THE ANALYST

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กรมวิทยาศาสตร์

A Monthly Publication
dealing with all branches
of Analytical Chemistry:
the Journal of the Society
of Public Analysts and
Other Analytical Chemists

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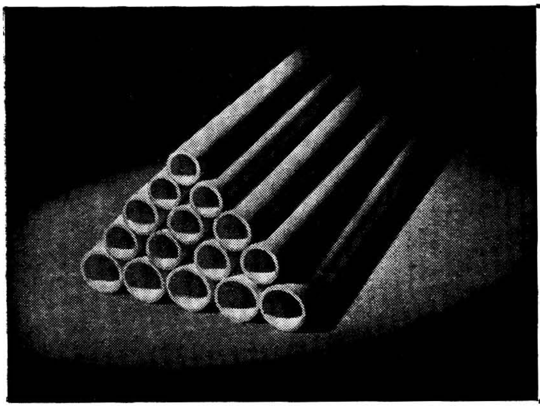

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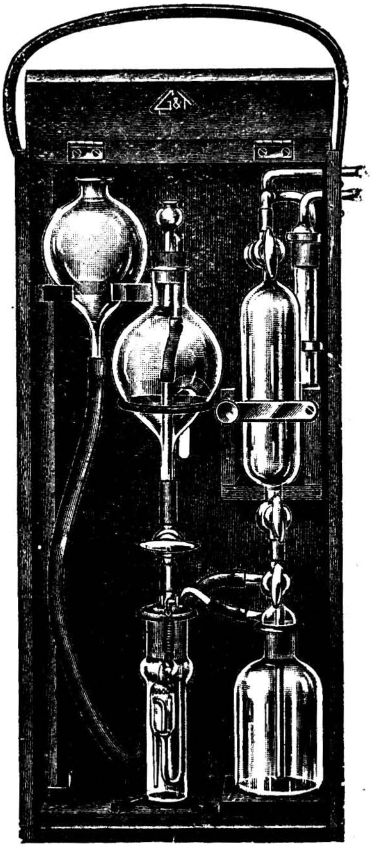

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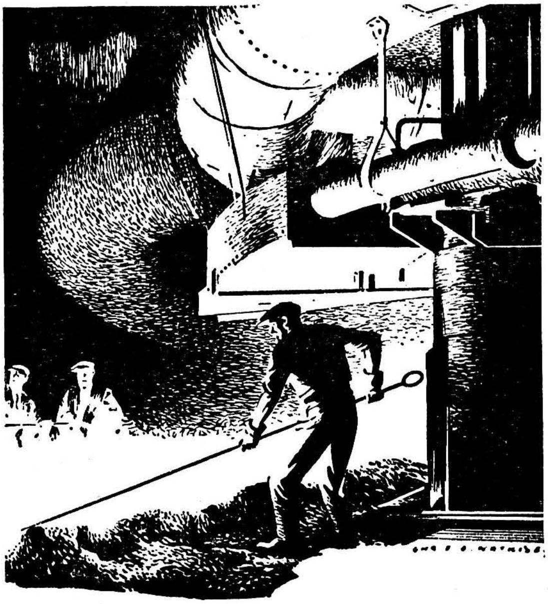

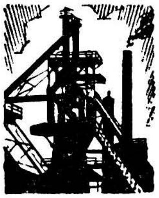

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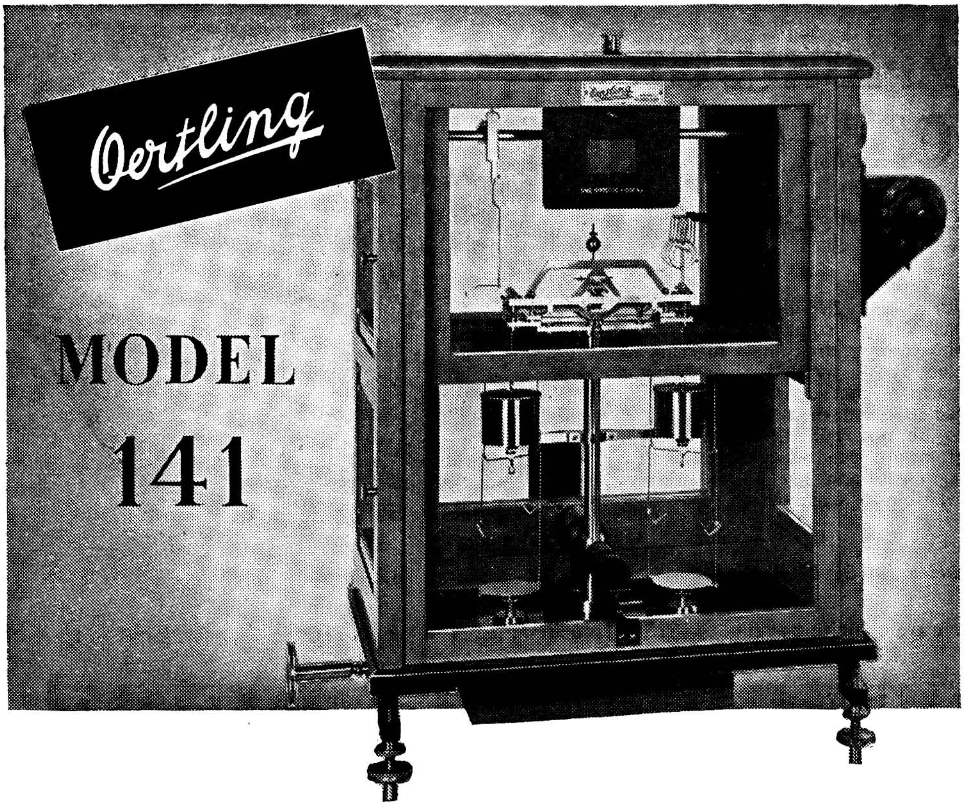

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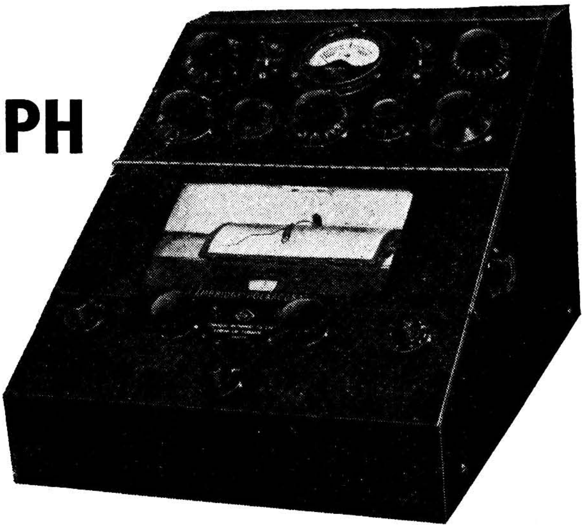

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JUNE, 1951 Vol. 76, No. 903

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

An Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, April 4th, 1951, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The chair was taken by the President, Dr. J. R. Nicholls, C.B.E., F.R.I.C.

The following papers were presented and discussed: "The Determination of the Relative Availability of the Nitrogen in Nitrogenous Fertilisers. Part II," by J. Hubert Hamence, M.Sc., Ph.D., F.R.I.C.; "The Determination of the Acidity of Milk," by E. I. Johnson and J. King, O.B.E., F.R.I.C.; "An Improved Volumetric Method for the Determination of Hydrogen Sulphide and Soluble Sulphides," by J. A. Kitchener, Ph.D., A. Liberman, B.Sc., Ph.D., D.I.C., and D. A. Spratt, B.Sc., A.R.C.S.

NEW MEMBERS

Doris Emily Butterworth; Arthur Leslie Davis, A.R.I.C.; Ann Taylor Dix; Eric Reginald William Fogden, B.Sc. (Lond.), A.R.I.C.; Alfred Edward Lambden, B.Sc. (Lond.); Ernest Charles Mills, A.R.I.C.; Richard Colin Norris, B.Sc. (Liv.), A.R.I.C.; Kenneth Alfred Palmer; Ronald John Thompson, A.R.I.C.; Geoffrey Alison Vaughan, A.R.I.C.

DEATH

We regret to record the death of
Walter Collingwood Williams.

SCOTTISH SECTION

An Ordinary Meeting of the Section was held in the Central Hotel, Glasgow, on Wednesday, March 7th, 1951, at 7 p.m. Mr. H. C. Moir presided and 32 members and friends were present. A number of scientific films were shown by Dr. H. Dryerre.

MICROCHEMISTRY GROUP

The Spring Meeting of the Group was held jointly with the Edinburgh and East of Scotland Sections of the Royal Institute of Chemistry and the Society of Chemical Industry in Edinburgh on Friday and Saturday, April 13th and 14th, 1951. The proceedings on the afternoon of the first day consisted of a Symposium on Newer Biochemical Methods and, by courtesy of Professor G. F. Marrian, F.R.S., were held in the Department of Biochemistry at the University of Edinburgh.

The papers presented were as follows: "Recent Developments in the Use of Isotope Techniques in Biochemistry," by Professor J. N. Davidson, M.D., D.Sc., F.R.I.C., F.R.S.E.; "The Fractionation of Plasma Proteins and its Clinical Significance," by C. P. Stewart, Ph.D., M.Sc.; "Amino-Acid Analysis," by G. R. Tristram, Ph.D. A discussion followed each paper.

On the following morning a visit was made to the Organon Laboratories Ltd., at Newhouse, Lanarkshire.

PHYSICAL METHODS GROUP

THE Thirty-first Ordinary Meeting of the Group was held at 6.30 p.m., on Tuesday, April 10th, 1951, in the Meeting Rooms of the Iron and Steel Institute, 4, Grosvenor Gardens, London, S.W.1. This meeting was organised by the Polarographic Discussion Panel, and Mr. J. Haslam, Chairman of the Panel, was in the chair.

The following papers were presented and discussed: "Vibrating Electrodes in Polarography. The Effect of Frequency and Amplitude of Vibration on Diffusion Current," by A. J. Lindsey, M.Sc., Ph.D., F.R.I.C., and E. D. Harris, M.Sc., A.R.I.C.; "The Polarographic Behaviour of Iodo-organic Compounds," by J. E. Page, B.Sc., Ph.D., F.R.I.C.; "Selected Applications of Polarography in Inorganic Analysis," by G. W. C. Milner, B.Sc., F.R.I.C., A.Inst.P.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held at 6.30 p.m. in the Meeting Room of the Chemical Society, Burlington House, London, W.1, on Monday, March 12th, 1951. Mr. N. T. Gridgeman was in the chair, and thirty-two other members and guests were present.

The following papers on "The Evaluation of Drugs in Man" were presented and discussed: "The Evaluation of Drugs in Man, with Special Reference to Antihistaminics," by Professor W. A. Bain; "Tests on Analgesic Drugs in Man," by Dr. C. A. Keele.

Obituary

ALEXANDER HENRY MITCHELL MUTER

ALEXANDER HENRY MITCHELL MUTER was born in Kennington in 1873. He was the elder son of Dr. John Muter, who was one of the founders and third President of the Society and part proprietor of *The Analyst* for several years. As he was intended to follow his father's career, he was sent to Germany to be educated according to the Victorian custom for boys intended to become chemists. On returning to London he entered the laboratory at Kennington, where his father had built up a large practice in the analysis of drugs, food and water, and held several appointments as Public Analyst. He took classes in chemistry at King's College, London, and passed the examinations for the Associateship and Fellowship of the Institute of Chemistry in 1896 and 1899 respectively. His first public appointment was to Tunbridge Wells in 1908. On the death of his father he succeeded to several appointments for which he had been deputy, including the Boroughs of Wandsworth and Lambeth, the Parts of Lindsey and the Metropolitan Asylums Board; and in later years he became Public Analyst to the Parts of Holland and Kesteven, Lincolnshire, and to the Borough of Colchester.

Muter acquired a large experience in the Courts. He prepared his certificates with great care; he took pains to appreciate and appraise both sides of a case and gave his evidence in moderate terms, conveying the impression that his function was to assist the Court only. He would never appear against another Public Analyst and held strong opinions against the etiquette of this practice. In 1912 he took into partnership Charles Hackman, with whom he had formed a friendship at King's College while studying for the examination of the Institute of Chemistry in Branch E. Hackman was an excellent analyst, very receptive to new ideas and, as befitted a pupil of Chaston Chapman, a stickler for accuracy, method and neatness. Though the friends were very unlike in temperament and outlook, their association proved a felicitous one during the long period of the partnership. One curious feature they shared in common was a dislike of publishing new work, with the natural result that someone else had to go over much the same ground before the results were available to the profession. Hackman trained for some years as an engineer and he had a flair for designing and constructing apparatus for speeding up routine work or for some special type of work. Hackman died in December, 1940, and Muter relinquished his daily attendance at the laboratory from that time.

Muter was a prominent Freemason for over half a century and attained high office in the fraternity.

In his youth, Muter played the violin in some South London orchestras. In those days meetings of the Society used to be followed by informal concerts; Bernard Dyer, in his 91st year, writing to Muter, recalled his playing at these functions. He had many hobbies, with strong preferences for out-door occupations in the country. An almost life-long motorist, he was one of the earliest members of the A.A. But gardening was his greatest delight. He built a house near Charing, some years ago, for week-end residence. In 1940, his house in Dulwich having become damaged in an air-raid, he retired to Charing. He continued to visit the laboratory every week, and on most Saturdays during the winter months came to town to carry out his masonic duties. He passed away on February 7th, 1951, after a few days' illness. His never-failing good humour, open-handed generosity and readiness to help his professional friends will long be remembered by those who were privileged to know him.

J. E. WOODHEAD

Polarographic Congress: Prague, 1951

A POLAROGRAPHIC Congress was held in Prague from February 4th to 8th, 1951, in honour of Professor J. Heyrovský's sixtieth birthday. It was not possible for the Society to be represented at the Congress, but the following letter of congratulation was sent to Professor Heyrovský, and his reply follows.

A report of the Congress is appended.

December 28th, 1950.

Dear Professor Heyrovský,

I write to convey to you the congratulations of the President, Council and Members of the Society of Public Analysts and Other Analytical Chemists on your recent attainment of your sixtieth birthday.

As you know, we, in this Society, have the highest admiration for the pioneer work that you carried out in establishing polarography as a tool of analytical chemistry, and we are proud to think that much of the early work was undertaken at University College, London, in 1924. Since then, of course, polarography has found applications in every field of analytical chemistry, and over 2000 original papers have been published on the subject.

We are glad to hear that you have been nominated as Director of the Polarographic Institute in which you will be able to apply polarographic methods to both pure and applied science; and we trust that you will be able to spend many happy years there working in the field of which you are a master.

Yours sincerely,

K. A. WILLIAMS,

Honorary Secretary.

March 4th, 1951.

Dear Dr. Williams,

Accept my belated thanks for your very kind congratulations on my sixtieth birthday and for your words of appreciation, consideration and encouragement, which you express in your letter as to the development of polarography.

In connection with my birthday we had the First International Polarographic Congress held in Prague, a report of which I enclose as it may interest the members of your Society I was very sorry that owing to the circumstances only a few visitors were able to come from abroad.

Please convey my warm thanks to your President, to the Council and to all members of the Society of Public Analysts.

With best regards,

Yours very sincerely,

J. HEYROVSKÝ.

REPORT OF THE CONGRESS

A SUCCESSFUL meeting of some 400 polarographists of Czechoslovakia and visitors from Poland, Hungary, Roumania and Bulgaria has shown the great interest in the new branch of science, polarography.

The Congress was organised by the Centre of Research and Technical Development in Prague. The President was Prof. J. Heyrovský, Director of the Central Institute of Polarography, and the Vice-President was Prof. R. Brdička, Director of the Physico-chemical Institute of the Charles University, Prague.

An evening session to welcome the visitors, held in the spacious rooms of the old palace of Sylva Taroucca, preceded the opening of the Congress. The Congress was inaugurated in the large theatre of the Purkyně Medical Institute on the morning of Monday, February 5th, by the Minister of Planning, Dr. J. Dolanský, and the Director of the Centre of Research and Technical Development, Prof. J. Fukátko. Minister Dr. Dolanský spoke on the cardinal tasks of science in building up socialism and Prof. Fukátko spoke on the important rôle of planning in scientific research. Then Prof. J. Heyrovský gave his review on the "Fundamentals of Polarography." The communications at the Congress were divided into eight reviews, each of about one hour's duration, in which the modern development of polarography was surveyed, and into discussions, of about fifteen minutes' duration, of new papers submitted to the Congress. The morning sessions were devoted to pure science and the afternoons to applied polarography. The following reviews were given: "Inorganic Analysis," by Vl. Majer; "Polarography of Organic Compounds," by Vl. Hanuš; "Organic Analysis," by P. Zuman; "Polarography in Biochemistry and Medicine," by F. Šantavý; "Instruments for Oscillographic Polarography," by J. Forejt; "Applications of Oscillographic Polarography," by J. Heyrovský; "Kinetics of Electrode Processes in Polarography," by R. Brdička.

There were 55 communications presented as follows—

On February 5th: "The Validity of the Nernst Formula in Deducing the Equation of the Polarographic Wave," by M. Kalousek and A. Tockstein; "Polarography in Concentrated Sulphuric Acid," by A. Vlček; "Study of the Discontinuities of Current on Polarographic Curves," by P. Valenta; "Some Examples of the Analysis of Alloys," by M. Spálenka; "Determination of Phosphates," by J. V. A. Novák; "Determination of Small Quantities of Thorium," by K. Komárek; "Determination of Alkalinity," by K. Komárek; "Experience in the Control of Steel Manufacture," by J. Korecký, F. Nademlejnský and B. Neliba; "Determination of Manganese by Means of Triethanolamine," by J. Mojžíš; "Determination of Gold," by F. Linhart.

On February 6th: "Determination of Oxygen, Benzene and Hydrogen Sulphide in Lighting-gas," by J. Prchlík; "Reduction of Hydrogen Peroxide Catalysed by Complexes of Iron with Catechol, Pyrogallol and Ascorbic Acid," by J. Doskočil; "Polarographic Analysis of Benzoic Acid and of Phthalic Anhydride," by B. G. Šimek, F. Majer and G. Šebor; "Polarography of Coumarine," by O. Čapka; "Polarography of Alkaline Products of Glucose," by J. Trnka; "The Reaction of Carbonyl Compounds with Primary Amines," by P. Zuman; "Cyanuric and Rubeanic Acid," by K. Suchý; "Some Complexes of Amino-acids with Metals," by R. Pleticha; "Determination of Phenol in Water and in Urine," by J. Roubal and J. Zdražil; "Determination of Pentosans," by R. Domanský; "Determination of Barbiturates by Titration with Mercuric Salts," by R. Kalvoda and J. Zýka; "Polarometric Determination of Unsaturated Compounds," by A. Blažek; "Determination of Diacetyl," by R. Pleticha; "Colchicine in Meadow-saffron during Growth," by J. Buchníček; "Applications in Paper Industry," by B. Sandholec; "Sulphydryl Compounds in Fruits," by P. Zuman.

On February 7th: "Hydrolysis of the Oxidation Product of Vitamin K_5," by E. Knobloch; "Ascaridol," by B. Bitter; "Muconic Acid in Bacteria," by A. Kleinzeller and Z. Fencl; "Polarography of Sterols," by J. Nosek; "Heart Poisons with a Five- or Six-membered Lactone Ring," by F. Šantavý; "Oxidation Products of Morphine," by F. Šantavý; "Determination of Oxygen in Blood," by M. Šimáně; "Biological Redox Indicators," by J. Doskočil; "A Contribution to the Brdička Filtrate Reaction in Serum," by J. Homolka and D. Krupička; "Determination of Thallium in Urine," by Z. Zábranský; "Studies of Peroxidase Reactions," by J. Doskočil; "The Electronic Polarograph," by K. Ezr; "Artificial Control of the Drop-time," by O. Nesvadba; "A New Apparatus for Oscillographic polarography," by J. Vogel; "Vibrations of Drops due to Currents of High Frequency," by W. Kemula.

