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.
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THE ANALYST

Editorial

ON December 17th, 1953, three Special Resolutions or groups of Resolutions were passed by an Extraordinary General Meeting of The Society of Public Analysts and Other Analytical Chemists. The first Resolution changed the name of the Society to "The Society for Analytical Chemistry." The second group of Resolutions modified the Memorandum of Association to delete the "professional" clauses and the third group altered the Articles of Association to provide for a Junior Membership for chemists aged between 18 and 27 years.

These are the latest steps in the development of the Society, and it is of interest to look back for a moment as well as forward into the future.

The Society of Public Analysts was founded at a meeting held on August 7th, 1874; Dr. Theophilus Redwood, whose portrait is the central feature of the Society's badge, became the first President. In those early days membership was restricted to practising public analysts and their assistants, but in course of time analytical chemists other than public analysts were also admitted as members. When, on August 7th, 1907, the Society was incorporated, its extended membership was recognised by the adoption of the title "The Society of Public Analysts and Other Analytical Chemists."

Even then there were doubts about the suitability of this title, and to-day the Society has far outgrown its original purpose of looking after the professional needs of the public analysts. The continued growth of the Society and the varied interests of its members have made it impossible for the Council to speak with one voice for all classes of analysts on professional matters, and the Council felt very strongly that its functions in the matter of the professional needs of analysts would be very much better discharged by the newly formed "Association of Public Analysts" and by the Royal Institute of Chemistry. The new Professional Association is receiving the full support of our Society, and financial and other help has been extended to them.

The membership of the Society has increased by over 50 per cent. during the last ten years. The spirit of the Society has not changed, but there is no doubt that the quarters into which its interest has been directed have altered. The developments in analytical chemistry have been so vast and striking in this period that the Society has had to grow in order to accommodate them. The inevitable feeling of regret at losing the words "Public Analysts" from the title of the Society after so many years will be softened by the knowledge that the public analysts themselves will still be with us. Whilst the Society's interest extends over the whole range of natural and manufactured products, there will still be the same platform for the discussion of investigations into the composition of food and drugs.

The growth of analytical chemistry was reflected in 1950, when the ever-increasing number of abstracts led to their being taken out of *The Analyst*. For four years *British Abstracts C*, published by the Bureau of Abstracts, was sent to all members and subscribers with *The Analyst*. With the closing of the Bureau, the Society is once more undertaking their production under the title of *Analytical Abstracts*.

The Council has for some time past considered the needs of young chemists and, with the object of encouraging them, a class of Junior Membership has been instituted. Junior Members will pay a considerably reduced subscription and no entrance fee, and will receive *The Analyst*. It is hoped they will attend meetings.

The Society for Analytical Chemistry will continue in the tradition, established in 1874 and upheld ever since then, of encouraging, assisting and extending the knowledge and study of all questions relating to the analysis, nature and composition of natural and manufactured materials generally. There is every intention that the character of *The Analyst* and of the ordinary meetings of the Society will be maintained unchanged.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

ORDINARY MEETINGS

AN Ordinary Meeting of the Society, organised by the Physical Methods Group, was held at 5 p.m. on Friday, October 23rd, 1953, in the Lecture Hall, Southampton University. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C.

The subject of the meeting was "Paper Electrophoresis" and the following papers were presented and discussed: "The Analysis of Inorganic Compounds by Electromigration and Electrochromatography," by F. H. Pollard, B.Sc., Ph.D.; "The Use of Paper Electrophoresis in the Study of Nucleic Acids," by Roy Markham, M.A., Ph.D.; "Paper-Strip Electrophoresis of Serum Proteins," by A. L. Latner, M.Sc., M.D., F.R.I.C.

The meeting was preceded by an afternoon visit to the Esso Refinery at Fawley.

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, November 4th, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C., and visitors included members of the Iron and Steel Institute, the Institution of Mining and Metallurgy, the British Iron and Steel Research Association and the British Non-Ferrous Metals Research Association.

The subject of the meeting was "The Determination of Niobium in Minerals and Mineral Dressing Products," and the following papers were presented: "The Absorptiometric Determination of Niobium in Some African Low-grade Minerals and Mineral Dressing Products," by G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P., and A. A. Smales, B.Sc., F.R.I.C. (A.E.R.E., Harwell); "The Absorptiometric Determination of Niobium in Some African Low-grade Ores," by A. E. O. Marzys, B.Sc., A.R.I.C. (Uganda Development Corporation Ltd.) (read by Dr. K. A. Williams, F.R.I.C., A.Inst.P.); "Spectrographic Determination of Niobium and Tantalum in Sukulu-type Soils," by C. S. Campbell, M.A., and D. Nicholas (Fulmer Research Institute Ltd.); "Inorganic Chromatography on Cellulose. Part XIV. A Shortened Method for the Determination of Niobium and Tantalum in Minerals and Ores," by R. A. Mercer and R. A. Wells, B.Sc., A.R.I.C. (Chemical Research Laboratory, Teddington); "The Colorimetric Estimation of Niobium and Tantalum with Pyrogallol," by E. C. Hunt, B.Sc., A.R.I.C., and R. A. Wells, B.Sc., A.R.I.C. (Chemical Research Laboratory, Teddington); "Inorganic Chromatography on Cellulose. Part XV. A Rapid Method for the Determination of Niobium in Low-grade Ores," by E. C. Hunt, B.Sc., A.R.I.C., and R. A. Wells, B.Sc., A.R.I.C. (Chemical Research Laboratory, Teddington).

Contributions to the discussion following the papers were made by E. A. Hontoir (Rio Tinto Co. Ltd.); L. E. Gardner (British Iron and Steel Research Association); W. Ramsden (British Non-Ferrous Metals Research Association); A. Bowes and T. Burchell (Murex Ltd.); A. R. Powell (Johnson, Matthey & Co. Ltd.); W. H. Bennett (Colonial Geological Surveys); B. Bagshawe (Brown-Firth Laboratories).

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, December 2nd, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C., and a Lecture on "Recent Advances in Medical Chemistry" was given by Professor C. H. Gray, M.D., D.Sc., M.R.C.P., F.R.I.C., and was illustrated by lantern slides and exhibits. The Lecture was followed by a discussion.

EXTRAORDINARY GENERAL MEETING

AN Extraordinary General Meeting of the Society was held in London on December 17th, 1953, the President occupying the Chair.

A Special Resolution was proposed that the name of the Society be changed to The Society for Analytical Chemistry and was carried by an overwhelming majority, there being 357 votes for the motion and 18 against. Of those present at the meeting, 48 voted for the motion and 3 against.

Special Resolutions were proposed for altering Clauses 3 (B) and 3 (J) of the Society's Memorandum of Association and for deleting Clauses 3 (C) and 3 (D). These were carried

by overwhelming majorities. Further Special Resolutions for altering Articles 5, 6, 10, 27 and 28 of the Society's Articles of Association were also carried by overwhelming majorities.

The effect of these alterations to the Memorandum and Articles of Association is (*a*) to remove the "professional clauses"; (*b*) to permit the transfer of a sum of money to the newly formed Association of Public Analysts, which will look after the professional interests of public analysts; (*c*) to establish the new class of Junior Membership of the Society and (*d*) to confirm the power of the Council of the Society to determine the subscription and entrance fee to be paid by members.

CHANGE OF EDITORSHIP OF *THE ANALYST*

THE Council has appointed Mr. J. B. Attrill as Editor of *The Analyst* from January 1st, 1954. Mr. F. L. Okell will remain on the editorial staff as Advisory Editor. Mr. B. J. Walby has been appointed Assistant Editor.

ANALYTICAL ABSTRACTS

WE are pleased to announce the appearance concurrently with this issue of *The Analyst* of the first number of *Analytical Abstracts*, dealing with all branches of analytical chemistry, published monthly by the Society. The Council has appointed Dr. Norman Evers as Editor and Mr. B. J. Walby as Assistant Editor.

NEW MEMBERS

Peter Frank Sedgeley Cartwright, B.Sc. (Lond.); Gordon Ivor Carver, B.Sc. (Lond.), A.R.I.C.; Alan James Dobbins; Edward C. Dunlop, A.B. (Westminster), M.S., Ph.D. (Illinois); Claude Douglas Essex, A.R.I.C., A.M.Inst.B.E.; Stanley Hill, M.A. (Cantab.), F.Inst.P., F.S.S.; Ahmad Mohammad Jawad, B.A. (Beirut); Einhart Kawerau, B.A., M.B., B.Ch., B.A.O., L.M., M.Sc., A.R.I.C.; Basheshar Dass Kochhar, D.Sc. (Punjab), F.R.I.C.; Stanley Vasa Gray Lindwall; Leonard Charles Sears Mallery; Richard McCotter, M.Sc. (Q.U.B.); Arthur Thomas Ness, B.S., Ph.D. (Washington); Frank Henry Singleton, B.Sc. (Lond.), A.R.I.C.; David Alan Tame, B.Sc. (Lond.), A.R.I.C.; Silvio J. Tassinari, M.S. (St. Michael's, Vermont); John Robert Windass, B.Sc. (Lond.), A.R.I.C.; Douglas Arnold Yoxall, B.Sc. (Lond.), A.R.I.C.

BIOLOGICAL METHODS GROUP

A JOINT Meeting of the Group with the Crop Protection Panel of the Agricultural Group of the Society of Chemical Industry, the Association of Applied Biologists and the Pharmacological Society was held on Friday, October 2nd, 1953, in the Large Chemistry Lecture Theatre, Imperial College, London, S.W.7.

The meeting, which was in two sessions, took the form of a Symposium on "Organo-Phosphorus Insecticides." Professor V. B. Wigglesworth, C.B.E., M.D., F.R.S., occupied the Chair at the morning session, which began at 10.30 a.m. The following papers were presented: "Insecticidal and Anti-Esterase Activity of Organo-Phosphorus Compounds," by Dr. K. A. Lord and Dr. C. Potter; "Toxic Action of Organo-Phosphorus Insecticides in Mammals," by Dr. J. M. Barnes. A discussion followed, which was opened by Dr. B. A. Kilby.

The afternoon session began at 2.15 p.m. and the Chair was taken by Dr. J. R. Nicholls, C.B.E., F.R.I.C. The following papers were presented: "The Behaviour of Organo-Phosphorus Systemic Insecticides in the Living Plant," by Dr. G. S. Hartley; "Some Hydrolytic Aspects of Organo-Phosphorus Compounds," by Dr. P. R. Carter; "Bio-Assay of Organo-Phosphorus Insecticides," by Mr. J. F. Newman. A discussion concluded the meeting.

Conductimetric Determination of Carbon in Metals

By J. E. STILL, L. A. DAUNCEY and R. C. CHIRNSIDE

The metal sample is burnt in oxygen in a normal combustion tube and resistance furnace. The carbon dioxide evolved is absorbed in sodium hydroxide or barium hydroxide solution and measured by the change in electrical conductance of this solution.

An improved loading device allows introduction of the sample and gives a good view of the combustion process, without admission of atmospheric carbon dioxide. A conductivity cell made of Perspex provides for continuous circulation of the absorbing solution and for measurement of the conductance at any time without stopping the oxygen flow. Except when the highest precision is required for extremely low carbon contents, sodium hydroxide solution is preferred to barium hydroxide solution. A special conductivity bridge, ordinary 50-cycle A.C. and a vibration galvanometer give high precision of measurement over a wide range; provision is made to compensate for the capacitance of the cell. The apparatus requires the minimum of maintenance, both in preparation for a series of analyses and in use.

The method gives adequate precision and accuracy on 1-g samples of standard steels and cast irons containing from 0·03 to 3 per cent. of carbon, the only change necessary over this range being an alteration in solution concentration. A wide variety of samples of metals and alloys, weighing from 0·1 to 3 g and containing from 8 per cent. down to 0·01 per cent. or less of carbon, have been analysed satisfactorily.

THE work described in this paper was initially undertaken in an attempt to determine carbon in pure iron at levels well below 0·02 per cent.

Although carbon determinations are by no means an everyday requirement in our laboratory, the range of samples submitted to us includes cast iron, many varieties of steel, nickel and nickel alloys, chromium, cobalt, tungsten and titanium alloys, and also iron and iron alloys that have been subjected to various experimental treatments such as decarburising. The infrequency and variety of the determinations has, in the past, raised two other problems besides that of attaining sufficient precision for very low carbon contents.

The first of these was the large proportion of time spent in setting up, testing and maintaining apparatus, which frequently amounted to more than that spent in analysing samples. This trouble was likely to be intensified if the determination of very low percentages required an apparatus of a type entirely unsuitable for the higher ranges.

The second subsidiary problem was to find a way of decomposing the sample so that the carbon was completely recovered. With the steady improvement in quality of combustion furnaces, tubes and boats, the direct combustion of even the most refractory of alloys has now become possible, although there are still a few alloys that need special treatment.

Attention had already been focused on the need, in the steel industry, for the determination of small percentages of carbon because of the stringent requirements of the electrical engineer for silicon steel of low carbon content. Two main techniques have been developed: that known as the "low pressure" method and that involving the use of a large sample, say 20 g, for the combustion, and subsequent weighing of the carbon dioxide on the usual solid absorbent.

In the low pressure method, the carbon dioxide produced from the combustion of 0·5 to 2 g of sample is condensed in a liquid oxygen trap; it is then expanded into a previously evacuated vessel of known volume and its pressure is measured. Modifications of this general method have been described by a number of workers[1,2,3,4,5,6,7,8,9] in the U.S.A. and in this country. It is fairly rapid and has for some years been established as the most accurate method available.

The large-sample method[10] is of obvious value when the sample material is freely available and the equipment is in regular use. It shares with the conventional combustion method from which it was developed the disadvantage of being inconvenient for occasional use, and it has the more serious limitation for our work that the provision of sufficient sample would be often difficult and sometimes impossible.

A third type of method,[11,12,13,14] less commonly used, involves conductimetric determination of the carbon dioxide produced in the combustion. For our purpose, this seemed to have advantages because—

(*i*) A conductivity cell and bridge would form a compact unit and would need little maintenance.

(*ii*) The sensitivity and precision of measurement would be related to the electrical techniques available, and ought therefore to be high.

(*iii*) The carbon dioxide could be measured as and when it is evolved, without disconnection and removal of the absorption vessel. This is an important feature in an apparatus used for a great variety of samples, as there may often be some doubt whether all the carbon dioxide has been liberated within a specified time.

One such method is that of Gardner, Rowland and Thomas.[14] Their apparatus includes a special device for introducing the sample without letting air into the combustion tube, and a conductivity cell made from a Friederich-type gas washing bottle with a long helical path for the gas bubbles. The carbon dioxide is absorbed in a weak solution of barium hydroxide containing a non-ionic wetting agent (Lissapol N).

Apparatus

Experience over a few months with apparatus similar in all but minor details to that described by Gardner *et al.* gave results generally supporting the claims made for it, and confirmed our view that this method offered a useful compromise between the simplicity of the large-sample gravimetric method, and the accuracy of the low pressure method.

Partly from this experience, and partly from consideration of our own special problems, a number of improvements in the apparatus and technique seemed desirable. For example—

(*i*) A reduction in the number of components in the oxygen purifying train would reduce maintenance work still further.

(*ii*) The arrangement for introducing the sample, although adequate in preventing the entry of carbon dioxide, could be improved by eliminating the greased joint and hence the risk of grease contaminating the boat, and by providing a clear view of the interior of the combustion tube.

(*iii*) As the temperature coefficient of conductance is about 2 per cent. per ° C, the extra expense and maintenance of a thermostat for the conductivity cell would probably be repaid by increased accuracy of measurement.

(*iv*) The conductivity cell could be improved in several ways, the most important one being the avoidance of the troublesome necessity for stopping the oxygen flow and blowing the solution back into the electrode chamber before each conductance reading. Besides the saving in time and trouble, this would obviate some minor faults such as the drying out of the electrodes between readings and the occasional erratic readings caused by persistence of gas bubbles in the electrode chamber. The provision of adequate circulation of absorbing solution in the helix would be another major improvement.

(*v*) The incomplete absorption of carbon dioxide noted by Gardner *et al.* (only 98 per cent. of the theoretical under the best conditions and for not more than 0·6 mg of carbon) seemed to merit further investigation, with possibly a radical change in the absorbing solution.

(*vi*) If the range of the method could be extended very considerably, say to as much as 30 mg of carbon, this would make it applicable to all our carbon-in-metals determinations instead of its being restricted to the very low values that were of primary interest when the work was started.

All these improvements were eventually effected, together with some minor ones that are apparent in the following detailed description of our apparatus.

Oxygen purifying train—

Four components are necessary, and sufficient, to supply the combustion tube with the required stream of pure oxygen: these are a control valve, a flowmeter, a preheater and a carbon dioxide absorption tube. In addition, a reservoir, as included in the apparatus of Gardner *et al.*, is useful for smoothing out variations of flow during combustion of the sample.

Control valve—This is a small medical-type needle valve, mounted close to the entrance end of the combustion tube. It is supplied with oxygen at a pressure of about 10 lb per sq. inch.

Flowmeter—This reads in litres per hour up to a maximum of 15.

Preheater—A silica tube 12 inches long and ½ inch in internal diameter is packed with platinised asbestos for a length of 6 inches; the packed section is maintained at 400° to 450° C in a small electric furnace.

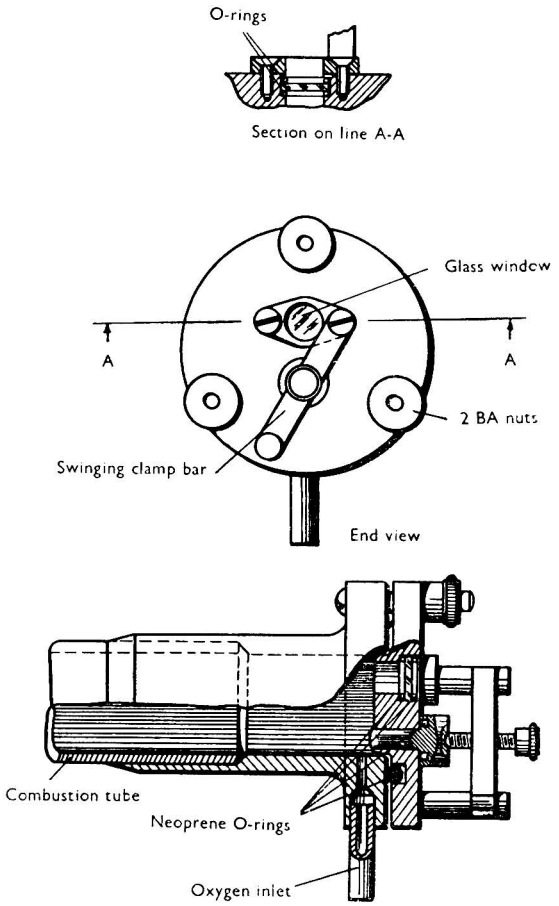

Fig. 1. Combustion tube closure

Reservoir—A 2½-litre bottle, with a three-hole rubber stopper, has an inlet tube passing to the bottom and an exit tube extending just below the stopper, which also carries an open mercury U-tube manometer to indicate the pressure applied to the combustion tube.

Purifying tube—A glass U-tube about 8 inches long and ½ inch in diameter is packed with soda asbestos, the first part being large granules to prevent choking of the tube, and the remainder 14 to 20 B.S.S. mesh.

All these components are connected with the minimum lengths of elastic PVC tubing,* with glass connecting tubes where necessary.

COMBUSTION TUBE AND FITTINGS—

The mullite combustion tube is 30 inches long, of 1-inch bore, and is without a reduced end or a side tube. The middle portion is heated in a platinum-wound resistance furnace 15 inches long.

* Supplied by Portland Plastics Limited.

The brass end-piece at the entrance end (Fig. 1) fits closely over the mullite tube; the joint is covered by a thin polythene sleeve, and this in turn by a longer rubber sleeve cut from a 1¼-inch cycle inner-tube of good quality. A second rubber sleeve over the whole adds to the firmness of the joint and protects the inner one from perishing. All other closures, including both those that are opened each time the apparatus is used, are effected by compressing neoprene O-rings against glass or brass surfaces.

On removal of the three large knurled nuts (2 B.A.), which run on stainless steel screws, the whole end-cap can be slid off the screws, allowing insertion of the boat with no danger of contamination. After the cap is replaced and the tube swept out with pure oxygen, the small knurled screw is loosened to enable the clamping bar to be swung aside and the plug beneath it to be removed. The small hole thus opened allows the insertion of a thin stainless steel rod for pushing the boat into the hot zone of the tube, while the oxygen supply escapes around the rod, preventing the entry of atmospheric carbon dioxide. The flat glass window gives a good view of this operation, and also of the subsequent combustion. The fitting has the additional advantage of incorporating the side tube for the delivery of oxygen, which makes the combustion tube easier to replace.

The exit end of the combustion tube is closed by a rubber bung carrying a glass tap; the bung is protected from radiant heat by a platinum baffle inside the tube about 6 inches from the bung. The replacement of this bung by another metal fitting incorporating a valve has been considered, but no trouble has yet been caused by the bung. Some form of tap is necessary at this point to prevent solution from being drawn out of the conductivity cell by a momentary reduction of the pressure in the combustion tube to below that of the atmosphere. Such a reduction in pressure can occasionally be caused by the combustion of a vigorously-burning sample, unless the reservoir is made inconveniently large.

A small glass tube containing a few grams of granular precipitated manganese dioxide is connected between the glass tap and the conductivity cell to retain oxides of sulphur produced in the combustion.

CONDUCTIVITY CELL—

The basic principle of a long helical gas passage on the outside of a cylindrical cell, with the electrode chamber in the centre, was retained as being the best arrangement of these main parts. In addition, the following requirements were kept in mind—

(*i*) The gas path should be longer than that in the Friederich wash-bottle used by Gardner *et al.* in case the incomplete absorption noted by them was caused by too short a path.

(*ii*) The electrode chamber must always be full of solution. This ensures that (*a*) readings of conductance can be taken at any time with the minimum of trouble, (*b*) the oxygen flow need not be stopped in taking readings, with consequent extension of the sweeping time, and (*c*) the electrodes do not dry out between readings.

(*iii*) The solution must circulate continuously between the absorbing helix and the electrode chamber. This ensures (*a*) continuous renewal of the absorbing solution in the helix, and (*b*) continuous mixing of the whole body of solution without a separate operation.

(*iv*) There must be no dead spaces where solution of a concentration different from that of the main bulk can accumulate.

(*v*) Gas bubbles must not pass through the electrode chamber, and any bubbles trapped there in filling the cell must be able to escape without clinging to horizontal electrode surfaces.

(*vi*) Although both the helix and the electrode chamber must be full of solution all the time, the total solution volume must be kept to a minimum to avoid reducing the sensitivity.

(*vii*) All solution that passes back into the gas entry tube when it is open to the air during the filling of the cell (or when the pressure in the combustion tube drops) must be expelled into the cell before the gas current starts, otherwise a serious error will be caused by absorption of carbon dioxide in this solution.

(*viii*) The cell must be robust, easy to dismantle, and extremely easy to empty and refill with fresh solution.

Material—It would be almost impossible to satisfy the above list of requirements with a glass apparatus, because of difficulties of fabrication. Perspex was chosen because of its transparency and workability; preliminary tests showed that this material was without measurable effect on the conductance of solutions of barium hydroxide or potassium chloride with which it was left in contact. Early fears that, under the conditions imposed on it, the material might not be sufficiently stable dimensionally have proved groundless.

General construction (see Fig. 2)—There are three separable parts, all made of Perspex. The outer wall is a piece of tube 2 inches in diameter and of $\frac{1}{8}$-inch wall thickness; this is

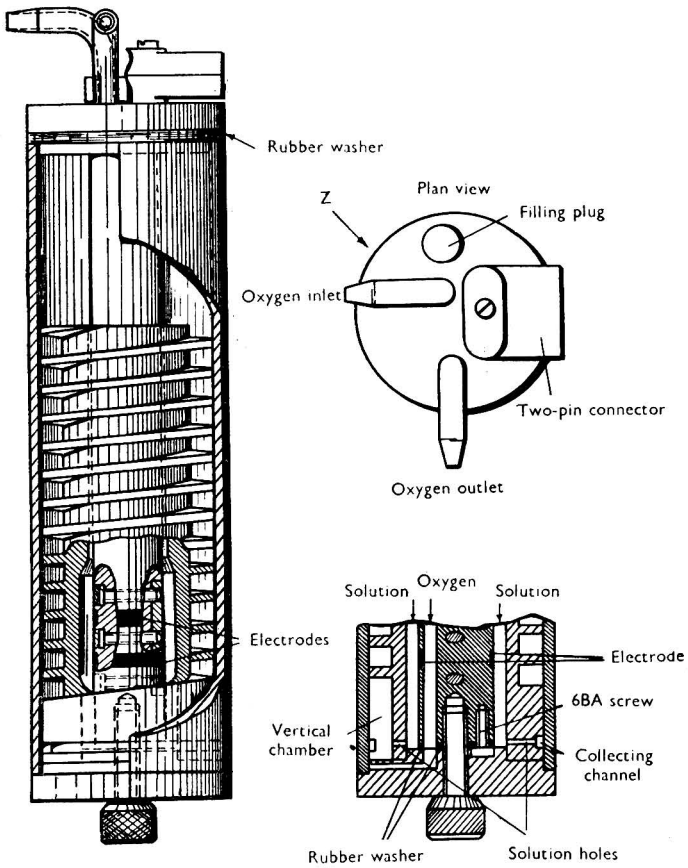

Fig. 2. Conductivity cell

cemented to the base. The annular member, on the outside of which is turned the helical bubble passage, is made from rod 1·75 inches in diameter; it is bored out centrally to a diameter of 1 inch for the lower 2 inches of its length to form the electrode chamber, and above this to $\frac{13}{16}$ inch in diameter. This member is fastened to the base with three 2 B.A. screws having their centres on a circle $1\frac{3}{8}$ inches in diameter, so that it can be removed, if necessary, to give access to the gas distributor holes. The remaining part is the lid, to which is cemented the central stem of $\frac{3}{4}$-inch rod carrying the electrodes.

To empty the cell, or to remove the electrodes, it is only necessary to take out the knurled stainless steel screw from the centre of the base; this screws into the central rod, and when tightened pulls it down into close contact with the base, a thin rubber washer between the

surfaces making a gas-tight fit. The length of the outer Perspex tube is so adjusted that when this bottom joint is tight the thicker and softer rubber washer under the lid is also slightly compressed; this ensures that no atmospheric carbon dioxide can enter.

Because the correct functioning and convenient operation of the cell depend very much on some of the smaller details of its construction, the making of some parts is described rather minutely below. Some care was taken to avoid any operations needing elaborate equipment; the only machine used in making the cell was a 3½-inch screw-cutting lathe.

Electrode system—The electrodes are two bands of platinised platinum ($\frac{3}{16}$ inch wide, 0·003 inch thick, $\frac{1}{4}$ inch apart) surrounding the central rod near its bottom end, and slightly recessed into its surface. The ends of these bands are folded over into a vertical milled slot, 1¼ inches long, $\frac{1}{4}$ inch wide and $\frac{1}{4}$ inch deep. They are secured by a slightly tapered block of Perspex forced into this slot and retained by three 6 B.A. stainless steel screws, both ends of each screw being afterwards covered with cemented Perspex plugs to protect them from the platinising solution.

Connections are made to the two-pin socket mounted on the lid through two $\frac{1}{8}$-inch holes drilled down the length of the centre rod to the levels of the electrodes. A little mercury in the bottom of each hole makes contact with the platinum through a $\frac{1}{16}$-inch horizontal hole, and two thick copper wires, soldered into the socket, dip into the mercury. Some cotton wool, forced into the holes around the wires, serves to retain the mercury if the cell is inverted.

Gas passages—The gas inlet to the cell is a third $\frac{1}{8}$-inch hole passing down the central rod. At the bottom of this, it communicates through a hole in the rubber washer with a passage in the base passing outwards to a point immediately under the bottom of the helix. Correct registering of these holes in assembly is ensured by a 6 B.A. cheese-headed screw in the bottom of the central rod, on the side opposite to the gas passage. The head of this screw locates in a recess in the base, and serves both to position the two parts and to hold the washer on to the rod during assembly.

