYST—Salary scale: £735 × £30–£825. Candidates must be Graduates or Associates or Fellows of the Royal Institute of Chemistry and have had some years' experience in the analysis of food and drugs, preferably in a Public Analyst's laboratory.

SECOND ASSISTANT ANALYST—Salary scale: £560 × £20–£640. Candidates should be Graduates or Associates of the Royal Institute of Chemistry. Experience in food and drugs analysis will be an advantage.

For the Assistant Analysts' posts preference will be given to candidates who are prepared to train for the Fellowship of the Royal Institute of Chemistry in Branch E.

J. K. HOPE,
Clerk of the County Council.

Shire Hall,
Durham.
February, 1955.

APPLICATIONS are invited for the following vacancies in the Analytical Dept. of Ozalid Co. Ltd., Loughton, Essex:—

(1) Young graduate required for long term analytical work connected with research problems.
(2) Junior Analyst, Inter. B.Sc. standard required for analysis of organic intermediate and finished products.

Apply in the first instance in writing, stating age, education, experience, etc., to Chemical Products Manager, Ozalid Company Limited, Lenthall Road, Loughton, Essex.

CHEMIST (SENIOR) wanted for charge of process laboratory of company manufacturing sintered hard metals, in Midlands. A good knowledge of chemistry, organising ability and capacity to control staff are necessary and some metallurgical experience is desirable. An age about 30 is envisaged, but applicants of other ages having the necessary qualifications will be considered. The company has an attractive pension scheme. Write Box No. 3889, THE ANALYST, 47, Gresham Street, London, E.C.2.

BOROUGH OF GRAVESEND

APPOINTMENT OF PUBLIC ANALYST

APPLICATIONS are invited for the appointment of Public Analyst of the Borough of Gravesend from persons qualified in accordance with the Public Analysts' Regulations, 1939.

The appointment will be on a part-time basis and the remuneration will be in accordance with the standard fees approved by the Joint Negotiating Committee for Public Analysts.

The appointment, which is subject to the approval of the Minister of Food, will be terminable on three months' notice on either side.

Applications, giving age, qualifications and experience, and the names of three persons to whom reference can be made, should be sent to the Medical Officer of Health, 3, Woodville Terrace, Gravesend, Kent, by not later than Saturday, the 26th March next.

Canvassing will disqualify.

F. W. HARRISON,
Town Clerk.

7th February, 1955.

ANALYTICAL ASSISTANT, aged 25–30, military service completed, required for general analytical work by important industrial concern. Experience in analysis of organic/inorganic materials desirable. Work interesting and varied. Good prospects of advancement. Pension scheme. Canteen facilities. Position permanent. Write, stating age, qualifications, experience and salary required, to Box No. 3887, THE ANALYST, 47, Gresham Street, London, E.C.2.

THE UNIVERSITY OF MANCHESTER

THERE is a vacancy in the Department of Chemistry for an Organic Micro Analyst in the Senior Technician grade. Salary at a point on the scale £490 per annum to £890 per annum, with membership of a Superannuation Scheme. Applications should be sent to the Chief Technician, The Department of Chemistry, The University, Oxford Road, Manchester, 13.

CHEMISTS, physico-chemical bias, interested in design, development and selling of equipment for laboratory use, State experience and salary required to Technical Director. Box No. 3888, THE ANALYST, 47, Gresham Street, London, E.C.2.

WANTED for works assay laboratory Eastern Seaboard U.S.A. Analytical chemist. Applicants must hold University Degree or equivalent qualification and have had four or more years' experience as works chemist. The work will be mainly inorganic analyses and applicants must be capable of working out rapid methods for the determination of the lesser known metals. Salary according to age and experience. Write, giving full particulars of age, education and experience to Box No. S.215, c/o Streets, 110, Old Broad Street, London, E.C.2.

NORTHERN RHODESIAN COPPER MINE has a vacancy for an Assistant in the Assay Laboratory. Applicants must hold Higher National Certificate with Chemistry as a major subject and should preferably have had some experience of assaying. Starting basic salary £68 per month plus fluctuating cost of living allowance now about £6 per month. Life Assurance and Contributory Pension Scheme. Cash Bonus Scheme which at present rate equal to 65 per cent. of basic salary. Outward passage paid. Apply, giving full details of age, marital status, qualifications and experience, to Nchanga Consolidated Copper Mines Limited, 11, Old Jewry, London, E.C.2.

THE RESEARCH DEPARTMENT of a leading industrial organisation situated in the South requires two graduates to undertake the expansion of an important research project connected with the raw materials of the industry.