On February 8th: "Catalysed Depolarisations in Inorganic Redox Systems," by E. Svátek; "The Rate of Dissociation of the Complex of Cadmium with Nitrilotriacetic Acid," by J. Koryta;

"Recombination of the Ions of Phenylglyoxylic Acid," by Vl. Hanuš; "Linear Systems of Electrode Reactions," by J. Koutecký; "Slow Electrode Reactions," by M. Smutek; "Irreversibility of Electrode Reactions," by A. Tockstein; "Generalisation of the Theory of Linear Diffusion Currents," by J. Plíva; "The Hydrogen Overvoltage with a Controlled Drop-time," by J. Kůta; "The Discontinuity on the Polarographic Curve in the Reduction of Nitrates," by J. Mašek; "A Study of the Complexes of Tervalent Chromium," by H. T. Arend; "Complexes of Sucrose with Ferric and Ferrous Ions," by M. Křivánek; "The Control of the Surface Adjustment of Metals," by T. Jelinek; "The Derivative Curves in Polarography," by J. Říha; "The Effect of Thymol and Gelatin on Polarographic Waves," by M. Drátovský and M. Ebert; "The Influence of Capillary Constants on Maxima," by J. Dvořák; "The Classification of Refined Sugars," by I. Vavruch.

The eight meetings were fully attended right to the end. The Congress ended with a social gathering in the Sylva Taroucca palace, when all who had attended expressed their appreciation in speeches and toasts. The guests were: Prof. W. Kemula, Prof. M. Michalski and J. Chodkowski of Warszaw University, Prof. L. Erdey and Mrs. Ajtai of Budapest, Prof. A. Pârvu and Prof. E. Macovschi of the University of Bucharest and Dr. A. Trifonov of the Institute of Technology, Sofia.

Reports of the communications and discussions will be published in full in three volumes of *Proceedings*, of which the first will also contain contributions, in English, French and German, that were not communicated at the Congress, the second a full critically revised bibliography of Polarographic papers from 1922 to 1950, and the third the reports of the meetings themselves. Single volumes will be on sale to those who did not attend the Congress.

Analytical Methods Committee

RECOMMENDATIONS OF THE MEAT EXTRACT SUB-COMMITTEE

Analysis of Meat Extract

THE Analytical Methods Committee has received the following Report from the Meat Extract Sub-Committee, and its publication has been duly authorised.

CONSTITUTION OF THE SUB-COMMITTEE

The Sub-Committee consists of: G. Taylor, O.B.E., F.R.I.C. (Chairman); R. Gordon Booth, Ph.D. (from December, 1949); Osman Jones, F.R.I.C.; the late E. C. Keeley, B.Sc., A.R.I.C. (until December, 1949); J. King, O.B.E., F.R.I.C.; G. Spall; R. G. Westall (nominee of Dr. E. C. Bate-Smith); H. G. Rees, B.Sc., Ph.D., A.R.C.S., D.I.C., F.R.I.C. (Honorary Secretary).

The Sub-Committee wish to place on record the loss sustained by the untimely death of Mr. E. C. Keeley on December 20th, 1949.

INTRODUCTION

The terms of reference of the Sub-Committee are: "to consider whether standard analytical methods are necessary for meat extracts and similar products and to carry out such investigations as may be deemed necessary."

The Sub-Committee agree that Standard Methods of Analysis are required and that they should be so formulated to give the maximum information as to the detection of possible adulterants.

They further consider, however, that it is desirable that a standard method for this purpose should be one that is both sufficiently accurate and precise for all commercial purposes and also capable of adoption in any analytical laboratory; on this basis, in fact, the experimental work has been designed and the suggested methods have been formulated.

The Sub-Committee do not regard the prescription of standards of composition for meat extracts and similar products as coming within their terms of reference. Nevertheless they have thought it desirable to define products that they regard as being within the general description of meat extracts and similar products, in order to indicate suitable methods of analysis. These definitions are given immediately below.

DEFINITIONS—

Meat extract—The product obtained by extracting fresh lean meat with boiling water and concentrating the liquid portion after removal of fat.

Meat stock—The product obtained by the extraction of meat trimmings, bones, rind and edible offal with boiling water and concentrating the liquid portion after removal of fat, with or without the addition of salt.

Bone stock—The product obtained by the extraction of fresh trimmed bones with boiling water, with or without pressure, and concentrating the liquid portion by evaporation after removal of fat, with or without the addition of salt.

Essence of beef—The product obtained by the extraction of minced beef with boiling water, such extraction being sufficiently prolonged to produce a jelly on cooling.

Meat juice—The fluid portion of fresh lean meat obtained by pressure and concentrated by evaporation at a temperature below the coagulating point of the soluble proteins.

Similar products—Products or composite products more or less simulating the characteristics of the various extracts obtainable from meat or bones but containing products other than meat, for example, yeast extract, hydrolysed protein, vegetable soup stock, meat extract cubes or gravy cubes and soup powders.

SCOPE OF THIS REPORT—

This Report of the Sub-Committee deals only with meat extracts as defined above, but is generally applicable to materials other than "similar products." Additional methods of analysis are necessary to cover these and, to a less extent, meat stock and bone stock, and it is the intention of the Sub-Committee to undertake further work for this purpose.

DISCUSSION AND EXPERIMENTAL WORK

To assess the quality and genuineness of meat extracts it was considered that the determinations listed below should satisfactorily serve the purpose for products of the nature of meat extracts, and accordingly only such determinations are dealt with in this Report—

Water.
Ash.
Chloride.
Total nitrogen.
Total creatine and creatinine (determined as creatinine).

Some consideration was also given to organoleptic tests, but although these are of considerable value to an observer with a trained palate, it was decided that they cannot be recommended for an analyst without this experience.

For the purpose of a more detailed analysis, particularly to assess and estimate adulterants or the addition of non-meat ingredients, it was thought that some or all of the further determinations specified below would be necessary—

Phosphate.
Fat.
Soluble and insoluble nitrogen.
Amino nitrogen.
Gelatin.
Tannic acid precipitate.
Nicotinic acid.
Starch.
Qualitative tests for extraneous ingredients.

PREPARATION OF THE SAMPLE FOR ANALYSIS—

Meat extracts contain significant proportions of constituents that tend to separate after the extract is filled into the containers; for this reason, and owing to their viscous condition when cold, the extracts are usually transferred slightly warm to the containers. On storage there is a tendency for creatine, together with some mineral matter, principally phosphate, to separate out. It is therefore necessary to mix the sample thoroughly before taking portions for analysis. A cautious warming will expedite the blending of pasty samples and any sediment must be thoroughly incorporated. However carefully such mixing is carried out there is still a danger that very small portions of the mixture do not accurately represent the bulk. Accordingly it was decided that a satisfactory method of overcoming this difficulty would be to take a portion of 10 ± 1 g dissolved in water to a bulk of 100 ml and to use aliquots of this solution for the various determinations. It is desirable that a specially calibrated pipette be used as it has been realised that use of a pipette with such a solution may lead to a slight error due to differences in viscosity and surface tension from those of water.

DETERMINATION OF WATER—

Considerable discussion ranged around the question of determining the water content of meat extract, since it was accepted from the beginning that such a determination must be an arbitrary one, there being no assurance that any method would yield a true figure for water, that is to say for free water or free and bound water as distinct from water produced by protein breakdown. The Sub-Committee were fortunate in having at their disposal copies of a recent monograph reprinted from the *Journal of the Council for Scientific and Industrial Research, Australia*,[1] in which the theoretical considerations are dealt with in considerable detail. They accepted the conclusions given in the monograph, *viz.*, that it is not possible to obtain a true value for the water content by any known method and that therefore some arbitrary method carefully defined and rigorously followed should be adopted.

As a basis for experimental work by members of the Sub-Committee, it was agreed that an empirical standard method could be based on the Society's method for sweetened condensed milk.[2] Comparative determination of loss of weight at 100° C were then made under various conditions of time, size and type of dish, weight of sample, and with and without sand. The results of this experimental work led the Sub-Committee to the conclusion that the method based on drying for a fixed time 1 g of meat extract after solution in water yielded results satisfactorily comparable with the method using sand and was therefore to be preferred on account of its simplicity.

A series of collaborative determinations was carried out by all the members of the Sub-Committee on two samples of meat extract having moisture contents of approximately 20 and 27 per cent. Statistical examination of these results by Dr. E. C. Wood showed that the variance between laboratories was very significantly more than the variance within laboratories. The precision of the method from both points of view is summarised in the following statistics—

Standard error, calculated from the within-laboratories variance = 0·096%
Standard error, calculated from the between-laboratories variance = 0·501%

This means that 19 out of 20 determinations of moisture on the sample, in the same laboratory, should differ from their mean by not more than 0·2 per cent.; but if one determination were made in each of 20 different laboratories, 19 out of 20 should differ from their mean by not more than 1·1 per cent.

DETERMINATION OF ASH AND CHLORIDE—

Ash—The direct ashing method was compared experimentally with a method involving charring, leaching out and complete ashing. As the results by the direct ashing method were found not to be significantly different from those by the alternative method, it was decided that the former should be recommended. Due care must be exercised to ensure that there are no losses due to decrepitation or volatilisation. The latter may be avoided by ensuring that a temperature of 550° C is not exceeded. As an alternative method when no temperature control is available for this purpose, details of a leaching-out process are included.

Chloride—Methods examined included determination of chloride obtained (*a*) by direct ashing, (*b*) by ashing and leaching and (*c*) directly in the extract solution. An ashing process is recommended, but it is essential to avoid fusion of the ash and possible loss by volatilisation.

DETERMINATION OF TOTAL NITROGEN—

The determination of nitrogen has been so thoroughly examined by various workers that the Sub-Committee considered it necessary only to survey the literature, particularly as regards the time of digestion and the type of catalyst, and to carry out collaborative work on given samples of meat extract by the accepted method.

TOTAL CREATINE AND CREATININE—

The method for the determination of total creatine and creatinine (recommended to be expressed as total creatinine) has been very thoroughly investigated in the laboratories with which members of the Sub-Committee are associated, since it is realised that this value is probably the most important index of quality of a meat extract and that on which analysts will largely base their conclusions. All stages of the process, including the method of hydrolysis, influence of volume, and concentration of caustic soda and picric acid on the development and stability of colour, have therefore been examined in great detail. The recommended method is based on the results of this investigation; it also incorporates as far as possible the techniques readily available for the evaluation of colour. As creatinine zinc chloride is now obtainable in a crystalline form of guaranteed purity, it is recommended as a standard. "Creatinine Zinc Chloride, 99 to 100% (standard for creatine and creatinine determinations)" may now be obtained from The British Drug Houses, Ltd.

RECOMMENDED METHODS OF ANALYSIS

All reagents should conform to recognised analytical standards.

Stock solution—A cautious warming will expedite the blending of pasty samples and any sediment must be thoroughly incorporated.

Take 10 ± 1 g of meat extract, accurately weighed, and dissolve with successive small quantities of hot distilled water to ensure solution of all soluble material; cool and make up to 100 ml. Shake the solution before taking aliquots for the various determinations.

DETERMINATION OF WATER—

Procedure—Pipette 10 ml of stock solution into a nickel dish approximately 3 inches in diameter, preferably fitted with a close-fitting lid (see Report of the Milk Products Sub-Committee[2]) and evaporate to apparent dryness on an open steam-bath, adjusting the level of the dish to ensure an even film. Transfer to a drying oven at 100° C, insulate the dish from the shelf and dry for 8 hours. Replace the lid, which has also been in the oven, before removal to the desiccator. Cool for 30 minutes before weighing.

DETERMINATION OF ASH—

Procedure—Evaporate 10 ml of the stock solution in a platinum dish on the steam-bath and char thoroughly over a low flame. Complete the ashing (preferably in an electric muffle) at a temperature not exceeding 550° C to avoid loss of volatile ash.

Alternative procedure—Evaporate 10 ml of the stock solution in a platinum dish on the steam-bath and char thoroughly over a low flame. Cool and extract with three portions of hot water (10, 5 and 5 ml). Decant through a filter-paper and wash the paper with a few ml of hot water. Return the filter-paper to the dish, dry on the steam-bath and incinerate the contents completely. After cooling, return the filtrate to the dish, evaporate it to dryness and heat at a temperature not exceeding 550° C, preferably in a muffle, until the weight is constant.

DETERMINATION OF CHLORIDE—

Procedure—Take 20 ml of the stock solution in a platinum dish and evaporate to dryness with 10 ml of 5 per cent. sodium carbonate. Ignite as thoroughly as possible at a temperature not exceeding dull redness. Extract with hot water, filter and wash. Return the filter-paper and residue to the dish and moisten with a few drops of carbonate solution, evaporate and ignite to a white ash. Dissolve in dilute nitric acid. Filter from any insoluble matter, wash thoroughly and add to the previous filtrate. Determine chloride by the Volhard method.

DETERMINATION OF TOTAL NITROGEN—

Digestion—Digest 5 ml of the stock solution with 25 ml of sulphuric acid, 10 g of anhydrous sodium or potassium sulphate and a catalyst (0·2 g of copper sulphate, 0·7 g of mercuric oxide or 50 mg of selenium).

The time of digestion should be 3 hours after clearing, irrespective of the catalyst used.

Distillation of ammonia—Dilute the digest with 100 to 200 ml of water, according to whether a steam-distillation or boiling method is used, and make it alkaline with 100 ml of 40 per cent. sodium hydroxide, free from carbon dioxide, with addition of sodium sulphide where mercury has been used as a catalyst. The method of distillation and absorption of ammonia shall be left to the discretion of the analyst.

Note—(*i*) The clear layer of a 40 per cent. solution of caustic soda is satisfactory for rendering the solution alkaline.

Note—(*ii*) Methyl red is a satisfactory indicator if the ammonia is absorbed in standard sulphuric acid.

Note—(*iii*) An appropriate blank determination should be carried out.

DETERMINATION OF TOTAL CREATINE AND CREATININE AS CREATININE—

Solutions required—

Hydrochloric acid—A 2 *N* solution.

Sodium hydroxide—A 2 *N* solution.

Picric acid—A 1 per cent. solution (see Note *ii*, below).

Stock creatinine zinc chloride solution—1·603 g of pure crystalline creatinine zinc chloride made up to 1000 ml with 0·1 *N* hydrochloric acid. This solution is stable for at least six months, and an aliquot should be diluted ten times, immediately prior to use, so that 1 ml ≡ 0·1 mg of creatinine.

HYDROLYSIS OF MEAT EXTRACT SOLUTION—Heat under reflux 10 ml of the stock solution of the extract with 10 ml of 2 *N* hydrochloric acid in a boiling water-bath for at least 2 hours, or autoclave for 20 minutes at 117° to 120° C. Cool the hydrolysed solution and add 10 ml of 2 *N* sodium hydroxide solution. Dilute to a volume of (*a*) 250 ml for Duboscq method, or (*b*) 500 ml for the absorptiometer method.

(*a*) *Duboscq and other visual methods such as Nesslerising*—Measure from a burette two aliquots of 7 ml and 10 ml from the 250-ml dilution (see Note *i*) into clean, dry 100-ml graduated flasks. Make each quantity up to 20 ml with distilled water, add 20 ml of 1 per cent. picric acid solution, then 2·5 ml of 2 *N* sodium hydroxide, and maintain at 20° ± 1° C for 15 minutes. Dilute to 100 ml with distilled water. Filter, rejecting the filtrate until the solution is clear and bright. Compare with a standard made up at the same time and under the same conditions from 20 ml of standard creatinine zinc chloride solution (equivalent to 2 mg of creatinine). The colour can be read immediately and is stable for 30 minutes.

Note—(*i*) These two aliquots will indicate the approximate percentage of creatinine; an aliquot can then be calculated which, after development of colour and dilution, will compare closely with the standard.

(*b*) *Absorptiometer method*—Measure an aliquot of 5 ml from the 500-ml dilution into a clean, dry 100-ml volumetric flask and make up to 20 ml with distilled water. Add 20 ml of 1 per cent. picric acid solution and 2·5 ml of 2 *N* sodium hydroxide; maintain at 20° ± 1° C for 15 minutes. Dilute to 100 ml with distilled water. Filter; reject the first few millilitres until the solution is clear and bright.

Readings are made in the absorptiometer with an Ilford filter No. 604 and a 1-cm cell; a reagent blank composed of all the reagents minus the creatinine is used. The colour can be read immediately and is stable for 30 minutes.

Prepare a standard curve covering a range of 0 to 1·0 mg of creatinine. Into clean, dry volumetric flasks measure quantities of 2, 4, 6, 8 and 10 ml of the standard creatinine zinc chloride solution. These flasks contain creatinine in amounts of 0·2 to 1·0 mg. Add distilled water to bring the volume of solution in each flask to 20 ml. To each flask add 20 ml of 1 per cent. picric acid solution and 2·5 ml of 2 *N* sodium hydroxide. Maintain at 20° ± 1° C for 15 minutes and dilute each volume to 100 ml.

Note—(*ii*): 1 *per cent. picric acid solution*—This strength is chosen owing to the difficulty of maintaining complete solution at a concentration of 1·2 per cent. during winter conditions. It should be standardised against 0·1 *N* sodium hydroxide, phenol red being used as indicator.

Note—(*iii*) In a determination where Nesslerising is involved it is essential that colour comparisons be carried out under optimum conditions in order to obtain good contrast and reliable results. These include employment of a good north light with no artificial light illuminating the Nessler tubes. Due consideration should be paid to the colour vision of the operator: it is desirable that this should be normal in the region of the colour to be observed.

REFERENCES

1. Riddle, A. R., *J. Council Sci. & Ind. Res., Australia*, 1944, **17**, 291.
2. Analytical Methods Committee, "Report of the Milk Products Sub-Committee," *Analyst*, 1927, **52**, 402.

Flame Photometers: A Description of Two Instruments

By L. BREALEY and R. E. ROSS

(*Presented at the meeting of the Physical Methods Group on Friday, October* 6*th*, 1950)

Two flame photometers are described; the first a simple instrument for the determination of sodium and potassium, and the second, a versatile general purpose instrument. Some results are given to show the effect of other ions upon that under examination.

During the past ten years, several flame photometers have been described.[1,2,3,4,5,6] These instruments have varied from the simple, which could be built easily in almost any laboratory, to complex commercial equipment. Many types of flame have been described, those most used being air or oxygen with illuminating gas or propane, or air - acetylene. For isolating light of the required wavelengths either filters or a monochromator have been used. Most types of light detector, from barrier layer photo-cells to photomultipliers have been used; commercial instruments are readily obtainable in America, but in this country they are sold only as accessories to other expensive equipment, and even these have not been available until recently; consequently, the majority of workers have built their own apparatus.

This paper describes two instruments that have been built in the laboratory. The first is simple and limited in its application to the determination of sodium and potassium; the second is capable of determining any element that will give suitable emission from a flame. In designing these instruments the following four points were regarded as fundamental requirements of any flame photometer—

(1) It should be robust and simple to operate so that it can be used by laboratory assistants as a routine instrument.
(2) It should give a steady final reading, free from drift and accurately repeatable.
(3) It should be sufficiently sensitive to give useful readings on very dilute solutions.
(4) It should be as free as possible from interference by ions other than that being determined.