The horizontal part of the passage in the base is $\frac{1}{16}$ inch in diameter and less than $\frac{1}{16}$ inch below the surface; it is drilled from the outside before the base is cemented to the outer wall of the cell, and the outer end of the hole is filled with a Perspex plug. At the required position for the gas distributor, a hole is made from the upper surface with a $\frac{3}{16}$-inch end mill, which just runs into this horizontal hole. (Care is necessary to ensure that no dead spaces that could retain even a small quantity of liquid are left in the gas passages). A recess, concentric with the $\frac{3}{16}$-inch hole and $\frac{1}{32}$ inch or less in depth, is now made with a $\frac{1}{4}$-inch end mill, and a $\frac{1}{4}$-inch diameter Perspex plug is cemented into this recess, cut off, and filed flush with the surface. The gas inlet is thus left closed by a thin disc $\frac{3}{16}$ inch in diameter.

A pattern of seven fine holes is then made in this disc by rotating an ordinary sewing needle in the lathe at a fairly high speed, and pressing the desired spot of the disc gently on to its point by means of a block or bracket fixed to the top slide or tool-post. The first hole is taken in a few thousandths of an inch at a time until it is just possible to blow through it when it is tested by mouth pressure. Then the other six are pierced to exactly the same depth, as measured by the graduations of the top slide feed screw. Drilled holes, even if made with the smallest drill commonly available, are too large to give small bubbles, but the above process is quite easy, and the holes can be cleared, if this should ever be necessary, with a similar needle mounted in the end of a thin brass rod, a little longer than the cell, that can be twirled between the fingers.

A pressure of less than 1 inch of mercury should be sufficient to pass 3 litres per hour of oxygen through the cell, most of this pressure being required to overcome surface tension. As only one or two of the holes are in use at this rate of flow, only a small increase in pressure is necessary to increase the flow to 10 litres or more per hour for rapid sweeping of the tube before the analysis.

Immediately above the gas distributor holes is a small vertical chamber about $\frac{3}{4}$ inch high, and of about the same cross-section ($\frac{7}{32}$ inch wide × $\frac{1}{4}$ inch deep) as the helical passage. This is cut with an end mill before the helix is turned and the helix itself starts from its upper end. This vertical part of the bubble passage ensures that when the first bubbles appear they rise into the helix instead of passing through the solution holes (mentioned below) into the electrode chamber. The helix is cut at 3½ turns per inch, and the bubble path is more than 55 inches long.

The gas inlet and outlet holes in the lid are provided with suitably directed nozzles made from $\frac{1}{4}$-inch Perspex rod drilled with a $\frac{1}{8}$-inch axial hole; such tubes are easily bent if held

a short distance above a small gas flame. A short length of glass tubing may be attached to the exit tube to ensure that no air has time to diffuse into the cell during the few seconds while the oxygen flow is stopped. A soda-lime guard tube is unnecessary, and also undesirable, because the moist oxygen will soon cause it to become choked, and excess pressure in the cell may then force solution into the holes carrying the electrode connections.

Solution circulation—The volume of solution put into the cell is such that when the helix is filled with gas bubbles there is $\frac{1}{4}$ inch or more of solution above the top of the annular member. The solution carried up the helix by the gas bubbles can thus pass across to the inner annular space surrounding the central rod and down this space into the electrode chamber. At the bottom of this chamber the annular member is drilled with four horizontal holes, nearly equally spaced around the circumference and arranged to clear the 2 B.A. fixing screws previously mentioned. One of these holes leads directly into the small chamber

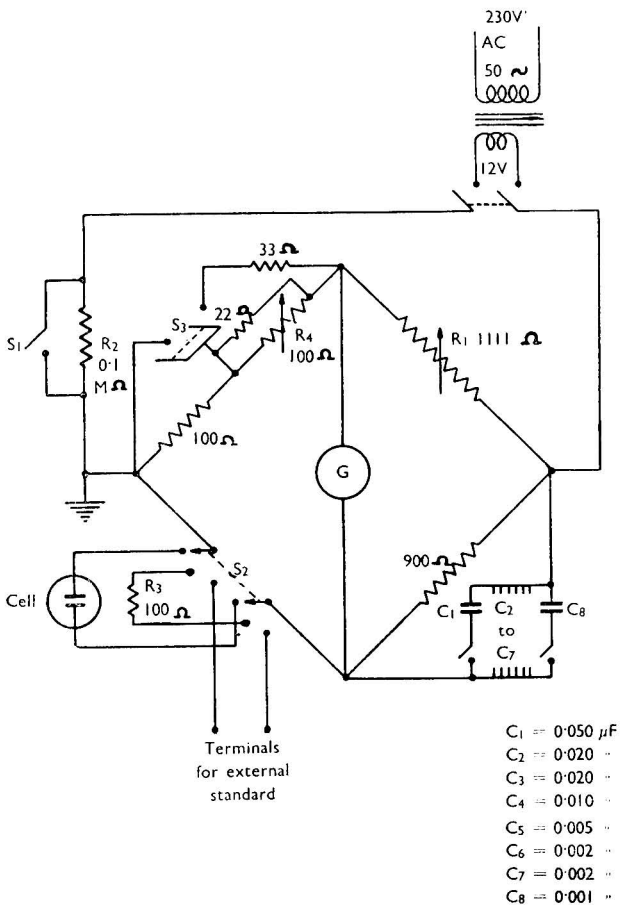

Fig. 3. Circuit diagram

below the helix, and the others into a collecting channel of about $\frac{1}{16}$-inch square cross-section communicating with this chamber.

About 45 ml of solution are required, and the whole of this is circulated through the cell about once a minute by the lift-pump action of the gas bubbles in the helix.

THERMOSTAT—

The cell is clamped by its upper end so that the part containing solution is immersed in water at 25° C. Any small thermostatically controlled bath at least 6 inches deep will serve; ours is a 1300-ml beaker, stirred by a stream of air from a sintered-glass diffuser and

heated by a home-made 50-watt glass immersion heater.[15] The control is a commercial mercury-in-glass contact thermometer with a suitable relay. A small glass cooling coil carrying mains water is used in hot weather to keep the thermostat functioning properly.

MEASURING BRIDGE—

This was specially made for the purpose, and comprises a control box containing the necessary switches, rheostats and so on, a four-decade resistance box of good quality for the actual measurements, and a galvanometer. The control box has flexible leads to the resistance box, to the conductivity cell, to the terminals of the galvanometer and also to its lamp transformer. The only external supply required is a connection to the A.C. mains.

Circuit—This is shown in Fig. 3. It is basically a simple A.C. bridge arranged to read directly in micromhos of conductance up to 11,110 on the resistance box, R_1. The balance point is detected by the 50-cycle vibration galvanometer, G (sensitivity 10 mm per microampere).* The power supply is 12 volts A.C., obtained from a small transformer, which was originally built into the control box. Some anomalous behaviour of the bridge was traced, after considerable trouble, to currents induced in the control box circuits by the magnetic field of this transformer, so the transformer was removed a short distance and connected to the control box by another flexible lead. Four additions to the simple bridge circuit are described in the sections that follow.

Sensitivity switch—This is a spring-off switch, S_1, which protects the galvanometer by inserting a large resistance, R_2, in the supply circuit while an approximate balance is attained.

Standardising switch—A three-way change-over switch, S_2, allows the bridge to be connected to the cell, to an internal standard conductance, R_3 (a good quality radio-type 100-ohm wire-wound resistor), or to an external standard by way of two terminals on the top of the control box. Standardisation is seldom necessary except when the range of the bridge is changed (see below); it is carried out by putting S_2 in the appropriate position, setting up the known value of the standard conductance on the resistance box (9452 micromhos for the 100-ohm internal standard at present in use) and bringing the galvanometer to zero by adjusting the standardising rheostat, R_4.

Range-changing switch—This is a double-pole switch, S_3, which, when closed, increases the conductance readings by a factor of ten; in other words, the resistance box then reads in tens of micromhos up to 111,100. The standardising connections of the bridge are altered at the same time so that the same rheostat, R_4, can still be used with adequate precision for standardisation (the internal standard being set up as 945 units, for instance). The setting of R_4 is different for the two ranges.

Capacity compensation—Two decades of capacitors, C_1 to C_8, giving a total of 0·110 microfarads in steps of 0·001, are provided for this purpose. They are connected as required to balance the capacity of the cell by eight small tumbler switches on the control box. Small silvered-mica condensers are used, specially supplied† to a tolerance of ± 1 per cent.; these are much smaller and cheaper than boxed decade capacitors, and are quite adequate for the purpose.

Performance—Up to about 4000 units, there is no difficulty in reading the conductance to the nearest unit; from about 7000 up to the maximum of 11,110 there may be uncertainty amounting to ± 1 unit. In fact, the precision of reading is better than the accuracy of the resistance box unless this is of quite exceptional quality. In terms of carbon content of the sample, the above figures mean that a small carbon percentage is read to the nearest 0·00005 per cent. on a 1-g sample, *i.e.*, to the nearest half microgram of carbon, and that throughout the range the error of the electrical measurement is insignificant compared with the total of errors from other causes.

REAGENTS

Barium hydroxide solution—A solution containing 5 g of $Ba(OH)_2.8H_2O$ per litre.
Dilute sodium hydroxide solution—A solution containing 5 g per litre.
Strong sodium hydroxide solution—A solution containing 50 g per litre.
Lissapol N‡—A 2 per cent. v/v solution.

* Model SS1 made by H. Tinsley & Co., Ltd.
† Supplied by the London Electrical Manufacturing Co., Ltd.
‡ Supplied by Imperial Chemical Industries Limited.

The exact strength of the solution in the conductivity cell need not be known; this is another advantage of the conductimetric method as regards maintenance. The solids can be weighed to the nearest 0·1 g, or to the nearest pellet of sodium hydroxide, and the solutions can be kept in corked bottles without special precautions.

ADJUSTMENT TO WORKING STRENGTHS—

The above strengths of solutions are all more than five times those used in the conductivity cell. In filling the cell, the necessary amount of alkali solution (about 8 ml) is put in by means of a graduated pipette, together with the necessary few drops of Lissapol solution; the cell is then counterpoised on a rough balance and filled up to the correct weight with distilled water. Two counterpoises are used; one equal in weight to the empty cell, and another equal to the weight of solution required. If a new determination of the cell constant shows it to have altered slightly, this second counterpoise can be adjusted so as to alter the volume of solution used and to keep the factor of the determination the same as before. Similarly, the cell counterpoise can be separately adjusted if any alteration is made affecting the cell weight without altering the constant, *e.g.*, fitting a new rubber washer.

ADDITION OF LISSAPOL—

The quantity of Lissapol solution required is not highly critical, and is best found by trial; it varies from 1 to 5 drops. Too little allows the bubbles to coalesce too early on their way up the helix, leading to alternate fast and slow bubbling and erratic conductance readings; too much causes persistence of the foam in the top part of the cell, and possible loss of solution through the gas exit tube. Rather less Lissapol is required for sodium hydroxide solutions than for barium hydroxide solutions.

PROCEDURE

It is assumed that sodium hydroxide solution is to be used in the cell; the modifications necessary when barium hydroxide solution is used are mentioned on p. 15.

FILLING THE CELL—

Remove the knurled screw from the bottom of the cell, and raise the lid a millimetre or so to allow the used solution to drain out. Replace the bottom screw and remove the filling plug from the lid. Put in the necessary few drops of Lissapol solution and about the right volume of sodium hydroxide solution to bring the conductance near to the top of the bridge range. Use the weak solution and the lower bridge range for less than about 3 mg of carbon, and the strong solution and the higher range for quantities between 3 and 30 mg. Place the cell on the pan of a rough balance and fill it with distilled water to balance the counterpoises already mentioned, with 2 or 3 drops in excess to allow for the evaporation that occurs during the analysis period. Replace the filling plug, put the cell in place in the thermostatically controlled bath and connect it to the combustion tube.

COMBUSTION OF THE SAMPLE—

Weigh a sufficient quantity (usually from $\frac{1}{2}$ to 2 g) into a suitably prepared boat, together with any flux or igniting material necessary (see p. 14). Remove the cap from the combustion tube, put the boat just inside, and replace the cap. Increase the oxygen flow to 8 or 10 litres per hour for 5 minutes, then reduce it to 3 litres per hour and measure the cell conductance as described below.

When the conductance has remained steady for 2 or 3 minutes, remove the small plug from the end cap of the tube, push the boat into the hot zone of the tube, and replace the plug. Increase the oxygen flow as necessary during the burning of the sample, observing the reservoir manometer as well as the burning process meanwhile. If, in spite of an increased oxygen supply, the reservoir pressure falls to atmospheric pressure or below, close the tap at the exit end of the combustion tube for a few moments to prevent solution from being sucked out of the cell.

When combustion ceases, set the oxygen flow at 3 litres per hour, and take the conductance reading when it has been steady for several minutes. This is usually 25 to 30 minutes after pushing the sample into the hot zone.

MEASURING THE CELL CONDUCTANCE—

To take a reading, see that switch S_2 is in the position connecting the cell to the bridge, and adjust the first two dials of the resistance box to give a minimum reading on the galvanometer. Then depress the sensitivity switch S_1 and adjust the resistance box and the condenser switches alternately so as to reduce the galvanometer reading to zero. When the conditions are unknown, several alternate adjustments may be necessary, but after some experience the correct capacity will be approximately known, and a very close balance can be attained quickly with the resistance box, the capacity being adjusted by a unit or two only to improve the sharpness of the zero. The capacity varies from about 0·02 microfarad with a weak barium hydroxide solution to 0·09 or more with strong sodium hydroxide solution.

CALCULATION—

When the conductance reading is steady, check the weight of the cell before refilling it. If it is found to be more than 0·1 g in error, *e.g.*, because of a long idling period before the analysis, apply the appropriate small correction to the result. As the volume of solution is about 45 ml, 0·1 g represents 1 part in 450.

Manipulations involving an unnecessarily large number of decimal places, particularly in the factor determinations described later, are avoided by considering the factor to be the number of micrograms of carbon per micromho change in cell conductance. This is about 0·9 for sodium hydroxide and about 0·5 for barium hydroxide solution. Then—

$$\text{Amount of carbon, per cent.} = \frac{\text{micromhos change} \times \text{factor}}{\text{sample weight in grams} \times 10^4}$$

In our experience, the factor remains constant for months at a time, even when the apparatus is not in use, provided that the cell is left filled with solution or with water.

EXPERIMENTAL

THE COMBUSTION PROCESS—

Although the primary object of the work described was the development of the method of measuring carbon dioxide rather than investigation of the combustion procedure, some attention had to be given to the latter, largely because of the considerable variety of samples encountered. No experiments have yet been made with a high-frequency furnace, but there seems to be no reason why the conductimetric method of measurement should not be used in conjunction with such a furnace if desired.

Combustion tube temperature—A temperature of about 1200° C was found suitable for cast irons, iron powders and carbon steels. Stainless steels and other alloy steels required at least 1250° C, and this temperature also appeared suitable for a considerable variety of alloys, including iron, nickel, chromium, cobalt and tungsten as major constituents. Titanium was found to give low and variable results by any method that caused vigorous combustion; carbon remained in the slag and was slowly given up over a period of more than 6 hours. However, this metal gave satisfactory and reproducible results under the following conditions. The sample was introduced when the tube was at 1100° C and the temperature was raised to 1300° C. The metal oxidised steadily without igniting, and evolution of carbon dioxide ceased after about one hour.

Combustion boats—These have been the source of considerable trouble, especially with low-carbon alloys where a low and consistent blank is particularly desirable. In general, boats of a relatively porous nature that do not crack on heating tend to give large and variable blanks, and non-porous boats, which give low blanks, tend to crack on heating or when the sample burns, so allowing the tube to be contaminated with slag. Our usual method is to use a small non-porous boat inside a larger one; it is then seldom that both fail badly enough for any slag to reach the tube.*

For low carbon contents, it has been found essential to preheat the boats to the operating temperature in oxygen in the combustion tube, or in a similar tube, for about an hour immediately before use, and to expose them to air for the minimum period required to put in the sample.

* The small boats are sold by A. Gallenkamp & Co., Ltd., the large ones by C. V. Brindley of Sheffield; both are said to be made of aluminous porcelain, but their textures differ considerably.

Fluxes and igniters—Some of the metals and alloys dealt with will burn well without any addition, and the results are not improved by the use of fluxing or igniting material. These samples include cast irons, iron powders, plain carbon steels if in a finely-divided condition, and also titanium.

Metallic lead and tin have both been found to be free from large blank errors, and results have been satisfactory with either or both of these additives on all the other alloys so far analysed. Lead appears to act primarily as a flux, and is most useful with iron-rich alloys. Tin acts mainly as an igniter, and is most useful for alloys containing much nickel, cobalt or tungsten; with high-nickel alloys it has a fluxing effect as well.

It was found impossible to get satisfactory blank values from analytical reagent grade lead foil by any method of cleaning with abrasives, acids or solvents. On the other hand, a very low blank value (of the order of 5 μg of carbon) is obtained by simply igniting the desired amount of lead (usually 0·7 g) to a bright red heat in a small refractory boat in a gas flame, and tipping the molten globule on top of the sample.

VARIABLES AFFECTING THE CALCULATION—

If the assumptions (not necessarily justified) are made that the whole of the carbon in the sample has been released into the gas stream as carbon dioxide, and that other gases and vapours that could alter the cell conductance are absent or are removed by the manganese dioxide tube, there still remain at least eight variables that can affect the relation between the weight of carbon dioxide released by the burning sample and the conductance change observed in the cell. All these have been separately investigated.

Oxygen blank—If oxygen is passed through the apparatus with the combustion tube cold, the only change in the conductance of the cell, after it has reached its correct temperature, should be a slow rise due to evaporation (see below). The theoretical rise in conductance is observed when the cell contains potassium chloride solution, but with barium hydroxide solution the rise is a little slower. If this difference is caused by carbon dioxide remaining in the oxygen, which is probable, the blank error from this cause does not amount to more than 0·5 μg of carbon during the period of an analysis.

Tube blank—The oxygen blank is always appreciably higher when the combustion tube is hot than when it is cold. The difference might be caused by traces of combustible matter escaping the preheater, but, as it is unaffected by an increase in the preheater temperature and it increases with the combustion tube temperature above about 1000° C, we believe it to be due to diffusion of carbon dioxide through the tube wall from the higher partial pressure in the atmosphere outside. The additional blank from this cause amounts to between 1 and 2 μg of carbon during the analysis period.

Boat blank—By use of suitable boats and correct pre-treatment as described above, this can be reduced to the equivalent of 4 or 5 μg of carbon.

Flux blank—If the lead is added in the molten condition as described, or if pure tin is cut up with a clean milling cutter and subsequently washed with acetone in a Soxhlet extractor, this blank is reduced to the same order as that for the boat.

These four blanks are conveniently determined together in an ordinary blank analysis; they are subtracted from the observed change in conductance before the remainder of the calculation. Their sum is of the order of 10 μg of carbon, or 0·001 per cent. on a 1-g sample; this is reasonably small even for samples containing little carbon, and becomes negligible in the higher ranges. The remaining variables affect the factor for the determination and cannot be included in a subtracted blank.

Evaporation from the cell—This could theoretically be avoided by saturating the oxygen with water vapour before introducing it into the combustion tube, but this expedient would involve further difficulties; for instance, the manganese dioxide tube and the connecting tubes would have to be kept warm to avoid condensation in them. The error produced is small, amounting to an increase in conductance of about 0·15 per cent. per hour, if the oxygen is assumed to be saturated on leaving the cell; it is easy to check the cell weight after an analysis, if necessary.

Absorption efficiency—Except when the strong barium hydroxide solutions mentioned below were used, we have no evidence that carbon dioxide is incompletely absorbed in the present cell. Although the theoretical conductance change is not quite attained even in weak barium hydroxide solution, the factor as determined is not altered by a decrease in oxygen flow-rate or by an increase in Lissapol concentration. These changes, giving respectively a

longer contact time and smaller bubbles, would be expected to improve absorption to a significant extent, if it were incomplete under normal conditions. Furthermore, with sodium hydroxide solutions containing a little less Lissapol than usual, it is possible to increase the oxygen flow to 6 litres per hour throughout the analysis, without measurably increasing the factor.

Cell constant—This has been determined over the whole available conductance range with potassium chloride solutions of nine different strengths from 0·001 to 0·4 *M*. When this work was first carried out, a large rise in the cell "constant" was observed as the concentration was increased; the cause of this was eventually found to be in the connecting leads to the electrodes, whose resistances were too high. The resistance was reduced to the lowest reasonable value, but there still remains a small error of the same nature, the cause of which is not certainly known. The cell "constant" rises steadily from 0·414 at a conductance of 350 micromhos to 0·457 at 110,000 micromhos. It should be noted that these are the extreme limits of the usable range of the bridge; not more than about one-tenth of this range is used for a single analysis.

A slight change in the cell constant sometimes occurs when the electrodes are re-platinised, but because the electrode stem does not have to be removed from the cell in normal use, this operation is seldom necessary. The only other occurrence that has been found to affect the cell constant is the accumulation of barium carbonate in the cell, which is caused by performing a number of analyses with barium hydroxide solution without washing out the carbonate with acid. Most of the carbonate is precipitated in the lower part of the helix, and none appears to adhere to the walls of the electrode chamber; nevertheless the constant changes slightly if more than two or three batches of barium hydroxide solution are used successively without acid rinsing. To avoid this effect, before each batch of barium hydroxide solution is emptied, about 0·5 ml of diluted hydrochloric acid (1 + 9) is put into the cell while the oxygen is still passing through it. This dissolves the carbonate in a minute or so, after which the cell is drained and washed out with distilled water before it is refilled.

Equivalent conductivity—Even if the cell "constant" were truly a constant, the conductance change would still not be proportional to the weight of carbon dioxide absorbed, over any considerable range of concentrations of barium hydroxide solution. With a change of concentration from 1 to 5 milli-equivalents per litre, the equivalent conductivity changes from 247·0 to 240·8. This represents a proportional change about twice that due to the alteration in cell constant over the same range. The two changes affect the factor in the same direction, so that the theoretical factor rises from 0·467 to 0·487 over this range.

When a similar calculation is made for sodium hydroxide solution the result is much different. It is assumed for this purpose that the conductivities of the hydroxide and carbonate, in a mixed solution, can be regarded as added together; this assumption may not be strictly justified, but it is largely borne out by experiment. The equivalent conductivities of both hydroxide and carbonate, like that of barium hydroxide, fall with increasing concentration, but that of the carbonate falls much the more rapidly. Hence the difference between the two equivalent conductivities (the quantity that is a measure of the carbon dioxide absorbed) rises with increasing concentration, and so affects the factor in the opposite direction to the effect of the cell constant change. As the two changes are of about equal magnitude, the "theoretical factor" (derived on the above assumption that the conductivities are additive) only alters from 0·946 to 0·938 over a tenfold increase in concentration (from 20 to 200 milli-equivalents per litre).

FACTOR DETERMINATION—

The factors for barium hydroxide solution and for both strengths of sodium hydroxide solution have each been determined in at least two ways. The methods used included heating small weighed amounts of calcium carbonate in the tube, burning small quantities of sucrose either weighed direct or weighed in the form of a dilute solution that was dried before burning, and evolving small amounts of carbon dioxide gas directly into the oxygen stream by measuring small volumes of standard sodium carbonate solution into a gas wash-bottle containing strong sulphuric acid through which the oxygen was passing. Platinum boats were used for heating sucrose or calcium carbonate in the combustion tube, because these give a negligible blank even with barium hydroxide solution.

The changes in the factors expected on theoretical grounds were generally realised in practice. The factor for barium hydroxide solution certainly changes with concentration

at approximately the rate predicted, but the average at a particular concentration is between 1 and 2 per cent. higher than that predicted; that is, the conductance change is always a little less than expected. With sodium hydroxide, on the other hand, the factor is about 4 per cent. lower than that predicted (this is not surprising in view of the assumptions that have to be made), but it is, as expected, constant within less than 10 parts per thousand over the whole range available.

COMPARISON OF BARIUM HYDROXIDE AND SODIUM HYDROXIDE SOLUTIONS—

Barium hydroxide solution containing about 1 g of the base per litre was used for all the earlier work on the method, because interest was mainly centred around attaining the highest possible precision in measuring very small carbon contents. Later, when it was required to extend the range upwards, a solution containing about 3·5 g of barium hydroxide per litre was used; the conductance of this solution was as great as could be measured at that time. To our surprise, the results with this solution were low and variable, and it appears that 1 g per litre is about the maximum concentration of barium hydroxide that will absorb carbon dioxide quantitatively under our conditions.

We regard 1 mg as being about the maximum amount of carbon that can be satisfactorily determined with barium hydroxide solution in the type of cell described here. Below this limit, barium hydroxide has the advantages of higher sensitivity (nearly twice that of sodium hydroxide) and somewhat higher precision of the electrical measurement (because the measurement is made in the lower part of the bridge range). Nevertheless, it is more troublesome to use, because the cell must be washed out frequently with acid instead of merely being drained and refilled, and the factor is more difficult to determine and to apply; instead of a single figure, a graph is required, which must be checked at a number of points. This graph is plotted with the factor as ordinate against the average conductance during the analysis as abscissa.

Sodium hydroxide is necessary for all analyses in which more than 1 mg of carbon is to be determined, and unless the highest possible precision is required, we also regard it as preferable to barium hydroxide in the lower ranges. It has an additional advantage in that by using a little less Lissapol than usual a steady oxygen flow can be maintained at as much as 5 or 6 litres per hour. This decreases the sweeping time after the combustion to 15 or 20 minutes, with only a small loss of precision in the conductance measurement.

TIME REQUIRED FOR ANALYSIS—

The time that elapses between placing a sample in the tube and taking the final reading varies from 25 to 40 minutes, except in the rare instances when the carbon dioxide is released relatively slowly. To this must be added the time required for bringing the cell to equilibrium

TABLE I

CARBON IN STANDARD STEELS AND CAST IRON

Carbon present according to B.A.S.		
Average, %	Range, %	Carbon found, %
0·029	0·0275 to 0·032	0·0289, 0·0283, 0·0283, 0·0285
0·365	0·360 to 0·370	0·361, 0·363, 0·363, 0·362
2·88	2·83 to 2·93	2·90, 2·89, 2·88, 2·89

with the thermostatically controlled bath and for pre-treating the boats. This can be as much as an hour if the solution is put into the cell at a temperature much below 25° C, and if the boats are to be ignited in the combustion tube used for the analysis, but the time can be much reduced by using solution and water at about the right temperature; the same boats can often be used for more than one analysis. Further time can often be saved by using the same solution for two or more analyses. (Barium hydroxide solution can be used down to 20 per cent., and sodium hydroxide down to 65 per cent., of the original conductance without loss of carbon dioxide.)

RESULTS—

The results shown in Table I were obtained on two standard steels and one standard cast iron from the Bureau of Analysed Samples Ltd.

All these determinations were made with sodium hydroxide solutions in the cell; the factors were the means of those from a number of determinations with both calcium carbonate and sucrose. Sample weights of both 0·5 g and 1 g were included in each set of four; no result has been discarded.

The method has been used for a great variety of metals and alloys containing from 8 per cent. of carbon down to less than 0·1 per cent.; sample weights have varied from 0·1 to 3 g. In several instances the results have been checked by independent gravimetric determinations, always with good agreement.

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The Determination of Silicon in Tungsten and Titanium Metal Powders, Carbide Sintering Alloys, Tungstic Oxide and Tungstates

BY B. BAGSHAWE AND R. J. TRUMAN

A method is described for the determination of silicon in such tungsten and titanium based compounds and materials as tungstic oxide, ammonium tungstate, tungsten metal and carbide, titanium metal and carbide and mixtures of them with molybdenum carbide, tantalum and cobalt. It depends upon calcination to oxide, fusion with sodium carbonate, extraction of the fusion product under conditions that prevent adsorptive and hydrolysis losses of silicic acid and determination by an adaptation of the molybdisilicic acid - molybdenum blue reaction. Optimum conditions for the application of this reaction to the materials under review are given, and results are shown that establish the validity of the method for a series of synthetic mixtures.