The work involved, which offers ample scope for initiative and imagination, will appeal to ORGANIC CHEMISTS and BIO-CHEMISTS who are interested in fundamental research on natural products and in applying the results of this research to the agricultural and manufacturing practices of the industry.

Applicants must have a 1st or 2nd Class Honours Degree and some post-graduate experience would be useful, although not essential.

The appointments will be to the regular staff and will be pensionable with starting salaries according to age and experience within the range £700–£1,100. Applications, giving full particulars, will be treated in confidence. Box No. T.6209, Haddon's, Salisbury Square, Fleet Street, London, E.C.4.

continued next page

HER MAJESTY'S OVERSEA CIVIL SERVICE

VACANCIES exist for Chemists in the Department of Chemistry, Federation of Malaya.

Qualifications:

Honours degree in Chemistry with Associateship or Fellowship of Royal Institute of Chemistry. Two years' specialised experience in some line (Forensic Science; Toxicology; Foods; Drugs and Water; Bacteriology; Pharmacy; etc.), relevant to the work in Malaya, or research experience indicated by Ph.D. or comparable post-graduate qualification. For pensionable employment age limit 25–30. For contract appointments age limit may be exceeded.

Duties:

General analytical and consultative work on liquors, foods, drugs, water and petroleum, etc. Clinical analysis and examination of exhibits for Court evidence. Inspection of petroleum carrying vessels and explosives. To make reports, give evidence in court, and assist in the training of subordinate staff.

Terms of Appointment:

(*a*) Pensionable with salary and pensionable overseas allowance from £1,004 to £2,072 per annum (incremental credits may be granted for experience). An additional non-pensionable overseas allowance for a married officer. A substantial non-pensionable cost of living allowance, according to family commitment is also payable to all officers.

At present total emoluments for a single man range from £1,218 per annum to £2,100; for a married man without children from £1,442 per annum to £2,499 per annum; and for a married man with dependent children from £1,533 per annum to £2,730 per annum.

(*b*) On Contract for three years (with provision for renewal) in above salary scale and with same allowances. Gratuity is payable at rate of £70 for each completed period of three months' satisfactory service on full pay provided officer's basic salary is £1,277 per annum, or above. For basic salaries below £1,277 per annum gratuity is payable at the rate of £50.

Apply in writing to the Director of Recruitment, Colonial Office, Great Smith Street, London, S.W.1, giving briefly age, qualifications and experience. Mention the reference number BCD 97/60/01.

AN EXPERIENCED ANALYST is required by A. Boake, Roberts & Co. Ltd., for their laboratory at Stratford. The applicants should be between 26 and 30, and must possess B.Sc. Special and/or A.R.I.C., qualifications. The work is interesting and varied and the prospects in an expanding company are good. The salary will depend initially on age, experience, and ultimately on ability. Applications, which will be treated in strict confidence, should be addressed to the General Works Manager, A. Boake, Roberts & Co. Ltd., Carpenters Road, London, E.15.

GRADUATE (CHEMIST OR PHARMACIST) required for analytical work in Control Laboratory of pharmaceutical manufacturers. The work offers a variety of experience in modern pharmaceutical analysis and control procedures. Previous experience will be an advantage, but applications are also invited from persons recently qualified. A non-contributory Pension and Life Assurance Scheme is in operation. Application forms from Eli Lilly & Company, Limited, Basingstoke, Hampshire.

GOLD COAST LOCAL CIVIL SERVICE

A VACANCY exists for a Government Chemist, Public Works Department, Gold Coast Local Civil Service.

Qualifications:

Honours degree in Chemistry and Associateship or Fellowship of Royal Institute of Chemistry, preferably in Branch I. Sound knowledge of Bacteriology and Biology of Water Supply required. Experience of practical operation of water purification plant essential.

Duties:

Supervision of purification of Government water supplies and investigation of new supplies.

Terms of Appointment:

On contract terms for two tours of duty (18–24 months each tour) with consolidated salary according to experience in the scale £1,030–£2,020 per annum plus gratuity of £37 10s. for each completed three months' resident service payable on satisfactory termination of contract. Outfit allowance between £30–£60 per annum, according to commencing salary. Free 1st class passages; quarters when available at rental of between £60–£90 per annum. Vacation leave at rate of 7 days for each completed month of resident service. Income tax at low local rates.

Apply in writing to the Director of Recruitment, Colonial Office, Great Smith Street, London, S.W.1, giving briefly age, qualifications and experience. Mention the reference number BCD 112/13/018.

Volume 80. No. 948, Pages 161-240 March, 1955

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

CONTENTS

Printed and Published for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. B. Attrill, 7-8 Idol Lane, London, E.C.3.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47 Gresham Street, London, E.C.2.

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