THE FIRST INSTRUMENT

Probably the most difficult parts of a flame photometer to design satisfactorily are the atomiser and burner. Many types of flame are available, from the very hot oxy-hydrogen to the comparatively cool mixture of air and coal gas. For the simpler of the two instruments a cool air - propane flame is preferable, as this type of flame has a low propagation velocity and can therefore be burned at an ordinary Meker type of burner without much risk of explosion by striking back. The atomiser and flame unit is shown in Fig. 1. The atomiser needs to be of a rigid structure if it is to be robust and give a constant spray; the one used was of the concentric type, similar to that on the Beckman instrument and described by Gilbert *et al.*[6]; from which it differs, however, in that the annular space between the outside of the sample capillary and the inside of the surrounding air nozzle is smaller and a somewhat higher air pressure is used; this gives rise to a denser spray and so introduces a larger sample into the flame. The larger droplets are removed from the airborne spray by the decreased air velocity in the expansion chamber, a 750-ml conical flask, and are run to waste from the bottom of the flask. The fine mist that remains in suspension is carried directly into the flame.

In order to achieve a steady reading it is of primary importance to have a flame that is perfectly steady and free from flicker; to ensure this, the gas and air must be supplied at constant pressure. The air supply is particularly important since any drop in pressure will, besides affecting the flame itself, result in a fall in the rate at which the solution is atomised. Both gases are supplied from cylinders, the propane through a needle-valve at a pressure of 9 inches water and the air through a two-stage regulator at 35 lb per square inch. In use, the flame is protected from draughts by a chimney, which also serves to prevent errors in the readings from extraneous light.

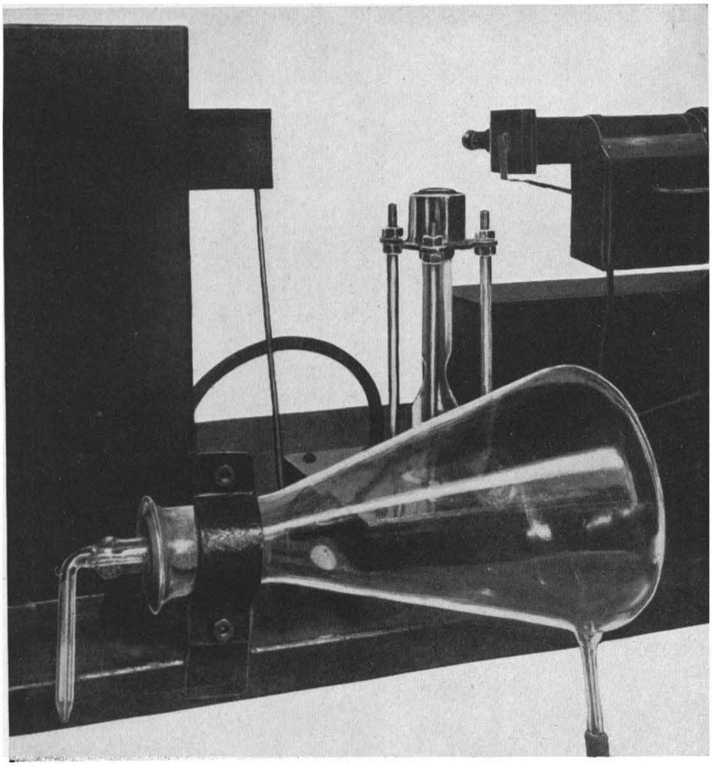

Fig. 1. Burner and atomising unit

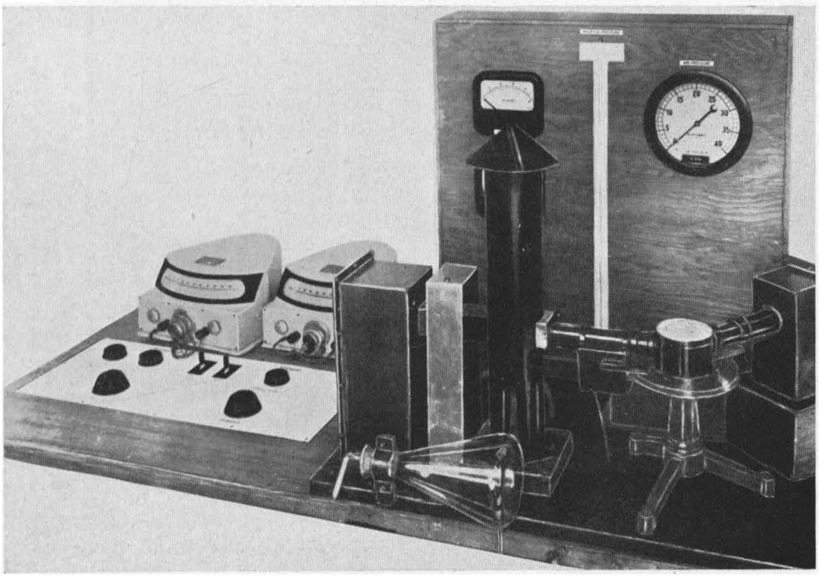

Fig. 3. The first instrument

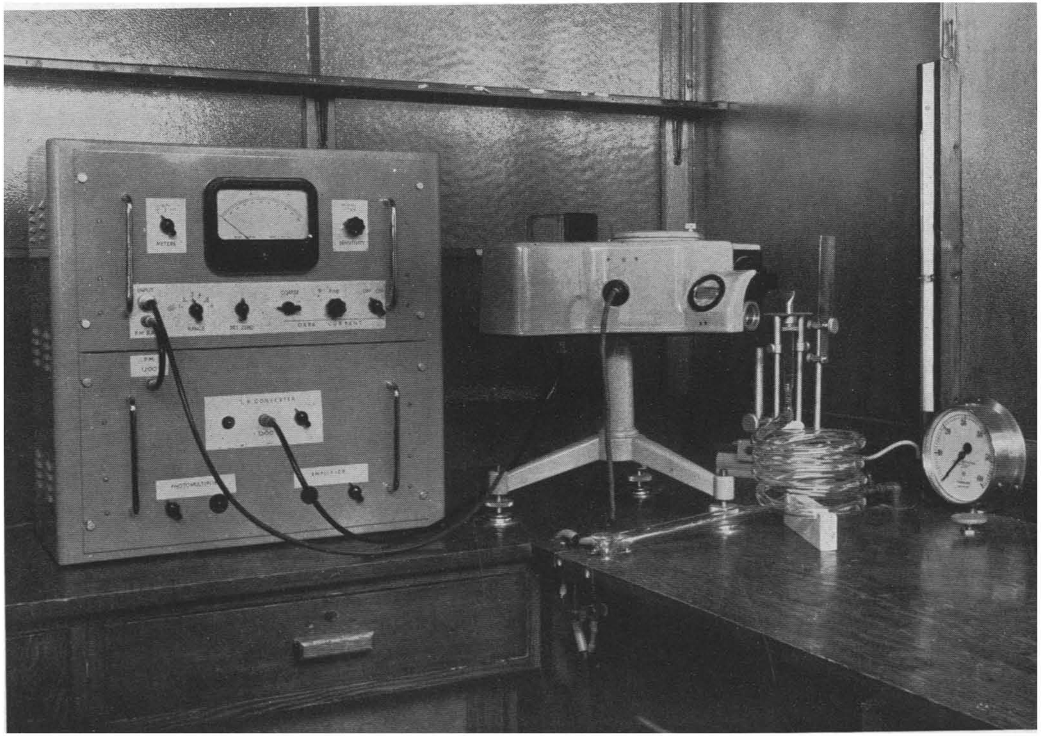
METERS
SENSITIVITY
INPUT
RANGE
SET ZERO
COARSE
FINE
DARK CURRENT
I.R. CONVERTER
AMPLIFIER

Fig. 4. The second instrument

The light from the flame emerges from two diametrically opposed windows in the chimney and passes into the units that separate and detect the light due to potassium and sodium respectively. The isolation of the potassium doublet 7660 and 7690 A is achieved by means of filters. Those used are Wratten 88A, with a Chance ON20 heat-absorbing filter included to cut down the background due to water molecular bands, which occur at about 10,000 A. As a further precaution a Chance ON16 (dense didymium) has been used since it has a strong absorption band at about 5890 A, the wavelength of the sodium doublet 5890 and 5896 A. This filter was included because, in many applications, we desired to determine potassium in the presence of large excesses of sodium, *e.g.*, potassium as an impurity in sodium salts. The use of filters in this application is permissible because the general flame background is fairly low in the region of 7500 A and even with low potassium concentrations, a reasonably high ratio of potassium line radiation to general background radiation is possible. With the filters used, the response from the background with water running through the instrument is perfectly steady and is the same as that from about 3 parts per million of potassium. It must be remembered, however, that several elements give lines that pass this filter system, for example, caesium 8521 A and 8594 A, rubidium 7800 A and 7948 A, barium 7450 A, 8300 A and 8730 A.

Several types of detecting circuits were tried; that giving the most satisfactory results and finally adopted for use comprised a "Cinema-Television" photomultiplier MS20 followed by a simple single-stage battery amplifier (see Fig. 2). The photomultiplier has an internal amplification of about 1000 and the amplifier gives a further amplification of ten. The output is measured on a sensitive spot galvanometer with a scale from 0 to 100 and a full-scale sensitivity of 2 microamps. With this system, full-scale deflection is easily obtained from a solution containing 10 p.p.m. of potassium. Provision is made to back off the dark current including flame background and the sensitivity is adjusted by means of an Ayrton shunt on the galvanometer. This shunt comprises a helical potentiometer, the total resistance of which is arranged to be the critical damping resistance of the galvanometer.

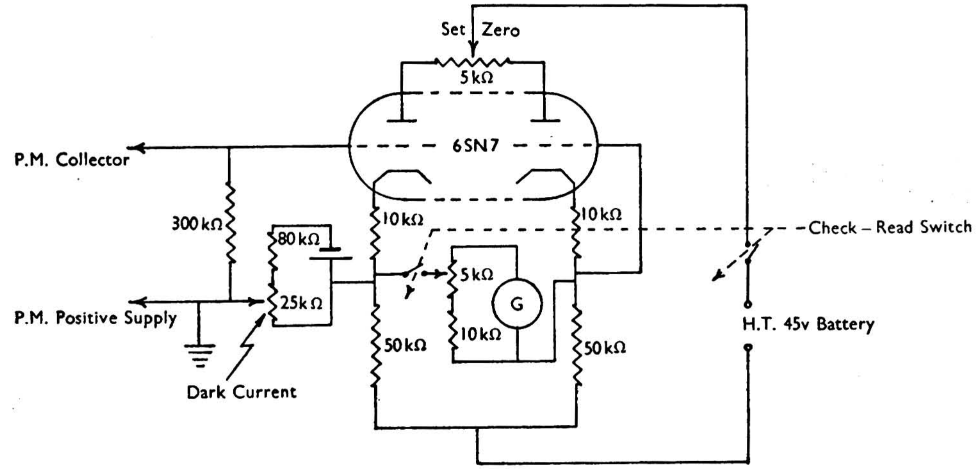

Fig. 2. Circuit of battery-operated D.C. amplifier
The valve filaments are supplied with current from a 6-volt constant-voltage transformer

In considering the method of isolation of the sodium doublet, the type of sample in which sodium was to be determined had to be taken into account. Since these samples were to contain, amongst other elements, fairly large concentrations of calcium, it was decided that the use of filters was likely to prove inadequate since the molecular bands associated with calcium at about 6030 A, 6240 A and 6480 A spread considerably and therefore produce considerable background in the region of the sodium doublet. For this reason it was necessary that some form of monochromator, no matter how simple, should be used. A small glass spectroscope was available from which the eyepiece was removed and replaced by an exit slit made from two razor blades positioned at the focal point of the sodium doublet. The entrance and exit slits were both fixed at a width of about 0·3 mm.

The detector first used was a Mazda 27M1 photomultiplier but a Government surplus RCA931A has recently proved satisfactory, although the dark currents of the latter are usually some three to ten times those of the former.

The output is measured directly on a second galvanometer similar to that used for potassium and provision is again made for backing off dark current and adjusting sensitivity. Both photomultipliers are supplied with 960 volts from eight dry batteries.

The complete instrument is shown in Fig. 3. It is built on a table at which the operator sits, all the controls being on a panel mounted in the table top. The water manometer for the propane and the pressure gauge on the air supply are mounted on a vertical panel at the back of the table. On the same panel is a voltmeter connected across the battery supplying the amplifier in the potassium circuit.

RESULTS—

The standard curves obtained from solutions of "Specpure" sodium and potassium chlorides are curvilinear over the range 0 to 50 p.p.m. and both follow precisely the same points. These points have been checked many times and are reproducible. When the instrument was set to read 100 with a solution containing 50 p.p.m. of sodium or potassium, the standard deviations of the points obtained with solutions containing 10, 20, 30 and 40 p.p.m. were all below 0·2 per cent.

Interference effects have been studied and a few are given in Table I. It can be seen that in general the error due to the effect of cations is small over a wide range of concentration while anions depress the readings, particularly those of potassium. It has been shown that there is no optical interference on potassium by sodium or calcium since solutions of the latter of concentrations up to 1 per cent. give no reading for potassium. Similarly, 1 per cent. solutions of potassium and calcium give no reading for sodium.

TABLE I

INTERFERENCE EFFECTS

	Sodium			Potassium		
Interfering substance, p.p.m.	Added, p.p.m.	Found, p.p.m.	Error, %	Added, p.p.m.	Found, p.p.m.	Error, %
K 100	30	30·0	0	—	—	—
K 1000	30	30·0	0	—	—	—
K 10,000	30	30·0	0	—	—	—
Na 100	—	—	—	30	30·0	0
Na 1000	—	—	—	30	30·0	0
Na 10,000	—	—	—	30	33·2	+10·1
Ca 100	30	29·2	− 2·8	30	30·0	0
Ca 1000	30	29·2	− 2·8	30	30·0	0
Li 100	30	30·0	0	30	30·0	0
Li 1000	30	31·0	+ 3·3	30	31·0	+ 3·3
Sr 100	30	30·0	0	30	30·0	0
Sr 1000	30	30·8	+ 2·7	30	30·0	0
0·01 *N* HCl	30	30·0	0	30	30·0	0
0·1 *N* HCl	30	26·6	−11·4	30	25·0	−16·6
N HCl	30	22·0	−27·0	30	13·2	−55·3
0·01 *N* H_2SO_4	30	30·0	0	30	30·0	0
0·1 *N* H_2SO_4	30	27·2	− 9·7	30	25·2	−16·0
N H_2SO_4	30	27·2	− 9·7	30	13·4	−55·3
0·01 *N* H_3PO_4	30	30·0	0	30	30·0	0
0·1 *N* H_3PO_4	30	26·6	−11·4	30	17·0	−43·0
N H_3PO_4	30	23·6	−23·1	30	17·0	−43·0

THE SECOND INSTRUMENT

In designing an instrument that will be as versatile as possible, it is necessary to consider it as three units. First, the flame must be of a sufficiently high energy to excite as many elements as possible, secondly, the method of isolating the required radiation must be such that any wavelength may be easily selected at will, and thirdly, the detecting and measuring device must be capable of giving readings for solutions varying from the very dilute to the more concentrated.

The second instrument, which was constructed with these points in mind, is shown in Fig. 4. The source unit consists of an atomiser, similar to the one on the first instrument, an expansion chamber and an air - acetylene burner of the Lundegårdh type. A spiral tube was used as the expansion chamber, because it was found that it gave a very much steadier flame than any of the other types tried, owing perhaps to the long distance from atomiser to burner. The spiral is constructed of ½-inch glass tubing and consists of four turns about 6 inches in diameter. The total distance taken by the spray from atomiser to burner is approximately 6 feet. Both air and acetylene are supplied from cylinders, the air at 30 lb per square inch through a B.O.C. two-stage regulator, and the acetylene first through a two-stage regulator, which drops the pressure to 30 lb per square inch, followed by a needle-valve, which is adjusted to give a final pressure of 16 inches of water.

The burner is placed at a distance of 4½ inches from the entrance slit of a Hilger D246 monochromator. This admirably fulfills the second condition stated above, as it is a high dispersion instrument calibrated from 2000 A to 3·5 μ, the wavelength required being selected by turning a calibrated drum.

Two detecting units are used. For wavelengths of more than about 6000 A a CV148 infra-red image converter is used, followed by a Mazda 27M1 photomultiplier, whilst for the shorter wavelengths a Mazda 27M3 photomultiplier is used. The D.C. electrical supplies to the image converter and either photomultiplier are obtained from valve-stabilised, mains-operated power packs. The housings containing the two units are easily interchangeable as they are secured by two screws to the existing fitting at the exit slit of the monochromator.

The photomultiplier output is amplified and read on a robust meter calibrated from 0 to 100. The amplifier used is a conventional valve voltmeter, so modified as to permit the use of photo-cell load resistors of very high values. Its basic circuit, shown in Fig. 5, is of the balanced bridge type, the output to the meter being taken from points A and B. For an input voltage across R1 of 1·5 volts, an output current of 1 milliamp is obtained, and the response is linear over this range, but to reduce grid current effects and to make quite sure that the response cannot become non-linear the input is limited to 1 volt. This is done by so arranging the shunt across the output meter that in no position is it possible to obtain a reading from more than 650 micro-amps. Seven sensitivity ranges are provided, by having seven input resistors ranging from 200,000 ohms to 200,000 megohms, the appropriate resistor being selected by means of a rotary switch. The input resistor so chosen forms the load resistor of the photomultiplier; and, since full-scale deflection can be obtained when 1 volt is developed across it, it is possible to obtain full-scale deflection from current output from the photomultiplier between 5 micro-amps and 5×10^{-6} micro-amps. When photomultipliers are used, this gives far more sensitivity than will be required, as it has been estimated that when a solution containing 10 p.p.m. of potassium is put into this apparatus, the photomultiplier signal current is about 0·05 micro-amp. The control R8 is used to reduce the grid

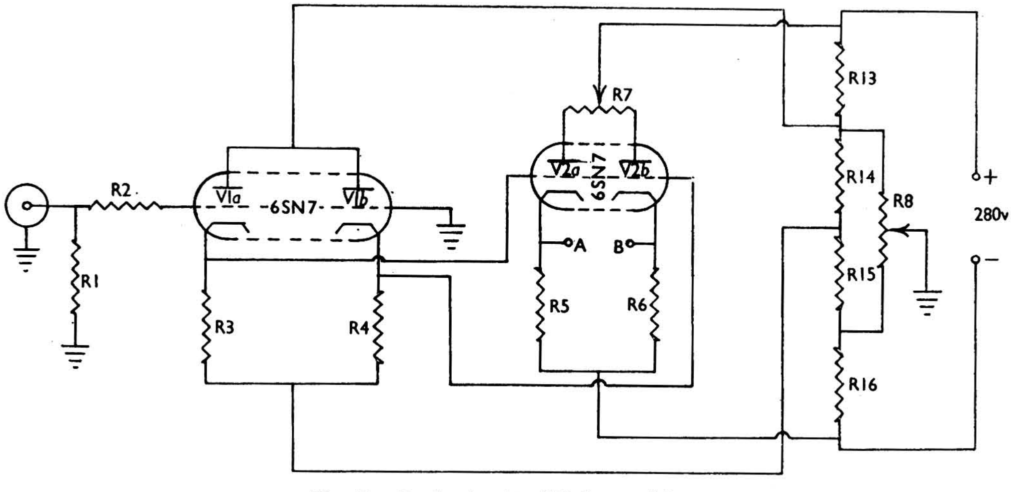

Fig. 5. Basic circuit of D.C. amplifier

current of valve V1*a* to a point where it is negligible and R7 is the "set zero" control. The 280-volt supply is valve stabilised to counteract fluctuations in mains voltage.

The flame photometer has proved easy to use and extremely stable in operation. Provided that the flame conditions are kept constant, no drift is encountered during a run of 2 hours or more, and once the instrument has been set up by means of standard solutions, it is unnecessary to put through the standards as a check more frequently than every 10 or 15 minutes.

The capillary of the atomiser dips into a small vessel of about 2 ml capacity which has a funnel side-arm for filling and a tap for emptying. This greatly facilitates the handling of the solutions and speeds up operations to an average of about 30 seconds per sample.