DURING recent years the need has arisen for an accurate means of determining the total silicon content of tungsten and allied metal powders, their carbides, and mixtures of these with other elements, such as those used in the manufacture of hard sintered alloys.

Small percentages of silicon and, particularly, sodium silicate can have a pronounced effect on the properties of hard metal carbides. The silicon can be responsible for excessive brittleness and porosity, and it can also interfere very considerably with the control of grain size of the finished material, which has to be controlled very closely to ensure that these products have their required physical properties.

The amount of silicon is usually small, ranging from a few tenths of 1 per cent. for some carbides to amounts below 0·01 per cent. for the purest tungstic oxide and for tungsten metal powders produced by reduction with hydrogen. It is virtually impossible to determine these small amounts of silicon with sufficient accuracy by the usual chemical separation methods, and results by these methods are usually of doubtful authenticity. Hydrolysis of silicic acid at low silica concentrations is incomplete, and as silica is usually a minor component of a major residue of hydrolysis, its evaluation in these large residues by measurement of the loss of weight resulting from hydrofluoric acid treatment is questionable, being further complicated by the high volatility of other oxide components of the residues, notably molybdic and, to a lesser extent, tungstic oxides. In addition, precipitation of silica is usually incomplete from solutions containing tartrate, citrate, and so on, as hydrolysis inhibitors for the other metals present.

We were led, therefore, to examine the possibilities of applying the molybdisilicic acid - molybdenum blue colour reaction to the materials under review.

Experimental

Preliminary experiments in roasting the metal or carbide to form the oxide and in fusing the oxide with sodium carbonate[1] were carried out with the object of producing in the acidified fusion extract a complex of tungsten with phosphate, oxalate or tartrate. All these reagents, however, were shown to interfere with the formation of the molybdisilicic acid complex. Further experiments with alkali carbonate fusions of pure tungstic oxide showed that the stability of the resulting alkali tungstate was such that its acidified solution could be manipulated without the occurrence of hydrolytic decomposition and precipitation of tungstic acid. The equivalent of 0·5 g of tungsten could be rendered stable in this manner after fusion with 4 g of sodium carbonate and extraction. The stability of these solutions is attributable to the formation of complex tungstates such as $Na_2W_4O_{13}$, which are not readily decomposed in weak acid solution. Hence we were able to make determinations by the molybdisilicic acid - molybdenum blue reaction in the presence of all the tungsten on aliquots of suitable size from fusions of 0·5-g samples of tungsten metal or oxide.

Effect of other elements—

The introduction of elements such as iron, cobalt and titanium into the tungsten based mixture causes low results owing to adsorption of silica in the precipitate from the aqueous fusion extract. Table I shows the results for a sample of tungsten metal powder containing 0·18 per cent. of silicon and iron in the form of ferric oxide, which was added before fusion with sodium carbonate. In each determination the iron precipitate was filtered from the aqueous extract of the fusion, again fused with sodium carbonate, and the silica was determined separately on both the primary and secondary extracts. The results show clearly that double fusion is insufficient to free the ferric hydroxide precipitate from occluded silica, the yield of silicon is never the maximum and the error increases with increasing iron additions.

Table I

Recovery of silicon from tungsten containing iron and 0·18 per cent. of silicon

Amount of iron, %	Silicon found: Single treatment, %	Silicon found: Repeat fusion, %	Silicon found: Total, %	Silicon not recovered, %
5	0·145	0·015	0·16	0·02
10	0·08	0·025	0·105	0·07
20	0·04	0·025	0·065	0·115

Losses of silica in the precipitate from the fusion extract are shown in Table II for tungsten based materials containing 5 per cent. of cobalt or 5 per cent. of titanium as titanium carbide. It is shown also that silica is lost independently of whether the precipitate containing the titanium or cobalt is filtered from the alkaline fusion extract or is filtered after its acidification.

TABLE II

LOSSES OF SILICA IN FUSION EXTRACTS

Sample	Silicon found in fusion extract; cobalt or titanium residue removed after acidification		Silicon found in fusion extract; cobalt or titanium residue removed before acidification	
	Single treatment, %	Repeat fusion, %	Single treatment, %	Repeat fusion, %
Tungsten metal + 5 per cent. of cobalt	0·16	0·02	0·165	0·02
Tungsten metal + 5 per cent. of titanium	0·17	0·02	0·15	0·05

Repeated fusions were avoided by digesting the acid fusion extract at boiling point for several minutes and oxidising with permanganate, when cobalt and most of the iron dissolve. Titanium and tantalum remain insoluble, but they do not occlude silica (see Tables IV and VI).

CONDITIONS FOR COLOUR DEVELOPMENT—

The conditions we finally derived for the formation of a stable molybdenum blue colour in solutions of samples rich in tungsten were similar to those put forward by Gentry and Sherrington for steels[2]; we utilised a combination of oxalic acid and ferrous sulphate to reduce the yellow molybdisilicic acid to molybdenum blue. We considered this reduction to be more suitable for solutions rich in tungstic acid than one based on stannous chloride, in which interference from the blue tungsten reduction product is possible.

We were able to confirm in general the conditions postulated by Gentry and Sherrington, but their claim for stability of the molybdenum-blue complex is one that requires some qualification. We noted a slow but progressive deterioration of colour, which limited the permissible period for reading at maximum intensity, and we found ammonium oxalate to be more effective than its equivalent of oxalic acid in promoting colour stability, although the colour was slightly less intense for a given concentration of silicic acid. We found that a particular solution with ammonium oxalate giving a reading of 0·830 units within 5 minutes of colour development showed no change over 15 minutes and that the reading had only fallen to 0·81 units after 60 minutes, whilst its counterpart solution with oxalic acid gave an initial reading of 0·995 units after 5 minutes and had fallen to 0·925 after 60 minutes. This degree of instability is equivalent in our application of the method to a loss of about 0·015 per cent. of silicon on a 0·2 per cent. silicon content over the 60-minute period, whilst with ammonium oxalate the equivalent error is reduced to 0·005 per cent. of silicon over the same period.

We found, as did Gentry and Sherrington, that colour development is not critically related to the concentration of ferrous ammonium sulphate used in the reduction, but we noted that the background colour produced in the compensating fraction increases with increase in ferrous ammonium sulphate concentration, and so for this reason we have limited the amount used to rather less than half the amount they specify. This amount has proved to be sufficient for full reduction over the whole range of products discussed in this paper.

METHOD

REAGENTS—

Sulphuric acid, sp.gr. 1·125—Add sulphuric acid, sp.gr. 1·84, to water, cool, and adjust with acid or water to a specific gravity of 1·125.

Potassium permanganate solution, 1 per cent.—Prepare the solution in cold water.

Ammonium molybdate solution—A 5 per cent. aqueous solution of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$.

Ammonium oxalate solution—A 3 per cent. aqueous solution.

Ferrous ammonium sulphate solution, 5 per cent.—Dissolve crystalline ferrous ammonium sulphate, $FeSO_4.(NH_4)_2SO_4.6H_2O$, in water containing 0·1 ml of sulphuric acid per 100 ml.

All reagent solutions should be freshly prepared in minimum amounts for each series of determinations, as solutions stored in bulk take up silica from the containing vessels. Only the purest of reagents are suitable for this class of work.

PROCEDURE—

Weigh 0·5 g of sample (B.S. mesh size <100) into a platinum dish and ignite the sample, cautiously at first, at a low temperature and finally at about 1000° C until the metal or carbide is completely converted into oxide. Roasting is facilitated if the sample is stirred at intervals with a platinum spatula or wire (Note 1).

Fuse the ignited residue with 4 g of anhydrous sodium carbonate and extract the fusion products in 50 ml of water in a nickel beaker, digest for 5 minutes, add 30 ml of sulphuric acid, sp.gr. 1·125, from a burette or pipette, and immediately transfer the solution to a 250-ml beaker made of resistance glass.

Stir briskly to facilitate removal of carbon dioxide (Note 2). Heat the solution, which at this stage should not exceed 120 ml, to boiling. If precipitates of iron, titanium, cobalt, tantalum and so on, are present at this stage, heat the solution at its boiling point for 5 minutes. This digestion is unnecessary for pure tungsten and molybdenum compounds.

Add 1 per cent. potassium permanganate solution, dropwise, until a slight excess is present, digest for a minute or two and then discharge the excess of permanganate by adding the minimum number of drops of 5 per cent. ferrous ammonium sulphate solution. Cool the solution, transfer it to a 200-ml graduated flask, add a small amount of macerated filter-paper pulp, make up to volume with water and mix. Collect about 100 ml of clear filtrate in a dry flask by passing the solution through a dry Whatman No. 40 filter-paper. If the filtered portion is cloudy, as it occasionally is with samples containing much titanium, re-filter through another Whatman No. 40 filter-paper. Take two 40-ml fractions by pipette from the clear filtered portion and transfer them to 100-ml calibrated flasks. Treat the two fractions separately as follows.

Test solution—Add 5 ml of 5 per cent. ammonium molybdate solution, mix and set aside for 5 minutes at 20° to 25° C. Add 10 ml of 3 per cent. ammonium oxalate solution, mix and dilute to about 90 ml with water. Add 2·5 ml of 5 per cent. ferrous ammonium sulphate solution, dilute exactly to the mark with water, mix and set aside for 5 minutes.

Compensating solution—Add 10 ml of 3 per cent. ammonium oxalate solution, mix, rinse the walls of the vessel with water, add 5 ml of 5 per cent. ammonium molybdate solution and dilute to about 90 ml with water. Add 2·5 ml of 5 per cent. ferrous ammonium sulphate solution, dilute exactly to the mark with water and mix.

Measure the difference of absorption between the test and compensating solutions on a photo-electric absorptiometer—a Spekker absorptiometer with a mercury-vapour lamp, 4, 2 or 1-cm cells and Ilford Spectrum yellow No. 606 and H697 or Calorex H503 filters, is suitable. The difference in readings is a measure of the silica from the sample plus minor amounts of silica introduced from the reagents or from glassware. The amount of extraneous silica introduced in this way is normally extremely small, if freshly prepared solutions of pure reagents are used. Correction for it is made by means of a blank fusion on 4 g of sodium carbonate and subsequent determination through all stages of the procedure.

Evaluate the corrected readings by reference to a standard calibration graph prepared from pure reagent solutions that have been passed through all stages of the procedure, as is done for the blank solution, but which contain suitable additions of standard silicate solution (Note 3).

NOTES ON PROCEDURE—

1. (*a*) Tungstic oxide requires only the minimum of ignition to be freed from water and traces of lower oxides.

(*b*) Ammonium paratungstate must be ignited cautiously in the initial stages until water and ammonia are completely volatilised.

(*c*) Finely divided metallic powders, such as tungsten and titanium powders, sometimes are oxidised violently, so the ignition should be carefully controlled in the initial stages to prevent mechanical loss of sample or explosive attack on the platinum dish. If the powders are liable to contain hydrogen, either free or as hydride, the ignition is best performed in a nickel crucible. This should be at the lowest temperature at which oxidation is complete, following which the sample must be transferred to a platinum dish before fusion with sodium carbonate.

(*d*) Molybdenum based materials, *e.g.*, metal, oxide, carbide and carbide mixtures containing molybdenum carbide as one of their components, should be finally ignited at

about 1000° C until the characteristic white fumes of molybdenum trioxide cease to be evolved. All or most of the molybdenum in molybdenum-based compounds is removed in this way.

2. The decomposition of fusion products from materials containing much titanium may be somewhat protracted and continued digestion after transfer of the acidified extract to the glass beaker may be necessary.

3. The standard silicate solution is prepared from pure calcined Brazilian quartz (100 B.S. mesh) as follows. Fuse 0·200 g of the prepared quartz in a platinum dish with 5 g of sodium carbonate. Extract with water in a 500-ml nickel beaker, dilute to the maximum capacity of the beaker and filter through a paper-pulp pad. Wash the filter with water containing a small amount of sodium carbonate, reject the filter-paper and residue, cool and make up the filtrate to exactly 1000 ml in a calibrated flask (1 ml of this solution is equivalent to 0·0002 g of silica). This solution is suitable for the calibration range of the 1-cm cell. For calibrations with the 2 and 4-cm cells a weaker solution is required; this can be prepared by diluting a suitable quantity of the silicate solution to one quarter of its original concentration.

For use in calibration tests prepare a series of blank 4-g sodium carbonate fusion extracts, and operate as in the initial stages of the procedure as far as the point at which the solution is transferred to a 200-ml calibrated flask. Then add suitable amounts of the appropriate silicate solution before adding macerated pulp and making up to volume. The number of calibration tests and the amounts of standard silicate solution to be added to each should be arranged to cover adequately the desired working range for the materials to be examined.

Take the series of calibration tests through all subsequent stages of the procedure as described in the foregoing method and correct for reagent silica and "picked-up" silica by means of a blank calibration test, *i.e.*, a test in which no addition of standard silicate solution is made.

Range—

The method for 0·1-g aliquots from an initial sample weight of 0·5 g gives the following approximate calibration ranges for 0 to 100 drum divisions—

Cell size	4	2	1
Range of silicon, per cent.	0 to 0·12	0 to 0·24	0 to 0·47

Relatively small amounts of silica, such as those found in pure samples of tungstic oxide, are, therefore, most accurately measured in a 4-cm cell, for which the accuracy of measurement is within $\pm$0·002 per cent. and amounts of silica as low as 0·01 per cent. can readily be determined.

Results

The greatest difficulty in assessing the quality of the values obtained from test samples whether as oxide, metal or carbide is associated with the complete absence of reference standards and the inadequacy of the classical methods for the purpose of standardising selected samples as reference standards. This means that any sample of tungstic oxide,

Table III

Silicon content of tungstic oxide

Test	Silicon added, %	Uncorrected absorptiometer reading	Silicon found, %	Silicon in tungstic oxide by difference, %
Solution containing no WO_3	0	0·075 (blank)	—	
	0·005	0·12	0·005	
	0·010	0·16	0·010	
	0·030	0·345	0·031	
Solution containing 0·5 g of WO_3	0	0·175 (blank)*	0·012	0·012
	0·005	0·220	0·017	0·012
	0·010	0·265	0·022	0·012
	0·030	0·420	0·040	0·010

* Value inflated owing to silicon in tungstic oxide.

for instance, that may be used for testing the method carries an inherent small, but unknown, silica content whose value is beyond measurement by any alternative method.

Table III shows that known additions of standard silicate solution can be determined by the method with the same precision and accuracy in the presence of the tungstic oxide as they are in pure solutions free from tungsten; from these values the inherent silica content of the tungstic acid is found to be 0·012 per cent. of silicon to close limits of accuracy.

Having established the validity of the method on pure tungsten-base material, we analysed samples of tungstic oxide and tungsten metal powder and used them as a basis for addition of cobalt, iron, titanium, and so on.

Table IV shows that the equivalent of 5 per cent. of cobalt, iron and titanium added to either tungstic oxide or tungsten metal samples does not affect the results.

TABLE IV

EFFECT OF COBALT, IRON OR TITANIUM ADDED TO TUNGSTEN-BASED SAMPLES

Specification	Silicon present, %	Silicon found, %
Tungstic oxide—		
Nothing added	0·15	0·15
5 per cent. of iron added	0·15	0·15
10 per cent. of iron added	0·15	0·145
Nothing added	0·06	0·06
5 per cent. of iron added	0·06	0·07
Tungsten metal—		
Nothing added	0·17	0·17
5 per cent. of iron added	0·17	0·17
5 per cent. of cobalt added	0·17	0·17
5 per cent. of titanium added	0·17	0·18

The stability of the molybdenum blue in tungsten-based solutions is illustrated by the figures in Table V, which show that there is no loss of colour over a period of 60 minutes.

TABLE V

STABILITY OF MOLYBDENUM BLUE IN TUNGSTEN-BASED SOLUTIONS

Sample	Absorptiometer readings after 5 minutes	Absorptiometer readings after 60 minutes	Silicon found, %
Tungstic oxide containing 0·15 per cent. of silicon and 5 per cent. of iron	0·69	0·69	0·15
Tungstic oxide containing 0·15 per cent. of silicon and 10 per cent. of iron	0·66	0·67	0·145
Tungstic oxide containing 0·06 per cent. of silicon and 5 per cent. of iron	0·35	0·35	0·07

In applying the method to titanium based materials, we are able to make use of a sample of titanium carbide, TiC, containing 0·4 per cent. of silicon, on which we established an accurate independent value gravimetrically by the method of sulphuric acid dehydration. The following values show that the proposed method gives results of comparable accuracy and reproducibility—

Silicon by gravimetric method, per cent.	0·39	0·40	0·40	0·40	0·41
Silicon by proposed method, per cent.	0·39	0·40	0·39	0·39	0·41

This sample of titanium carbide and samples of tungsten carbide powder, WC, and molybdenum carbide, Mo_2C, were used as bases for the preparation of a series of synthetic carbide mixtures on which the validity of the method was finally proved. The results are shown in Table VI.

TABLE VI

RESULTS BY THE PROPOSED METHOD ON SYNTHETIC MIXTURES OF CARBIDE

Mixture	Silicon present, %	Silicon found, %
Tungsten carbide (WC)	0·25	0·250
Titanium carbide (TiC)	0·40	0·405
Molybdenum carbide (Mo_2C)	0·025	0·025
60 per cent. of TiC + 40 per cent. of Mo_2C	0·255	0·252
50 per cent. of TiC + 50 per cent. of Mo_2C	0·21	0·19
20 per cent. of TiC + 80 per cent. of Mo_2C	0·10	0·105
80 per cent. of TiC + 20 per cent. of Co (containing 0·10 per cent. of Si)	0·34	0·335
40 per cent. of TiC + 40 per cent. of MoC + 20 per cent. of Co	0·19	0·18
80 per cent. of WC + 20 per cent. of Ta as Ta_2O_5 (containing 0·004 per cent. of Si)	0·20	0·19
60 per cent. of WC + 20 per cent. of TiC + 20 per cent. of Ta	0·23	0·23
50 per cent. of WC + 20 per cent. of TiC + 20 per cent. of Ta + 10 per cent. of Co	0·225	0·22

Typical values for silicon in various production samples were as follows—

Tungstic oxide	0·009 to 0·017 per cent. of silicon
Ammonium paratungstate	0 to 0·007 per cent. of silicon
Hydrogen reduced tungsten metal	0·005 to 0·08 per cent. of silicon
Carbon reduced tungsten metal	0·16 to 0·18 per cent. of silicon
Tungsten carbide	0·005 to 0·007 per cent. of silicon
Titanium metal	0·04 to 0·31 per cent. of silicon
Titanium carbide	0·40 per cent. of silicon
Molybdenum carbide	0·025 per cent. of silicon

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The Fractionation of Urinary Neutral 17-Ketosteroids by Adsorption and Partition Chromatography

By E. R. COOK, S. R. STITCH, A. E. HALL and MARY P. FELDMAN

The fractionation of urinary neutral 17-ketosteroids by adsorption and by partition chromatography is described, and the close similarity of certain fractions obtained by both methods is illustrated. Despite the influence of the method of hydrolysing the 17-ketosteroid conjugates upon the chromatographic pattern, the constancy of the patterns for the urines from some subjects over a period of weeks is shown by each fractionation procedure.

A method for separating urinary 17-ketosteroids into eight fractions by adsorption chromatography on columns of alumina has been described by Dingemanse, Huis in't Veld and de Laat,[1] and by Dingemanse, Huis in't Veld and Hartogh-Katz.[2] The procedure is somewhat lengthy for clinical purposes, and micro-modifications of the technique have been described by Zygmuntowicz, Wood, Christo and Talbot,[3] by Pond[4] and by Wilkins and Carlson.[5] Rubin and Rosenkrantz[6] use silica gel columns, and the principle of partition chromatography has been applied to the separation of 17-ketosteroids by Jones and Stitch.[7] The partition chromatographic method requires no change of eluting solvent system and can be used with an automatic fraction collector. This technique has been compared with the longer but otherwise satisfactory method of Pond.[4]

Adsorption chromatography

Reagents—

Benzene—Analytical reagent grade benzene is unreliable in quality for chromatographic requirements, and the solvent must be washed with water and fractionally distilled, the middle fraction being then stored over anhydrous analytical reagent grade sodium sulphate until required.

Carbon tetrachloride—Hopkin and Williams technical grade carbon tetrachloride gives satisfactory reagent blanks after being washed with water and fractionated, the middle fraction being then dried with anhydrous analytical reagent grade sodium sulphate.

Other reagents are purified by the methods described by Cook.[8]

Procedure—

Samples of urine are hydrolysed by the method of Callow, Callow, Emmens and Stroud.[9] Add 37 ml of concentrated hydrochloric acid and 50 ml of carbon tetrachloride to 250 ml of urine and heat under reflux for 1 hour on a bath of vigorously boiling water. Cool, remove the carbon tetrachloride and repeat the procedure with two more 50-ml quantities of carbon tetrachloride. Combine the extracts and wash them once with 30 ml of 2 *N* sodium hydroxide, once with 30 ml of 2 *N* sodium hydroxide saturated with sodium hydrosulphite (shake very hard until the organic solvent layer is decolorised), once more with 30 ml of 2 *N* sodium hydroxide, and three times with 30 ml of distilled water. Dry over anhydrous sodium sulphate, filter, distil to dryness and apply suction from a water pump to remove the last traces of carbon tetrachloride. Dissolve the residue in 10 ml of benzene, use a pipette to put duplicate 0·4-ml aliquots into test tubes, evaporate the aliquots to dryness in an oil-bath at 100° to 105° C, and again apply suction. Estimate the steroid in the dry residue by means of the Zimmerman[10] reaction as modified by Callow, Callow and Emmens.[11]

An aliquot of the extract containing from 1·5 to 2·0 mg of 17-ketosteroid is then chromatographed by the method of Pond.[4] It is found necessary to decrease the vacuum applied to the columns from 30 cm of mercury to 10 to 15 cm of mercury and to increase very slightly the volume of eluting liquid, developing the columns with—

5 × 10 ml of benzene (including the extract volume),
14 × 10 ml of benzene containing 0·05 per cent. of ethyl alcohol,
12 × 10 ml of benzene containing 0·1 per cent. of ethyl alcohol,
12 × 10 ml of benzene containing 0·5 per cent. of ethyl alcohol,
5 × 10 ml of benzene containing 1·0 per cent. of ethyl alcohol,
1 × 10 ml of ethyl alcohol.

The eluates are evaporated to dryness and the residue is determined by the Zimmerman reaction. Under these conditions, reproducible chromatograms can be obtained (Table I), although only one batch of alumina has so far been used.

By analogy with the method of Dingemanse, Huis in't Veld and Hartogh-Katz,[2] it appears probable that the 17-ketosteroids eluted in each peak are—

Fraction I—Hydrolytic artefacts 3β-chloro-androst-5-en-17-one, and androst-3:5-dien-17-one (formerly 3-chloro-Δ^5-androstenone-17 and $\Delta^{3:5}$-androstadienone-17, respectively).

Fraction II—3:5-*cyclo*Androsten-6-ol-17-one (*i*-androstan-6-ol-17-one).

Fraction III—The β-hydroxy-17-ketosteroids, androst-5-en-3β-ol-17-one and androstan-3β-ol-17-one (dehydro*iso*androsterone and *iso*androsterone).

Fraction IV—Androstan-3α-ol-17-one (androsterone).

Fraction V—Testan-3α-ol-17-one (etiocholanolone).

Fraction VI—Androstan-3α:11α-diol-17-one (11-hydroxyandrosterone).

Fraction VII—Testan-3α:11α-diol-17-one (11-hydroxyetiocholanolone).

Fraction VIII—Unidentified.

Our own studies with pilot substances confirm the elution of these 17-ketosteroids in fractions I, II, III, IV and V of Pond chromatograms, but the 11-hydroxy-17-ketosteroids have not been available for pilot studies of fractions VI and VII.

Partition chromatography

The technique used is that of Jones and Stitch,[7] which is briefly described here.

An aliquot containing 5 mg of the crude total 17-ketosteroids is purified by the Girard T separation described by Reiss, Hemphill, Gordon and Cook,[12] and approximately 2·5 mg of the ketonic fraction are evaporated to dryness and dissolved in 0·2 ml of pure alcohol and 5 ml of the mobile phase described below. This solution is then loaded on a previously prepared silicic acid column containing nitromethane as stationary phase, and the chromatogram is developed with a mobile phase consisting of 3 per cent. v/v of chloroform in light petroleum, boiling range 60° to 80° C, saturated with nitromethane. The eluate is collected in 5-ml fractions by the automatic receiver changer of Grant and Stitch.[13] The fractions are evaporated to dryness in an oil-bath at 100° C and then reduced pressure is applied from a water pump to remove the last traces of nitromethane; the dry residue is estimated by the Zimmermann reaction. Studies with pilot substances show that 3β-chloro-androst-5-en-17-one is eluted in peak I, 3:5-*cyclo*androsten-6-ol-17-one and androstan-3α-ol-17-one in peak III, androstan-3β-ol-17-one in peak IV, androst-5-en-3β-ol-17-one and testan-3α-ol-17-one in peak V, and androst-5-en-3:17-dione in peak VI.

3:5-*cyclo*Androsten-6-ol-17-one is only encountered in extracts of neutral urine and may be an artefact formed by the decomposition of androst-5-en-3β-ol-17-one sulphate (Jones and Stitch[7]). There is also a partial separation of androst-5-en-3β-ol-17-one and testan-3α-ol-17-one in peak V, especially if the β-17-ketosteroid content is high.

The colorimetric determination of 17-ketosteroids

Crude urinary extracts contain non-specific chromogenic material that intensifies the colour produced in the Zimmermann reaction. These chromogens are almost entirely non-ketonic in nature; either they can be removed by the Girard T chemical separation (Girard and Sandulesco,[14] Talbot, Butler and MacLachlan,[15] Pincus and Pearlman,[16] Cook[8] and Bray[17]) or their interference can be allowed for by means of a correction factor, which makes use of the different absorption maxima shown by the non-ketonic fraction and pure 17-ketosteroids (Gibson and Evelyn,[18] Fraser, Forbes, Albright, Sulkowitch and Reifenstein,[19] Talbot, Berman and MacLachlan,[20] Engstrom and Mason,[21] Hamburger[22] and Allen[23]).

Jones and Stitch[7] use a purified 17-ketosteroid extract for fractionation, and correction for interfering chromogens is then unnecessary. The non-specific material present in the eluates from the Pond technique must be allowed for by colour correction methods; to reduce the calculations required to a minimum, a nomogram has been constructed and has been described in detail by Cook and Rooks.[24]

Procedure—

Add 0·4 ml of alcoholic 1 per cent. *m*-dinitrobenzene solution and 0·2 ml of alcoholic 2·5 *N* potassium hydroxide to the dry residue in the test tube. Make up a similar reagent

blank. Shake the tubes thoroughly, stopper them firmly and incubate them in the dark at 25° ± 0·1° C for 60 minutes. Dilute the reaction mixture with 10 ml of absolute alcohol, and determine the colour extinction against the reagent blank in a photo-electric absorptiometer with an Ilford No. 604 spectrum green filter. An additional reading must be made with an Ilford No. 601 spectrum violet filter for eluates from the Pond columns. The 17-ketosteroid content of each tube can be calculated by reference to a standard graph or a nomogram.

Results

The reproducibility of chromatograms formed by each chromatographic method was examined by carrying out duplicate fractionations of the same urinary extracts; the results, as Table I shows, were in close agreement.

Table I

Reproducibility of chromatographic patterns obtained by the methods of Pond[4] and Jones and Stitch[7]

Urine	Chromatographic technique	Distribution of 17-ketosteroids in fraction							
		I %	II %	III %	IV %	V %	VI %	VII %	VIII %
A	Pond	4·4	—	19·5	39·0	24·8	11·4	—	0·9
A	Pond	4·9	—	19·9	37·2	26·1	11·0	—	0·9
A	Dingemanse *et al.*[1,2]	5·1	—	19·4	33·4	32·0	8·6	—	1·5
B	Pond	3·2	42·9	2·4	27·3	15·9	7·4	0·2	0·7
B	Pond	3·9	42·8	2·7	27·2	14·1	7·6	0·5	1·2
C	Jones and Stitch	41·5	7·6	20·0	—	26·8	4·1		
C	Jones and Stitch	44·0	5·9	16·7	—	26·7	6·7		

Other urine specimens were divided into aliquots, which were then separately hydrolysed and extracted by the method of Callow, Callow, Emmens and Stroud,[9] previously described, and the extracts were chromatographed by both adsorption and partition techniques. In some instances urine aliquots intended for fractionation by the adsorption chromatographic method were extracted by the neutral procedure of Dingemanse, Huis in't Veld and Hartogh-Katz[2] before acid hydrolysis. Table II shows the chromatographic patterns obtained for ten urines.

Table II

Distribution of 17-ketosteroids in chromatograms prepared by the adsorption technique of Pond and the partition technique of Jones and Stitch

Urine	Fraction by Pond technique								Fraction by Jones and Stitch technique					
	I %	II %	III %	IV %	V %	VI %	VII %	VIII %	I %	II %	III %	IV %	V %	VI %
A1	5·0	4·8	2·9	63·0	17·6	6·2	—	0·5	20·6	3·2	48·0	—	23·1	5·1
A2	4·8	10·0	2·3	58·8	16·7	7·0	—	0·4	14·6	3·0	51·5	—	25·0	5·9
A3	6·1	9·2	2·9	58·5	13·4	9·9	—	—	24·8	4·7	40·5	—	24·9	5·1
B	10·2	4·2	1·4	37·7	36·3	9·3	—	0·9	25·1	11·4	32·5	—	31·0	—
C	3·3	—	9·9	19·2	51·0	12·9	1·7	2·0	7·6	14·5	24·0	—	51·2	2·7
D	4·4	—	8·9	30·9	35·2	16·8	1·6	2·2	15·7	14·0	38·2	—	28·2	3·9
E	7·4	5·4	6·1	27·2	39·1	10·5	2·0	2·3	10·8	2·9	28·8	—	51·0	6·5
F1	9·6	—	13·7	34·6	24·9	11·3	1·9	4·0	8·5	7·5	33·6	—	42·5	7·9
F2	14·5	—	18·1	28·6	29·4	8·1	—	1·3	15·8	5·1	32·8	—	40·9	5·4
F3	13·4	2·1	12·7	32·4	26·4	9·9	—	3·1	17·8	6·0	37·3	—	31·6	7·3

Discussion of results

It is well known that the severe acid hydrolysis necessary to liberate free 17-ketosteroids from their water-soluble conjugates may cause considerable destruction or transformation, especially of androst-5-en-3β-ol-17-one (formerly called dehydro*iso*androsterone) mostly by the substitution of the 3β(OH)-group with chlorine (Talbot, Ryan and Wolfe,[25] Bitman and Cohen,[26] Landau, Knowlton, Lugibihl and Munson,[27] Butenandt and Dannebaum,[28] Butenandt, Dannebaum, Harrish and Kundszus[29] and Venning, Hoffman and Browne[30]).

Transformations may take place (*a*) during the process of deconjugation, (*b*) in the hot aqueous acid suspension of the free steroid and (*c*) to a very slight extent by the action of aqueous acid on an organic solvent containing the extracted free 17-ketosteroid.

Many methods, differing widely in their hydrolysis and extraction conditions, have been published for the determination of total 17-ketosteroids, and we have found that five of these techniques give similar results. However, the technique of hydrolysis and extraction becomes important when fractionation is required, and Table III shows the difference in chromatographic patterns that can be obtained by different hydrolysis procedures. The figures for urines A and B in Table III show clearly that the method of independent hydrolysis followed by solvent extraction, used by many workers, produces a high proportion of artefact material, mostly by attack on the β-17-ketosteroids in fraction III (Dingemanse technique).

TABLE III

INFLUENCE OF THE HYDROLYTIC METHOD ON THE CHROMATOGRAPHIC PATTERN

Urine	Method of hydrolysis of urine	Chromatographic technique	Distribution of 17-ketosteroids in fraction							
			I %	II %	III %	IV %	V %	VI %	VII %	VIII %
A	(*a*) Heat under reflux with 15 per cent. v/v of conc. HCl for 15 minutes, cool, extract with ether	Dingemanse *et al.*	18·9	—	16·3	35·4	18·6	8·1	—	2·7
A	(*b*) Add 15 per cent. v/v of 25 per cent. v/v HCl and heat under reflux for two periods of 6 hours with 40 per cent. v/v of benzene	"	12·0	—	26·1	34·8	17·6	8·3	—	1·2
B	(*a*) As in A (*a*)	"	26·6	—	5·5	37·1	26·0	4·5	—	0·3
B	(*b*) Add 15 per cent. v/v of conc. HCl and heat under reflux for three periods of 1 hour with 20 per cent. v/v of carbon tetrachloride	"	11·1	—	17·9	36·4	25·9	7·8	—	0·9
C	(*a*) Reflux neutral urine for two periods of 6 hours with 40 per cent. v/v of benzene, then hydrolyse as in B (*b*). Combine extracts	Pond	3·7	41·2	2·0	27·3	17·6	7·6	—	0·6
C	(*b*) As in B (*b*)	"	15·1	—	25·1	26·9	18·3	13·2	—	1·4

Alternative and gentler methods of hydrolysis by the use of β-glucuronidase preparations are still being studied; at present they require a minimum of 50 to 60 hours for complete deconjugation of the 17-ketosteroid glucuronides (Buehler, Katzman, Doisy and Doisy,[31] Bitman and Cohen,[32] Buehler, Katzman and Doisy,[33] Kinsella, Doisy and Glick[34] and Cohen[35]). Cohen[35] states that these extracts contain considerably less non-specific chromogenic material than do extracts from acid-hydrolysed urines. Recently, Henry and Thevenet[36] and Henry, Thevenet and Jarrige[37] have described the total hydrolysis of urinary steroids and of the potassium salt of androst-5-en-3β-ol-17-one with enzyme preparations from the edible snail (*Helix pomatia*) and shown that the artefact peak was absent when steroid extracts prepared by this method were chromatographed on alumina. Stitch and Halkerston[38] have shown by partition chromatography that there is a marked absence of artefact material with a concomitant increase of the fraction containing androst-5-en-3β-ol-17-one and testan-3α-ol-17-one in ketosteroid extracts from urine hydrolysed by enzyme preparations of the common limpet (*Patella vulgata*). These workers also indicated the presence in these preparations of a sulphatase able to split sulphates conjugated through alcoholic hydroxyl groups, such as occur in the 17-ketosteroid sulphates.

However, although Beher and Gaebler[39] have demonstrated that abnormal constituents of pathological urines may introduce considerable losses of 17-ketosteroids during the course

of boiling with acid, it seems probable that for some time acid hydrolysis will remain the method of choice for clinical work. We have preferred to use the slightly longer lower-temperature (about 78° to 80° C) simultaneous hydrolysis and extraction procedure of Callow, Callow, Emmens and Stroud[9] in order to reduce transformations by process (*b*) to a minimum, and also to decrease the quantity of non-specific chromogens present in the extract (Cook[8]).

Dingemanse, Huis in't Veld and Hartogh-Katz[2] have shown that by heating neutral urine with benzene under reflux, it is possible to obtain 3:5-*cyclo*androsten-6-ol-17-one, an acid-labile 17-ketosteroid that is easily converted by hydrochloric acid into 3β-chloro-androst-5-en-17-one and thence to androst-5-en-3β-ol-17-one by heating. Mason and Engstrom[40] suggested on theoretical grounds that 3:5-*cyclo*androsten-6-ol-17-one may be produced from the sulphate of androst-5-en-3β-ol-17-one, and this has since been confirmed by Jones and Stitch.[7] It may be that 3:5-*cyclo*androsten-6-ol-17-one is produced as an artefact by the Dingemanse, Huis in't Veld and Hartogh-Katz[2] technique of neutral hydrolysis. The figures for urine C in Table III show the chromatographic patterns obtained from a urine hydrolysed (*a*) by the Dingemanse neutral procedure and then by Callow acid hydrolysis and (*b*) by Callow acid hydrolysis. The absence of the β-17-ketosteroids in fraction III (Pond technique) after neutral extraction is noticeable, and in such chromatograms it is probably more correct to add fractions II and III together in order to get an approximation to the β-17-ketosteroid value. This figure is undoubtedly lower than the true content of the urine specimen, since artefact peaks, corresponding to the transformation products of β-17-ketosteroids, occur in the chromatograms obtained by both adsorption and partition techniques.

When comparing the parallel chromatograms in Table II, it must be remembered that 3:5-*cyclo*androsten-6-ol-17-one does not normally appear in fraction III of the Jones and Stitch chromatograms, and this peak is then equivalent to fraction IV of the Pond method. There is excellent agreement between the two sets of values, except for urines A1 and A3. Fraction V of the Jones and Stitch procedure, in which is eluted testan-3α-ol-17-one and androst-5-en-3β-ol-17-one, can be compared only with the total of fractions II, III and V of the Pond technique, and here the agreement is not so pronounced, especially for urine D. However, in view of the inherent difficulties of chromatography and the use of two different chromatographic principles, the general agreement is remarkably close.

TABLE IV

CONSTANCY OF CHROMATOGRAPHIC PATTERN OF 17-KETOSTEROID EXTRACTS OF URINE COLLECTED FROM SOME MALE SUBJECTS AT DIFFERENT TIMES

Subject	Method of hydrolysis	Chromatographic technique	Days	Distribution of 17-ketosteroids in fraction I %	II %	III %	IV %	V %	VI %	VII %	VIII %
A	Neutral procedure of Dingemanse *et al.*,[2] followed by Callow *et al.*[9]	Pond	0	7·8	2·2	1·5	35·2	44·8	7·1	1·4	—
			22	3·1	—	5·4	36·6	45·0	8·8	—	1·1
			56	10·2	4·2	1·4	37·7	36·3	9·3	—	0·9
			61	4·1	—	7·9	35·1	40·7	10·4	0·9	0·9
B	"	"	0	3·6	41·4	1·9	23·8	18·8	9·7	0·3	0·5
			85	3·5	42·6	4·4	22·0	14·0	13·0	—	0·51
C	Callow *et al.*[9]	"	0	9·8	—	16·0	31·5	32·6	8·7	—	1·5
			25	12·8	—	14·5	34·4	29·0	7·3	—	2·0
			29	10·2	—	13·3	32·9	32·1	9·4	—	2·2
D	"	Jones and Stitch	0	20·6	3·2	48·0	—	23·1	5·1		
			2	14·6	3·0	51·5	—	25·0	5·9		
			25	24·8	4·7	40·5	—	24·9	5·1		
E	"	"	0	17·3	4·1	33·2	2·1	31·3	12·0		
			120	21·1	5·1	30·0	—	33·1	10·7		

Comparatively little is known of the variations in chromatographic patterns found for normal subjects. Zygmuntowicz *et al.*[3] have published data for 6 men and 4 women, and Robinson and Goulden[41] have reported the percentage composition of ketosteroid excretion patterns of 19 normal men. The determination for one subject was repeated after 12 months and no change in pattern was observed. Dingemanse, Huis in't Veld and Hartogh-Katz[2] have given the ranges found in repeated determinations for 13 women and 14 men. They reported their values in milligrams of the particular fraction per 24 hours and stated that the

daily variations in excretion patterns for a single subject were so large that repeated determinations on one individual had the same significance as the same number of determinations on different subjects. Our own work shows that the individual pattern of normal and some mental patients, reported as percentage distribution of the various fractions in the 24-hour specimen, remains remarkably constant over many weeks, as illustrated in Table IV. It is hoped to report this work in more detail elsewhere.

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

June 29th, 1953

A Semi-micro Wet Combustion Method for the Determination of Carbon

By E. E. ARCHER

The method involves digesting the sample with a wet combustion mixture, aspirating the evolved gases by a slow stream of air through a heated silica tube containing a silver spiral into an evacuated Buchner flask containing barium hydroxide solution, neutralising the excess of barium hydroxide to thymolphthalein and titrating the precipitated barium carbonate directly by means of standard acid with bromophenol blue as indicator. A correction is made for the amount of acid needed to change the acidity of the solution from that required for the colour change of thymolphthalein to that for the change of bromophenol blue. Thus the carbon dioxide evolved is directly estimated, without isolation of the precipitated barium carbonate, and errors caused by volatile mineral acids are avoided.

The method is applicable to hydrocarbons, aliphatic carboxylic acids, alcohols, ketones and various sulphur and chlorine compounds. Results are quantitative for glucose. Dry sucrose give low and irregular results, but results are quantitative with aqueous solutions of sucrose. Aqueous solutions, in general, can be successfully analysed when they contain not less than 0·5 per cent. of carbon.

Many methods have been described for the determination of carbon by wet combustion. A comprehensive review of the literature is given in a paper by Houghton.[1]

Weak mixtures of sulphuric acid and dichromate as used by some authors[2,3] permit simple gravimetric or titrimetric estimation of the evolved carbon dioxide, but many substances are incompletely oxidised. Stronger oxidation mixtures[1,4,5] increase the range of substances oxidised completely, but special precautions[1] or difficult techniques[4,5] are necessary to avoid interference from evolved acids. In the method described a simple double titration technique avoids this interference. An evacuated flask[6] containing barium hydroxide is used for absorbing evolved carbon dioxide; titration is carried out in the precipitation flask, so that transference errors are avoided.

In accordance with statements in the literature, it was found in this laboratory that simple mixtures of dichromate and sulphuric acid did not completely oxidise many substances, notably aliphatic acids, and although the addition of silver[2] and ceric[7] salts effected some improvement, the range of substances completely oxidised was still limited. It was decided to use the powerful oxidation mixture devised by Van Slyke and Folch,[4] as this completely oxidised a wide range of substances. However, volatile hydrocarbons were not completely oxidised and it was necessary to place a heated silica tube after the main reaction flask.[3] To avoid bleaching of the indicators by halogens a silver spiral was placed in the silica tube.[8]

The researches of Boivin[9] showed that glucose, fructose, lactose and sucrose in the dry state gave low results in wet combustion methods owing to the production of carbon monoxide. Van Slyke and Folch[4] claimed quantitative results for dry dextrose; the method described below also gave quantitative results for dextrose. On the other hand, results were low and irregular for dry sucrose, despite the use of the auxiliary furnace; thus it seems probable that carbon monoxide is evolved so rapidly that insufficient oxygen is present to oxidise it to carbon dioxide. Results were quantitative for sucrose when it was added as a dilute solution.

Method

Apparatus—

The apparatus is shown in Fig. 1. The combustion apparatus is a modification of that used for alkoxyl estimations by Shaw.[10] The bulb on the inlet is of 10-ml capacity, and the inlet from the Winchester quart bottle packed with soda lime should be sufficiently wide to admit a 1-ml pipette.

The Winchester bottle packed with soda lime allows a rapid stream of air freed from carbon dioxide to be drawn through the apparatus.

No. 17 January, 1954

THE SOCIETY FOR ANALYTICAL CHEMISTRY

BULLETIN

The Physical Methods Group of The Society for Analytical Chemistry.

In recent years many classical methods of analysis have been supplemented by methods involving the use of electrical, optical, thermal and other physical properties. Workers in this field have therefore had to deal with entirely new problems connected with the application of these techniques. This article has been written to acquaint analytical chemists with the aims and activities of the Physical Methods Group which must be of vital interest to all chemists using or considering the use of such techniques.

Towards the end of 1943 the Council of the Society convened a meeting at which it submitted to the members draft proposals for the formation of Groups within the Society. Members were reminded that the Memorandum of Association set forth as one of the objects of the Society that it was "to encourage, assist and extend the knowledge and study of analytical chemistry." There having been general agreement that these objects might be more fully implemented by the formation of Groups along the lines suggested by the Council, the first Group came into being in October, 1944, and incorporating as it did the Microchemical Club, became known as the Microchemistry Group. Subsequently a Sub-Committee set up by the Council to examine responsible requests from members for the formation of new Groups recommended the formation of a Physical Methods Group and a Biological Methods Group, and these were inaugurated in February and October, 1945, respectively.

The Physical Methods Group, at the beginning of its life, determined on a policy that would be primarily "educative." An attempt was to be made to answer the questions that analysts were asking or formulating in their minds about the use of physical methods as applied to analytical problems—their utility, their precision, their cost.

To this end, each of the early meetings of the Group was devoted to some particular physical method, and experts in these methods were invited to discourse on their subjects. For example, among the earliest of these papers was one on "The Use of Infra-Red Spectra for Analysis" by Dr. H. W. Thompson, F.R.S., of Oxford, and one on "Partition Chromatography" by Dr. R. L. M. Synge, F.R.S., to whom the Nobel Prize has recently been awarded for his work on this subject.

The high quality of these early papers and the subsequent enthusiastic discussions led the Committee of the Group, and the Council of the Society in its turn, to believe that a further useful purpose would be served in publishing the proceedings of some of those meetings in the form of monographs, a belief that was confirmed by the very considerable sale of those monographs.

With this pioneer work accomplished, the Committee of the Group was able to re-shape its policy, and a glance at the pages of *The Analyst* over the last five or six years will indicate the amount of original work that has been presented at meetings of the Group. It may justly claim to have made a definite contribution to the widening of the Society's interests.

In the eight years of the life of the Group it has grown to a membership of 437, and has settled down to a regular annual programme of five or six meetings, two of which are normally held in the provinces. These two meetings are usually organised as joint meetings with local groups of other societies or specialised discussion panels. Group meetings have been held in Manchester, Liverpool, Edinburgh, Cardiff, Newcastle, Nottingham, Sheffield, Cambridge (2), Birmingham (2), Tees-side, Swansea and Ipswich. In addition the Group has had the privilege of organising two meetings of the Parent Society on subjects coming within the special orbit of the Group. The proceedings of these successful meetings, dealing with Methods of Moisture Determination and with Chromatography, have been published in full in *The Analyst.*

In the 41 Group meetings held up to May, 1953, over 110 papers have been presented. Most of these have eventually appeared in *The Analyst*. The following list of a few of the papers, classified into subjects, will indicate the very wide scope of the meetings held by the Group.

(1) SPECTROSCOPY (EMISSION AND ABSORPTION)—

"Determination of vitamins A and A_2 by photoelectric spectrophotometry with some remarks on general principles," by R. A. Morton, Ph.D., D.Sc., F.R.S., F.R.I.C., and A. L. Stubbs.

"Modern aids to spectroscopy," by B. S. Cooper, B.Sc., F.Inst.P.

"Applications of spectrographic analysis to soil investigations," by R. L. Mitchell, B.Sc., Ph.D., F.R.I.C.

"Spectrographic analysis of rare and high purity materials," by D. M. Smith, B.Sc., A.R.C.S., D.I.C., F.Inst.P.

"The rapid determination of sodium and potassium in rocks and minerals," by G. H. Osborn, F.R.I.C., A.M.Inst.M.M., and H. Johns, B.Sc.

"The scope of infra-red analysis," by N. Sheppard, M.A., Ph.D. (Cantab.).

"The applications of infra-red spectroscopy to the analysis of polymer composition," by H. A. Willis, B.Sc.

"Infra-red spectrometry in the petroleum industry," by H. Powell, Ph.D.

(2) ABSORPTIOMETRY AND FLUORIMETRY—

"Some applications of fluorimetry in vitamin analysis," by E. Kodicek, Ph.D., M.D.

"The use of high-absorbancy reference standards in absorptiometry," by H. M. N. H. Irving, M.A., D.Phil. (Oxon.), F.R.I.C.

(3) X-RAY ANALYSIS (CRYSTALLOGRAPHY)—

"The Barker Index: a means of identifying crystals from their shape," by R. C. Spiller, M.A.

"Micro-analysis using X-ray diffraction techniques," by H. P. Rooksby, B.Sc., F.Inst.P.

(4) RADIOCHEMISTRY AND MASS SPECTROMETRY—

"Radioactivation analysis—some glimpses of its scope," by A. A. Smales, B.Sc., F.R.I.C.

"Paper chromatography of radioactive penicillin," by E. Lester Smith, D.Sc., F.R.I.C., and D. Allison.

"Microdetermination of Na and K by activation analysis," by R. D. Keynes, M.A.

"Determination of abundance ratios of non-radioactive isotopes," by E. R. Roberts, A.R.C.S., Ph.D., D.I.C., and E. R. S. Winter, A.R.C.S., Ph.D., D.I.C.

(5) ION EXCHANGE RESINS—

"Some newer applications and techniques of cation and anion exchange resins in chemical analysis," by G. H. Osborn, F.R.I.C., A.M.Inst.M.M.

(6) CHROMATOGRAPHY—

"The chromatographic estimation of vitamin A in whale liver oil," by N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson, B.Sc., Ph.D., F.R.I.C., and J. P. Savage, B.Sc., A.R.I.C.

(7) ELECTROPHORESIS—

"The technique of moving boundary electrophoresis," by R. A. Kekwick, D.Sc.

"Electrophoresis in the analysis of serum proteins," by N. H. Martin, M.A., M.B., M.R.C.P., F.R.I.C.

(8) POLAROGRAPHY—

"An outline of general principles of polarographic analysis," by W. Cule Davies, D.Sc., Ph.D., F.R.I.C.

"Biochemical applications of polarographic analysis," by J. E. Page, B.Sc., Ph.D., F.R.I.C.

"The application of the cathode ray oscillograph to polarography," by J. E. B. Randles, M.A., B.Sc., and L. Airey, B.Sc., A.R.I.C.

"Applications of mercury drop control to differential and derivative polarography," by L. Airey, B.Sc., A.R.I.C., and A. A. Smales, B.Sc., F.R.I.C.

(9) REVIEW LECTURES AND SPECIAL SUBJECTS—

"A mid-century review of physical methods of analysis," by H. M. N. H. Irving, M.A., D.Phil. (Oxon.), F.R.I.C.

"Physical methods in the titanium pigment industry," by F. R. Williams, Ph.D., F.R.I.C.

In addition, meetings have been held on the subjects of electrometry, electrography and rheology.

No. 19 March, 1954

THE SOCIETY FOR ANALYTICAL CHEMISTRY

BULLETIN

ANNUAL GENERAL MEETING, MARCH 3rd, 1954

THE Eightieth Annual General Meeting of the Society was held at 4.30 p.m. on Wednesday, March 3rd, 1954, in the Meeting Room of the Royal Society, Burlington House, London, W.1. The Chair was occupied by the President, Dr. D. W. Kent-Jones, F.R.I.C. The financial statement for 1953 was presented by the Honorary Treasurer and approved, and the Auditors for 1954 were appointed. The Report of the Council for the year ending March, 1954, was presented by the Honorary Secretary and adopted.

The Scrutineers reported that the following had been elected Officers for the coming year—

President—D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.

Past Presidents serving on the Council—Lewis Eynon, G. W. Monier-Williams, J. R. Nicholls and George Taylor.

Vice-Presidents—C. A. Adams, A. J. Amos and T. McLachlan.

Honorary Treasurer—J. H. Hamence.

Honorary Secretary—K. A. Williams.

Honorary Assistant Secretary—N. L. Allport.

Other Members of Council (for the ensuing two years)—D. C. M. Adamson, J. Haslam, C. L. Hinton, A. G. J. Lipscomb, R. E. Stuckey and C. Whalley.

A. L. Bacharach, R. C. Chirnside, Miss Mary Corner, D. C. Garratt, H. W. Hodgson and H. M. N. H. Irving, having been elected members of the Council in 1953, will, by the Society's Articles of Association, remain Ordinary Members of the Council for 1954.

T. W. Lovett (Chairman of the North of England Section), R. S. Watson (Chairman of the Scottish Section), A. M. Ward (Chairman of the Microchemistry Group), A. A. Smales (Chairman of the Physical Methods Group) and L. J. Harris (Chairman of the Biological Methods Group) will be *ex-officio* members of the Council for 1954.

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, April 7th, 1954

AN Ordinary Meeting of the Society, organised by the Biological Methods Group, will be held on Wednesday, April 7th, 1954, at the Wellcome Research Foundation, Euston Road, London, N.W.1. The Chair will be taken by Professor J. H. Burn, M.A., F.R.S., and the meeting will be in the form of a symposium on "The Comparison of Chemical and Biological Estimation of Drugs in Quantitative Pharmacology" and will deal with the estimation of digitalis preparations, vitamin D and adrenaline by both chemical and biological methods.

The meeting will be in two sessions, the first session beginning at 2.30 p.m. with the Chairman's introduction, which will be followed by two papers on "Digitalis"—

"Chemical Methods," by Dr. J. M. Rowson.

"Biological Methods," by Mr. G. A. Stewart.

After a break for tea, the second session will begin at 4.30 p.m., and the following papers on "Vitamin D" will be presented—

"Chemical Methods," by Dr. J. Green.

"Biological Methods," by Dr. M. E. Coates.

The meeting will conclude with the following two papers on "Adrenaline"—

"Chemical and Biological Methods for the Estimation of Adrenaline and Noradrenaline," by Dr. G. B. West.

"Routine Methods used in the Quantitative Estimation of Adrenaline," by Dr. G. F. Somers.

Ordinary Meeting of the North of England Section, April 10th, 1954

AN Ordinary Meeting of the North of England Section will be held at 2 p.m. on Saturday, April 10th, 1954, at the City Laboratories, Liverpool.

Joint Meeting of the Scottish Section with the Aberdeen and North of Scotland Sections of the Royal Institute of Chemistry, the Chemical Society and the Society of Chemical Industry, April 1st, 1954

A JOINT Meeting of the Scottish Section with the Aberdeen and North of Scotland Sections of the Royal Institute of Chemistry, the Chemical Society and the Society of Chemical Industry will be held in Aberdeen on Thursday, April 1st, 1954, when an address on "Some Applications of the Newer Techniques in Analytical Chemistry" will be given by Dr. J. R. Nicholls, C.B.E., F.R.I.C.

Ordinary Meeting of the Physical Methods Group, April 6th, 1954

AN Ordinary Meeting of the Physical Methods Group will be held at 6.30 p.m. on Tuesday, April 6th, 1954, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The subject of the meeting will be "Mass Spectrometry" and papers will be presented by Dr. R. I. Reed, Dr. G. P. Barnard and Dr. F. Hageman.

PAPERS ACCEPTED FOR PUBLICATION IN *THE ANALYST*

THE following papers have been accepted for publication in *The Analyst*, and are expected to appear in the near future. It is not possible to enter into correspondence about any of them.

"The Absorptiometric Determination of Niobium in Some African Low-Grade Minerals and Mineral Dressing Products," by G. W. C. Milner and A. A. Smales.

> A method is described for the determination of 0·5 per cent. to at least 16 per cent. of niobium pentoxide in head samples and mineral dressing fractions produced during treatment of Sukulu soils (mainly magnetite, apatite and quartz) and Nigerian granite (mainly quartz and cryolite). After chemical attack on the sample, the niobium is separated from the bulk of the other materials by precipitation with tannic acid and cinchonine with silica as carrier, and is finally determined absorptiometrically with potassium thiocyanate. The separation steps need not be quantitative because radiometric correction for losses is made by incorporating niobium-95 tracer in the procedure.

No. 16 January, 1954

THE SOCIETY FOR ANALYTICAL CHEMISTRY

BULLETIN

FORTHCOMING MEETINGS

Joint Meeting of the Society and the Royal Institute of Chemistry, January 20th, 1954

A JOINT Meeting of the Society and the Royal Institute of Chemistry will be held at 6 p.m. on Wednesday, January 20th, 1954, in the Lecture Theatre of the **Institution of Electrical Engineers,** Savoy Place, Victoria Embankment, London, W.C.2. The President of the Royal Institute of Chemistry, Sir Harry Jephcott, M.Sc., Ph.C., F.R.I.C., will take the Chair, and the subject of the meeting will be "The Determination of Alcohol in Blood and Urine."

A report on this subject has been presented by a Panel of Analysts appointed by the Royal Institute of Chemistry to assist the Alcohol and Road Accidents Committee of the British Medical Association in the preparation of their Report on "Recognition of Intoxication" (about to be published).

At this meeting the Report of the Panel will be surveyed in a paper by D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C., and G. Taylor, O.B.E., F.R.I.C.

Prints of the paper will be available at the meeting.