RESULTS—

A few interference results are shown in Tables II, III and IV. These make it clear that although the hotter air - acetylene flame is desirable from the point of view of exciting more elements than a cooler flame, the interference effects of cations at any rate upon the alkali metals is more severe. The anionic effect, however, is perhaps a little less serious.

TABLE II

INTERFERENCE OF VARIOUS IONS IN THE DETERMINATION OF POTASSIUM

		Potassium		
Interfering ion	Concentration, p.p.m.	Added, p.p.m.	Found, p.p.m.	Error, %
$Na^{\cdot}$	10	5	5·08	+ 1·6
	100	5	6·40	+28·0
$Ca^{\cdot\cdot}$	10	5	4·96	− 0·8
	100	5	5·05	+ 1·0
$Mg^{\cdot\cdot}$	10	5	4·98	− 0·4
	100	5	5·02	+ 0·4
$NH_4^{\cdot}$	20	5	5·00	0
	200	5	4·98	− 0·4
PO_4'''	25	5	4·92	− 1·6
	250	5	4·90	− 2·0
SO_4''	50	5	4·98	− 0·4
	500	5	4·96	− 0·8
HCl	0·001 *N*	5	4·98	− 0·4
"	0·01 *N*	5	4·90	− 2·0
"	0·1 *N*	5	4·73	− 5·4

TABLE III

MUTUAL INTERFERENCE OF SODIUM, POTASSIUM AND CALCIUM

	Percentage error on 6 p.p.m. of—		
Interfering ion, p.p.m.	K	Na	Ca
K 10	—	0	+ 5·0
K 100	—	+5·8	+10·0
Na 10	+ 1·6	—	+ 3·3
Na 100	+28·0	—	+10·2
Ca 10	− 0·8	0	—
Ca 100	+ 1·0	+4·2	—

TABLE IV

EFFECT OF MINERAL ACIDS ON DETERMINATION OF POTASSIUM

Percentage error on 5 p.p.m. of potassium			
Acid	0·001 *N*	0·01 *N*	0·1 *N*
HCl	−0·4	−2·0	− 5·4
H_2SO_4	−0·4	−0·8	− 4·6
H_3PO_4	−1·6	−2·0	−24·0

DISCUSSION—

Of the original aims all have been fulfilled on both instruments except the fourth, that there should be freedom from interference by ions other than the one being determined. The experience of other workers and of ourselves is that these interference effects present the greatest difficulty in this type of work. Parks *et al.*[7] have surveyed the effect of many anions and cations on the results obtained for potassium. They used a source unit which burned coal gas and found that most substances depressed potassium readings.

Higher temperatures such as are obtained in the air - acetylene flame have been found by other workers, Riem[8] and Domingo and Klyne,[4] to cause an enhancement effect when alkali metals mutually interfere. This agrees with our findings and it was checked that, on our instruments, this effect was not due to light from the interfering elements getting past the optical systems. The effect therefore occurs in the flame and is a true enhancement such as is encountered in all types of emission spectroscopy.

The anionic effect is usually a depression of the reading and is reported by Parks *et al.*[7] and Berry *et al.*[3] Phosphate seems to give most trouble in this respect, and it would appear that the hot flames give the better results.

A systematic study of as many types of flame as possible is indicated as a profitable line of investigation.

ACKNOWLEDGMENT

The authors are much indebted to the directors of Boots Pure Drug Co., Ltd., for permission to publish this paper and to Mr. P. T. Turner for the determination of interference effects.

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The Determination of Potassium in Fertilisers by Flame Photometry

By L. BREALEY

(*Presented at the meeting of the Society on Wednesday, October 4th,* 1950)

A description is given of the procedure for testing, calibrating and using the flame photometer described in the preceding paper, p. 334.

The degree of interference from ions other than potassium that may be found in mixed fertilisers receives special attention.

The method is suitable for determining potassium within the range of 3 to 8 parts per million in the test solution. For fertilisers containing from 8 to 12 per cent. of potassium the results show satisfactory agreement with the official method.

FLAME photometric methods for the rapid determination of the concentration of certain elements in solution are becoming increasingly popular in spite of the difficulty of obtaining suitable ready-made equipment. The basic principle of the method is simple and straightforward; the solution containing the element under investigation is atomised and introduced into a non-luminous flame, the emitted light being passed through filters or a monochromator in order to isolate the required radiation. The selected waveband then falls on a suitable photo-electric device and the current output is measured. Several workers have designed suitable equipment for this kind of work, descriptions of which have been given by Barnes *et al.*,[1,2] Boon,[3] Domingo and Klyne[4] and Mitchell.[5] The apparatus described by these workers varies from the simple to the complex, depending upon the purpose for which it is designed.

The following is a brief description of the apparatus that was made in this laboratory and used in the work now described. An air - acetylene flame is used. The acetylene is delivered direct to the burner, which resembles that designed by Lundegårdh[6]; the air supply is first used to atomise the sample solution and the resulting fine spray passed through a spiral to the burner. The air and acetylene pressures are read on two manometers. The light from the flame enters a monochromator where it is dispersed, and the wavelength of light which emerges from the exit slit is selected by the drum. The emergent light falls on a photosensitive element consisting of an infra-red converter and photomultiplier. The output from the latter is amplified to be read on a meter calibrated from 0 to 100.

A full description of this and a simpler instrument is the subject of the preceding paper, see p. 334. In use the required wavelength is selected, distilled water placed in the vessel and, after a few seconds, the reading due to the dark current of the photo-cell and the flame background is brought to the zero mark by the "backing-off" control. A standard solution containing the largest quantity of potassium used for the most suitable standard curve is then put into the receiving vessel, and the reading adjusted to 100 by means of the sensitivity control. The solutions under investigation are then passed through, one at a time, and the readings noted. These readings are then converted to parts per million by reference to a curve previously produced from a set of standard solutions.

One of the chief difficulties of flame photometric methods is that cations and anions present in the analytical solution often give rise to interference in the intensity of the light emitted by the element under investigation. This phenomenon is dealt with at length by Barnes *et al.*,[1] Shapiro and Hoagland,[7] Domingo and Klyne[4] and by Parks, Johnson and Lykken.[8] The experience of these investigators and of ourselves is that these effects vary considerably with different types of flame. One way of overcoming this difficulty is to use standard solutions that are similar in composition to the analytical solution, but this complicates the method and is to be avoided if possible. It is essential, therefore, that before any analytical work is undertaken a thorough investigation should be made, by means of the apparatus that is to be used, into the effects of the other ions that are likely to be present.

For determining potassium in fertilisers, the official method must be followed up to the point of getting the sample into solution. This includes leaching with hydrochloric acid and subsequent dilution to 500 ml; so that, in addition to the substances in the original sample, there is also hydrochloric acid present in the final solution. Accordingly, the effect of different concentrations of hydrochloric acid, phosphate, sulphate, calcium, ammonium, magnesium and sodium on the intensity of the potassium emission was investigated. It was known from previous experience that these interferences are reduced by using very dilute solutions, so the final concentration of potassium was brought within the range of 2 to 10 parts per million. A series of standard solutions was prepared, containing respectively 2, 4, 6, 8 and 10 parts per million of potassium as potassium chloride. The photometer was then set to zero with water in the spray vessel, and the sensitivity control adjusted so that a meter reading of 100 was given by the 10 parts per million standard. Readings were then taken of the other four standards and a curve plotted showing meter readings against concentration. This standard curve was checked repeatedly against different solutions and found to be reproducible.

A series of solutions were then prepared each containing 5 parts per million of potassium plus varying quantities of other ions. The results from this series of experiments is shown in Table I.

TABLE I

THE EFFECT OF INTERFERING IONS

All solutions contain 5 parts per million of potassium

Interfering ion	Concentration	Photometer readings		Potassium found	Error, %
Na	10 p.p.m.	47·0	48·0	5·08	+1·6
	100 „	61·0	62·0	6·40	+2·8
Ca	10 p.p.m.	46·0	46·0	4·96	−0·8
	100 „	47·5	46·5	5·05	+1·0
Mg	10 p.p.m.	46·0	46·5	4·98	−0·4
	100 „	46·5	47·0	5·02	+0·4
NH_4	20 p.p.m.	46·5	46·5	5·00	0
	200 „	46·0	46·5	4·98	−0·4
PO_4	25 p.p.m.	45·0	46·0	4·92	−1·6
	250 „	45·0	45·5	4·90	−2·0
SO_4	50 p.p.m.	46·0	46·5	4·98	−0·4
	500 „	46·0	46·0	4·96	−0·8
HCl	0·001 *N*	46·0	46·5	4·98	−0·4
	0·01 *N*	45·0	45·5	4·90	−2·0
	0·1 *N*	43·0	43·5	4·73	−5·4

The two fertilisers so far examined are typical of many commercial products, being well balanced and containing organic nitrogen, ammonium sulphate, superphosphate, bone meal and potash. The potassium content lies within the range 5 to 10 per cent. and is present as sulphate, chloride or manure salts. In the final test solution the major ions likely to be present in addition to potassium are calcium, sodium, magnesium, ammonium, phosphate, sulphate and chloride. Of these only calcium, ammonium and phosphate are likely to approach the same order of concentration as the potassium. In any event, as the final solution will contain about 3 to 8 parts per million of potassium, it is impossible that any ion can be present in a concentration exceeding 100 parts per million, indeed, the total content of the final solution cannot exceed 100 parts per million. The concentration of hydrochloric acid cannot exceed 0·005 *N*. The most serious interference is that from sodium, but as this will be present only in very small amount—certainly less than 5 parts per million—it is clear that there will be no significant error in the results given by this instrument.

As a final check on the method, six samples each of two types of fertiliser were taken. These were ground, 10 g charred, taken up in 10 ml of concentrated hydrochloric acid, boiled with 300 ml of water, filtered and diluted to 500 ml, exactly as laid down in the official method.

A portion of this solution was then examined chemically by the cobaltinitrite-perchlorate method[9] and another portion examined in the flame photometer.

The instrument was switched on and the flame lit, the acetylene pressure was adjusted to read 16 inches and the air 30 lb per square inch. The entrance slit length was set at 5 mm and the width of both entrance and exit slits at 0·20 mm. The wavelength drum was turned to read 7665 A and the position of the potassium doublet, which is not resolved at these slit widths, located by spraying a solution containing about 5 parts per million of potassium and adjusting the drum until a maximum reading was obtained. The whole equipment was then allowed to warm up for half an hour.

The fertiliser solution was diluted in two stages, 5 ml to 100 ml, and again 5 ml to 100 ml and potassium standard solutions containing 10 and 5 parts per million of potassium were prepared.

The spray vessel was rinsed twice with water; then with water running through the spray the dark current control was adjusted until the meter reading was steady at zero. The vessel was emptied, rinsed twice with the 10 parts per million potassium standard by spraying for a few seconds at each rinse; then, with this standard running through the spray, the sensitivity control was adjusted so that the meter read 100. The spray vessel was not rinsed with water again, but the 5 parts per million standard and the 12 diluted fertiliser solutions were put through the instrument one at a time, giving the container and atomiser two rinses with the test solutions. The whole procedure from the point of setting the dark control for water was repeated twice. The triplicate readings for the fertilisers were averaged, and converted to parts per million from the standard curve. The final results are shown in Table II.

TABLE II

FLAME PHOTOMETRIC AND CHEMICAL DETERMINATIONS OF POTASSIUM IN FERTILISERS

Sample	Readings			Average	Potassium, p.p.m.	% Potash	
	1	2	3			F.P.	Chemical
G1	29·0	29·5	29·0	29·2	3·35	8·07	8·12
G2	27·0	28·0	27·5	27·5	3·18	7·66	7·73
G3	32·0	32·0	31·0	31·7	3·64	8·77	8·61
G4	38·0	37·0	37·0	37·3	4·15	10·00	10·27
G5	29·0	28·0	29·0	28·6	3·30	7·95	7·96
G6	29·5	29·5	29·5	29·5	3·40	8·20	8·20
T1	45·0	44·5	44·0	44·5	4·83	11·65	11·58
T2	40·0	39·0	39·0	39·3	4·32	10·40	10·29
T3	47·0	47·0	47·0	47·0	4·07	12·25	11·93
T4	39·5	39·5	39·0	39·3	4·32	10·40	10·53
T5	32·5	33·5	33·0	33·0	3·75	9·05	8·83
T6	36·5	37·5	36·5	36·8	4·10	9·88	9·74

The advantage of flame photometry over the official chemical method is speed; this is of importance, particularly in manufacturing control. The time taken for the dilutions, readings and calculation of results for these 12 samples was about 40 minutes.

SUMMARY

1. A description is given of the work done, and the preliminary results obtained, in an investigation of flame photometry for the routine control of potash in fertilisers.

2. The results on the two types of fertiliser so far examined indicate that reproducible results can be obtained that agree well with those by the official Fertilisers and Feeding Stuffs method, and save a considerable amount of time.

3. The influence of interfering anions and cations likely to be present in the analysis solution has been shown to be negligible. The necessity for investigating these interferences with the particular apparatus used in the determination is emphasised.

The author is indebted to the Directors of Boots Pure Drug Co., Ltd. for permission to publish this paper and to Miss B. M. Wright for the chemical analyses.

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DISCUSSION

THE PRESIDENT drew the author's attention to a material known as "potash nitrate" that was now on the market. He noticed the author's table of interfering substances did not include the effect of the nitrate ion.

MR. BREALEY said, in reply to the President, that the influence of the nitrate ion on the determination of potassium had not been studied as it was not present in the samples under examination. He added that the influence of other ions on the element being determined was the most difficult problem in this work. The type of flame used was always a significant factor and if in any particular application one kind of flame proved unsatisfactory, it might be found that a cooler or a hotter flame would be better. He had tried an air - propane flame in addition to the one described and it had been found, in general, that with the cooler flame there was less interference from other cations, but more from anions than with the hotter air - acetylene flame.

MR. A. A. SMALES said that he entirely agreed with the author that the effect of other elements on the emission of a given element was very important in flame spectrophotometry. There were two general methods of overcoming this difficulty in a particular estimation: (*i*) dilution to the point of disappearance, or at least insignificance, of the effect, and (*ii*) the addition of a spectroscopic "buffer," *i.e.*, a fairly strong solution of a salt, which "swamped" effects due to possible variations in the amounts of other elements in the samples.

The author had used the first of these methods, which was satisfactory when a major constituent was being determined, but owing to the necessity for working at low concentrations it could lead to difficulties from contamination. He suggested that the author should try the second method to overcome the effect of, for example, sodium, which had been mentioned.

MR. R. K. MATTHEWS asked whether, in connection with the presence of substances likely to interfere in the determination of potassium, the author was of the opinion that the potash content of soils could be satisfactorily assessed by the method described.

MR. BREALEY replied that Dr. R. L. Mitchell in Aberdeen was successfully using an instrument similar to the one described for soil potash determinations.

MR. J. HASLAM asked if the author had any experience of the rapid determination of potassium in rocks and minerals by his flame photometric method.

MR. BREALEY replied that in the short time he had been using the apparatus he had not done any work on mineral deposits.

MR. R. N. WOODWARD asked what was the approximate cost of the apparatus. Could it be cheapened by the use of colour filters and a barrier-layer type of photo-cell?

MR. BREALEY replied that the instrument used cost approximately £700. An earlier model, suitable only for the determination of sodium and potassium, had been built for between £50 and £60. Domingo and Klyne[1] described a simple apparatus for sodium and potassium determination in biological materials. This instrument used colour filters and either barrier-layer or simple photo-emmissive cells. In his opinion filters could be used satisfactorily for potassium determinations, but for sodium a monochromator was necessary, particularly if calcium was likely to be present in the sample under examination.

MR. G. C. COLLINS enquired as to the concentrations in which the various interfering elements might be present in the determination of potassium in fertilisers and also if the author could recommend a technique for determining sodium in presence of calcium.

MR. BREALEY said that with the dilutions described in the paper, the concentration of the interfering elements or groups could not exceed 100 p.p.m. Sodium would be present only in minor quantities, perhaps of the order of one-tenth of the amount of potassium. Phosphate and calcium would be present to about one to three times the concentration of potassium. With regard to a technique for the determination of sodium in the presence of calcium, in his opinion a monochromator was required in order to isolate a band sufficiently narrow to reduce to a minimum the background due to calcium. This background was considerable; for the fringe of the calcium molecular bands at 6000 A to 6200 A overlap the sodium lines.

REFERENCE

1. Domingo, W. R., and Klyne, W., *Biochem. J.*, 1949, **45**, 400 (see reference 4 above).

Microphotometric Determination of Carboxyhaemoglobin in Blood

By H. B. SALT

(*Presented at the meeting of the Society on Wednesday, February 7th,* 1951)

A simple method is described for the determination of carboxyhaemoglobin (HbCO) in the presence of oxyhaemoglobin (HbO_2) in blood by means of a photo-electric absorptiometer with a narrow band light of peak transmission at 6000 A. The HbO_2 is estimated in terms of extinction increment brought about by reduction with sodium dithionite ($Na_2S_2O_4$). In this method, the HbCO remains unaltered. The total pigment in another portion of the sample is converted to HbO_2 and then estimated in a similar manner. The fractional proportions of HbO_2 and HbCO are computed and converted into absolute values from a knowledge of the concentration of total haemoglobin. The analytical method will detect as little as 4 per cent. of HbCO and enable determinations of higher proportions of HbCO to be made with an accuracy within ±2·5 per cent.

DIFFERENCES in light absorption by oxyhaemoglobin (HbO_2) and carboxyhaemoglobin (HbCO) have been used as a basis for elaborate spectrophotometric methods for estimating these substances when present together in blood.[1,2] Hill[3] has used similar principles in a method involving light absorption measurements at different wavelengths by means of vacuum photo-cells and an electrometer. A spectrophotometric method for detection of HbCO in blood, described by Heilmeyer[1], made use of the reduction of HbO_2 to Hb with sodium dithionite ($Na_2S_2O_4$) and measurement of light absorption values at three wavelengths. In this procedure, HbCO was unaffected by sodium dithionite. Another method, quoted by Snell and Snell,[4] included the use of sodium dithionite and the determination of light absorptions at two wavelengths with a visual step-photometer.

In the simple method now described, the necessary measurements are made with a photo-electric absorptiometer and the use of only one narrow waveband region, the pigments being differentiated by reduction with sodium dithionite. The photometer may be of any design, provided it allows the use of plane-parallel optical cells of 1·0 cm optical depth and 6·0 ml working capacity and is sufficiently sensitive to permit the use of narrow wavebands such as are selected by the Ilford series of spectral filters, which yield transmittance values of an accuracy within 0·5 per cent.

DEVELOPMENT OF THE METHOD

Light absorption measurements made by using solutions of HbO_2, HbCO and dithionite-reduced Hb, with each of the Ilford spectral colour filters Nos. 601 to 609, showed that the orange colour filter No. 607, of peak transmission 6000 A, provided a waveband of light that was absorbed by HbCO and by Hb with maximal differentiation, whereas the absorptions by HbCO and by HbO_2 were equal.

The photometer described by Salt[5] was used in all the experiments. It was found to be a further advantage to combine three thicknesses of heat-absorbing glass (Chance ON13) with the No. 607 colour filter, the effect being to absorb much of the red wavelengths otherwise partially transmitted by the orange filter and thereby narrow the selected waveband. This arrangement was additional to the one piece of heat-absorbing glass used ordinarily in the photometer; the resulting filter combination provided a narrow spectral orange waveband.