Ordinary Meeting of the Society, January 29th, 1954

AN Ordinary Meeting of the Society, organised by the Microchemistry Group, will be held at 7.15 p.m. on Friday, January 29th, 1954, in Room 73 of the Sir John Cass College, Jewry Street, Aldgate, London, E.C.3.

At this meeting a film entitled "Old Masters of Microchemistry" will be shown and the following papers will be presented—

"Organic Ion Exchange," by L. Saunders, B.Sc., Ph.D., F.R.I.C.

"Inorganic Ion Exchange," by G. H. Osborn, A.M.I.M.M., F.R.I.C.,

From 3 p.m. to the close of the meeting there will be an Exhibition of Microchemical Apparatus in the Laboratories of the College.

Annual General Meeting of the North of England Section, January 30th, 1954

THE Annual General Meeting of the North of England Section will be held at 2 p.m. on Saturday, January 30th, 1954, at the Engineers' Club, Manchester.

This will be followed by an Ordinary Meeting of the Section, at which the President, Dr. D. W. Kent-Jones, F.R.I.C., will present a paper entitled "The Society for Analytical Chemistry."

Annual General Meeting of the Scottish Section, January 20th, 1954

THE Nineteenth Annual General Meeting of the Scottish Section will be held at 12.45 p.m. on Wednesday, January 20th, 1954, in the Rhul Restaurant, 123 Sauchiehall Street, Glasgow.

Annual General Meeting of the Michrochemistry Group, January 29th, 1954

THE Tenth Annual General Meeting of the Microchemistry Group will be held at 7 p.m. on Friday, January 29th, 1954, in Room 73 of the Sir John Cass College, Jewry Street, Aldgate, London, E.C.3.

Ordinary Meeting of the Physical Methods Group, February 9th, 1954

AN Ordinary Meeting of the Physical Methods Group will be held at 7 p.m. on Tuesday, February 9th, 1954, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The subject of the meeting will be "Coulometric Analysis," and the following papers will be presented—

"The Principles of Coulometric Analysis," by E. Bishop, B.Sc., A.R.T.C., A.R.I.C.

"An Automatic Coulometric Titrimeter," by N. Bett, B.Sc., G. Morris, Ph.D., F.Inst.P., and W. Nock, M.A., Grad.I.E.E.

"Some Apparatus and Techniques for Semimicro Coulometric Analysis," by G. Packman, M.Sc., A.R.I.C.

Extraordinary General Meeting of the Polarographic Discussion Panel, February 9th, 1954

AN Extraordinary General Meeting of the Polarographic Discussion Panel will be held at 6 p.m. on Tuesday, February 9th, 1954, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

NOTICE

New British Chemical Standards

THE Bureau of Analysed Samples, Ltd., of Newham Hall, Middlesbrough, announce the following new standard samples, each of which has been analysed by eight or more metallurgical analysts—

MAGNESIUM ALUMINIUM ALLOYS

	B.C.S. No. 262 (D.T.D. 300) %	B.C.S. No. 263 (D.T.D. 165) %
Copper	0·03	0·13
Magnesium	10·57	4·23
Silicon	0·10	0·14
Iron	0·18	0·41
Manganese	0·06	0·50
Zinc	0·05	0·05
Chromium	0·06	0·34
Titanium	0·10	0·05

LOW CARBON STEELS

	B.C.S. No. 264 %	B.C.S. No. 265 %
Carbon	0·037	0·047
Manganese	0·36–	0·44–
Nitrogen	0·013	0·020

These low carbon steels, together with B.C.S. No. 230, form a new series of steels standardised for nitrogen.

The Bureau also announce a replacement of a low carbon Ferro-chromium No. 203/1, and the standardisation of B.C.S. No. 163 for phosphorus (0·049 per cent.) to provide a figure close to the specification limit of 0·05 per cent.

Supplies of these standards are obtainable direct from the Bureau or through the usual laboratory suppliers.

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"The Absorptiometric Determination of Niobium in Low-Grade Ores," by A. E. O. Marzys.

The absorption of light in the near ultra-violet by the reduced niobium - thiocyanate complex in an organic medium is made a basis for the determination of niobium in low-grade ores and minerals. The respective merits of a water - acetone mixture and of ether as solvents are described. The method has been adapted for use with a Hilger Spekker absorptiometer provided with a mercury-vapour lamp. Four modifications of the main procedure are described for use in the presence of certain interfering elements.

The results for a variety of African soils and minerals are compared with those found by gravimetric methods. The precision of the method is as good as, and probably better than, that of gravimetric methods when applied to complex soils and rocks of low niobium content. The speed and ease of manipulation are greatly increased.

By suitable adjustment of the amounts of material used, the method can, in the absence of vanadium, be extended to all niobium minerals. The ratio of niobium to tantalum in mixed oxides can be rapidly found by determining niobium and titanium colorimetrically and calculating the tantalum content by difference.

"The Polarographic Determination of Fluoride. Part II: The Determination of Fluoride in Bromine, Hydrochloric Acid and Hydrobromic Acid," by Miss J. S. Beveridge, B. J. MacNulty, G. F. Reynolds and E. A. Terry.

The application of the method developed in Part I to the determination of fluoride in bromine, hydrochloric acid and hydrobromic acid is described. In the application discussed the cathode-ray polarograph appears to be superior to the conventional type of instrument.

"Automatic Recording of Ion Concentration in Flowing Solution," by J. A. Lewis and K. C. Overton.

An apparatus, consisting essentially of two polarographs in opposition, automatically and continuously records the concentration in flowing or stationary solutions of metallic ions for which discrete and well-formed polarographic waves are available. The instrument can also be used as an ordinary polarograph and for differential or derivative polarography.

"An Improved Method for the Analysis of Gaseous Mixtures on the Micro Scale," by G. H. Bush and R. J. Loneragan.

The various micro methods of gas analysis are reviewed briefly and a modification of the apparatus and method used by Sutton, but in which dry reagents are used, is described. The method, which employs a simply constructed capillary burette used at constant pressure, enables a rapid analysis of a mixed gas to be carried out on a volume of about 0·1 ml with a precision comparable with that obtained on the macro scale.

"A Semi-Micro Gas Analysis Apparatus for the Estimation of the Permanent Gases," by G. J. Minkoff and N. V. V. Parthasathi.

A semi-micro gas-analysis apparatus to analyse samples of from 0·5 to 3 ml of gas has been developed for the determination of carbon dioxide, hydrogen, oxygen, carbon monoxide, methane and nitrogen. No liquid or solid absorbents are used and no excess gases have to be introduced to complete the estimations. The basic features of the method are the combination of oxygen with hydrogen and carbon monoxide in the presence of copper oxide at 285° C and the subsequent removal of excess oxygen by copper, also at 285° C. The water and carbon dioxide formed are frozen out separately in solid carbon dioxide and liquid-air traps, and the pressure of the gas, in a constant volume, is measured at each stage. Should oxygen not be in excess, however, it is first made to combine with hydrogen and carbon monoxide in the presence of copper at 285° C, and the remaining hydrogen and carbon monoxide is then passed over the copper oxide. The pressures of the water and carbon dioxide formed indicate the original concentrations of the three gases. Methane is oxidised over the Arneil catalyst at 600° C, and the pressure of the remaining nitrogen is measured directly.

"The Separation and Determination of Gallium," by G. W. C. Milner, A. J. Wood and J. L. Woodhead.

From a study of the extractability of the halides of gallium from acid solutions with organic solvents, the chloride was found to be more readily extracted than either the bromide or the iodide. In addition, several organic solvents proved to be as efficient as ether for extracting gallium chloride from solution. Diethyl ether was preferred in the analysis of gallium - uranium mixtures. The extracted gallium was then precipitated with camphoric acid after buffering the solution with a formic acid - ammonium formate buffer of pH 3·3, this precipitate being separated by filtration through a sintered-glass crucible, washed, dried and finally weighed. The factor for converting the weight of precipitate to weight of gallium proved to be 0·213. The determination of amounts of extracted gallium less than about 3 mg was more satisfactorily accomplished by a potassium ferrocyanide titration with 3:3′-dimethylnaphthidine as indicator.

"The Determination of O:O-Diethyl O-*p*-Nitrophenyl Thiophosphate Residues in Tomatoes," by R. Buckley and J. P. Colthurst.

A method is described for the separation and colorimetric determination of parathion residues in tomatoes. Residues on the surface are removed by washing the fruit with alcohol, plant pigments are oxidised with hydrogen peroxide and the parathion is hydrolysed to *p*-nitrophenol, which is determined colorimetrically. Residues in the whole fruit are determined by extraction of the macerated tomato with *n*-hexane, removal of plant pigments by oxidation and extraction and colorimetric determination of *p*-nitrophenol after hydrolysis.

"The Micro-determination of Picric Acid in Picrates," by P. R. W. Baker.

Three methods for the determination of picric acid in organic picrates, on a micro scale, have been examined. Neither Bolliger's method of titration with methylene blue, nor the method of titration with sodium hydroxide, is of universal application. The macro method of Busch and Blume involving precipitation with nitron, gives satisfactory results on a micro scale, and is applicable to all the compounds examined. The solubility of the precipitate and the effect of chloride upon its formation have also been examined.

The officers of the Group will always welcome suggestions from members for special facilities to be offered so that a new technique can receive special attention.

All members of The Society for Analytical Chemistry are eligible for membership of the Group without extra subscription fee. Applications for registration as a member of the Group should be sent to Mrs. D. V. Hicks, Secretary, The Society for Analytical Chemistry, 7–8, Idol Lane, London, E.C.3.

THE SOCIETY FOR ANALYTICAL CHEMISTRY

FORMERLY THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry by holding periodical meetings, by promoting lectures, discussions and conferences, and by the publication of a journal devoted to analytical chemistry; to study questions relating to the analysis, nature and composition of natural and manufactured materials generally; and to promote, or assist to promote, the efficiency and the proper administration of the laws relating to the control and composition of such materials generally.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society who shall provide written testimony of their personal knowledge as to his scientific and professional fitness. If the Council in their discretion think fit, such testimony may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so elects. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

The Entrance Fee for Ordinary Members is £1 1s. and the Annual Subscription is £2 2s. Junior Members are not required to pay an Entrance Fee and their Annual Subscription is £1 1s. No Entrance Fee is payable by a Junior Member on transferring to Ordinary Membership. The Entrance Fee (where applicable) and first year's Subscription must accompany the completed Form of Application for Membership. The Society's official year runs from March to March, but the financial year begins on January 1st and subscriptions are due on that date.

Scientific Meetings of the Society are usually held on the first Wednesday in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. The Annual General Meeting is usually held in London on the first Friday in March. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the official organ of the Society, is issued monthly to all Ordinary and Junior Members, and contains reports of the proceedings of the Society, original papers and notes, information about analytical methods, Government reports and reviews of books. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, the Society for Analytical Chemistry, 7–8, Idol Lane, London, E.C.3.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England Section and the Scottish Section were formed to promote the aims and interests of the Society among the members in those areas. Members of the Society residing in England or Wales north of Birmingham become members of the North of England Section and those resident in Scotland members of the Scottish Section.

The Microchemistry Group, the Physical Methods Group and the Biological Methods Group have been formed within the Society to further the study of the application of microchemical, physical and biological methods of analysis. All members of the Society are eligible for membership of the Groups.

There is no extra subscription for membership of a Section or Group. Application for registration as a member should be made to the Secretary.

The Sections and Groups hold their own meetings from time to time in different places.

The silica tube, which is supported by a sheet-iron shield, ensures that volatile substances not readily oxidised in a wet combustion process are completely converted to carbon dioxide before reaching the absorption flask. Two spirals can conveniently be mounted in one shield and, if a Y-piece is connected to the outlet of the soda-lime tower, two sets of apparatus can be used side by side. Volatile hydrocarbons gave results about 5 per cent. too low when the silica tube was omitted. For many substances its use is unnecessary, but in this work it was retained throughout. If the substance under examination contains chlorine or bromine, a few strands of silver wire are put in the bend of the silica tube to absorb

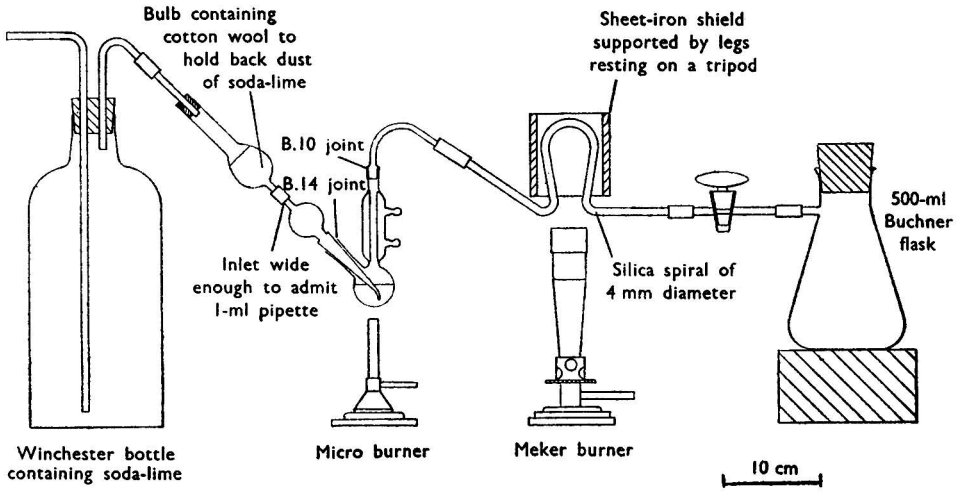

Fig. 1. Apparatus assembly for determination of carbon by the wet-combustion procedure (drawn to scale)

the halogen, which otherwise would bleach the indicators used in the titration procedure.

A long jet is fitted to a 25-ml burette, so that a rubber bung that fits the Buchner flask can be slid over it.

REAGENTS—

Barium hydroxide solution—Dissolve 12 g of barium hydroxide in 100 ml of hot water; make up to 1 litre with cold boiled water. Keep the solution in an aspirator bottle fitted with a tap at the bottom and a soda-lime tube at the top. If the solution is left overnight, any sediment of barium carbonate will conveniently settle below the level of the tap outlet. Connect the tap to a 20-ml automatic pipette, the overflow of which is connected to a trap closed by means of a soda-lime tube. When the pipette is not in use, close its tip with a rubber cap.

Combustion mixture—Pour 85 ml of sulphuric acid, sp.gr. 1·85, and 15 ml of phosphoric acid, sp.gr. 1·75, into a 250-ml conical flask fitted with a ground-glass stopper. Add 20 g of phosphorus pentoxide, 10 g of chromium trioxide and 1 g of potassium iodate. Heat the mixture to 150° C, stir at this temperature for 2 minutes, cool and stopper the flask.

Hydrochloric acid, 0·05 N—Dilute standard *N* acid with boiled and cooled distilled water.

Alcoholic thymolphthalein, 0·2 *per cent.*—Dissolve the solid in 95 per cent. ethanol.

Alcoholic bromophenol blue, 0·1 *per cent.*—Dissolve the solid in 95 per cent. ethanol.

PROCEDURE—

This varies slightly according to the nature of the sample. Normally sufficient sample is taken to give about 5 mg of total carbon.

Non-volatile solids and liquids—Add 10 ml of combustion mixture to the combustion flask and assemble the apparatus as in Fig. 1, lubricating the ground-glass joints with a little phosphoric acid. Remove the Buchner flask, evacuate the apparatus by means of a water pump, close the tap, and re-assemble the apparatus. Open the tap to aspirate carbon

dioxide-free air through the apparatus. Repeat the operation to eliminate all carbon dioxide from the system and to fill the Buchner flask with carbon dioxide-free air. From the automatic pipette add 20 ml of barium hydroxide solution to the Buchner flask, replace the bung, and again evacuate.

Weigh the sample in a weighing spoon that can be lowered through the condenser attached to the combustion flask. Break the joint leading to the silica tube, carefully lower the weighing spoon into the combustion flask, and replace the silica tube. Open the tap on the Buchner flask carefully so that a slow stream of air is aspirated through the apparatus. Heat the combustion flask fairly vigorously with a micro-burner, but avoid heating enough to cause heavy white fumes to pass the condenser. After 10 minutes remove the flame and increase the air flow so that in about another 5 minutes the pressure inside the Buchner flask is again atmospheric. After 15 minutes, with occasional swirling, the flask is ready for titration.

For each batch of combustion mixture an "indicator blank" must be prepared. Take 10 ml of the combustion mixture through the procedure exactly as above. Remove the bung from the Buchner flask, add a few drops of 0·2 per cent. alcoholic thymolphthalein and close the flask with the bung attached to the 25-ml burette containing 0·05 *N* hydrochloric acid. Titrate the solution to the thymolphthalein end-point, remove the burette, and add 1 ml of 0·1 per cent. bromophenol blue. Titrate until a reddish-yellow colour is reached. The difference between the two titrations is referred to as the "indicator blank." In practice this is about 0·6 ml of 0·05 *N* hydrochloric acid, or slightly more than would be needed to take 20 ml of barium hydroxide solution directly delivered from a pipette through the same indicator changes. This difference is thought to be caused by traces of organic matter in the combustion mixture—possibly from the paraffin wax used to seal bottles of phosphorus pentoxide.

Titrate the sample in exactly the same way as the "indicator blank," until the barium carbonate has completely dissolved. Near the end-point replace the rubber bung in the flask, and, closing the tap, shake the flask well to dissolve any carbonate sticking to the sides. When the end-point is near, allow the flask to stand for 5 minutes before making the final adjustment. The end-point is reached when the sample solution and "indicator blank" exactly match. The match is best made by comparing the colours of the two solutions, viewed horizontally, against a white background. It is convenient to refill the burette after reaching the thymolphthalein end-point, as the value of the first titration is disregarded.

Then

$$\text{the percentage of carbon in sample} = \frac{(\text{titre} - \text{"indicator blank"}) \times 6 \times 100}{20{,}000 \times \text{weight of sample taken.}}$$

Volatile liquids—The sample is weighed in a piece of capillary tubing, about 2½ inches long, drawn out to a very fine point at both ends. B.D.H. capillator tubing is ideal for the purpose.

Weigh the empty tube. Manipulate the sample by means of a capillator bulb so that it occupies the middle section of the tube, leaving none at either end. Remove the capillator bulb, taking care not to break the fine tip and, after wiping the tube with filter-paper, re-weigh the tube. Temporarily break the connection to the soda-lime tower, drop the tube down the side-arm of the combustion flask and completely assemble the apparatus as before.

Aspirate a slow stream of air through the apparatus, and at first heat fairly strongly. As the air in the capillary warms up, the sample liquid is forced up the tube until it reaches the tip. It is then vaporised by the incoming air stream. At this point increase the air stream slightly to prevent the carbon dioxide, which is rapidly evolved, from sucking back. When the reaction has subsided somewhat, close the tap temporarily so that the combustion mixture is sucked back into the side-arm and the capillary is completely immersed in the hot combustion mixture. This ensures complete reaction of any less volatile constituents.

The rest of the procedure is exactly as described for non-volatile solids and liquids.

Aqueous solutions—Temporarily break the connection to the soda-lime tower, and run 1 ml of the sample from a pipette down the side-arm. Then proceed as before.

It was found that with solutions of formic acid the reaction was extremely rapid, leading to losses of carbon dioxide whilst the sample was being added. This was avoided by first running a few drops of water down the side-arm to form a "buffer layer" between the combustion mixture and sample solution.

Experimental

To test the absorption of carbon dioxide under the conditions described, a solution of pure sodium carbonate was used. The apparatus was set up as usual except that no combustion mixture was added to the flask. After the apparatus had been swept free from carbon dioxide, 10 ml of the solution were placed in the flask via the condenser by means of a pipette. The condenser was reconnected and 1 ml of water and then 1 ml of 5 *N* hydrochloric acid were added through the side-arm by means of a pipette. The carbon dioxide scrubber was reconnected, and air was aspirated through the system exactly as described for a sample, the mixture being gently boiled.

In these experiments 0·4373 g of sodium carbonate was made up to 100 ml, so that each 10-ml aliquot contained 0·04373 g of sodium carbonate.

On calculating the amount of absorbed carbon dioxide back to its equivalent weight of sodium carbonate in three experiments, the amounts of sodium carbonate recovered were 0·04380, 0·04377 and 0·04370 g. Hence, within normal experimental limits, the recovery of carbon dioxide is 100 per cent.

Results

Table I shows the range of substances examined and the accuracy of the results obtained.

Table I

THE DETERMINATION OF CARBON BY THE PROPOSED METHOD

Sample	Carbon found, %	Carbon theory, %
Hydrocarbons—		
n-Heptane	83·7, 83·2	84·0
3-Methylpentane	83·1, 83·1, 82·8	83·7
Naphthalene	92·9, 93·6, 93·8	93·8
Carboxylic Acids—		
Acetic acid (by direct weighing)	40·5, 39·9, 40·2	40·0
Formic acid (dilute aqueous solution)	26·0, 25·9, 25·9	26·1
Butyric acid (dilute aqueous solution)	54·6, 54·6	54·6
Carbohydrates—		
Glucose	39·8, 39·8	40·0
Sucrose (weighed dry)	34·6, 34·4	42·0
Sucrose (aqueous solution)	42·0, 42·1	42·0
Various—		
Ethanol (dilute aqueous solution)	51·9, 51·9	52·2
Acetone (dilute aqueous solution)	62·5, 61·8	62·1
Sulphanilic acid	41·4, 41·2	41·6
Polyvinyl chloride	38·4	38·4
αα-Dichloropropionitrile (experimental sample)	29·4, 28·9	29·0

The author wishes to thank Mrs. E. Jacquet, who assisted in working out the method, and the Directors of The Distillers Company Limited for permission to publish this article.

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RESEARCH AND DEVELOPMENT DEPARTMENT
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June 23rd, 1953

An Improved Copper Reduction Method for the Micro-determination of Reducing Sugars

By HAROLD G. WAGER

Errors in the method for the micro-determination of reducing sugars proposed by Nelson are shown to derive from (*i*) oxidation of the copper reagent by dissolved oxygen during heating to give reducing compounds that interfere with the determination, (*ii*) re-oxidation of the cuprous ions formed and (*iii*) instability of the blue colour formed by the arsenomolybdate reagent. These can be avoided by carrying out the whole test under oxygen-free conditions and recording the extinction coefficient at a standard time after adding the arsenomolybdate reagent.

The use of paper chromatography for the qualitative separation of sugars in extracts of plant tissues or plant products is now well established, but for the quantitative determination of the sugars so separated a good and convenient micro-method is required. If, as not infrequently occurs, one of the sugars is present in small amounts relative to the other sugars, the determination must be reliable over a wide range of concentration.

The estimation could be carried out by a micro technique of the Linderstrom-Lang[1] type, but a less specialised method was required. The colorimetric method proposed by Nelson[2] seemed suitable for the quantitative determination of sugars eluted from paper chromatograms, but it was found to be subject to serious errors, especially at low concentrations of sugar. The causes of some of these errors are shown below, and an improved procedure is suggested by which 100 μg of reducing sugar can be estimated to ± 1 per cent. and 10 μg to ± 3 per cent.

The modification of the Shaffer - Hartmann[3] copper reduction method proposed by Somogyi[4] in 1937 was adapted for use as a micro-colorimetric method by Nelson.[2] The cuprous ions formed by the reaction with sugars were allowed to react with an arsenomolybdate reagent and the blue colour so formed was measured photometrically. In 1945, Somogyi[5] proposed another copper reagent and recommended it for use with the arsenomolybdate reagent in the micro-method.

A further paper by Somogyi[6] has appeared since the work described below was done; in it he criticises the copper reagent proposed in his 1945 paper on the grounds that it interferes with the stability of the colour developed by the arsenomolybdate reagent, and he proposes in its place a reagent, buffered by sodium carbonate and bicarbonate, which therefore is of similar type to the Maskell and Narain copper reagents used in the present work. He further states that "tartrate measurably reduces copper, especially when the reagent is heated," but apart from this no reference to the effect described below is made.

Experimental

Two copper reagents have been used—

(1) The reagent described by Somogyi.[5] With this reagent the water blank value slowly increased with time.

(2) The modified Shaffer - Hartmann copper reagent developed by Maskell and Narain* with the potassium iodate and oxalate omitted. These compounds are only required for the iodometric estimation of the cuprous ion. This reagent was found to be stable.

* Professor E. J. Maskell has kindly contributed the following brief description of his method. "The modified Shaffer - Hartmann technique developed by Maskell and Narain in 1932 and described in R. Narain's Ph.D. thesis, Cambridge, 1932, is unpublished except for a brief statement by Gawadi.[7] The essential features of the modified technique are (1) the use of a copper reagent of lower alkalinity, which improved linearity and reduced the sensitivity to non-sugars, and (2) the mixture of copper reagent and sugar solution is, immediately before being boiled, swept out for 10 minutes with a stream of nitrogen gas, thereby greatly reducing reoxidation by the reduced copper oxide during boiling. The copper reagent consists of 0·02 *M* copper sulphate, 0·1 *M* sodium carbonate, 0·2 *M* sodium bicarbonate, 0·05 *M* sodium potassium tartrate, 0·1 *M* potassium oxalate and 0·0033 *M* potassium iodate."

The arsenomolybdate reagent described by Nelson[2] was used for the development of the molybdate blue colour.

The tests were made with 2 ml of the copper reagent and 5 ml of sugar solution in a 6 × $\frac{5}{8}$-inch test tube with a 25-ml calibration mark. The tube with the mixed solution was plunged into a bath of boiling water for 10 minutes, and then cooled in running cold water, 1 ml of arsenomolybdate reagent was added, the volume was made up to 25 ml and the extinction coefficient of the molybdate blue was determined, a deep red filter such as Ilford No. 608 being used.

RESULTS—

Considerable variations in the extinction coefficient of both the water blanks and tests with 100 μg of glucose were found in tests with the same solution of the Somogyi copper reagent on successive days, and results are shown in Table I. The variation was approximately constant at all the concentrations of glucose tested and therefore the percentage errors in determinations of 5 to 10 μg of glucose were very large.

TABLE I

EXTINCTION COEFFICIENT OF THE WATER BLANK AND OF 100 μg OF GLUCOSE: SOMOGYI COPPER REAGENT

Date	Water blank	100 μg of glucose	Average increase for 100 μg of glucose
8.12.48	0·106, 0·108	0·552, 0·552	0·445
10.12.48	0·092, 0·098	0·562, 0·562	0·467
10.12.48	0·110, 0·108	0·533, 0·545	0·435
13.12.48	0·127, 0·115		

Possible causes of these variations seemed to be—

(*i*) The formation of reducing compounds by oxidation of the copper reagent.
(*ii*) The re-oxidation at some stage of the cuprous ions formed during the oxidation of the sugar.

To test the first possibility, water blank tubes were prepared with the Somogyi copper reagent, and oxygen, air or nitrogen was bubbled through the liquid for 10 minutes before heating. The results of this experiment are shown in Table II. The values of the extinction coefficient of the water blank are clearly dependent on the amount of oxygen in the liquid. Heating the reagent in the presence of oxygen, therefore, appears to lead to the production of reducing substances that reduce either the cupric ion or the arsenomolybdate complex.

TABLE II

EXTINCTION COEFFICIENT OF THE WATER BLANK AFTER EQUILIBRATION WITH NITROGEN, AIR OR OXYGEN: SOMOGYI COPPER REAGENT

Gas used for equilibration	Extinction coefficient
Nitrogen	0·044, 0·042
Air	0·128, 0·127
Oxygen	0·305, 0·308

Lower and less variable values for the water blank and for tests with glucose might therefore be expected if oxygen was removed from the test liquids by a stream of nitrogen gas before heating (this technique was used by Maskell and Narain, *cf.* Gawadi[7] and footnote, p. 34). Many tests of the effectiveness of oxygen removal by a stream of nitrogen gas were made with the Somogyi copper reagent; the extinction coefficient of the water blank was always low but was still variable; most values fell between 0·02 and 0·03, but values as high as 0·057 were recorded.

In consequence of this variability, the possibility of the re-oxidation of the cuprous ion during cooling or during the addition of the arsenomolybdate reagent was investigated. The mixture of copper reagent and water or sugar solution was freed from oxygen by a stream of nitrogen and was maintained under nitrogen both during the heating and during the cooling, and nitrogen was blown into the top of the test tube while the arsenomolybdate reagent was added. The effect of this treatment was to increase the value of the extinction coefficient

of the water blank to about 0·06. Re-oxidation of cuprous ions must, therefore, have been occurring in the previous tests. This re-oxidation could be demonstrated by admitting a little air to the tubes before adding the arsenomolybdate reagent, when the value of the extinction coefficient fell again to 0·02.

The reliability of the method with both reagents and with the modified technique just described was studied and results for the Maskell and Narain copper reagent are shown in Table III. This table shows that the estimations made in 1949 agree very closely with those made in 1951, that 100 μg of glucose can be estimated to within ± 1 per cent., and that a control sugar sample in each run is unnecessary; such controls were required in the Nelson technique. A similar series of observations, which are not quoted, made with the Somogyi copper reagent showed that the extinction coefficient of the water blank increased slightly on successive days and for some unknown reason the scatter of values was greater; in 14 tests the range of the increase in the extinction coefficient of 100 μg of glucose over that of the water blank was 0·479 to 0·522.

TABLE III

EXTINCTION COEFFICIENT OF WATER BLANK AND OF 100 μg OF GLUCOSE: MASKELL AND NARAIN COPPER REAGENT, UNDER NITROGEN THROUGHOUT

Each figure is the average of two determinations in one experiment

Date	Water blank	Increase over water blank for 100 μg of glucose	Date	Water blank	Increase over water blank for 100 μg of glucose
20.1.49	0·073	0·591	13.4.51	0·051	0·591
21.1.49	0·065	0·580	18.4.51	0·041	0·584
21.1.49	0·065	0·585	23.4.51	0·056	0·584
25.1.49	0·052	0·586	23.4.51	0·060	0·582
26.1.49	0·061	0·579	26.4.51	0·064	0·591
31.1.49	0·060	0·592	26.4.51	0·065	0·594
1.2.49	0·050	0·592	1.5.51	0·056	0·587

With the proposed method the extinction coefficient is directly proportional to the concentration of glucose over the range of 0 to 100 μg, as shown in Table IV. This table also shows that a reasonably reliable estimate of as little as 10 μg of glucose is possible with the modified technique.

TABLE IV

EXTINCTION COEFFICIENT FOR VARIOUS CONCENTRATIONS OF GLUCOSE: MASKELL AND NARAIN COPPER REAGENT, UNDER NITROGEN THROUGHOUT

Each value is the average of two determinations in one experiment

Glucose, μg	Increase of the extinctions coefficient over the water blank for glucose
10	0·059, 0·059
20	0·119, 0·119
100	0·591, 0·594

An estimate of the reducing bodies formed, and of the extent of re-oxidation of the cuprous ion, can be made by comparing the extinction coefficients of solutions heated in equilibrium with either air or nitrogen and then cooled either under air or nitrogen (see Table V). The high value of the water blank of the Somogyi reagent when heated with air is, presumably, caused by oxidation of the reagent, and as there is only a slight increase in value when it is cooled under nitrogen, only a small amount of re-oxidation can be occurring as a result of the reduced solubility of oxygen caused by the presence of sodium sulphate. In the Maskell and Narain reagent, on the other hand, the presence of oxygen at any stage leads to much oxidation of the cuprous ion, as instanced by the low water-blank values when air is present at any stage. Further, the higher value given by 100 μg of glucose with the nitrogen - air treatment than with the air - nitrogen treatment shows that this re-oxidation of the cuprous ion is greater during the heating than the cooling phase. The production

of reducing bodies by direct oxidation of the reagent seems to be less in the Maskell and Narain reagent than in the Somogyi reagent; this may be related to the different pH values of the two reagents.

TABLE V

EXTINCTION COEFFICIENTS OF WATER BLANKS AND 100 μg OF GLUCOSE UNDER DIFFERENT CONDITIONS OF AERATION

Each value is the mean of two determinations in one experiment

Gas under which solution was		Maskell and Narain copper reagent		Somogyi copper reagent	
heated	cooled	Water blank	Increase for 100 μg of glucose	Water blank	Increase for 100 μg of glucose
Air	Air	0·013, 0·112	0·444, 0·449	0·121, 0·126	0·439, 0·434
Air	Nitrogen	0·026, 0·024	0·452, 0·443	0·143, 0·131	0·443, 0·476
Nitrogen	Air	0·024, 0·048	0·561, 0·527	0·044, 0·034	0·476, 0·471
Nitrogen	Nitrogen	0·056, 0·060	0·584, 0·582	0·067, 0·070	0·488, 0·502

The blue colour produced by the arsenomolybdate reagent increases with time, as shown in Fig. 1. This rise in intensity of colour is undoubtedly one of the major sources of error

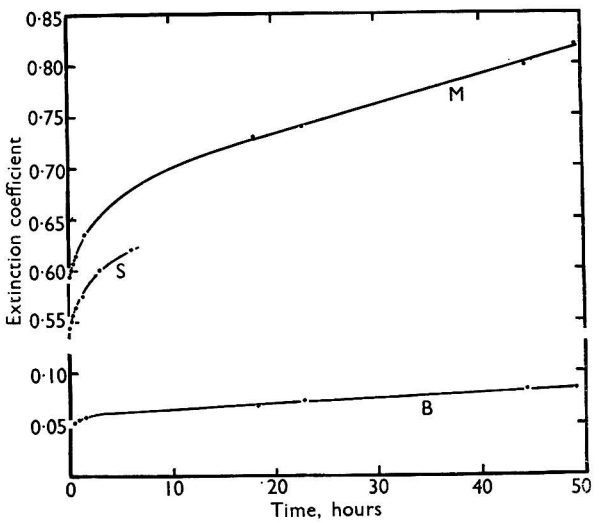

Fig. 1. Change of extinction coefficient of molybdate blue with time, measured through a deep-red filter. The arsenomolybdate reagent was added at zero time. Curve M, 100 μg of glucose tested with the Maskell and Narain copper reagent; curve B, water blank value of the Maskell and Narain copper reagent; curve S, 100 μg of glucose tested with the Somogyi copper reagent

remaining in the method. Modifications to the conditions of colour development were tried, but no increase in stability resulted. The use of a phosphomolybdate reagent was found to give a stable colour, but with the various conditions tested, an intensity of colour, resulting from added glucose, greater than about one-third of that produced by the arsenomolybdate reagent could not be obtained, and this led to a serious decrease of sensitivity. It was finally decided to determine the extinction coefficient at an exactly standard time after adding the arsenomolybdate reagent. This slowed the determinations, but the accuracy was considerably increased. The results in Tables III, IV and V were recorded with this technique.

PROPOSED METHOD

APPARATUS—

A convenient apparatus for carrying out the method is shown in Fig. 2. A series of units, only one of which is shown complete, are linked together by two systems of T-pieces.

The test tubes stand in a rack that can be put successively into boiling water and into cold running water without stopping the flow of nitrogen.

PROCEDURE—

Place 5 ml of sugar solution and 2 ml of copper reagent by pipette into each of the graduated test tubes and fix them to the rubber bungs. Pass nitrogen into the liquid for about 10 minutes through the T-pieces, D, the capillary resistances, E, inserted to equalise the flow in all the test tubes, and the tube, F, of narrow bore to ensure a stream of fine bubbles.

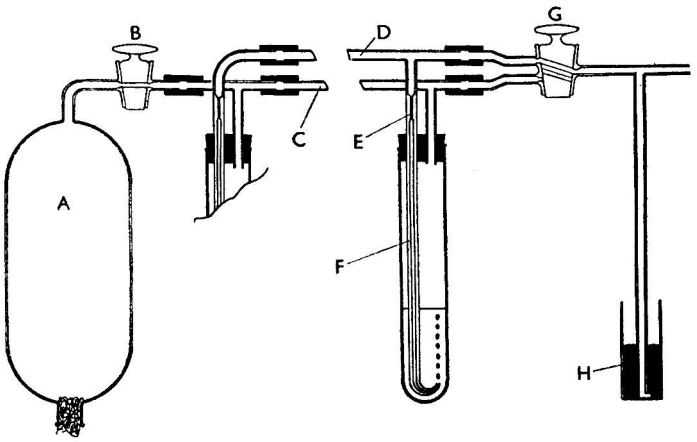

Fig. 2. Apparatus for heating and cooling under nitrogen

The nitrogen escapes through the second system of T-pieces, C. After the liquids have been freed from oxygen, turn the two-way tap, G, to allow the stream of nitrogen to flow over the tops of the tubes. Put the rack with the tubes into boiling water for exactly 10 minutes, and then transfer it to cold water for 3 minutes. The immediate contraction in volume occurring on plunging the tubes into cold water is made good by nitrogen from the vessel, A. Then shut the tap, B, when nitrogen escapes through the pressure regulator and overflow, H. Lift each bung in turn just sufficiently to insert a pipette and add 1 ml of the arsenomolybdate solution; replace the bung and shake the tube. During this addition the whole stream of nitrogen escapes from the test tube, so effectually preventing entry of oxygen. Add the arsenomolybdate reagent to successive tubes at exactly 2-minute intervals and measure the extinction coefficient exactly 24 minutes later. These times are arbitrary, but they have been found convenient for eight or ten tubes and must be kept standard, as the duration of standing affects the calibration. Once the arsenomolybdate has been added, oxygen has no further effect and the liquids are made up to volume in air.

I have used the Maskell and Narain technique for the semi-micro estimation of sugars for many years, and I should like to thank Professor E. J. Maskell for having given me particulars of it.

The work described in this paper forms part of the programme of the Food Investigation Organisation of the Department of Scientific and Industrial Research. Mr. J. Howe carried out the experimental work.

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LOW TEMPERATURE STATION FOR RESEARCH IN
BIOCHEMISTRY AND BIOPHYSICS
UNIVERSITY OF CAMBRIDGE
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DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

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The Determination of Iron and Copper in Single Serum Samples

By S. VENTURA* and J. C. WHITE

A method is described for the spectrophotometric determination of iron and copper in single samples of serum. The metallic ions are liberated from protein with 6 *N* hydrochloric acid and determined as the 2-2′-dipyridyl-ferrous complex and as copper diethyldithiocarbamate, the latter being extracted into a mixture of ether and amyl alcohol.

In the study of iron and copper metabolism, it is convenient to measure the amount of metals in single samples of serum. Satisfactory splitting of the metallic ions from protein is an essential prelude to their spectrophotometric or colorimetric determination as coloured complexes. The ferrous-ion complex with 2-2′-dipyridyl and copper diethyldithiocarbamate prove most suitable, as they can be separated readily.

Experimental

Separation of iron and copper ions from serum proteins—

Both metals are bound to protein in the serum. The iron-binding protein or siderophyllin has been identified as a β_1-globulin in the IV–7 fraction; but although this fraction binds both iron and copper *in vitro, in vivo* it appears to be concerned only with binding of iron (Surgenor, Koechlin and Strong[1]).

A variety of methods have been used for freeing the metallic ions. Dejardin and Lambrechts[2] used sodium citrate, Kitzes, Elvehjem and Schuette[3] and Cartwright, Jones and Wintrobe[4] used warm trichloroacetic acid and Jones[5] nitric acid. Hydrochloric acid at different strengths and temperatures is frequently used (Barkan and Walcher,[6] Agner[7] and Von Porat.[8] Ventura and King[9] have shown that simple 6 *N* hydrochloric acid extraction of serum, as used by Heilmeyer and Plotner[10] for the determination of serum iron, gives maximal reproducible values for both iron and copper (Tables I and II).

Table I

Effect of concentration of hydrochloric acid on splitting of iron and copper from serum proteins

Each value is the mean of five determinations

Concentration of hydrochloric acid, *N*	Iron released after a reaction time of				Copper released after a reaction time of 10 minutes, μg per 100 ml
	5 minutes, μg per 100 ml	10 minutes, μg per 100 ml	15 minutes, μg per 100 ml	20 minutes, μg per 100 ml	
2	51	59	63	64	101
4	89	97	104	107	113
6	106	117	117	118	124
8	97	98	100	100	114

Table II

Differences in amounts of iron and copper split from serum by various reagents and 6 *N* hydrochloric acid acting for 10 minutes

Means of five samples

Reagent	Iron, %	Copper, %
6 *N* Hydrochloric acid	100	100
Nitric acid	91 (86–95)	92 (88–98)
Sodium citrate	85 (75–93)	85 (76–91)
Trichloroacetic acid†	104 (96–110)	101 (98–103)

† By heating the serum to 90° C and then treating it with trichloroacetic acid by the method of Kitzes *et al.*[3] for 3 minutes.

* Present address: Clinica Medica, Pavia, Italy.

PRECIPITATION AND SEPARATION OF THE PROTEINS—

Deproteinisation after liberation of the metallic ions is commonly carried out with 20 per cent. trichloroacetic acid. Separation of the protein precipitate can be effected by centrifugation or filtration. The former method does not always yield an optically clear supernatant liquid, and for filtration, papers washed in iron-free *N* hydrochloric acid must be used. Five successive determinations on the same serum sample by both methods gave average values of 118 μg of iron per 100 ml (standard deviation 1·48; coefficient of variation 1·25 per cent.) by filtration and 119 μg per ml (standard deviation 1·84; coefficient of variation 1·55 per cent.) by centrifugation.

FORMATION OF THE COLOURED IRON COMPLEX—

The iron in the protein-free solution may be determined as ferric thiocyanate or as the stable complexes of ferrous iron with 2-2′-dipyridyl or *o*-phenanthroline (Table III). Here, where copper is determined simultaneously, the 2-2′-dipyridyl reaction has proved to be the best method.

TABLE III

RECOVERY OF IRON ADDED TO SERUM

Iron added to sample, μg per 100 ml	No. of samples	Method: 2-2′-Dipyridyl Recovery, %	2-2′-Dipyridyl Coefficient of variation, %	*o*-Phenanthroline Recovery, %	*o*-Phenanthroline Coefficient of variation, %	Thiocyanate Recovery, %	Thiocyanate Coefficient of variation, %
10 to 50	9	85·9	6·32	92·6	3·78	97·4	2·58
75 to 150	9	96·6	2·59	98·9	1·08	98·9	1·26
175 to 300	9	99·4	1·01	99·7	0·84	99·5	2·02

With a solution containing 300 μg per 100 ml of iron^{III}, we have found that for complete reduction to iron^{II}, 2 per cent. hydroquinone in 0·1 per cent. aqueous ascorbic acid solution is more reliable than sodium dithionite ($Na_2S_2O_4$) (Hill[11]), hydrazine sulphate (Barkan and Walcher[6]), hydroxylamine hydrochloride (Jones[5]), sodium pyrosulphite (Agner[7] and Von Porat[8]), or hydroquinone alone (Heilmeyer and Plotner[10]).

The coloured complex with 2-2′-dipyridyl forms between wide pH limits (pH 3 to 9; Snell and Snell[12]), but the intensity and rate of formation vary. Standardisation is effected by buffering to a pH of 4·4.

FORMATION OF THE COLOURED COPPER COMPLEX—

The yellow-green of the complex of copper with sodium diethyldithiocarbamate does not vary greatly in intensity between pH values of 4·4 and 10·0. At pH 4·4 interference from ferrous ions is eliminated if these are fixed as the stable complex with 2-2′-dipyridyl.

TABLE IV

MEAN DENSITIES AND STANDARD DEVIATIONS OF THE COPPER - SODIUM DIETHYLDITHIOCARBAMATE COMPLEX BY A SINGLE EXTRACTION FROM VARIOUS SOLVENTS, EACH CONTAINING 365 μg OF COPPER PER 100 ml

Ten determinations at a pH value of 4·4

Solvent	Ether	Amyl alcohol	Mixture of ether and amyl alcohol (2 + 1)	Mixture of ether and amyl alcohol (2 + 5)	Mixture of ether and amyl alcohol (1 + 1)
Mean density ..	0·285	0·247	0·275	0·270	0·268
Standard deviation ..	0·00205	0·00155	0·00268	0·00139	0·000641

The copper complex can be extracted from the mixture with solvents of low polarity. Amyl alcohol (Parker and Griffin[13]) and *iso*amyl alcohol cannot be used for the extraction at a pH of 4·4 as the iron complex is also partly removed. Ether extracts the copper complex

selectively, but several extractions are required, and, if a high concentration is present, some may still remain in the aqueous phase. A mixture of equal volumes of ether and amyl alcohol removes the greater part of the copper complex in a single extraction. The optical densities of the resulting extracts are reproducible under standard conditions (Table IV). None of the iron complex is removed and the intensity is not affected. With the *o*-phenanthroline - iron complex, however, some fading occurs.

The formation of the respective complexes of iron and copper proceeds independently, and the molar extinction coefficients and absorption curves in the separated fractions are the same as when determined independently. Recovery of added copper and iron from the mixtures is shown in Tables III and V.

TABLE V

RECOVERY OF ADDED IRON AND COPPER FROM SERUM BY COMBINED DETERMINATION

Metal added to sample, μg per 100 ml	Recovery of iron, %	Recovery of copper, %
50 (6 samples)	96·9–101·0	93·0–101·0
100 (6 samples)	96·7–101·0	97·3–98·6
Mean	98·4	98·3

STOCK STANDARD SOLUTIONS—

Iron—Dissolve 0·863 g of ferric ammonium sulphate and 5 ml of concentrated sulphuric acid in distilled water and make up to 1 litre. Each millilitre contains 100 μg of iron.

Copper—Dissolve 0·1179 g of AnalaR copper sulphate, $CuSO_4.5H_2O$, in distilled water and make up to 1 litre. Each millilitre contains 30 μg of copper.

METHOD

PROCEDURE—

Mix 4 ml of serum with 2 ml of 6 *N* hydrochloric acid and set the mixture aside for 10 minutes. Then add 4 ml of 20 per cent. trichloroacetic acid, leave for another 10 minutes and filter.

Add to 5 ml of filtrate in a separating funnel, 1 drop of *p*-nitrophenol solution. Carefully add concentrated ammonium hydroxide solution dropwise until the colour is yellow-green. Adjust the pH value by back titration with 0·1 *N* hydrochloric acid until colourless, add 0·5 ml of sodium acetate and acetic acid buffer solution (pH 4·4) and then 0·5 ml of 2 per cent. hydroquinone in 0·1 per cent. ascorbic solution.

After shaking, add 5 drops of 1 per cent. 2-2′-dipyridyl solution, when the red colour develops at once. Five minutes later, add 1 ml of 0·1 per cent. sodium diethyldithiocarbamate solution and 5 ml of a (1 + 1) mixture of ether and amyl alcohol. Extract the copper complex by shaking for 1 minute. Separate the phases. If the mixture of ether and amyl alcohol is deeply coloured, extract a second time.

Measure the optical density of the copper complex at 440 mμ on a spectrophotometer. Make the aqueous layer containing the red iron complex up to 10 ml and determine its optical density at 520 mμ on the spectrophotometer. Simultaneously put a blank solution and 4 ml of a standard containing 1·5 μg of iron per ml and 1·5 μg of copper per ml through the same procedure.

APPLICATION

The method described has the advantage of requiring only a small serum sample, but allows accurate separation and determination of the iron and copper contents. 2-2′-Dipyridyl for determination of iron has been suggested frequently (Hill,[11] Allport[14] and Snell and Snell[12]). It is not the most sensitive reagent for iron (Woods and Mellon[15] and Sandell[16]), but the complex is stable and is not extracted from the aqueous phase under the conditions used. We have found a molecular extinction coefficient (ϵ) of 8950 for the coloured cation at 520 mμ and at a pH of 4·4, after 5 minutes. The complex contains 3 molecules of 2-2′-dipyridyl co-ordinated with 1 atom of ferrous iron (Feigl[17]). The figures given by Heilmeyer and Plotner[10] indicated that ϵ was about 8630. There is close adherence to Beer's law,

even with concentrations of less than 100 μg of iron per 100 ml of serum, which give optical densities of less than 0·032 in the final five-fold diluted coloured solution. With the copper complex, $Cu(S.CSN.C_2H_5)_2$, there is similarly a regular relation between concentration and density.

The method has been used mainly with a Beckman spectrophotometer, at a slit-width of 0·4 mm. With the low densities frequently encountered in pathological sera, accuracy can be maintained best by use of this, or a similar sensitive spectrophotometer. A photoelectric absorptiometer is not so well suited to this purpose, but we have obtained good results with a Hilger "Biochem" absorptiometer and a Chance OGRI filter and also with a Hilger Spekker absorptiometer, a 4-cm cell for higher densities and an Ilford No. 604 spectrum green filter (transmission 10 per cent. at 520 mμ). With the second of these instruments 30 ml of coloured solution are required and the serum sample must be 12 ml in volume. Attention to the cleanliness of the optical cells is essential, so they must be washed several times in water doubly distilled from glass apparatus, and then drained before use.

Representative results are shown in Table VI (see also Ventura and White[18]).

TABLE VI

REPRESENTATIVE VALUES FOR IRON AND COPPER DETERMINED IN SINGLE SERUM SAMPLES IN HEALTH AND VARIOUS ANAEMIAS

Group	No. of cases	Iron, μg per 100 ml	Copper, μg per 100 ml
Normal males	10	115–141 (Mean, 125; standard deviation, 7·8)	109–124 (Mean, 116; standard deviation, 5·5)
Iron-deficiency anaemia	8	29–85	91–278
Haemolytic anaemia	4	69–175	44–174
Pernicious anaemia..	2	130–191	174–181
Other blood disorders	13	32–190	134–237

We are grateful to Professor E. J. King for his interest and help in this work, and to Dr. G. H. Beaven for criticising the manuscript.

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POSTGRADUATE MEDICAL SCHOOL
DUCANE ROAD
LONDON, W.12

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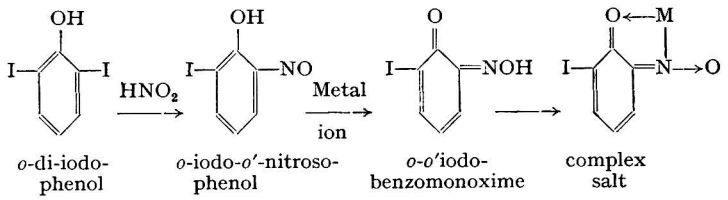
The Colorimetric Determination of 3:5-Di-iodotyrosine

By N. R. MOUDGAL, L. K. RAMACHANDRAN and P. S. SARMA

The specific property of *o*-di-iodophenols of forming stable coloured complexes with metal ions has been made the basis of a sensitive and accurate method for determining 3:5-di-iodotyrosine. The method enables the evaluation of di-iodotyrosine in 500 mg of a natural or artificial iodoprotein with an accuracy of $\pm 2{\cdot}8$ per cent.

Komant[1] recently showed that, when one of the iodo groups of di-iodotyrosine is replaced by a nitroso group, the resulting compound can form metallic chelates. The reactions taking place have been represented by him to be as follows—



The metal complex was found to be stable in a mixture of sodium acetate and acetic acid; the colour formed depended upon the metallic salt used, cobalt giving green, nickel red, mercury, copper and molybdenum violet. Of the various complexes examined by Komant, the cobalt one was found to be the most stable. This communication embodies the results of an investigation carried out in an attempt to adapt this qualitative colour test to the specific colorimetric determination of di-iodotyrosine in iodoproteins, after initially separating thyroxine from di-iodotyrosine by butanol fractionation.

Experimental

Reagents—

Standard 3:5-di-iodotyrosine solution—Dissolve 108·4 mg of B.D.H. 3:5-di-iodotyrosine dihydrate in 100 ml of water made alkaline by the addition of 4 drops of 10 *N* sodium hydroxide solution to give a solution containing 1 mg of di-iodotyrosine per ml.

Hydrochloric acid, N.

Sodium nitrite solution—A freshly prepared 1 per cent. solution.

Cobalt chloride solution, 1 per cent.—Prepared from B.D.H. recrystallised cobalt chloride.

Saturated sodium acetate solution—A filtered solution.

Acetic acid, 20 per cent. v/v.

Procedure for developing colour—

To aliquots of the test solution, add 0·5 ml of *M* sodium chloride solution and make up the volume to 5 ml. To this, add 0·2 ml of *N* hydrochloric acid and 0·2 ml of 1 per cent. sodium nitrite solution and keep the tubes in an incubator at 38° C for 30 minutes. At the end of this period, take them out and add 0·05 ml of 1 per cent. cobalt chloride solution and then 5 ml of a mixture of three volumes of saturated sodium acetate solution and two volumes of 20 per cent. acetic acid. Prepare a blank solution by substituting distilled water for the test solution. Measure the colour with the aid of a blue filter in a Lumetron colorimeter.

Stability of the colour—

The intensity of colour does not decrease even when the solution is kept for over an hour. Nevertheless it is advisable to compare the solutions within 30 minutes of developing the colour.

SUITABILITY OF THE FILTER—

A 0·6-ml portion of standard solution containing 0·6 mg of di-iodotyrosine was taken and colour developed as described above. The spectral characteristics of the cobalt colour complex were studied on a Beckman spectrophotometer. The graph obtained is shown in Fig. 1, the maximum absorption being at 390 to 395 mμ. For routine analysis with the Lumetron colorimeter the blue filter was used, as it gave the maximum absorption.

INFLUENCE OF AMOUNT OF REAGENT AND OF TIME AND TEMPERATURE ON COLOUR PRODUCTION—

Glacial acetic acid is not suitable as it gives low transmission readings. By calculation, 0·042 ml of 1 per cent. cobalt chloride solution is sufficient to form a complex with 1 mg of di-iodotyrosine. It was found that on keeping the tubes in an incubator at 38° C for 30 minutes nitrosation was accelerated. The transmission readings observed under different conditions for 0·4 mg of di-iodotyrosine are recorded in Table I.

TABLE I

TRANSMISSION READINGS OBSERVED UNDER VARIOUS CONDITIONS FOR 0·4 mg OF DI-IODOTYROSINE

Amount of *N* HCl, ml	Amount of 1 per cent. $NaNO_2$, ml	Temperature, ° C	Time of incubation, minutes	Amount of 1 per cent. $CoCl_2$, ml	Transmission, %
0·1	0·2	38	30	0·05	62·0
0·2	0·2	38	30	0·05	57·0
0·3	0·2	38	30	0·05	58·5
0·2	0·3	38	30	0·05	60·5
0·2	0·2	38	30	0·10	57·5
0·2	0·2	70	10	0·05	61·5
0·2	0·2	100	10	0·05	72·0

According to the above observations, the procedure suggested above produces maximum colour.

EFFECT OF SODIUM CHLORIDE ON COLOUR PRODUCTION—

As neutralised alkali hydrolysates of proteins used for analysis contain sodium chloride, it seemed advisable to study the effect of sodium chloride on colour production. In general, when sodium chloride is added to the assay solution, the absorption increases, thereby giving a lower transmission. The effect was studied of sodium chloride concentrations of 0·009 *M* to 1·62 *M*, *i.e.*, from 0·00585 g to 1·0 g in final volumes of 10·45 ml. The decrease in transmission for the whole range tested was the same, thus showing that the presence of an excess of sodium chloride would not bring about a further decrease in transmission readings. The transmission reading for 0·3 mg of di-iodotyrosine without the addition of sodium chloride is 63·5 per cent. On addition of sodium chloride anywhere in the concentration range 0·009 *M* to 1·62 *M* it is 58·5 per cent.

EFFECT OF ADDED AMINO-ACIDS ON COLOUR PRODUCTION—

Table II shows the effect of casein hydrolysate on colour intensity. Various amounts—25, 50 and 100 mg—of casein hydrolysate (in terms of total solids) were added to 0·5, 0·7 and 0·9 mg of di-iodotyrosine. The presence of 25 mg of casein hydrolysate did not affect the recovery of di-iodotyrosine. At higher concentrations of di-iodotyrosine, 50 mg of casein hydrolysate produce a slight increase in the recovery.

Tyrosine by itself does not answer the colour test. Large amounts of tyrosine (4 to 5 mg) do not affect the di-iodotyrosine recovery.