The method depends on the principle that HbO_2 and HbCO have the same light extinction (E) values for narrow spectral orange light, whereas Hb has an E value 2·41 times as great. When a solution of blood containing HbO_2 and HbCO, of extinction value E_B, is reduced with sodium dithionite, the extinction value increases to E_R proportionately to the content of HbO_2 in the mixture. The component of E_B due to the HbCO is unchanged by sodium dithionite, so that the component due to the HbO_2 ($EHbO_2$) thoretically may be derived from the relation—

$$EHbO_2 = (E_R - E_B)/(2{\cdot}41 - 1{\cdot}0)$$

This relationship presupposes simple proportionality between concentrations of each of the three pigments concerned and the appropriate E values. In practice, as may be seen from Fig. 1, curvilinear relationships were found for HbO_2 and Hb, although simple proportionality was demonstrated for HbCO. When ($E_R - E_B$) values were plotted against the concentrations of HbO_2 from which they were derived, a simple proportionality was observed, although the constant in the equation differed slightly from the theoretical value. In practice, it was found that the results obtained from analyses of diluted blood containing varying proportions of HbO_2 and HbCO, when calculated on the basis of this relationship, were not consistently accurate.

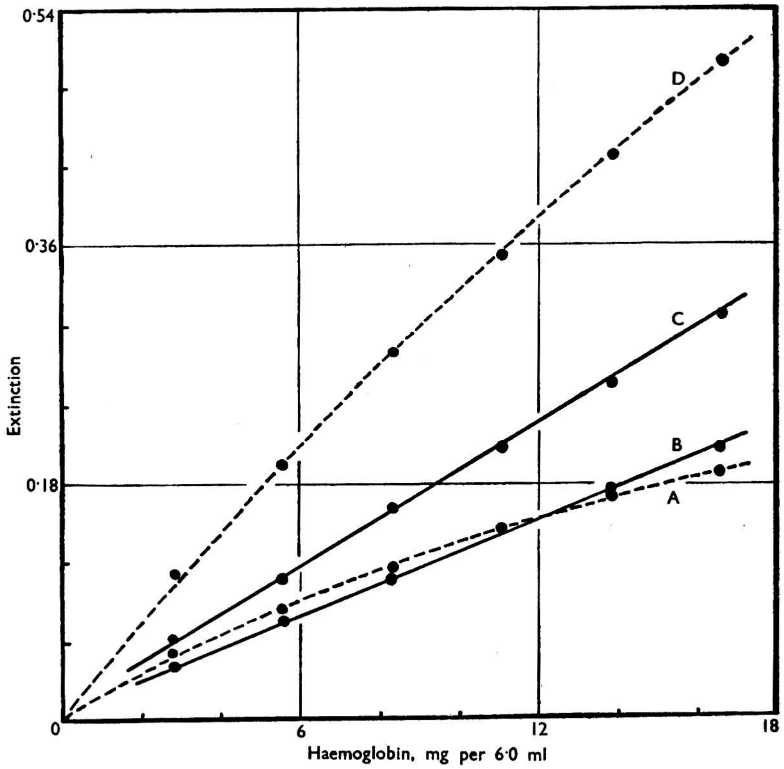

Fig. 1. Relationship between haemoglobin, mg per 6·0 ml, and extinction values. Curve A, HbO_2; curve B, HbCO; curve D, sodium dithionite ($Na_2S_2O_4$) - reduced Hb; curve C, values in D minus values in A, *i.e.* ($EHb - EHbO_2$)

Attempts to establish an empirical relationship between $EHbO_2$ and ($E_R - E_B$) values for standard solutions containing known mixtures of HbO_2 and HbCO proved to be difficult. Saturation of whole blood with carbon monoxide (water-washed coal gas) before dilution was unsatisfactory; saturation of diluted blood with carbon monoxide left an excess dissolved in the water, which converted more pigment to HbCO when the solution was mixed with a solution of HbO_2. It was shown experimentally that, when HbO_2 and HbCO standard solutions were prepared separately and their E values determined separately, the E values could be appropriately added together to yield extinctions corresponding to actual mixtures of HbO_2 and HbCO when present together in the same solutions, but this intricate method of standardisation was inconvenient.

Ultimately, it was found that when the HbO_2 present was determined in terms of extinction increment ($E_R - E_B$) brought about by reduction with sodium dithionite, and the total haem pigment, after initial conversion to HbO_2, was determined similarly by means of another equal sample, the ratio provided a good estimate of the fractional proportion of HbO_2 originally present. The HbCO fraction was derived by simple difference and the fractional proportions were converted into absolute values from a knowledge of the concentration of total haem pigment.

Since the HbO_2 content of a sample is related to the increment in extinction ($E_R - E_B$) brought about by reduction with sodium dithionite, in a manner independent of the absolute quantity of total pigment present, this quantity theoretically need not be controlled, but it was established experimentally that best results were obtained when 6·0 to 12·0 mg of haem pigment were present in 6·0 ml of solution.

METHOD

For ordinary blood specimens, prepare the sample by diluting 0·1 ml of blood to 12·0 ml with 0·007 *N* ammonia. For anaemic blood specimens of total haemoglobin content between 6·0 and 12·0 g per 100 ml, take double the quantity of blood; similarly, for severely anaemic specimens of total haemoglobin content below 6·0 g per 100 ml, take four times the quantity of blood. These adjustments in sampling are not essential but will ensure maximum accuracy in the determination.

Divide the sample into two portions of 6·0 ml each and transfer one portion to an optical cell. Determine the extinction value E_B for the diluted blood at 1·0 cm optical depth, using the narrow spectral orange waveband. Add a few granules of solid sodium dithionite, but avoid an excess, which may cause a turbidity. Mix to dissolve the dithionite and determine the extinction value E_R for the reduced blood sample. To ensure that reduction is complete, add a second small quantity of dithionite, still avoiding turbidity, mix and check that E_R has the same value.

Pass oxygen through the other portion of the diluted blood sample to convert all the pigment into HbO_2. Complete conversion will be ensured by bubbling oxygen through the solution in daylight at room temperature for 30 minutes at the rate of about six bubbles per second from a glass jet 1·0 mm in diameter. This procedure uses nearly 2 litres of oxygen and is adequate for the quantity of haemoglobin chosen, even if all is initially present in the form of HbCO. Transfer the solution to an optical cell, determine the extinction value $EHbO_2$, reduce the solution with sodium dithionite and determine the new extinction value EHb, in a manner similar to that described above.

Calculate the oxyhaemoglobin in the sample as a fraction of the total pigment from the ratio—

$$(E_R - E_B)/(EHb - EHbO_2)$$

Subtract this value from unity to obtain the fractional proportion of carboxyhaemoglobin in the sample.

If the total haemoglobin content of the blood is not previously known, determine the total pigment from the value EHb by means of a standard reference graph in which extinction values for known amounts of dithionite-reduced Hb are plotted against the concentrations (see Fig. 1, D). Calculate the absolute quantities of HbO_2 and HbCO in the sample from the fractional proportions and the total pigment concentration.

SENSITIVITY AND ACCURACY

The method is capable of detecting with certainty as little as 4 per cent. of HbCO in the presence of 96 per cent. of HbO_2. This compares favourably with other techniques currently used in clinical chemistry.

Wikoff and Carson[6] have compared the sensitivity of twelve methods (none of which involves sodium dithionite or photometry) for the detection of carbon monoxide in human blood, with the conclusion that tests using tannic acid or pyrotannic acid will detect 4 per cent. of HbCO, whereas the Hartridge reversion spectroscope will detect amounts only as low as 8 per cent. The remaining tests were still less sensitive.

It has been shown by analyses of samples, in which the total haemoglobin varied between 6·0 and 12·0 mg and the proportion of HbCO varied from 4 to 100 per cent. that the percentage proportions of the two pigments can be estimated by the microphotometric method to an accuracy within ±2·5 per cent.

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DISCUSSION

MR. P. J. GOODE asked whether it was considered necessary to take any special precautions to prevent oxygenation of the blood before taking the first extinction measurement.

MR. SALT replied that if the blood sample, which need not exceed 0·1 ml, was taken by finger-prick and diluted immediately, the solution need only be kept tightly stoppered and in the dark, until the analysis can be performed. Venous blood was better collected with sodium citrate as anti-coagulant rather than with potassium oxalate, other precautions being as before. As carbon monoxide has an affinity for haemoglobin about 210 times as great as the affinity of oxygen, there was very little chance of loss during the brief period occupied by the subsequent absorptiometric analysis.

MR. T. P. WHITEHEAD asked whether the determination as described could be made with a Spekker absorptiometer and the appropriate filters, or even with the single-cell type of colorimeter.

MR. SALT replied that the determination could certainly be made with the modern form of Spekker absorptiometer, or with any single- or double-cell instrument, in which the Ilford series of spectral filters and accurate plane-parallel optical cells were used.

DR. K. A. WILLIAMS drew attention to the practice of using round test tubes instead of plane parallel-sided cells for absorptiometric work. This practice had been advocated on the grounds of economy but led to inaccurate results, and was therefore to be deplored. He was pleased to see that Mr. Salt's economically constructed apparatus had not followed this trend, but made use of a properly constructed photometric cell.

MR. SALT agreed, and added that it had become evident in his personal experience that the greater cost of optical cells as compared with that of round test tubes was more than justified by the gain in accuracy in all absorptiometric methods. There was also the advantage that optical data and not merely arbitrary "colour comparisons" could be recorded. The present micro-procedure for carboxyhaemoglobin required optically accurate cells, and it would be helpful if manufacturers could be discouraged from designing instruments that did not possess the degree of precision demanded by modern analytical methods.

The Determination of Strontium in Sea Water by a Combination of Flame Photometry and Radiochemistry

BY A. A. SMALES

(*Presented at the meeting of the Physical Methods Group on Tuesday, January 3rd,* 1950)

A novel combination of flame spectrophotometry and radiochemistry is described and applied to the solution of an old problem, namely, the determination of strontium in sea water.

A measured amount of strontium chloride containing ^{89}Sr is added to the sea water and the fraction of the total strontium that is co-precipitated with barium sulphate on addition of barium chloride is measured by the decrease in the activity of the filtrate and also by estimating, by means of flame spectrophotometry, the amount of strontium that must be added to the filtrate in order to re-adjust the strontium emission to what it was before the precipitation by barium chloride. The original strontium content of the sea water can then be calculated by means of a simple equation.

Some evidence is also presented on the effect of the other constituents of sea water on the strontium emission in a propane - oxygen flame.

IN spite of a large volume of literature on the chemistry of sea water,[1] including much on strontium, few references appear to have been made to the determination of this element in sea water, and even fewer give details of the method used. Desgrez and Meunier,[2] by crystallisation of calcium and strontium sulphates on evaporation of the sea water, followed by spectrographic determination of the strontium in the dried material, found 13·5 mg per litre in a sample from the English Channel. This figure was confirmed by Thomas and Thompson (reported by Thompson and Robinson[3]) in Pacific Ocean water. Ramage[4] reported 40 to 50 mg per litre, but this has been generally regarded as high and ignored in the literature. Noll,[5] who used X-ray spectroscopy, gives a value of 6·2 mg per litre, Miyake[6] found 14·4 mg per litre by a gravimetric method and Vinogradov[7] gives a value of 8 mg per litre. There is no definite evidence to show whether the proportion of strontium varies widely with position, although on general grounds such variations would not be expected; it seems more probable that differences in the analytical methods lead to the apparent variations.

When a request was made for a determination of strontium in the sea water used in some fish experiments being carried out by the Ministry of Agriculture and Fisheries Research Laboratory at Lowestoft, the method of Desgrez and Meunier[2] was tried, but it proved extremely difficult to obtain consistent results with it. An alternative method was therefore sought and, as the generally accepted figure of Sverdrup,[1] 13 mg per litre, should be within the sensitive range of direct flame photometry, it seemed likely that such a method would be applicable.

The work described shows how this method was applied, and how the difficulty of obtaining true standards for comparison was overcome by the use of radiochemistry. It is of a preliminary nature and may require further experimental substantiation before its validity can be firmly established.

APPARATUS AND REAGENTS

The Beckman flame photometer attachment with the model DU spectrophotometer was used; as this is fully described in the manufacturers' catalogue, it needs no detailed explanation. It is worth noting, however, that the horizontal pre-heating chamber ensures that only a dry dust stream enters the flame, which in this work was oxygen - propane, while the dipping jet assembly permits rapid replacement of the sample. Since radio-active solutions (although extremely weak) were used, an extension chimney consisting of 3-inch piping was fitted to the top of the existing chimney and connected with the laboratory fume exit. No further description of the instrument is necessary, except that the flame is used as the light source in place of the tungsten or hydrogen lamp that is used with the Beckman monochromator and that the sensitivity and time constant are increased by the replacement of a 2000-megohm input load resistor in the photo-tube by one of 10,000 megohms. The instrument is used as for absorption spectrophotometry, *i.e.*, as a null instrument, the transmission-scale variable resistance being used to balance the photo-cell output.

Radio-active strontium chloride solution was prepared by irradiating strontium carbonate with slow neutrons in the Harwell pile and dissolving it in the minimum of hydrochloric acid, the solution being allowed to stand for several days before use in order to allow short-lived radio-active components to decay. A β-ray absorption and decay curve determined with the aid of an end-window Geiger counter demonstrated effectively the radiochemical purity of the product ($^{88}\mathrm{Sr} \xrightarrow{n,\gamma} {}^{89}\mathrm{Sr}$; half life 55 days, maximum β-energy 1·5 MeV). Although almost carrier-free tracer strontium might be desirable, this is only conveniently obtained from fission products and contains a major proportion of ^{90}Sr, which, because of its long half life, has a much lower health tolerance value; in addition the presence of the daughter ^{90}Y would be a disadvantage for this work. Liquid counting was carried out with a glass-walled (35 mg per sq. cm) Geiger counter with 10-ml samples and the usual power pack, scaler and timing units.

FLAME PHOTOMETRY—

Oxygen and air pressure—Preliminary experiments with a solution of inactive strontium chloride (10 μg of strontium per ml) established that an oxygen pressure of 35 inches of water, an air pressure of 12 lb per sq. in. and sufficient propane to give a flame approximately midway between the explosive and the luminous gave suitable conditions, *i.e.*, reasonable stability and sensitivity for strontium. The air pressure necessary varies, of course, with the particular jet used; in these experiments 12 lb per sq. in. was not quite enough to fully saturate the flame.

Gas pressure—One minor practical disadvantage of the Beckman flame photometer is that the gas pressure can only be read on the manometer provided *before* turning on the oxygen supply, and so no visible control of gas is available during operation except the rather coarse one of flame appearance. This means that standard conditions are difficult to reproduce from day to day; as recommended by the makers of the photometer, it was found essential each time the apparatus was used to carry out tests with standards as well as with the samples. One possible way that has been used to ensure approximately standard conditions is to set the air and oxygen pressures and then, while feeding a standard solution into the flame, to adjust the gas pressure to give a chosen reading on the transmission scale.

Wavelength setting—The exact position of the wavelength control setting is best found by slowly turning this control in the appropriate range while the flame is being fed with a solution containing the element under investigation; the slit should, for preference, be narrow. The position of maximum intensity is noted. In this work the strontium line used was at 4607 A, but the wavelength setting is so acute that visual setting on this wavelength, even with a calibrated wavelength scale, is useless and the procedure described above must always be followed.

Slit width—For the work described, the instrument was used with all controls at maximum sensitivity except for the slit width, which was usually set at a value between 0·15 and 0·05 mm. As the slit width is increased the flame background increases (as measured with distilled water feeding into the flame) and a compromise between sensitivity and selectivity is necessary. For example, at a slit width of 0·05 mm very little radiation other than that of the desired strontium and, of course, the continuous flame background, is apparent, while at 0·15 mm stray radiation is definitely noticeable when sea water is being used, but sensitivity is, of course, greater. The term "sensitivity" is used in this paper in the sense of *difference* in reading between the emission from a sample and the flame background, rather than as the *ratio* of these readings.

EFFECT ON STRONTIUM EMISSION OF OTHER ELEMENTS IN SEA WATER

A number of authors have discussed the possible sources of error in flame photometry; a summary of this work is given by Leyton.[8] The usual procedure adopted to avoid these errors is to calibrate the photometer with solutions approximating in composition to that under test.

Accordingly, an artificial sea water was prepared as described by Lyman and Fleming,[9] except that strontium was omitted, and with this as a base further solutions containing known amounts of strontium were prepared in order to plot a calibration curve relating photometer reading to strontium content.

However, little useful information was gained by this method because the artificial sea water gave a much greater photometer reading than did the distilled water flame background. The greater reading might have been due either to stray radiation, *e.g.*, from the large amount of sodium or calcium, or to strontium impurity in the reagents used for the artificial sea water. If stray radiation was the cause, the sample would presumably behave in the same way, *i.e.*, the high reading would be unimportant; if the reagents were at fault, accurate determination in the sample would not be possible, owing to uncertainty in the position of the intercept on the strontium-content axis of the standard curve.

There were two possible ways of investigating this problem: (*a*) by comparing the photometer reading for the artificial sea water on the strontium 4607 A line with the reading on either side of the strontium line; when this was done the comparison clearly showed the presence of some strontium in the artificial sea water; (*b*) by treating the artificial sea water, slightly acidified with hydrochloric acid, with barium chloride solution in order to co-precipitate at least some of the strontium; the filtrate, which would remain essentially the same in terms of major constituents, might then be used as the base solution. (In fact, the concentration of chloride in the filtrate would very slightly increase and that of sulphate would very slightly decrease.) This procedure also showed that some strontium was originally present as an impurity, as the photometer reading for the filtrate was lower than before the precipitation, although still higher than a distilled water flame background. Hence some uncertainty remained, although a possible way out of the difficulty would be repeated co-precipitation of strontium on barium sulphate.

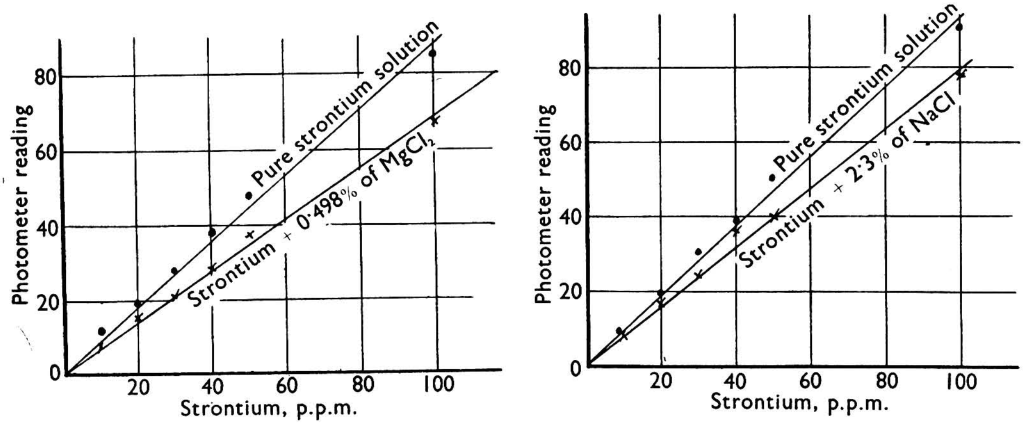

Fig. 1. Graph showing effect of sodium and magnesium on strontium emission (slit width 0·05 mm)

Before this means of purification could be accepted as sound, however, it was essential to prove that the strontium flame emission was independent of small changes in acidity and in concentration of chloride, sulphate and possibly calcium. It was felt that it might also be useful to consider the effect of the other major constituents of sea water simultaneously, since one of the possible sources of error mentioned by Leyton[8] is the mutual interference between elements in the flame.

It can be seen from Fig. 1 that there is some diminution of the strontium emission by sodium and magnesium when present in amounts similar to those in sea water, but small changes in acidity and in concentration of chloride, sulphate and calcium were shown to be without effect. No real evidence of true increased strontium emission was found, although Fig. 2 shows an apparent increase, which, however, is shown to be a stray light effect when allowance is made for the photometer reading with no strontium added.