It was found that 3:5-di-iodothyronine and 3:3′:5-tri-iodothyronine could easily be nitrosated. On addition of cobalt chloride, no colour characteristic of the cobalt complex was formed, whereas the golden yellow colour observed during nitrosation still persisted. 3:3′:5-Tri-iodothyronine behaves similarly to thyroxine in that it is precipitated in acidic media. One milligram of 3:5-di-iodothyronine with and without cobalt chloride gave transmission readings 69·0 and 73·0 per cent., respectively, indicating that in the presence of di-iodothyronine an interfering colour may be formed, even though it may not be due to the complex formation.

CALIBRATION CURVE—

A linear relationship between optical density and concentration of di-iodotyrosine has been observed for 0·1 to 0·9 mg in a final volume of 10·45 ml.

It has been shown earlier that sodium chloride in the medium decreases the transmission reading, so it was decided to add a fixed amount of sodium chloride for the whole range of 0·1 to 0·9 mg of di-iodotyrosine. The linear relationship between optical density and concentration was maintained even on addition of sodium chloride. The concentration of

TABLE II

EFFECT OF ADDED CASEIN HYDROLYSATE ON THE RECOVERY OF DI-IODOTYROSINE

Di-iodotyrosine, mg	Casein hydrolysate, mg	Di-iodotyrosine found, mg	Recovery, %
0·50	25·0	0·50	100
0·50	50·0	0·53	106
0·50	100·0	0·55	110
0·70	25·0	0·70	100
0·70	50·0	0·73	104
0·70	100·0	0·76	109
0·90	25·0	0·90	100
0·90	50·0	0·92	102
0·90	100·0	0·98	109

sodium chloride was always of the order of 0·0476 *M*. Fig. 2 represents the calibration graphs obtained with and without addition of sodium chloride. The calibration graph is obtained as follows: for each concentration in the range of 0·1 to 0·9 mg of di-iodotyrosine, 0·5 ml of *M* sodium chloride solution is added before the initial volume is made up to 5 ml. The colour is developed as described earlier. The final colour of the solution is yellowish-green.

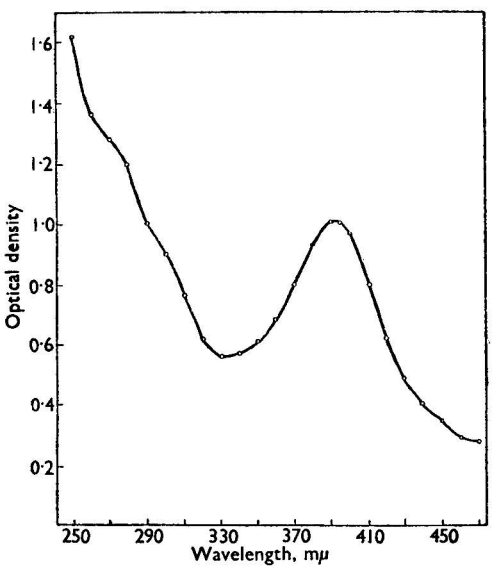


Fig. 1. Spectral transmittance of the cobalt complex (0·6 mg of diiodotyrosine)

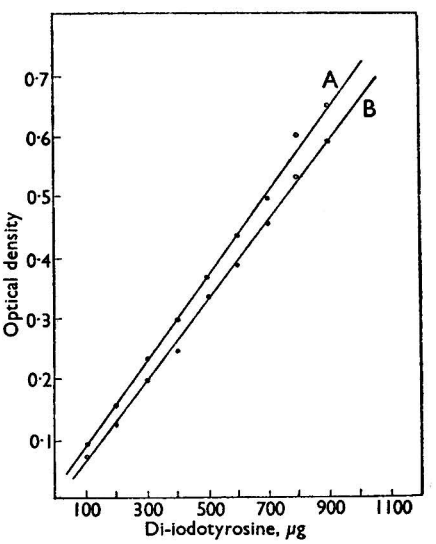


Fig. 2. Calibration graphs. A, with 0·5 ml of *M* sodium chloride solution; B, without sodium chloride solution

ACCURACY OF THE METHOD—

Ten separate analyses of the same standard solution led to an average recovery of 97 per cent., the average deviation from the mean being ±2·8 per cent. The strength of the standard solution was 0·6 mg of di-iodotyrosine in a final volume of 10·45 ml.

METHOD

PROCEDURE FOR ARTIFICIAL AND NATURAL IODOPROTEINS—

The hydrolysis and fractionation of artificial and natural iodoproteins is done according to the method of Roche and Michel.[2]

Hydrolyse 200 to 500 mg of the natural or artificial iodoprotein with 4 to 10 ml of 5 *N* sodium hydroxide in an oil-bath at 115° to 120° C for 8 hours. After hydrolysis, transfer the hydrolysate completely to a 50-ml graduated centrifuge tube or stoppered cylinder. To this, add 6 to 3 ml of 10 *N* sodium hydroxide so that, after making up the solution to 20 ml with distilled water, the final concentration is 4 *N* with respect to sodium hydroxide. Add 20 ml of *n*-butanol to the alkali medium, and shake it once every 15 minutes for 2 hours. At the end of 2 hours, centrifuge the medium and carefully draw off the butanol layer with a fine pipette or capillary tube. To the alkali layer, add 8 ml of 10 *N* hydrochloric acid and, after stirring well, filter the solution through a fluted filter-paper. Make the washings and the filtrate up to 100 ml. Use 1 to 4-ml aliquots containing 0·5 to 0·7 mg of di-iodotyrosine for the determination. After making up the aliquots to 5 ml with distilled water, develop the colour as described above. Sodium chloride is not added as it is already present in the medium.

As the hydrolysate is slightly coloured, the blank value is obtained by making an aliquot equal to that prescribed above up to 5·4 ml with distilled water. Add all the other reagents except hydrochloric acid and sodium nitrite as usual.

RESULTS AND DISCUSSION

Recovery experiments made by adding 5 mg of di-iodotyrosine to each 100 mg of a Boots iodinated casein sample just before hydrolysis gave an average recovery of 83 per cent. The recovery increased as the amount of added di-iodotyrosine was decreased; average recoveries were 90 and 99 per cent. when 3 mg di-iodotyrosine and 1·8 mg of di-iodotyrosine, respectively, were added to 100 mg of the sample. Borrows, Hems and Page[3] have observed a similar lowering effect in connection with the thyroxine recoveries. The recovery experiments so far reported are all with respect to added di-iodotyrosine. The possibility that the di-iodotyrosine added to the medium is destroyed to a greater or lesser extent than the bound di-iodotyrosine should not be excluded. The destruction of iodinated tyrosine derivatives during hydrolysis has been stressed by earlier workers[3,4,5] as being the major drawback of all chemical methods.

The main advantage of the method outlined above is its specificity towards di-iodotyrosine. Unlike other methods, it makes it unnecessary to correct for the presence of amino-acids such as tyrosine. Komant points out that even though di-iodotyramine and di-iodohordenine answer the test they are not found free in nature. Thyroxine, which answers the test, is removed by the butanol fractionation method. Borrows *et al.*[3] have questioned the total absence of di-iodotyrosine in the butanol layer after fractionation. The possibility of any interference from 3:5-di-iodothyronine and 3:3′:5-tri-iodothyronine can be ruled out as they must be present in appreciable amounts in the alkali layer in order to produce any effect, and, further, as they are structurally similar to thyroxine, they may be extracted with thyroxine into the butanol layer. The technique and test used ensures specificity towards di-iodotyrosine.

When 5 mg of di-iodotyrosine were added to 500 mg of an iodoprotein, recovery was accurate within ±2·8 per cent.

When using 500 mg of an iodoprotein for hydrolysis, the least one can measure by this method is 1 per cent. of di-iodotyrosine.

We gratefully acknowledge the gift samples of 3:5-di-iodothyronine and 3:3′:5-tri-iodothyronine supplied by Glaxo Laboratories Limited, Greenford, England.

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UNIVERSITY BIOCHEMICAL LABORATORY
MADRAS 25, INDIA

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The Determination of Lithium in Magnesium - Lithium Alloys by Internal-Standard Flame Photometry

By (the late) A. M. ROBINSON and T. C. J. OVENSTON

The use of a double-beam flame photometer of simple design for the determination of 11 to 14 per cent. of lithium in magnesium - lithium alloys is described. The internal-standard technique is used, and its general application to the determination of alkali metals as major constituents is discussed. In the example given, the internal standard selected is potassium, which, when added in amounts to give a comparatively large concentration, minimises errors caused by variations in alloy composition. Accuracy is to within ±1 per cent. for 11 to 14 per cent. of lithium.

Sodium and small amounts of potassium in the alloys do not interfere, and silver, zinc, aluminium and cadmium, if totalling not more than 10 per cent. of the alloy, also have no effect.

It is now generally accepted that flame photometric methods for the determination of small amounts of alkali metals offer outstanding advantages over other methods, particularly in speed and sensitivity, and often in accuracy too. But the value of the flame photometric technique for the determination of alkali metals present as major constituents is not so generally recognised.

The principal drawback to this technique lies in the dependence of the emission intensity of the element to be determined on the other constituents present. Preliminary separation is precluded if the advantage of speed is to be retained. This effect can cause variable errors, which become more serious with major-constituent analyses in materials of widely different compositions. The effect can be reduced by working with more dilute solutions[1] or by the addition of an excess of buffering salts[2]; it can also be reduced by using the internal-standard technique.[3]

Despite this drawback, the determination of alkali metals present in high proportions can frequently be made by flame photometric methods with at least as good an accuracy as that attainable by the standard methods. The most suitable subjects are those in which the other constituents of the material to be analysed are constant and present in fairly constant amounts; satisfactory calibration graphs can then be prepared with synthetic mixtures. A particular example is the determination of lithium in magnesium - lithium alloys, for which Strange[4] has recently published a method based on the use of the Beckman flame photometer. In his method the emission intensities of the samples are measured directly and related to a calibration graph prepared at the same time from standard lithium solutions. A high order of accuracy and reproducibility is claimed.

Notice of a method for this determination by the internal standard technique had previously been given by us.[5] But because this technique appears to offer certain advantages over that of direct measurement, particularly for the determination of major constituents, we are giving details of our own method. The principles illustrated in this method can readily be applied to similar determinations of other alkali metals, provided a suitable internal-standard element is available.

Method

The flame photometer was as described in our earlier paper.[5] The method was designed for alloys containing 11 to 14 per cent. of lithium. As only small amounts of sodium and potassium can be tolerated in these alloys, it was possible to use potassium as the internal standard element. The EEL barrier-layer photo-cells used were comparatively weak in response at 766 to 770 mμ, which allowed large amounts of potassium to be added to the sample solutions. This makes insignificant small variations in the true potassium contents of the samples and provides additional buffering salt for the solutions. The lithium and potassium radiations in the sample and internal-standard beams, respectively, were isolated by the liquid-filter combinations already described.[5]

REAGENTS AND STANDARDS—

All solutions must be prepared with doubly-distilled water, which should be as free as possible from lithium and potassium. The purity of the chemicals used for standards is not highly critical: the magnesium metal must contain not more than 0·02 per cent. of lithium and should be reasonably free from potassium; traces of potassium and magnesium can be tolerated in the lithium sulphate, and traces of lithium can be tolerated in the potassium sulphate.

Sulphuric acid, 12 N—Prepare this from analytical reagent quality acid.

Standard magnesium solution—Prepare a solution containing 3500 parts of magnesium per million by dissolving 1·750 g of pure magnesium metal in 37 ml of 12 *N* sulphuric acid and making it up to 500 ml with water. The final acidity is 0·6 *N*.

Standard lithium solution—Prepare a solution containing 500 parts of lithium per million by dissolving 2·305 g of pure lithium sulphate monohydrate in water and making it up to 500 ml with water.

Standard potassium solution—Prepare a solution containing 16,000 parts of potassium per million by dissolving 35·655 g of pure potassium sulphate in water and making it up to 1 litre with water.

PROCEDURE—

Dissolve 1·0 g of the alloy in 20 ml of 12 *N* sulphuric acid and dilute to 250 ml with water. Set the solution aside for half an hour to permit any undissolved particles to settle. Take a 10-ml aliquot of the clear solution in a 100-ml calibrated flask, add 10 ml of standard potassium solution and dilute to the mark with water. This solution, which is now ready for measurement, is 0·06 *N* in sulphuric acid and contains 0·04 per cent. of the alloy.

Prepare standards on the basis of a 0·04 per cent. solution of the alloy in 0·06 *N* sulphuric acid, representing alloy compositions of 11 to 14 per cent. of lithium, in increments of 0·5 per cent. For this purpose mix appropriate volumes, as indicated in Table I, of standard lithium and standard magnesium solutions, add 10 ml of standard potassium solution and dilute to 100 ml with water.

TABLE I

RELATED VOLUMES OF STANDARD LITHIUM AND MAGNESIUM SOLUTIONS FOR PREPARATION OF STANDARD ALLOY SOLUTIONS

Volume of lithium solution, ml	Volume of magnesium solution, ml	Amount of lithium in "alloy," %
8·8	10·18	11·0
9·2	10·12	11·5
9·6	10·06	12·0
10·0	10·00	12·5
10·4	9·94	13·0
10·8	9·88	13·5
11·2	9·82	14·0

With the lithium and potassium filters in position in the sample and internal-standard beams, respectively, transfer the standard and sample solutions to the atomiser consecutively and record the potentiometer readings after allowing 30 seconds for the flame to attain equilibrium. Record three readings for each solution, closing the photo-cells and off-setting the potentiometer between each reading, and record the mean. Plot potentiometer readings for the standards against lithium content of the "alloy" and from the calibration graph so obtained read the lithium contents of the samples. A calibration graph for lithium contents of 11 to 14 per cent. was found, for practical purposes, to be a straight line passing through the origin.

EXPERIMENTAL

EFFECT OF VARIATION IN ACIDITY—

It was necessary to check the effect of the small variations in the final acidity of the solutions that occurred with variation in alloy composition. The method of preparation of the standard "alloy" solutions involves an over-all change in acidity from 0·059 *N* for 14 per cent. lithium alloy to 0·061 *N* for 11 per cent. lithium alloy.

By means of synthetic standards, the acidity was varied from 0·03 *N* to 0·09 *N* without any noticeable error being introduced. The small variations found in practice are therefore insignificant.

EFFECT OF VARIATION IN POTASSIUM CONTENT OF ALLOY—

With potassium as the internal-standard element, variations in potassium content of the samples, if sufficiently large, could lead to appreciable errors. As the final solution contains 1600 parts of added potassium per million, a variation of 16 p.p.m. in potassium concentration would produce an error of 1 per cent. in the potentiometer reading, assuming a linear relationship. Measurements made with extra additions of potassium up to 40 p.p.m. proved that this linearity exists.

One per cent. of potassium in an alloy is equivalent to a final concentration of (1600 + 4) parts of potassium per million and would produce a negative relative error of 0·25 per cent. in the lithium result, that is, −0·03 per cent. calculated on the alloy for the lithium contents being considered. In fact, for metallurgical reasons, the potassium content would not be expected to exceed 0·01 per cent. of the alloy, so that errors from this source would be negligible.

EFFECT OF SODIUM AND POSSIBLE ADDITIVES—

Synthetic alloy solutions were made up to include, besides the normal amounts of lithium and potassium, as much as 40 p.p.m. of sodium, silver, zinc, aluminium and cadmium (*i.e.*, up to an equivalent of 10 per cent. of the alloy); the amount of magnesium being reduced appropriately. With none of these solutions was the lithium determination affected. In fact, considerably greater concentrations of sodium were found to be without measurable effect on the determination, largely because of the efficiency of the liquid filters used for absorbing the sodium radiation. Hence no precautions are needed to prevent sodium contamination.

REPRODUCIBILITY—

The reproducibility of the method is illustrated by the replicate determinations of lithium in three alloy samples recorded in Table II.

TABLE II

REPLICATE ANALYSES OF THREE ALLOYS

	Amount of lithium, per cent. (to nearest 0·05 per cent.)		
	Sample 1	Sample 2	Sample 3
	12·95	12·25	11·9
	12·95	12·35	11·85
	13·0	12·35	11·8
	12·9	12·35	11·75
	12·85	12·2	11·85
	13·0	12·3	11·75
	13·05	12·4	11·8
	13·0	12·25	11·9
	13·0	12·3	11·8
	12·95	12·3	11·8
Mean values ..	12·96(5)	12·30(5)	11·82
Standard deviations	0·058	0·060	0·054

ADAPTATION OF METHOD FOR OTHER LITHIUM CONTENTS AND FOR INSTRUMENTAL DIFFERENCES

In this method the concentration of the internal-standard element was chosen to give an almost full-scale reading on the potentiometer for lithium contents in the 11 to 14 per cent. range. By alteration of the amount of internal standard the lithium range can also be altered. Thus by reducing the final potassium concentration to 150 p.p.m. or increasing it to 3500 p.p.m. the maximum of the lithium range may be altered to 1 or 30 per cent., respectively. By arranging for the expected lithium content to correspond to an almost full-scale potentiometer reading in this way, the best possible accuracy is attained; this would normally be within 1 per cent.

The same effect can be obtained by putting a variable aperture in the path of the internal-standard beam.

The concentration of internal-standard element required for any particular application depends on the relative responses of the two photo-cells and on the transmittances of the filters. Therefore the appropriate concentration of potassium for the determination of lithium by the method proposed may require considerable adjustment when other flame photometer systems are used, particularly with different optical filters. However, this should present no difficulty. With photo-cells having a high response in the red part of the spectrum, the appropriate concentration of potassium might be reduced to a value so low that small variations of potassium content might become significant. Then it would be better to retain a high potassium concentration and use the variable aperture method of adjustment.

Conclusions

Alkali metals when present as major constituents can frequently be determined by means of flame photometry with no loss of accuracy compared with classical methods, with a speed that is much greater because no preliminary separations are necessary. Once the alloys have been dissolved and the solutions have been left for half an hour, the determinations only take a few minutes. Although this technique is of greatest value for the analysis of classes of materials having closely defined composition, the effect of fairly large variations in composition may frequently be rendered insignificant by means of the internal-standard technique and by the use of relatively high concentrations of the internal-standard element.

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Admiralty Materials Laboratory
Holton Heath
Poole, Dorset

June 30th, 1953

Notes

THE ABSORPTIOMETRIC DETERMINATION OF TELLURIUM IN TELLURIUM - LEAD ALLOYS

Lead containing 0·04 to 0·06 per cent. of tellurium[1] has an especially good chemical resistance, an increased strength and hardness and a fine grain. Several methods for the accurate determination of such a small amount of tellurium have been proposed, of which that described by Evans[2] is undoubtedly the quickest and most accurate. This method involved the separation of lead as the chloride and separation of tellurium by reduction with sodium hypophosphite; elemental tellurium was then oxidised to the quadrivalent state with standard iodine solution.[3] All published procedures suffer, however, from two disadvantages: the long time required for dissolving the large weight of lead sample and the necessity of separating the lead before precipitating the tellurium.

Johnson and Kwan[4] and, independently, Geiersberger and Durst[5] have proposed the absorptiometric determination of traces of tellurium by formation and suitable measurement of the potassium iodotellurite complex. Johnson and Kwan's method is a basis for this absorptiometric determination of tellurium in tellurium - lead alloys. No preliminary separation of lead is necessary.

The determination of tellurium as the iodotellurite complex—

The iodotellurite complex is reddish-yellow and has absorption maxima at 335 and 285 mμ.[4] The concentrations of potassium iodide and hydrochloric acid necessary for formation of the complex are critical; Johnson and Kwan's recommendations were followed. These authors also stated that iodotellurite solutions showed negligible change, for up to 20 minutes, in the transmittance measured on a Beckman model DU spectrophotometer. On a Spekker absorptiometer

we found that there was a continuous increase in the optical density of the complex, even when the flask was kept protected from light.

The Spekker absorptiometer drum difference readings were recorded for six separate solutions of 250 μg of tellurium taken through the calibration procedure described below but with different development times for the colour and 2-cm cells being used for the measurement. After allowance for the blank, readings after 5 and 5½ minutes, respectively, were 0·752 and 0·742, readings after 9½ minutes were 0·768 and 0·761, and after 15 minutes they were 0·780 and 0·765. The increase of reading with time appears to be caused by the instability of the complex and not by liberation of iodine from potassium iodide, as a blank determination was made with each reading. If the solution was kept in an open cell, increase of reading with time was great, even between consecutive readings on the absorptiometer. Reproducible results in preparing a calibration graph were only attained by strict adherence to the standardised procedure given below. The 50-ml flasks used must either be of amber glass or be protected from light by thick brown paper or black paint.

Method for preparation of calibration graph

Reagents—

All reagents should conform to recognised analytical standards.

Standard tellurium solution—Add 0·3357 g of dry potassium telluribromide[4,6] to about 50 ml of 0·2 *N* hydrochloric acid. Dissolve the white residue by warming gently, cool and make up to 250 ml with 0·2 *N* hydrochloric acid in a calibrated flask; 1 ml of this solution contains 250 μg of tellurium.

This procedure must be followed closely, otherwise in the presence of water a white residue is produced, which does not redissolve easily. The solution itself deposits a white precipitate after a few weeks, so it should be used as soon as possible after preparation.

Potassium iodide solution, 2 M—Store in an amber bottle away from direct light.

Hydrochloric acid, 2 N—Standardise this solution by titration.

Procedure—

Place suitable volumes of standard tellurium solution to cover the range 50 to 175 μg of tellurium in a series of 50-ml calibrated flasks and dilute with water to 25 ml. Into one flask run from pipettes 5 ml of 2 *N* hydrochloric acid and then 10 ml of 2 *M* potassium iodide solution. Make up to the mark with water and shake well, simultaneously starting a stop-watch, and place the flask in darkness. Into a similar 50-ml flask containing 25 ml of water put the same amounts of potassium iodide solution and hydrochloric acid. Make up to the mark, shake well and transfer the requisite amount of solution to a 4-cm Spekker cell. Use this for a blank reading in a Spekker absorptiometer, model H570, with Calorex H503 heat-resisting filters and Ilford No. 601 spectrum violet filters, using a 1·00 water-to-water setting. *Exactly* 5 minutes after starting the watch measure the absorption of the test solution in a similar 4-cm cell. Repeat the procedure for each amount of tellurium, using a separate blank solution each time. Plot the results as a calibration graph, which should take the form of a straight line passing through the origin.

Application of the method to lead alloys

Solution of lead—The acetic acid - hydrogen peroxide solvent proposed by Hamilton[7] was used; 0·25 g of tellurium - lead alloy dissolved in about 1 minute. Later results show that no tellurium was lost by this procedure.

Precipitation and determination of tellurium—The method tried was precipitation of tellurium in the elemental state by stannous chloride, solution of the precipitate in nitric acid to give tellurite, and determination of tellurium in this solution by the iodotellurite method after suitable adjustment of acidity and addition of potassium iodide. The precipitation was effected without preliminary lead separation by dissolving lead chloride in hot solution with an excess of saturated sodium chloride solution, with probable formation of compounds of the type $NaPbCl_3$ and Na_2PbCl_4.

Interference of copper—

Copper interferes in the iodotellurite determination by oxidising iodide to iodine in acid medium. Although 0·06 per cent. is present in tellurium - lead alloys, its possible effect was at first discounted because Johnson and Kwan claimed good separations of tellurium[IV] from copper and iron by the stannous chloride reduction. But it was observed that recoveries of 150 μg of

tellurium added to 0·25 g of lead in presence of 150 μg of copper (added as copper sulphate) were high, values found being 163, 160, 160 and 175 μg. Recoveries in the absence of copper were much better; when 125 μg of tellurium was added to 0·25 g of lead, 129 μg was found, and when 150 μg was added, 155 and 151 μg were found.

Crossley[8] determined tellurium in tellurium - copper alloys by complexing copper with thiourea before the stannous chloride reduction; copper forms a stable soluble complex in acid solution. Lead forms an insoluble complex, but did not interfere in this method. Tellurium gives a coloured thiourea complex, but is reduced to the element by stannous chloride. Addition of a small quantity of thiourea successfully prevented copper interference.

Method

Apparatus—

The filtration apparatus is shown in Fig. 1. The filter tube is of grade 1 porosity and is used in conjunction with asbestos.

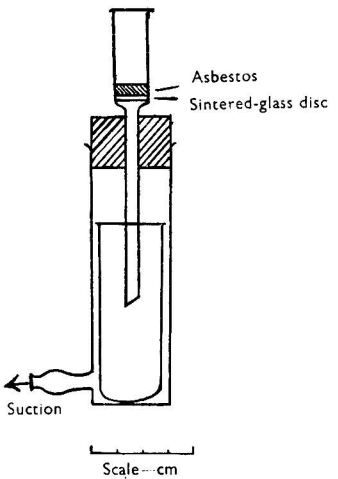

Fig. 1. Filtration apparatus

Reagents—

The following special reagents in addition to those in the calibration procedure are required.

Acetic acid - hydrogen peroxide solution—Freshly prepare a mixture of 2 volumes of 30 per cent. w/v hydrogen peroxide, 1 volume of glacial acetic acid, sp.gr. 1·05, and 2 volumes of water.

Sodium chloride solution—A saturated solution in water.

Stannous chloride solution—Dissolve 12·29 g of stannous chloride in 50 ml of concentrated hydrochloric acid, sp.gr. 1·18; store in an amber bottle.

Thiourea—Pure solid.

Asbestos—"Powminco," grade A, for Gooch crucibles.

Procedure—

Weigh 0·25 g of tellurium - lead alloy into a 50-ml Pyrex-glass beaker. Cover with a well-fitting watch glass and add 2 ml of acetic acid - hydrogen peroxide mixture from a glass dropper. Cool if necessary to moderate the reaction; after a minute or so swirl thoroughly and warm slightly. Add 10 ml of saturated sodium chloride solution, followed by 2 ml of concentrated hydrochloric acid. Heat to incipient boiling (vigorous effervescence), then place on a steam-bath for about 10 minutes until all solid has dissolved. Wash the cover glass and sides of the beaker with hot water and add 0·01 g of thiourea. Leave for 5 minutes, then add 4 ml of stannous chloride solution and set the beaker on the bath for a further 15 to 20 minutes to ensure complete precipitation and neutralisation of the peroxide.

Meanwhile prepare the filtration apparatus for use, using a pad of asbestos about 5 mm thick. Wash the asbestos with hot diluted hydrochloric acid solution (1 + 1) to remove any iron present. Separate the precipitated tellurium by filtration with gentle suction, and wash the beaker and precipitate with about 30 ml of hot water. Dissolve the tellurium on the filter with successive small portions of hot concentrated nitric acid, sp.gr. 1·42, 3 ml in all, adding a little acid to the beaker to dissolve any traces of tellurium therein. Wash the asbestos and beaker with two 3-ml portions of approximately *N* nitric acid. Collect the acid filtrate in a suitable tube placed below the filter. Boil the filtrate for 5 minutes to expel oxides of nitrogen, cool and neutralise to the phenolphthalein end-point with diluted ammonium hydroxide solution (1 + 1). Transfer to a 50-ml calibrated flask and determine tellurium as in the calibration procedure. For a blank use a solution that has been taken through the procedure from the nitric acid stage, to compensate for iron in the reagents.

Results

Recoveries of known additions of tellurium to 0·25 g of AnalaR lead to which 0·06 per cent. (150 μg) of copper had been added as copper sulphate were satisfactory, as shown by the following figures—

Tellurium added, μg ..	175	150	125	125	100	100	75	50
Tellurium found, μg ..	173	146	131	122	106	97	81	53

The method is inapplicable in the presence of antimony but this element is not normally present in tellurium - lead alloys. When a sample of antimonial lead containing 3 per cent. of antimony and the equivalent of 0·05 per cent. of tellurium was analysed by the proposed procedure, the result was very high, probably because of the presence of the iodoantimonite ion derived from adsorbed antimony. The possible interference of selenium, arsenic and bismuth on the procedure was not studied, as the method was only to be used for simple lead - tellurium alloys made according to British Standard Specification No. 334.

Typical results for a triplicate analysis of a commercial sample of tellurium - lead alloy were 0·046, 0·046 and 0·045 per cent. of tellurium; this is satisfactory precision. The whole analysis can be carried out in about 2½ hours. In general, the accuracy is not as good as that of Evans' method, but the procedure is much more rapid and the accuracy is adequate for control purposes. The mean error over the range of tellurium covered (0·02 to 0·07 per cent.) is about 3·5 per cent.

The author thanks the Directors of British Enka Limited for permission to publish this note.

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Research and Development Department
British Enka Limited
Aintree, Liverpool, 9

E. G. Brown
March 3rd, 1953

THE DETERMINATION OF FORMALDEHYDOGENIC STEROIDS

One of us[1] has shown that the so-called neutral steroid extract obtained after the hydrolysis of urine with hot dilute mineral acid and used in the determination of the 17-ketosteroids contains material that reacts with periodic acid to liberate formaldehyde. The 17-deoxy steroids, deoxycorticosterone and corticosterone, when added to urine, could be recovered quantitatively in such extracts as judged by the amount of formaldehyde liberated on reaction with periodic acid.

The extract was dissolved in 1 ml of ethanol, 5 ml of periodic acid reagent and 0·5 ml of concentrated sulphuric acid were added and the mixture was diluted to 15 ml with water. The mixture was heated to boiling and the first 9 ml of distillate were collected. The formaldehyde content of the distillate was determined colorimetrically by the chromotropic acid reaction (see Tompsett and Smith[2]) and the steroid content was calculated from a graph prepared from deoxycorticosterone acetate that had been subjected to the same procedure.

Experiments have since been carried out to determine the possible interference, if any, of fatty residues that may be present in the urinary extracts. In these experiments, the reactant mixture was evaporated to dryness, the distillate being collected in 3-ml portions. Formaldehyde was determined in each portion of the distillate by the chromotropic acid reaction. Results obtained with deoxycorticosterone acetate, tri-olein and a urinary extract are shown in Table I.

Table I

Recovery of formaldehyde in distillate fractions

Substance	Quantity used	Formaldehyde in distillate fractions				
		1st, μg	2nd, μg	3rd, μg	4th, μg	5th, μg
Deoxycorticosterone acetate	1 mg	16	22	21	10	7
Tri-olein	24 mg	1	1	1	2	19
Urinary extract	from 100 ml of urine	20	24	18	13	37
β-Carotene*	4 mg	2	2	2	2	12
Vitamin-A acetate*	7 mg	78	92	61	24	6

* Iodine liberated during distillation procedure was neutralised by addition of sodium sulphite to the distillate.

With deoxycorticosterone acetate, the recovery of formaldehyde in the first 9 ml of distillate amounted to 78 per cent. of the total, which was equal to the theoretical amount. With tri-olein, the first 9 ml of distillate contained but minimal amounts of formaldehyde, by far the greatest amount appearing in the last 3 ml of distillate. The total recovery of formaldehyde was considerably less than the theoretical expectation. Lecithin behaved in a similar manner. The figures for the urinary extract indicate that such extracts do contain substances producing formaldehyde with periodic acid and related to olein or lecithin. Basing the results on the first 9 ml of distillate minimises the effect of such non-specific fatty substances. It is evident, however, that the distillation must be carried out under controlled conditions.

DISTILLATION—

The apparatus consisted of a 50-ml round-bottomed flask, neck length 160 mm, attached by means of an india-rubber stopper to a water-cooled condenser of length 200 mm. Glass tubing used had an external diameter of 5 mm. The flask was placed on an asbestos-covered gauze on a tripod stand. Heating was by an ordinary bunsen burner, the height of the flame being adjusted so that the mixture in the flask (15 ml of water containing 5 ml of periodate reagent and 0·5 ml of concentrated sulphuric acid) was brought to the boil within 3½ to 4 minutes. The distillation was carried out in a fume chamber. The distillate was collected in a 10-ml conical centrifuge tube graduated in tenths of a millilitre.

NON-SAPONIFIABLE SUBSTANCES—

The influence of the non-saponifiable substances, vitamin A and carotene, was then examined. When vitamin-A acetate was subjected to the distillation procedure with periodic acid as outlined above, formaldehyde was recovered in the distillate. The recovery of formaldehyde in portions of the distillate followed the same pattern as that for deoxycorticosterone acetate. On the assumption that this formaldehyde was derived from the terminal primary alcohol group, the recovery was only about 50 per cent. of the theoretical. When carotene was subjected to the same treatment, formaldehyde was recovered in the distillate but the pattern resembled that obtained with tri-olein. When either of these substances was added to urine, no additional formaldehyde could be detected in the neutral steroid extracts. The ability to produce formaldehyde on reaction with periodic acid was destroyed by the hydrolytic treatment. Experiments have shown that with vitamin A, part at least of the formaldehyde is derived from products of the reaction with hot dilute mineral acid. Certain of these products can be separated by distillation from an aqueous solution or suspension.

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BIOCHEMICAL LABORATORY
NORTHERN GENERAL HOSPITAL
EDINBURGH

D. C. SMITH
S. L. TOMPSETT

June 30th, 1953

Apparatus

A MERCURY-PRESSURE SAFETY VALVE

FOR a long time mercury has been used for sealing an apparatus so that whilst air cannot enter, other gases, evolved in or deliberately passed through the apparatus, may escape under any desired mercury pressure. But an annoying drawback of these mercury valves is that a fall in temperature, or other change, may cause mercury to be drawn back into the apparatus. This applies not only to the simple mercury-in-test-tube type but also to the more elaborate mercury-on-sintered-glass seal. A valve has now been designed which, by trapping the mercury after it has been accidentally drawn back, prevents it entering the apparatus.

The valve, shown in Fig. 1, is divided into two compartments: the upper one, A, contains mercury, the lower one, B, which has a ground-glass joint, is fitted into the apparatus to be sealed (with the usual spring attachment, if necessary). Gas passed through or evolved in the apparatus leaves by the route indicated by the arrowed dotted line, the mercury in A being forced down in the inner cylinder, C, through the orifice, D, into the outer cylinder of A. Gas escapes when its pressure becomes greater than that of the (adjustable) head of mercury.

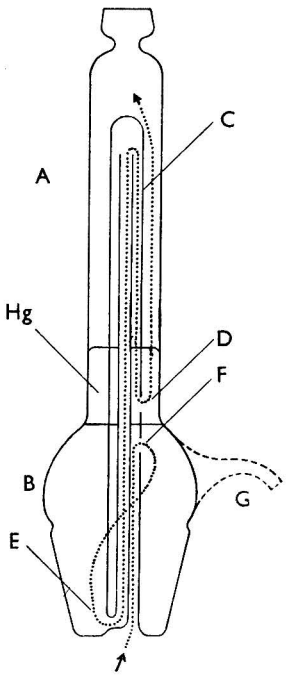

Fig. 1. The valve

If mercury is accidentally drawn back, it rises in C, overflows down the centre tube and out at E into B, which is large enough to hold it so that it does not rise to the height of the orifice, F. It will be clear that even after being drawn back, the mercury in B serves as a second seal.

To reset the valve it is only necessary to lift it from the apparatus, attach a water-pump to the top of the valve and apply suction; mercury will then be withdrawn from B and re-enter A.

The siphon and sintered-glass valves formerly used in the author's air-free carbon dioxide (or hydrogen) generator[1] have now been replaced by this new valve. With the earlier valves, mercury was rarely drawn back except during the preliminary setting-up of the apparatus; and even when it did occur, it could be ignored, as mercury has no deleterious action on the reagents used or on the gases being generated. Having to recharge the valves with mercury is, however, wasteful and a nuisance. Further, the sintered valve in time becomes choked with a basic mercury compound, although it can easily be cleared with diluted hydrochloric acid (1 + 1). The new valve obviates these disadvantages.

The valve, when used at the top of the generator, is modified by a side-arm sealed into B (as indicated by the broken line at G) to provide for connection with the central fitting. This side-arm, G, is useful, as mercury that has been drawn back can be poured from B through it, and then returned to the upper chamber, A, without the use of suction. It may usefully be made part of the standard valve, being capped when not required for removal of mercury.

I am greatly indebted to Quickfit and Quartz Ltd. for their collaboration in designing this valve.

REFERENCE

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CHEMISTRY DEPARTMENT
THE UNIVERSITY, GLASGOW
S. HORWOOD TUCKER
May 29th, 1953

Ministry of Food

STATUTORY INSTRUMENTS*

1953—No. 1820. The Public Health (Preservatives etc. in Food) (Amendment No. 2) Regulations, 1953. Price 2d.

These Regulations, which came into operation on December 16th, 1953, re-enact the Public Health (Preservatives etc. in Food) (Amendment) Regulations, 1953 (S.I., 1953, No. 1610; Analyst, 1953, 78, 737), with the inclusion, as required by law, of an expression of the Ministers' opinion that certain provisions are necessary for preventing danger to health or otherwise for protecting purchasers.

1953—No. 1827. The Soft Drinks (Revocation) Order, 1953. Price 2d.

This Order, which came into operation on December 20th, 1953, revokes the Soft Drinks Order, 1947 (S.R. & O., 1947, No. 2756; Analyst, 1948, 73, 33), as amended (S.I., 1948, No. 1291, and S.I., 1951, No. 137; Analyst, 1951, 76, 244).

1953—No. 1828. The Food Standards (Soft Drinks) Order, 1953. Price 4d.

This Order, which came into operation on December 20th, 1953, should be read with the Food Standards (General Provisions) Order, 1944 (S.R. & O., 1944, No. 42; Analyst, 1944, 69, 49), as amended (S.R. & O., 1944, No. 654; Analyst, 1944, 69, 247), and replaces with certain modifications the standards for soft drinks hitherto prescribed by the Soft Drinks Order, 1947, as amended (see S.I., 1953, No. 1827, above).

The principal changes are—

(a) certain medicated and glucose beverages are exempt from the Order;

(b) the standards, with certain modifications, apply to soft drinks for consumption by diabetics, and to ginger beer and herbal and botanical beers;

(c) the description of drinks made from whole fresh oranges has been modified;

(d) references to non-alcoholic wine and non-alcoholic cider and non-alcoholic perry are omitted.

British Standards Institution

AMENDMENT SLIP†

A PRINTED slip bearing amendments to a British Standard has been issued by the Institution, as follows—

PD 1719—Amendment No. 1 (October, 1953) to B.S. 733:1952. Density Bottles.

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to members of the Society, and can be obtained from the Secretary, The Society for Analytical Chemistry, 7–8 Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee FCC/4—Solvents and Allied Products. CR(FCC)7645—Draft B.S. for Cyclohexylamine.

Draft Specification prepared by Technical Committee DAC/18—Milk Can Washing Machines. CR(DAC)7898—Draft B.S. for Milk Can Washing Machines.

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

† Obtainable from the British Standards Institution, Sales Department, 2, Park Street, London, W.1.

Book Reviews

STARCH AND ITS DERIVATIVES. Volume I. By J. A. RADLEY. Third Edition. Pp. xii + 510. London: Chapman & Hall Ltd. 1953. Price 65s.

This work has increased in size to the extent that the third edition is to consist of two volumes, of which the first, here reviewed, is equal in size to the whole of the second edition. The present volume is concerned with the structure and reactions of starch, and with amylases and their action on starch.

Marked differences in quality exist between the various chapters of this volume. The descriptions of the biological function of starch, of its structure and of its fractionation, are comprehensive, logically arranged and sustain the reader's interest. "Starch and the hydrogen bond" is clear and concise. Other chapters fail to reach this high standard and some are relatively poor, Chapter IV, "Some physical properties of starch," and Chapter V, "The swelling and gelatinisation of starch," for example, being little more than an indiscriminate collection of verbatim abstracts.

The section dealing with amylases and their action on starch comprises the chapters included in Part V of the second edition and about 20 per cent. of newer material, which has not been well assimilated. A clearer picture might have been obtained if the earlier work had been treated more critically or ignored where there is doubt (as suggested in Chapter XXIV) about the analytical methods used.

The author has had the valuable assistance of numerous specialists in the preparation of many chapters and he has succeeded in welding together their individual contributions with the minimum of repetition. The bibliography is extensive and the supplementary references, which appear together with a short abstract at the end of certain chapters, are an excellent innovation.

C. H. F. FULLER

FERROUS ANALYSIS. MODERN PRACTICE AND THEORY. By E. C. PIGOTT. Second Edition Revised. Pp. xxviii + 690. London: Chapman & Hall Ltd. 1953. Price 84s.

This is a revised edition of the book originally published in 1942 as "Chemical Analysis of Ferrous Alloys and Foundry Materials."

It is divided into five sections: I, "Analytical Techniques" (50 pages); II, "The Constituents of Iron and Steel" (485 pages); III, "Microchemical Analysis of Iron and Steel" (30 pages); IV, "Alloys and Ores" (32 pages); V, "Refractory Materials" (30 pages); there is also an Appendix (31 pages) and an Index (19 pages).

Section I describes the recent physical apparatus devised as supplementary to the analytical methods described in Section II. Some of this is useful when traces of elements have to be estimated for which the methods in Section II would be too laborious or not sensitive enough; some of it is designed for rapid repetitive analysis. Most of it is expensive and careful consideration would be necessary to decide whether its installation was justifiable. The tables provided in this section form a useful summary of the possibilities of these instruments.

Section II is the most important in the book and it is extremely well done, but the inclusion of the interesting metallurgical data appears to be out of place in an analytical treatise. Some reference to the preparation of sample drillings for analysis might well have been made.

Section III deals with microchemical analysis. As the greatest possible information is frequently required on very small amounts of material, this is a subject likely to become of increasing importance in the future.

Section IV deals with non-ferrous alloys and ores. It is difficult to understand why non-ferrous material should find a place in a book on ferrous analysis. The omission of this section and also of the metallurgical information in Section II would have reduced the cost of the book without impairing its analytical value.

A. T. ETHERIDGE

MALEIC ANHYDRIDE DERIVATIVES. REACTIONS OF THE DOUBLE BOND. By L. H. FLETT and W. H. GARDNER. Pp. x + 269. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1952. Price 52s.; $6.50.

The importance of maleic anhydride in the production of alkyd resins, maleinised drying oils and co-polymers is well known, but it is also employed in the manufacture of many other organic substances. It can be used widely as a building block in chemical synthesis and is ideal for this purpose, as any atom in the molecule can be made to react under suitable conditions. The authors of this book say that they found the characteristic reactions of maleic anhydride so fascinating

that they were induced to collect them together into their simplest book form, to make reference easy, and so to extend their use.

They have presented 116 different types of chemical reactions, each representative, and they have grouped them in seven chapters, headed: hydrocarbons, halogens and their compounds, hydrogen, metallic compounds, compounds containing nitrogen, compounds containing oxygen and sulphur compounds. They consider energy and catalysts in a final eighth chapter.

The format is simple. Each preparation is described on two facing pages, with a simplified equation and a brief description of the method. There are comments, a note of the reaction products and possible uses of the product; and these are followed by references to the literature. This is a most interesting and clearly set out reference book. K. A. WILLIAMS

A GUIDE TO FILTER PAPER AND CELLULOSE POWDER CHROMATOGRAPHY. By J. N. BALSTON and B. E. TALBOT. Edited by TUDOR S. G. JONES. Pp. 145. London: H. Reeve Angel & Co. Ltd.; Maidstone, Kent: W. & R. Balston Ltd. 1952. Price 8s.

Paper chromatography is one of the most important techniques developed in recent years for the separation of mixtures of substances. The method is simple, does not usually require elaborate or expensive apparatus, and can accomplish separations that are extremely difficult to achieve by other means. In broad terms, paper chromatography depends on the use of a cellulose adsorbent in conjunction with an organic solvent or organic solvent mixture and is carried out on paper strips or sheets or columns of cellulose powder. With strips and sheets of paper, the test solution is placed as a spot on the paper and the solvent is allowed to diffuse through the paper, whereby individual substances are separated into bands or spots detectable by spraying with suitable reagents. With columns of cellulose powder, the sample solution is absorbed on a portion of pulp, which is then placed at the top of a prepared column of cellulose powder and eluted with the organic solvent.

This book gives an excellent account of how to carry out separations by this technique and of what has already been done by paper chromatography. The first part (14 pages) deals with methods and materials and the second part (103 pages) with applications. The second part is mostly concerned with separations of organic and biological materials and covers a very wide range. It also includes such special items as the use of alumina and silica impregnated papers, acetylated paper, reversed phase chromatography and electrophoresis on paper. A short section on applications to inorganic separations is also included. This practical guide to paper chromatography contains some illustrations, a large number of references and is generally well produced and free from errors. It is cordially recommended to all chemists, including students, as providing a useful introduction to an important and rapidly developing branch of chemical science. Its modest price should also be an encouragement to its purchase.

F. H. BURSTALL

CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY. Volume II: STEROID METABOLISM AND ESTIMATION and Volume V: BIO-ASSAY OF ANTERIOR PITUITARY AND ADRENOCORTICAL HORMONES. Edited by G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., B.Ch., and MARGARET P. CAMERON, M.A., A.B.L.S. Pp. xx + 429 and xvi + 228. London: J. and A. Churchill Ltd. 1952 and 1953. Price 35s. and 25s.

That admirable organisation, the Ciba Foundation, has put biochemists and endocrinologists everywhere under an obligation by the excellent series of colloquia they have sponsored in London, and no less by the valuable records of the proceedings, which are now published in permanent form.

There may be some readers of *The Analyst* not yet familiar with the aims and purpose of the Ciba Foundation. As explained by Dr. G. E. W. Wolstenholme in the prefaces to the volumes here under review, the Ciba House in Portland Place, London, provides an international centre where those engaged in chemical or medical research may stay during their visits and take part in the meetings, the duration of which range from a few hours to several days. The Foundation has now existed for over three years, and about 20 international conferences have already been held. The Foundation ranks officially under English law as an educational charity. It is administered independently of any trade connections, by four distinguished trustees, Dr. E. D. Adrian, P.R.S., Lord Beveridge, Lord Horder and Mr. Raymond Needham, Q.C., the funds being provided by the Ciba company of Switzerland.

An attractive feature of the Ciba conferences, as Dr. Wolstenholme remarks, is the "house-party" atmosphere that prevails at them. Frank and informal exchanges of information and opinion on current topics are encouraged rather than the formal presentation of completed investigations. Notwithstanding this, there has been a widespread demand that the proceedings should be made more generally available. This series of volumes is therefore now being issued, happily with a minimum of editing. Thus, the benefits of these group discussions are made accessible to a wider public.

The two volumes forming the subject of this review will be of special interest to members of the Biological Methods Group of the Society for Analytical Chemistry. Indeed, one may go further and say that the account of these two meetings forms an indispensable work of reference to all interested in biological assays, as well as providing a good deal of stimulating mental fare. A glance at the lists of the contributors and participants in the discussions is sufficient to show that they include most of the leading authorities on these topics, not only from Britain but also from overseas. All bio-analysts throughout the world are in debt to the eminent contributors, to the Editor and to the sponsoring body. LESLIE J. HARRIS

RICHMOND'S DAIRY CHEMISTRY. Revised by J. G. DAVIS, D.Sc., Ph.D., F.R.I.C., and F. J. MACDONALD, A.R.I.C. Fifth Edition. Pp. viii + 603. London: Charles Griffin & Co. Ltd. 1953. Price 60s.

This recent edition of a well-established book, whilst missing the obsolete matter of earlier editions, retains the more important information included in the fourth edition. The first twelve chapters constituting Part I are entitled General and Theoretical, the final thirteen constituting Part 2, Analytical Methods. Since the third edition of 1920 great advances have been made in dairy science, and the research worker has been greatly helped by the publication from 1939 of *Dairy Science Abstracts* prepared by the Commonwealth Bureau of Dairy Science. Nevertheless, dairy chemists will welcome the monumental compilation of references to original work in the list of references; these number upwards of 1200 and cover the literature up to 1952. One of the most interesting features of the book is the account of the variation in the composition of cows' milk during the last 20 years. Abnormalities and general changes, both in composition and freezing point depressions, have been reviewed with care and discrimination as to authenticity. The chapters dealing with the analytical methods include many useful modern methods, such as tests for estimating the degree of heating of milk and methods for the determination of metals in dairy products. This revised "Richmond" is indispensable to the dairy chemist. J. KING

FLUORESCENCE OF SOLUTIONS. By E. J. BOWEN, M.A., D.Sc., F.R.S., and FRANK WOKES, Ph.D., B.Sc., F.R.I.C., Ph.C. Pp. viii + 91. London and New York: Longmans, Green & Co. Ltd. 1953. Price 25s.; $4.00.

Modern analytical chemistry necessitates increasing use of physical methods, and the average analyst finds difficulty in acquiring sufficient theoretical knowledge for the intelligent use of modern instruments. Fortunately, few analysts have to carry out fluorimetric assays other than those of thiamine and riboflavine, but this book caters for a wider application.

The authors have concentrated on bringing "out the high-lights of fluorescence theory avoiding mathematical proof" in reasonably simple and accurate terms. Some criticism may be made, however, of their having defined the sensation of colour in terms of hue, tint and intensity (page 47) rather than in the accepted terms of colour physics (B.S. 233 and B.S. 1611 of 1953).

The first six chapters deal with theoretical concepts, and the remainder of the book deals with details for carrying out quantitative fluorimetry, based on the practical experience of Dr. Wokes and his collaborators. The last chapter, Fluorimetric Assays, on the determination of thiamine and riboflavin, shows the necessity for a most careful approach to the subject, and the book may be profitably studied by all analysts engaged in such assays. J. KING

Publications Received

CHIMIE ANALYTIQUE. APPLIQUÉ À LA MÉTALLURGIE. Third edition by R. CHADELLE and C. VANDAEL. Pp. 468. Paris: Masson & Cie; Liège: Sciences et Lettres. 1953. Price 2800 fr. (French); 400 fr (Belgian).

ECONOMIC CROPS. Volume III. COCOA. By EILEEN M. CHATT. Pp. xvi + 302. New York and London: Interscience Publishers Inc. 1953. Price $8.50; 58s.

STANDARD METHODS OF CLINICAL CHEMISTRY. Volume I. By the American Association of Clinical Chemists. Editor-in-Chief, MIRIAM REINER. Pp. xii + 142. New York: Academic Press Inc.; London: Academic Books Ltd. 1953. Price $4.50; 36s.

AN ADVANCED TREATISE ON PHYSICAL CHEMISTRY. Volume IV. PHYSICO-CHEMICAL OPTICS. By J. R. PARTINGTON, M.B.E., D.Sc. Pp. xl + 688. London and New York: Longmans, Green & Co. Ltd. 1953. Price 80s.

INDUSTRIAL INORGANIC ANALYSIS. By ROLAND S. YOUNG, Ph.D., F.R.I.C. Pp. viii + 368. London: Chapman & Hall Ltd. 1953. Price 36s.

PHYSICAL CONSTANTS OF HYDROCARBONS. Volume V. PARAFFINS, OLEFINS, ACETYLENES AND OTHER ALIPHATIC HYDROCARBONS (REVISED VALUES). By GUSTAV EGLOFF. American Chemical Society Monograph Series No. 78. Pp. x + 524. London: Chapman & Hall Ltd.; New York: Reinhold Publishing Corp. 1953. Price 160s.; $20.00.

THE JOURNAL OF ANALYTICAL CHEMISTRY OF THE U.S.S.R. IN ENGLISH TRANSLATION. Volume VII, No. 1, January–February, 1952. Pp. 80. New York: Consultants Bureau, 152 West 42nd Street, New York 18, N.Y. 1953. Annual subscription $80.00; Single issue $15.00.

ELECTROANALYTICAL CHEMISTRY. By JAMES J. LINGANE. Pp. x + 448. New York and London: Interscience Publishers Inc. 1953. Price $8.50; 68s.

STANDARDISATION OF MILK FOR CHEESEMAKING. Bulletin No. 332, Agricultural Research Institute Series No. 24. By H. L. NEETHLING. Pp. ii + 15. Pretoria, South Africa: Department of Agriculture. 1953. Price 3d.

ORGANIC SYNTHESES. Volume 33. Editor-in-Chief: CHARLES C. PRICE. Pp. vi + 115. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1953. Price $3.50; 28s.

CALENDAR OF THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN, 1953–1954. Pp. vi + 300. London: The Pharmaceutical Press. 1953. Price 12s. 6d.

STARCH AND ITS DERIVATIVES. Volume II. By J. A. RADLEY, M.Sc., F.R.I.C. Third Edition. Pp. xii + 465. London: Chapman & Hall Ltd. 1953. Price 65s.

DENTAL PRACTITIONERS' FORMULARY 1952. FIRST AMENDMENT 1953. Pp. 8. London: The Pharmaceutical Press; The British Medical Association. 1953. Price 6d.

Papers for Publication in *The Analyst*

THE Editor welcomes Papers and Notes for insertion in *The Analyst*, whether from members of the Society or non-members. They are submitted to the Publication Committee, who decide on their suitability for insertion or otherwise.

A copy of the current Notice to Authors, last published in full in *The Analyst*, 1953, **78**, 507, can be obtained on application to the Editor, *The Analyst*, 7–8, Idol Lane, London, E.C.3. All Papers submitted will be expected to conform to the recommendations there laid down and any that do not may be returned for amendment.

A few copies of the tabulated "Nomenclature of Vitamins," reprinted from *The Analyst*, 1953, **78**, 72, are also available.

MINISTRY OF SUPPLY, Royal Ordnance Factories at BISHOPTON, Renfrew, and PEMBREY, Carmarthen, require SENIOR ASSISTANTS (SCIENTIFIC) to carry out routine chemical analysis without supervision, and to supervise others engaged on this work. Some posts inlude individual work on more complicated routine chemical procedures, and experimental work under supervision. Qualifications: Minimum of School Cert. with a science credit, but O.N.C. (Chemistry) may be an advantage. Experience of laboratory work essential. Experience of analysis of explosives and acids desirable. Min. age 27. Salary within range £497–£675. Women less. Posts unestablished. Send postcard, quoting V.R.1161/AA, to Secretary, Ministry of Supply, Est. 3 (c) 1, Carlton Hotel, Haymarket, London, S.W.1, for application form.

GLAXO LABORATORIES LTD., Greenford, are interested in recruiting two, or perhaps more, qualified Analysts who should have had several years experience either with a Public Analyst or in an industrial laboratory concerned with Pharmaceuticals or related products. The positions are pensionable and the salaries offered will be commensurate with age and experience. Apply in writing to Senior Personnel Officer, Glaxo Laboratories Ltd., Greenford, Middlesex.

CHEMIST (graduate or equivalent) wanted to assist in establishing and eventually to control a technical research and development section in a bread and confectionery bakery. A keen interest in production problems is essential and some experience of food processing (not necessarily baking) is desirable. Applicants should be about 26–30 years old. Starting salary will be in the order of £650 to £700 per annum, and assistance will be given with housing if required. All enquiries will be treated in the strictest confidence. Apply by letter to Warburtons Limited, Back O'th' Bank Bakery, Bolton.

EVANS MEDICAL SUPPLIES LTD. Vacancies occur in the Analytical Department of the Pharmaceutical Research and Development Unit for Analysts with A.R.I.C., B.Sc., or suitable Pharmaceutical qualifications. Experience in the analysis of Pharmaceuticals would be an advantage. A superannuation scheme and five-day week in operation. Write giving age, qualifications and experience to the Pharmaceutical Development Director, Evans Medical Supplies Ltd., Speke, Liverpool, 19.

BRITISH ELECTRICITY AUTHORITY
MIDLANDS DIVISION

ASSISTANT CHEMIST is required in the Divisional Laboratory at Birmingham. National Joint Board service conditions superannuable appointment, salary within Schedule C, Grade 8 of the agreement, commencing in range £501 to £639, ultimately rising to £694.

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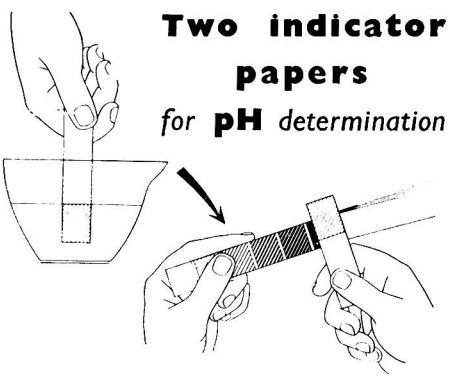

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