The preparation of a strontium-free artificial sea water was then attempted by adding slowly to 250 ml of the boiling, acidified, artificial sea water 50 mg of barium chloride ($BaCl_2.2H_2O$) dissolved in a small quantity of water and then allowing the precipitate to stand at least overnight before the barium sulphate was filtered off. Separate radiochemical experiments indicated that up to 70 per cent. of the strontium could be co-precipitated in this manner from an artificial sea water, but that yields might occasionally be only 10 per cent., particularly if the barium chloride was added quickly.

With this solution, after two barium sulphate precipitations, the strontium content of the sea water sample was indicated as 10 p.p.m. (mg per litre), as shown in Table I.

TABLE I

STRONTIUM IN SEA WATER DETERMINED BY COMPARISON WITH THE PURIFIED BASE SOLUTION

	Photometer reading
Artificial sea water, after two $BaSO_4$ precipitations—	
No added strontium	8·2
5 p.p.m. of strontium added	9·7
10 p.p.m. of strontium added	12·1
Sea water sample	12·2
Sea water sample, after two $BaSO_4$ precipitations	8·6

Nevertheless no real knowledge of the number of precipitations necessary to remove strontium completely could be obtained other than by using the tracer strontium in the artificial sea water itself, and even then at least two and often more precipitations were necessary. In addition there was always the possibility that the constitution of the real sea water samples was not completely identical with that of the artificially prepared sample, although this was guarded against to some extent, as shown in Table I, by precipitating barium sulphate from the sample of real sea water.

Further consideration of the possible uses of tracer strontium suggested a much simpler method of overcoming the difficulty.

COMBINATION OF THE TWO TECHNIQUES OF FLAME PHOTOMETRY AND RADIOCHEMISTRY

As mentioned earlier, the partial purification of artificial sea water by co-precipitation of strontium on barium sulphate gives a filtrate that, from the flame photometry aspect, is essentially the same as the original, except in strontium content. If real sea water is similarly treated with barium chloride, part of the strontium is removed, but in other respects the filtrate is still essentially sea water.

Now it is possible to measure the *fraction* of the strontium so removed, *e.g.*, by use of a radio-active tracer. If the filtrate after partial precipitation of strontium is examined in the flame photometer it will, of course, give a lower reading at the strontium line than did the original sea water. But, by adding weighed amounts of strontium to the filtrate until the reading is the *same* as that given by the original sea water, a relationship can be found between the *fraction* of the original strontium removed by precipitation and the equivalent mass of strontium; a simple calculation then gives the original concentration of strontium in the sea water. In other words the flame photometer is simply used as a null instrument to overcome the difficulties of the presence of strontium in the reagents, mutual interference and differences in surface tension and viscosity between standard and sample.

Let the original sea water contain x μg of strontium per unit volume. To this add weightless tracer strontium without altering the volume. Then remove a fraction p of the total strontium by co-precipitation on barium sulphate, again without altering the volume. Suppose a μg of strontium per unit volume must now be added to the filtrate in order to restore the reading on the flame photometer to that found for the original sea water. Then obviously $px = a$, and since p is measured by radiochemistry and a is measured by flame photometry, x can be calculated.

In practice, although weightless strontium tracer, *i.e.*, ^{90}Sr, half life 20 years,[10] obtained from fission products, may be used, as pointed out earlier it was decided to use ^{89}Sr in preference. Since this is obtained by slow neutron irradiation of inactive strontium, the tracer solution contains a significant mass of strontium and this necessitates a slight extension of the mathematical treatment but does not invalidate the simple argument presented above; in fact it makes possible an additional arithmetical relationship that serves as a useful check.

DETAILS OF METHOD—

Consider a volume of sea water containing x μg of strontium per 25 ml, to which y μg of strontium per 25 ml in the form of a radio-active strontium chloride solution is added without sensibly affecting the volume.

Carry out a barium sulphate precipitation on part of this mixed solution after slightly acidifying it with hydrochloric acid, allow the precipitate, barium sulphate and part of the strontium as strontium sulphate, to settle overnight, and then filter. Measure the activity of the original solution and the filtrate, under comparable conditions, in a liquid counter and express the fraction of the activity removed as p; on the assumption that complete exchange has taken place, this will also be the fraction of total strontium removed.

Now to 25-ml portions of the filtrate, add various increasing measured amounts of strontium, again without sensibly affecting the volume, so that the solutions cover the range of strontium content from that present in the filtrate to above that of the solution before precipitation, and take the flame photometer readings, under standard conditions, of these solutions together with readings for (*i*) the enriched sea water before precipitation, and (*ii*) the original sea water. Plot a graph showing the relation between the number of micrograms of strontium added per 25 ml and the flame photometer readings. From this graph find the amount of strontium that must be added to 25 ml of the filtrate to give the same photometer reading as the "enriched" sea water, say a μg.

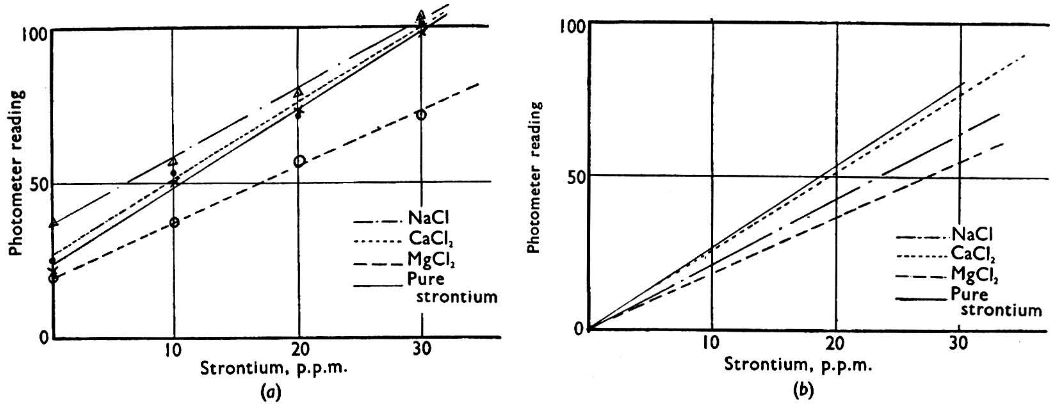

Fig. 2. Effect of sodium, magnesium and calcium on stronium emission (slit width 0·15 mm). Graph (*a*), absolute readings; graph (*b*), corrected for reading of solution containing no strontium

Then we have—

$$p(x + y) = a$$

$$i.e., \ x = \frac{a}{p} - y$$

and since a, p and y are known, x may be calculated.

The additional arithmetical relationship mentioned earlier may be obtained from the extra measurement so far unused, *i.e.*, the flame photometer reading for the original sea water. Dependent on the extent of co-precipitation of the strontium with the barium sulphate, *i.e.*, the value of fraction p, and on the amount of added strontium, y, the final strontium content of a 25-ml portion of the filtrate, after precipitation, may either be greater or less than that of a similar volume of original sea water. If it is less, as is usual, then the difference can be found from the graph plotted as explained above, since flame photometer readings of both were recorded. Call this difference b μg. The strontium content of the filtrate can also be expressed as a fraction of the original "enriched" content; since a fraction p was removed then $(1 - p)$ must remain, *i.e.*, $(1 - p)\ (x + y)$ is the strontium content of the filtrate, in units of μg per 25 ml. The strontium content of the original sea water is x μg per 25 ml and the difference between these is equal to b. Thus we have the relationship—

$$x - (1 - p)(x + y) = b$$

$$\text{or } x = \frac{b + y(1 - p)}{p}$$

It is possible for *b* to be either zero or negative, *e.g.*, when there is more strontium in the filtrate than in the original sea water and the value of *b* must be obtained by extrapolation, but provided the correct value and sign are included in the formula this does not affect the argument.

The procedure depends on achieving complete exchange between added tracer strontium and the inactive strontium present in the sea water. On general grounds this would appear to be ensured as strontium in both solutions is likely to be present as Sr¨ ions. To confirm that this exchange does in fact occur, a synthetic solution was prepared containing the major elements of sea water, 2·3 per cent. of sodium chloride, 0·5 per cent. of magnesium chloride and 0·4 per cent. of sodium sulphate, together with 50 p.p.m. of strontium and allowed to "age" to simulate as closely as possible the conditions of a sea water. This solution was examined together with two samples of North Sea water, collected by the Fisheries Research Laboratory at Lowestoft, and three samples of Atlantic Ocean water from depths of 600, 1200 and 2000 fathoms. After addition of the tracer strontium the solutions were allowed to stand for some hours before barium sulphate was precipitated by the dropwise addition

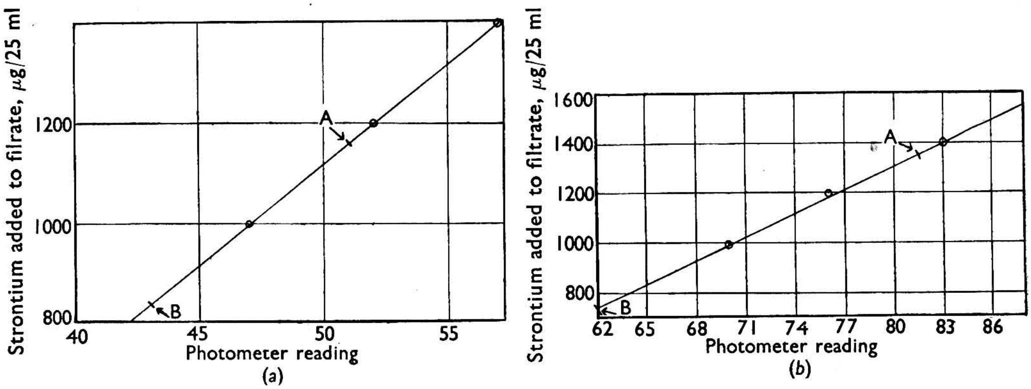

Fig. 3. Reading for synthetic solutions containing 50 p.p.m. of strontium. Graph (*a*), Sr* added, $y = 320$ μg per 25 ml; fraction of activity removed, $p = 13/18$; slit width 0·07 mm. Graph (*b*) Sr* added, $y = 640$ μg per 25 ml; fraction of activity removed, $p = 5/7$; slit width 0·08 mm

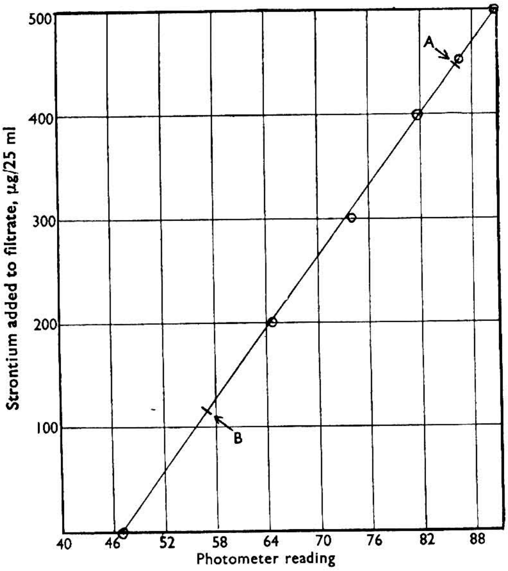

Fig. 3. Lowestoft sea water (1). Sr* added, $y = 320$ μg per 25 ml; fraction of activity removed, $p = 49/62$; slit width 0·15 mm

to the boiling solution of a small amount of hydrochloric acid (1 to 2 ml of 10 N acid per 250 ml of solution) followed by 20 mg of barium chloride, $BaCl_2.2H_2O$, dissolved in 5 ml of water. Boiling was continued until the original volume was reached and the precipitate was allowed to stand overnight before filtering through a Whatman No. 40 filter-paper.

RESULTS—

The radiochemical evaluation of p is recorded in Table II; an example of the flame photometric data is shown in Figs. 3 and 4, and all the values of a and b obtained from similar graphs, together with the calculated value of x, are recorded in Table III.

TABLE II

RADIOCHEMICAL EVALUATION OF THE FRACTION OF STRONTIUM PRECIPITATED, p

Water	Sr* added, y, µg per 25 ml	Activity before precipitation, corrected for background (∿10 c.p.m.), counts per 10 min. per 10 ml	Activity after precipitation, corrected for background, counts per 10 min. per 10 ml	Fraction of strontium removed, p
Synthetic solution A ..	320	3600 ± 2%	1000 ± 3%	13/18
Synthetic solution B ..	640	7000	2000	5/7
Lowestoft 1	320	6200	1300	49/62
Lowestoft 2	320	6000	2400	3/5
Atlantic, 600 fathoms ..	320	5600	1600	5/7
Atlantic, 1200 fathoms ..	320	5650	2000	365/565
Atlantic, 2000 fathoms ..	320	5650	1500	415/565

TABLE III

FLAME PHOTOMETRIC EVALUATION OF FACTORS a AND b

Water	Values taken from graphs		Values of x calculated from equations		Strontium content found	
	a, µg per 25 ml	b, µg per 25 ml	$x = \frac{a}{p} - y$ µg per 25 ml	$x = \frac{b+y(1-p)}{p}$ µg per 25 ml	from a, p.p.m.	from b, p.p.m.
Synthetic solution A ..	1160	840	1280	1290	51	52
Synthetic solution B ..	1350	750	1250	1300	50	52
Lowestoft 1	445	115	245	220	10	9
Lowestoft 2	340	35	245	270	10	11
Atlantic, 600 fathoms..	390	70	226	224	9	9
Atlantic, 1200 fathoms	360	40	238	240	10	10
Atlantic, 2000 fathoms	435	80	270	225	11	9

It is not claimed that the method is highly accurate, but the difference between the previously accepted figure of 13 to 13·5 p.p.m. and the figure of 10 p.p.m. obtained by the method described seems to be significant.

From the flame photometry or spectrochemical viewpoint this technique of combination with radiochemistry to ensure similarity between sample and standards has obvious applications when a part of the element to be determined may be removed without essentially altering the composition of the solution so far as major elements are concerned. This differential or null method of overcoming difficulties caused by impurities might also be extended to other elements.

The assistance of Mr. W. T. Fullwood in much of the experimental work and of Mr. G. M. Spooner of the Marine Biological Laboratory, Plymouth, in bringing to the notice of the author some of the less easily accessible literature references is acknowledged. Thanks are due to the Director, A.E.R.E., for permission to publish.

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The Determination of Potash by Measurement of its Radio-Activity

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A method is described for the determination of potassium in potash salts and fertilisers by measurement of the radio-activity of powdered samples. A description of the apparatus and its calibration, together with suggestions for checking its reproducibility, is included.

As no preliminary preparation is required other than grinding the samples, the method is rapid. The results, although they are not of the highest order of accuracy, can be obtained in from one to two hours. The coefficient of variation (S.D. expressed as a percentage of the mean) for pure potassium chloride is 1·45 and the standard deviation of the Geiger count from the results of chemical analysis on 60 routine samples of fertiliser, ranging from 14·5 to 16·0 per cent. of K_2O, was 0·28 with a coefficient of variation of 1·84.

The method has proved of value in works process control but, as some fertilisers contain radio-active elements other than potassium, it must be applied with caution.

IN 1943 a description appeared[1] of a method for determining potash in fertiliser by measuring the radio-activity. The fertiliser was brought into solution, and introduced into an annulus, between an outside jacket and a very large Geiger - Müller tube, made of glass, connected to a counting circuit of the usual type. We, therefore, made enquiries as to availability of equipment, but found that suitable Geiger tubes were not to be obtained in this country. Although potassium is γ-active as well as β-active, the radio-activity is very weak and a tube of large area was required.

We later discussed the subject with Professor F. Paneth and members of his staff at Durham, who suggested that the radiation from the solid should give a measure of the K_2O content, provided that the distance from the solid to the window of the Geiger tube remained constant, that the area of solid was sufficiently large and that the solid was in a layer thicker than the "critical depth." An ordinary Geiger tube, mounted just above the sample, in a lead castle of conventional type, could then be used for measuring the radiation. Experiments, with carefully prepared mixtures of pure potassium chloride, ammonium sulphate and ammonium phosphate, proved that this was so. While this work was in progress a description appeared[2] of a scheme similar to ours, but rather less convenient.

The power pack and counting circuit have been modified as experience has been gained; Geiger tubes of 2 inches in diameter have been specially made for us by the General Electric Company to replace the original 1 inch tubes; these increase the rate of counting fourfold. The sample holder also has been modified. The apparatus is fully described below.

COUNTING RATES AND "BACKGROUND"—

Even in the absence of the sample, and inside the lead "castle," which keeps out much stray radiation, the Geiger tube will register a certain number of "counts." These are due partly to radio-active dust (always present) in the laboratory atmosphere and partly to cosmic rays. These background counts cannot be distinguished from the true radio-active counts and a blank count must be made from time to time, which is to be deducted from the gross count, to obtain the net count due to radio-activity in the sample. Unfortunately the rate of the background counts is not constant (it was not found practicable to devise a coincidence circuit to eliminate the background), but varies somewhat from minute to minute, and also exhibits a (smaller) long-term variation so that background in successive weeks might well vary from 34 to 40 counts per minute. This uncontrollable random variation limits the precision of the method and it has been found[3] that the standard deviation is equal to the square root of the number of counts. Thus, if the total number of counts was 25, the deviation would be 5, that is 20 per cent. of the number of counts; if it was 100, the deviation would be 10 = 10 per cent. and for 10,000 it would be 100 or 1 per cent. of the number of counts. It is, therefore, clear that although accuracy increases with the number of counts, one soon reaches the region where, for small gains in accuracy, the time consumed is increased intolerably, *e.g.*, to obtain a standard deviation of 0·5 per cent., one would need 40,000 counts, and for 0·2 per cent. deviation, 250,000. It has been our aim to approach a standard deviation of 0·5 per cent., which is about as good as that of chemical analysis of fertilisers. There are other uncontrollable sources of error, in particular, traces of other radio-active substances present in some fertilisers, which make it improbable that it is worth while trying to improve much upon this accuracy, which is adequate for many purposes, *i.e.*, a net count of more than 10,000, preferably 40,000.

With a 1-inch Geiger tube in our apparatus, pure potassium chloride gave a gross count of about 107 counts per minute, the background being 8 counts per minute. A 2-inch tube gave figures of about 590 per minute and 34 per minute respectively. Pure potassium chloride is equivalent to 63·1 per cent. of K_2O, so that to obtain 10,000 counts from 40 per cent. potash salts, one would require with the 2-inch tube to count for half an hour; a mixed fertiliser containing 10 per cent. of K_2O would require 2 hours, this compares favourably with the quickest chemical method of comparable accuracy, which takes two and a half hours. But as no weighing or chemical manipulation is required the time spent in actual manipulation is only about 5 minutes per sample.

APPLICABILITY OF THE METHOD—

The method is more restricted than was expected, because of the widespread presence of other radio-active elements in many fertilisers. It is applicable to fertilisers of the type made at Billingham, but not to fertilisers containing superphosphate made from rock containing radio-active elements, and it should only be used after most careful investigation. In the proved absence of excessive amounts of extraneous radio-active elements it gives speedy results of moderate accuracy, and in our hands has proved its worth. The electronic part of the apparatus has, however, not been quite trouble-free and it would be necessary to improve its reliability before absolutely reliable operation in a plant routine testing laboratory can be expected.

SIZE OF SAMPLE—

It has been found most convenient to have the sample in a tray with a recess 5 cm in diameter and 4 mm deep; the tray is accurately positioned in the castle, perpendicularly below the window (4·3 cm diameter) of the Geiger tube. This area is large enough to fill the window of the counter and the depth is in effect "infinite." The maximum range of β-particles in potassium chloride is 0·35 g per sq. cm. This means that a depth of greater than 2 mm (critical depth) is sufficient. Any ordinary fertiliser material will be within the range of 4 mm, the depth of the sample cell.

The sample should be more or less dry and should be ground to about 60-mesh size, as is usual for analytical samples.

APPARATUS—

The apparatus is in 6 units.

(1) *The Geiger - Müller tube*—Fitted with a window not more than 0·0025 inch thick, to minimise absorption of β-particles.

(2) *Pre-amplifying unit.*

(3) *Lead castle*—For housing the counter, with 4 cm of lead shielding in every direction.
(4) *Scaling unit*—Capable of registering up to 2000 counts per minute.
(5) *High voltage power pack*—1000 to 2000 volts.
(6) *Sample trays.*

(1) THE GEIGER - MÜLLER TUBE—

The body of the Geiger - Müller tube is a 2-inch diameter copper tube 2½ inches long, with one end closed except for a small hole and the other open and provided with a flange. The flanged end is covered by a sheet of duralumin 2·5 thousandths of an inch thick, sealed on with a thermo-setting plastic cement. The small hole carries a glass bead through which a tungsten electrode is sealed. The body contains a mixture of 90 per cent. of argon and 10 per cent. of alcohol at a pressure of 0·1 atmosphere.

When the tube is in operation the central tungsten wire is at about 1500 volts positive to the body, which is earthed. β-Particles enter through the thin duralumin "window," and as each one reaches the gas space the electrons produced by ionisation of the gas are accelerated in the strong electric field and produce a momentary discharge. This discharge is transmitted to the pre-amplifier in the form of a negative pulse.

The construction of a Geiger - Müller tube is not to be lightly undertaken; ours are purchased from the General Electric Company's Research Laboratory at Wembley.

(2) THE PRE-AMPLIFIER—

The pre-amplifier is a single valve, resistance-capacity coupled, placed within a few inches of the Geiger - Müller tube in order to reduce stray capacities. Its voltage gain is in the region of 10 and its output power is adequate for transmission along a hundred feet of screened cable. It draws its power for filament heating and anode supply from the scaling unit. The pre-amplifier is made by Messrs. Dynatron of Maidenhead.

(3) THE LEAD CASTLE—

The lead castle is simply a lead box to protect the Geiger - Müller tube from stray radiation and cosmic rays. It is made in five main parts and a door, so that when dismantled it is reasonably light to handle. It provides a 4-cm thickness of lead all round, contains a shelf for the Geiger - Müller tube and has slots for locating the sample tray. The whole unit is made by the E.R.D. Engineering Co., Ltd., Slough. Although it is designed specifically for the 1-inch G.E.C. tube, the modifications necessary for fitting the 2-inch tube are simple. Two brass plates 3 inches square and bored 2⅛ inches are fitted in place of a shelf and screwed ring.

(4) THE SCALING UNIT—

The scaling unit is a somewhat complicated electronic device for counting the pulses generated by the Geiger - Müller tube, and passed forward by the pre-amplifier. The pulses arrive at irregular intervals, and are sometimes in the form of a multiple discharge in which a number of very closely spaced pulses are produced by only one β-particle. Direct registering by means of an electromagnet operating a mechanical counter is impracticable because many of the pulses are too close together to be distinguished by such a device. On the other hand it is necessary to prevent the occasional multiple discharges being counted as more than a single particle.

The scaling unit used for potassium counting is designed to ignore all but the first pulse of a multiple pulse, but to accept all pulses separated by more than one thousandth of a second. The pulses are then "scaled down" in number by a factor of 10, and only every tenth pulse is passed forward to the electromagnet and ratchet counter registering tens, hundreds, thousands, etc. (the units are shown by the neon lights). The maximum rate handled by the ratchet mechanism is 3 per second, or about 200 per minute, corresponding to a count of 2000 per minute.

The design of the scaling unit follows closely the system described by Rotblat and Sayle,[4] except that the scaling down factor is 10 instead of 100, and some of the extra refinements have been omitted. The unit is mains operated, and has a conventional unstabilised power pack, which also serves the pre-amplifier. The first two valves "shape" the pulse received into a 30-volt square-topped pulse lasting 1/1000 of a second, and during this pulse the unit ignores any other signals received from the pre-amplifier. The square-topped pulse is fed to a "scale of two" unit consisting of a double diode and a double triode, connected so that if one half of the triode is conducting the other is not. When this system receives a pulse the two halves change roles, and at the second pulse they change back again, and so on. When they change back at the second, fourth, sixth, etc., pulse they pass another pulse on to another "scale of two" unit, which in turn passes on pulses at fourth, eighth, sixteenth pulses. This system of dividing by two at each unit could be carried on indefinitely, but in the apparatus used for this work a special electronic switch is arranged to bypass the second and third scale of two units at alternate operations of the fourth (and last) unit. In this way the fourth unit becomes a scale of 10 instead of a scale of 16, and the arithmetic is made simpler for the operator.

The mechanical counter thus registers the tens, hundreds, thousands, etc., and the units are indicated by neon lights connected to the scale of two circuits.

The first two valves (the "pulse shapers") can be converted by operating a switch into an oscillator generating pulses every second, or alternatively every tenth of a second. These pulses are used for routine tests of the scaling circuit and mechanical counter.

(5) THE HIGH VOLTAGE POWER PACK—

The high voltage power pack provides the 1500-volt supply for the Geiger - Müller tube. Different tubes require different voltages, and the power pack is adjustable between 1000 and 1800 volts; it is stabilised to maintain the correct output during wide variations of the mains voltage.

A transformer and half-wave rectifier valve provide about 2500 volts, which is smoothed by condensers and resistances. A micro-ammeter reading to 200 μA in series with 10 megohms serves as an output voltmeter, and a pair of valves serve as a shunt load to draw off surplus current if the voltage rises above the pre-set figure. A standard 120-volt H.T. battery provides a reference voltage to control the valves.

The scaling unit and the H.T. power unit described above were made up from surplus Government material, but versatile scalers and power packs can be obtained commercially which provide all the facilities needed for potassium counting and can also be used for higher or lower counting rates and can provide H.T. voltages over a wider range.

(6) SAMPLE TRAYS—

The sample trays are brass plates 6 mm thick, with a central circular recess of 5·8 cm in diameter and 4 mm deep. They are flanged so as to slide into any one of four slots machined in the base of the castle, which permits the selection of four distances between the Geiger window and the sample. Usually the top slot is used.

PROCEDURE

Connect the H.T. power pack and the counting unit to the mains and to the Geiger tube. Connect the tube to the pre-amplifier and the latter to the counting circuit. Adjust the H.T. voltage to that specified by the makers of the tube in use. (This varies from tube to tube, and is usually about 1500 volts. The specified voltage should not be exceeded by more than 50 volts.)

Test the scaling unit by the internal impulse for fast and slow counting rates. If this is correct, close the castle and proceed to ascertain the "background," over several hours.

CALIBRATION—

Dry AnalaR potassium chloride, after grinding to pass a 60-mesh sieve, and fill the sample tray. Tamp down the sample lightly with a large clean glass surface, level off carefully with a spatula and brush off any fragments outside the depression. Insert into the top slot of the castle, read the scale, start the stop-clock and switch on the scaling unit. Allow to count for 20 to 30 minutes. Calculate the gross counts per minute. Repeat three times and take the average. This represents counts for 63·1 per cent. of K_2O.

Prepare mixtures of AnalaR potassium chloride, pure ammonium sulphate and pure ammonium phosphate, by grinding together in a mortar weighed quantities of the materials. The exact amounts are immaterial, but it is convenient to have the K_2O content in regular steps, *e.g.*, 50, 40, 25 and 15 per cent. of K_2O. Each mixture should be counted four times for long enough to give more than 10,000 counts each time and the average taken. The values, plotted on a graph of percentage of K_2O against counts per minute, should lie on a straight line, which if produced will coincide with the background value at "0 per cent. K_2O."

From our experience this checking is probably unnecessary, and a straight line graph can be drawn from "background" to 63·1 per cent. of K_2O. To do this the pure potassium chloride point must be ascertained with the greatest of care. The value obtained is a property of the Geiger tube in use, because of the variation in sensitivity to radiation from tube to tube. An average figure has already been given.

Tubes do not last for ever, and must be checked from time to time. We have found that permanent standards are very useful. These are glass discs made to fit into the sample trays. One is a uranium glass, which gives a count of about 500 per minute with an average Geiger tube, the other two are potash glasses, with counts of 350 and 200 per minute respectively. The use of these standards is a great help in ensuring reproducibility. As uranium radiation is more penetrating than potassium radiation, two Geiger tubes may not have the same ratio of counts for uranium and potassium, and a third potash glass standard of higher K_2O content might be preferable.

Note—The Geiger tubes do not function in a satisfactory manner at a temperature of less than 10° C, and films of moisture on high tension equipment are never beneficial. It is therefore essential to have the equipment in a dry warm room, preferably about 20° to 22° C. Costrell[5] gives a general account of the theory of Geiger - Müller counters.

DETERMINATION OF K_2O IN SAMPLES, *e.g.*, POTASH SALTS—

If damp, dry the sample at 105° C, grind to approximately 60 mesh, charge into the sample tray and proceed as described for pure potassium chloride. Allow to count for at least 10,000 counts, preferably for 20,000. Calculate the gross counts per minute and read the K_2O content from the graph, or calculate from the factor.

It is advisable to check the background for half an hour first thing in the morning and last thing at night, as a check on the cleanliness of the inside of the castle. A test piece should be used as a check about every 48 hours for half an hour or so.

Note—It has been found that when a Geiger tube is reaching the end of its life, it may diminish in sensitivity when used continuously and recover, or partially recover, if left unused. Thus, a sample could show, say, 250 counts per minute in the morning and if it were left in position for some hours, the rate could drop to 200 counts per minute or even less. This source of error should be guarded against, especially with old Geiger tubes.

DETERMINATION OF K_2O IN BILLINGHAM FERTILISERS MADE FROM MOROCCO ROCK—

Because Morocco phosphate rock contains traces of elements that are immensely more radio-active than potash, errors are likely to be higher in the determination of K_2O in the fertilisers than in potash salts, although but little of the extraneous radio-active elements find their way into the finished product. The magnitude of the effect can be seen from the following figures obtained under comparable conditions.

Pure potassium chloride	$\sim$500 to 550	counts per minute (net)
C.C.F. containing $\sim$15% K_2O ..	$\sim$150	„ „ „
Morocco rock	170 to 240	„ „ „

It is difficult to differentiate between the radiation from the two sources, but provided that the product is reasonably uniform, a suitable allowance can be made.

So long as the method of manufacture and the raw materials remain unchanged, the contribution made by radio-active substances other than potash will be a function of the P_2O_5 content of the fertiliser, as the extraneous radio-activity is associated with the P_2O_5, and hence the P_2O_5 content may be used to calculate a correction. The calculation of the K_2O content from the Geiger counts may be generalised as—

$$\text{Percentage of } K_2O = (\text{Net counts/min.} - \%\ P_2O_5 \times \text{radio-activity of Morocco rock} \times a) \times b$$

where the radio-activity of the rock is expressed as net counts per minute and a and b are constants; b indicates the net response of the Geiger tube to potash and must be determined for each tube. As long as similar Geiger tubes are in use, changes in a will not be great from tube to tube; and as the term including a is a second order correction, no great error will result if a is taken as constant for all similar Geiger tubes. With recent deliveries of

Morocco rock and the present supply of tubes, *a* has a value of 0·004. For a typical 2-inch Geiger tube, the formula then becomes—

$$\text{Percentage of } K_2O = (\text{Net counts/min.} - \%\ P_2O_5 \times 0{\cdot}7) \times 0{\cdot}105$$

RESULTS

STANDARDISATION WITH PURE POTASSIUM CHLORIDE—

In the earlier part of our work, the same tube was standardised with pure potassium chloride fifteen times, the tests being spaced over a month, each count being continued until between 20,000 and 30,000 impulses had been recorded. The range was from 625 to 666 net counts per minute, the average 653, and the standard deviation from the mean 9·51 counts per minute. This gives a *coefficient of variation* (*i.e.*, S.D. expressed as a percentage of the mean) of 1·45 per cent.

K_2O IN FERTILISER SAMPLES—

A range of fertiliser samples with a K_2O content of 14·5 to 16·0 per cent. was analysed with the results shown in Table I.

TABLE I

DETERMINATION OF K_2O BY CHEMICAL ANALYSIS AND GEIGER COUNTER

Net counts per minute	K_2O by chemical analysis, %	K_2O by Geiger counter, %	Difference	Net counts per minute	K_2O by chemical analysis, %	K_2O by Geiger counter, %	Difference
154	15·0	15·3	+0·3	155	15·2	15·4	+0·2
151	15·3	15·0	−0·3	155	15·3	15·4	+0·1
153	15·0	15·2	+0·2	156	15·3	15·5	+0·2
154	15·3	15·3	0·0	152	14·8	15·1	+0·3
151	14·9	15·0	+0·1	154	15·1	15·3	+0·2
153	14·8	15·2	+0·4	152	14·9	15·1	+0·2
153	15·0	15·2	+0·2	153	15·3	15·2	−0·1
155	15·3	15·4	+0·1	161	15·3	15·8	+0·5
155	15·2	15·4	+0·2	161	15·6	15·8	+0·2*
154	15·1	15·3	+0·2	161	15·0	15·8	+0·8
152	14·7	15·1	+0·4	157	15·0	15·4	+0·4
150	14·8	14·9	+0·1	155	15·4	15·2	−0·2
146	14·5	14·5	0·0	156	15·2	15·3	+0·1
155	15·2	15·4	+0·2	156	15·7	15·3	−0·4
151	15·1	15·0	−0·1	159	15·9	15·6	−0·3
152	15·2	15·1	−0·1	158	15·6	15·5	−0·1
158	15·6	15·7	+0·1	156	15·7	15·3	−0·4
149	15·0	14·8	−0·2	155	15·4	15·2	−0·2
154	15·3	15·3	0·0	158	15·3	15·5	+0·2
156	15·1	15·5	+0·4	152	15·3	14·9	−0·4
155	15·3	15·4	+0·1	154	15·7	15·1	−0·6
154	15·3	15·3	0·0	157	15·6	15·4	−0·2
163	15·7	16·2	+0·5	150	15·1	14·7	−0·4
153	15·3	15·2	−0·1	153	14·6	15·0	+0·4
151	14·9	15·1	+0·2	158	15·4	15·5	+0·1
150	14·9	14·9	0·0	156	15·0	15·3	+0·3
147	14·6	14·6	0·0	151	14·6	14·8	+0·2
150	15·1	14·9	−0·2	156	15·2	15·3	+0·1
154	15·3	15·3	0·0	155	15·7	15·2	−0·5
155	15·1	15·4	+0·3	157	15·4	15·4	0·0
Average					15·19	15·25	0·22

Standard Deviation of Geiger count from chemical analysis 0·28

"Coefficient of variation" 1·84

* A new Geiger tube was put in at this point in the series.

NOTES ON TABLE I—

(1) Each sample was analysed less than 24 hours after mixing.

(2) The K_2O was chemically determined by Perrin's modification of the chloroplatinate method.[6] The standard deviation of this method is not known (but is believed to be small), and hence the standard deviation of the Geiger results cannot be separated between the chemical and physical tests.

(3) All the results in Table I have been obtained by counts of about 20,000.

(4) Evidence is accumulating that the radio-activity of fertiliser samples increases with time. A batch of 19 samples were tested by Geiger count twelve months after manufacture. All the results were high, the average bias being +1·8. By counting the same sample at intervals results were as follows—

Date	4.8.49	8.8.49	17.8.49	7.9.49	17.11.49
Net counts/min.	164	167	169	176	190

Hence the method can only be applied to fresh samples.

APPLICATION TO OTHER SAMPLES

Once the absence of other radio-active elements is established, rapid approximate determinations of potash can be made. Even potash contents as low as 1 or 2 per cent. have been estimated and as short a time as 10 minutes counting indicates the order of the amount present; the errors are, of course, rather large; they can be roughly calculated from the deviations given on page 356, but in practice these deviations are likely to be exceeded. Some results for muriate of potash are shown in Table II.

TABLE II

MURIATE OF POTASH

Date	K_2O by chemical analysis, %	K_2O by Geiger counter, %
1.12.48	40·4	40·0
17.12.48	54·0	53·7
8.3.49	41·6	41·8
7.4.49	58·5	60·0
20.5.49	50·3	50·5
7.6.49	51·4	52·8
8.6.49	50·8	51·2
30.6.49	40·1	39·3
5.7.49	49·6	48·9
12.7.49	41·0	40·1
22.8.49	59·1	58·7
9.9.49	60·0	60·7

All the analyses were made on dried samples.

(Minimum number of counts = 20,000.)

In the above series of determinations the standard deviation of the counter method from the chemical method is 0·78, and the coefficient of variation is 1·57. This is rather higher than the standard deviation given for fertiliser samples in Table I, but the "coefficient of variation," *i.e.*, the standard deviation expressed as a percentage of the mean, is lower. As the muriate of potash is free from other radio-active elements, this might be expected.

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Radiometric Assay in Tracer Experiments

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The assay of radio-active isotopes at tracer levels of activity is briefly discussed in the light of sensitivity, precision and convenience. Experimental procedures adopted for tritium (^3H), carbon-14 and for bromine-82 are described.

In simple radio-active tracer technique a suitably labelled compound is introduced into the system under investigation. After a time, during which chemical or purely physical changes take place, samples are taken and assayed radiometrically. The radio-activities determined then provide data on the final concentrations and locations of the molecules originally labelled. Many factors have, of course, to be taken into consideration; for example, whether or not there has occurred isotope exchange with an unlabelled compound, when the activity as measured may no longer be significantly associated with the original molecule at all.

Ideally one should be able to assay a radio-active isotope simply by bringing a suitable detector to a fixed point near the sample, whether it be solid, liquid or gaseous. Few isotopes lend themselves to reliable assay in this manner, particularly at the levels of activity usually associated with the samples of a tracer experiment, *e.g.*, 0·001 μc (microcuries). The method adopted will depend upon the nature and energy of the radiations associated with the decay of the isotope, and upon the sensitivity, precision or degree of convenience required.[1] Up to the present time the Geiger - Müller counter has generally been found to be the most suitable detector in tracer work (*cf.* Libby[3] and others). This paper briefly describes some typical assay procedures developed in the course of radio-active tracer experiments.

In these experiments carbon-14 has been used to label methyl bromide in a study of its chemical action on the protein of wheat under the conditions of fumigation against insect pests. By treating the resulting methylated protein constituents with such reagents as sodium hydroxide and hydriodic acid, followed by the determination of the ^{14}C-labelled methyl groups in the volatile products, it has been possible to identify the principal protein groups methylated. Provision has been made for the assay of tritium so that it could be used as an auxiliary tracer for methyl groups. Bromine-82 has been used to label a bromine analogue of DDT that was then injected into normal and "DDT-resistant" houseflies. The enormous sensitivity of the radio-active tracer technique combined with paper partition chromatography enabled unchanged DDT and its metabolites to be identified and determined in single flies.

TRITIUM, ^3H

This isotope decays with a half-life of about 11 years and emits a negative β-particle of maximum energy 0·017 MeV.[2] The maximum range, r, of the β-particle calculated from the empirical equation[3] $r = E^{5/3}/150$, is 0·75 mg per sq. cm, E being measured in KeV. This means that if the tritium were assayed in a 1-mg sample spread over 1 sq. cm below a thin window counter, more than 90 per cent. of the emitted β-particles would be absorbed in the sample alone. Tritium has, therefore, usually been assayed as a gas or vapour introduced into a quartz ionisation chamber attached to a Lauritzen electroscope[4] or into the sensitive volume of a Geiger - Müller counter itself.[5] In Melander's method the tritium is assayed as tritium-labelled hydrogen inside the counter at a pressure of 9 cm of mercury together with methane at a partial pressure of 1 cm of mercury. Melander's apparatus was relatively simple, all gas manipulations taking place from higher to lower pressures and an ordinary rotary oil-immersed vacuum pump being used for evacuation. This method has been found to give satisfactory results, but provision has been made for transfer of gas from lower to higher pressures by means of a Töpler pump and for the recovery of active samples.

The laboratory-made gas counter shown in Fig. 1 consists of a brass tube, D, of internal diameter 2·5 cm and length 15 cm, the ends C being turned to take standard B 29 Pyrex cones. The counter is attached at A to the high-vacuum assembly by means of a standard B 10 joint. The anode, E, is 42 S.W.G. tungsten wire attached at one end to the glass bridge, B, and at the other end to a platinum lead, H, sealed in at G after the tungsten wire has been drawn taut. The relevant part of the high vacuum assembly is shown in Fig. 2. The principal manifold is exhausted at B, through the usual cold traps, by an oil diffusion pump backed by a rotary oil pump. The active hydrogen sample, prepared by reducing the corresponding water sample,[4] enters the system at A and is stored in one of the reservoirs R2, R3, etc. Residual gas is transferred from the manifold to the reservoir by means of the Töpler pump, TP, residual pressures being indicated by a Pirani gauge at P. The Töpler pump is operated by opening the reservoir to the air via a drying tower at L or to an auxiliary vacuum pump at W. Pressures of gas samples are indicated by the mercury manometers M1, M2, etc. The reservoir R1 is charged with inactive hydrogen, which is used for dilution and washing out. The suitably diluted sample is admitted to the counter, GMC, through tap T5, to a pressure of 9 cm of mercury. Methane is added from a cylinder via the "doser" bulb, D, to a pressure of 1 cm of mercury. Not less than 10 minutes are allowed for the sample to mix properly before counting is begun. The counter is connected at X and Z to conventional extra-high tension, pre-amplifying, quenching and scaling circuits. After counting, the sample is pumped by the Töpler into the oxidation unit, OU, which can be evacuated at G by the auxiliary vacuum pump. Oxygen is admitted from a cylinder through tap T18 to about twice the pressure of the hydrogen - methane sample. The total pressure within OU before ignition is never allowed to exceed 30 cm of mercury. The mixture is flashed quietly by bringing the 39 S.W.G. platinum filament to red heat for a few seconds. The water produced may be trapped in TR and sealed off after pumping away unwanted carbon dioxide; alternatively, it may simply be absorbed in the soda lime tube, S. Active gas samples are never passed into the laboratory atmosphere. Between assays the counter and manifold are flushed out with inactive hydrogen and methane; after a series of assays they are pumped out at high vacuum. As used, the counter showed a background of 60 counts per minute, but this figure could no doubt be reduced by surrounding the counter with similar counters connected in anti-coincidence.[6] The threshold was +1500 V, the plateau extending for a little over 30 V. It was therefore found advisable to check the plateau limits with an external radium source just before each count and to operate the counter at the mid-point of these limits. The absolute activity of the samples assayed was not known and it was not possible to estimate the total counting efficiency. Theoretically, every disintegration within the sensitive volume of the counter that does not occur during the quench period should give rise to a recorded pulse, so that a high counting efficiency would be expected. The geometry of the sample is dictated by the shape of the counter and is therefore constant, an important factor in precision or reproducibility. Melander quotes a figure of ±2 per

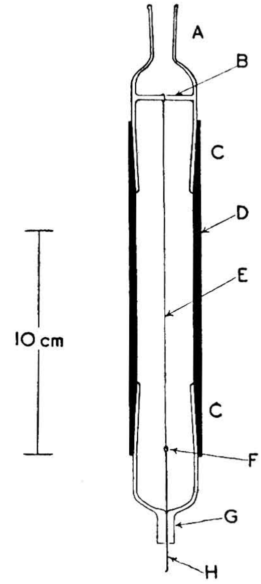

Fig. 1. Internal gas counter for tritium assay

cent. in his original paper.[5] The manipulation tends to be tedious; it was not possible to deal with more than one prepared active sample per hour.

CARBON-14

Carbon-14 decays with a half-life of 6400 years[7] and emits a negative β-particle of maximum energy 0·154 MeV.[8] The maximum range is about 28·5 mg per sq. cm, so that losses by self-absorption and absorption in the window-foil of the counter may seriously reduce the total counting efficiency for externally-mounted solid samples. Like tritium, carbon-14 has been assayed as carbon dioxide inside an ionisation chamber[9] and within the sensitive volume of a Geiger - Müller counter in admixture with carbon disulphide to improve the gas counting characteristics.[10] Assay by the latter method has been estimated to be more than 95 per cent. efficient.[11] Solid samples can be assayed inside demountable counters through which flows a suitable gas mixture.[12,13] In this way window-foil absorption losses are avoided and certain geometrical effects are reduced. A helium - ether flow mixture was found to give better counting characteristics than a helium - alcohol mixture,[13] but the higher threshold voltage (over 3000 V) is a disadvantage, because such high stabilised voltages are not always available. The use of a helium - alcohol mixture flowing through a demountable counter electronically quenched by an external circuit has been found to be very satisfactory. In a routine work this has proved a useful compromise between the efficient gas-counting and the convenient external-sample techniques. Carbon-14 has been assayed directly in mounted tissue specimens,[14] but in order to achieve a high degree of reproducibility it is better to prepare samples in some standard chemical form, *e.g.*, as barium or calcium carbonate, although this necessitates preliminary combustion or wet oxidation. Calcium carbonate has the advantage of a higher carbon content than the barium salt, self-absorption being correspondingly less for a given activity. The following procedure has been developed for the assay of carbon-14 at tracer levels of activity.

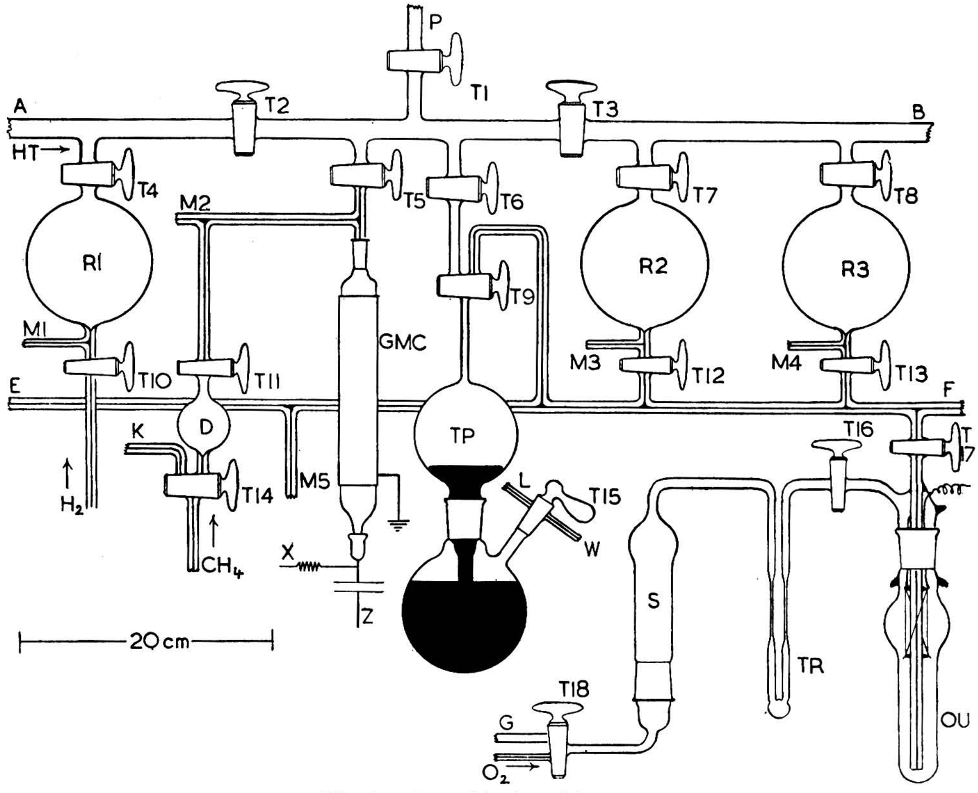

Fig. 2. Assembly for tritium assay

Carbon dioxide resulting from a wet or dry oxidation is purified and adsorbed, in conditions that preclude the presence of atmospheric carbon dioxide, in a train of three specially-designed units of the type shown in Fig. 3. Each unit is first flushed out with entraining gas, *e.g.*, nitrogen or a helium - oxygen mixture, that is free from carbon dioxide. The entraining gas enters at P, passes through bulbs E and D and leaves at C, which is a small indicator bubbler charged with aqueous barium hydroxide. When flushed out, bulb D is disconnected at O and the opening is immediately stoppered. The joint N is attached to a burette, J, containing the required reagent, free from carbon dioxide. The tip of the burette is normally protected from carbon dioxide by the soda-lime tube L. The required volume of reagent is added, tap T2 being opened to release the displaced atmosphere in D. The unit is quickly re-assembled and reflushed. The reagents are transferred from bulb D to bulb E, which contains a sintered glass bubbling-plate, while the entraining-gas passes through the system, entering each unit at P and leaving at Q. The transfer of the reagent is assisted by applying a slight positive pressure in D through the soda-lime tube B by means of the bellows, A. Reagents and washings are finally sucked into flask F, which may be exhausted at S with taps T3 and T6 closed. To wash the apparatus, D is filled to the neck, N, with distilled water free from carbon dioxide, or with any specially required washing agent. By exhausting at S, opening taps T4 and T2 and closing tap T3, the top half of the washing agent swills round the head above O and drains into F. By closing tap T2 and opening tap T3 the lower half of the washing agent sprays the barrel of E from the pin-holes of the small bulb at the bottom of the capillary extension vertically below T3. During the operation of washing, tap T1 is opened to B and the bellows, A, is detached. Silicone grease is used for lubricating joints and taps.

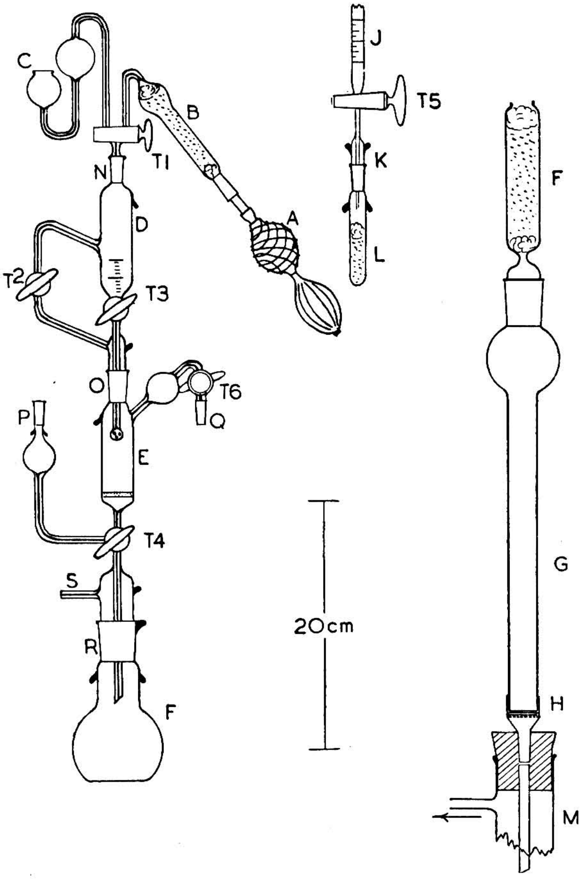

Fig. 3. Absorption of C^*O_2 and precipitation as CaC^*O_3

For the purification and absorption of the carbon dioxide resulting from a wet or dry oxidation, the first unit is charged with *N* alkaline sodium permanganate and the second unit with *N* alkali containing 0·3 *M* hydrazine. The third unit is charged with *N* sodium hydroxide alone. When the absorption of carbon dioxide is complete, an excess of sulphuric acid is added to the alkaline reagent in the first unit and the second unit is acidified with hydrochloric acid, the flow of entraining gas being maintained. When oxygen is used in the extraining gas, *e.g.*, during the dry combustion of methyl iodide labelled with ^{14}C and ^{131}I recovered from the labelled methylation of protein by a modified Baernstein method, the hydrazine is not added in the second unit until a few minutes before acidification. Sulphur dioxide and halogens are absorbed in the first bubbler, but some free halogens may reach the second bubbler where they will be reduced. On acidification all the labelled carbon dioxide passes over to the third bubbler where it is absorbed, the alkaline washings being finally sucked into F. Excess hydroxide in the final washings of the third unit is largely removed by boiling with 5 *N* ammonium chloride, any excess of this reagent being carefully avoided. The carbonate is precipitated from the hot solution by the dropwise addition of slightly alkaline calcium chloride. The precipitate is filtered off at the pump in the filter-stick G. The soda-lime tube, F, excludes atmospheric carbon dioxide during the initial stage of the filtration when the suspension is still alkaline. The precipitate is collected on a tared 1·75-cm Whatman No. 50 filter-paper backed by two Whatman No. 4 filter-papers. These are pressed flat by the rim of G, which is clamped firmly and vertically in the small Büchner funnel, H. "Creeping" of the calcium carbonate is prevented by the addition of absolute alcohol during the filtration process. The precipitate is finally washed with ether and then G is released from the clamp and gently raised from H. The precipitate remains as a clean circular disc; by selection of the right internal diameter for the tube G the disc has an area of 1·50 sq. cm. The disc is dried under an infra-red lamp and weighed. A drop of a neutral solution of cellulose nitrate in redistilled amyl acetate (prepared from fresh "Durofix") is added to the disc, which is re-dried while the edge of the filter circle is held flat in a small brass cup. The disc, which can now be handled without fear of damage, is re-weighed and mounted in the brass cup, F, of the demountable counter shown in Fig. 4.

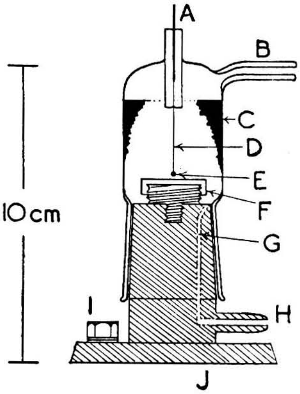

Fig. 4. Demountable flow-type counter for soft β-assay

The counter shown in Fig. 4 was designed for simplicity of construction and manipulation; it has functioned reliably and reproducibly for several months. The brass base, J, carries a standard B 29 solid brass cone. The counter envelope is a B 29 Pyrex socket closed at one end, as shown. The short glass capillary, A, carries a short platinum wire lead connected within the capillary to the 42 S.W.G. tungsten anode, D. The anode terminates in a 2-mm Pyrex bead, E, which clears the surface of the brass cup by 1 mm. The cathode surface, C, is prepared by painting the inside of the glass envelope with "Aquadag 660 B" followed by baking at 200° C. The cathode surface extends to the lower rim of the socket, so making electrical contact with the brass base, which is earthed at I. During counting, a helium - alcohol vapour mixture enters at B and leaves the counter through G and H. The operational assembly is shown in Fig. 5. Commercial helium from a cylinder enters at tap T2 and passes through the bubbler, A, containing absolute alcohol cooled in iced-water. The gas, saturated with alcohol vapour in equilibrium with the liquid at 0° C, passes through a packed wool-dust filter and through the spiral B in which it is brought to equilibrium with room temperature. The rate of flow of the emergent gas is indicated on the flowmeter E. The counter is flushed out for 5 minutes at 5 ml per second before use, but the gas flow is reduced to one or two bubbles per second in F during counting. The counter operates in conjunction with conventional extra-high tension, quenching and scaling circuits. The threshold of a typical counter of this design was +1500 V and the "flat" plateau extended over a range of at least 200 V. When screened by $1\frac{1}{4}$ inches of lead, the mean background rate was 30 counts per minute. By starting with a ^{14}C-labelled sodium carbonate solution it was possible for one person to make about 12 assays in one day.

It was of interest to compare the efficiency of assay by this method with the carbon dioxide gas-counter technique. A sample of calcium carbonate whose absolute activity was known from a comparison with a calibrated ^{14}C standard was assayed by the demountable counter technique described, and as an external sample mounted below a commercial counter having the thinnest end-window, 1·8 mg per sq. cm, that was available. The sample was then converted to carbon dioxide and assayed in a typical gas-counter assembly. With a precipitate 1 mg per sq. cm thick, the demountable counter was 29 per cent. efficient, the thin end-window counter was 10 per cent. efficient and the efficiency of the gas-counter was 65 per cent. The efficiency of the gas-counting could, however, have been increased by using a counter with a higher sensitive-volume to total-volume ratio than was available at the time.

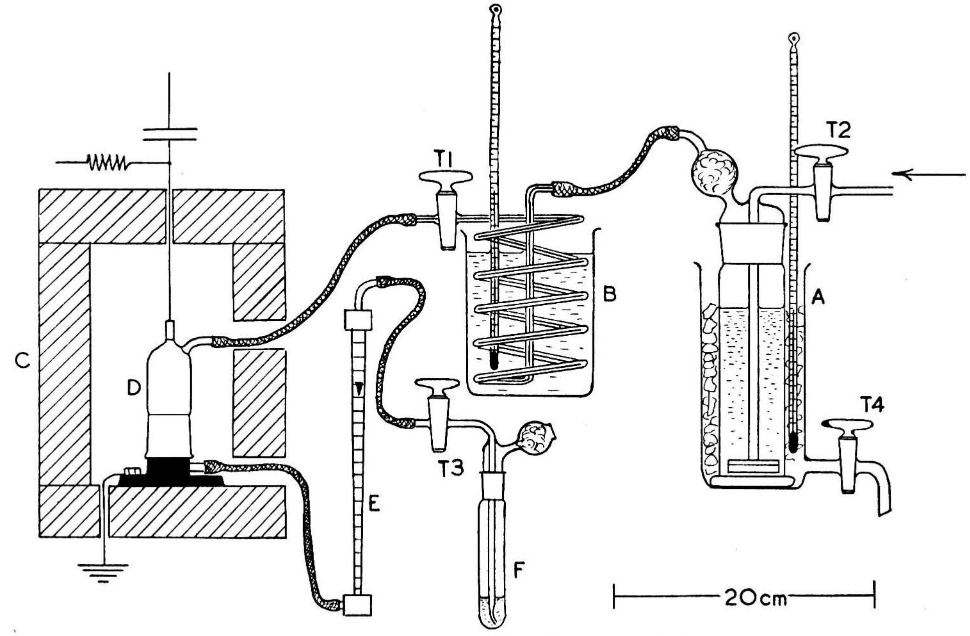

Fig. 5. Assembly for soft β-assay using demountable flow-type counter

In a series of assays made on a purely routine basis with the demountable counter, the coefficient of variation for all results was less than ±5 per cent. The random nature of the decay accounted for 1 per cent. of this variation.

BROMINE-82

This isotope decays with a half-life of 34 hours[2] and emits a negative β-particle of maximum energy 0·465 MeV and γ-rays of energies 0·545, 0·787 and 1·350 MeV in cascade.[2] The maximum range of the β-particles is about 150 mg per sq. cm, while the γ-rays can be detected through several centimetres of lead. Samples with an activity of the order of millicuries can be compared accurately at a distance (*e.g.*, 50 cm) from a shielded γ-ray counter or ionisation chamber, self-absorption corrections being unnecessary. The results of some measurements made with a γ-ray counter agreed to within 1 to 2 per cent. with those obtained by counting the much diluted samples in a liquid counter of the type described by Veall.[15] This type of counter proved extremely useful in tracer experiments with this isotope because solutions or suspensions[16] can be assayed directly. For high-efficiency counting, the bromine of the labelled substance is separated chemically and precipitated as the silver bromide, carrier bromide being added when necessary, and the precipitate is mounted on an alkathene-faced lead disc below a thin end-window counter.[17] The coefficient of variation for all results by this method is less than ±2 per cent. on total counts of 10,000. With a counter of window thickness 1·8 mg per sq. cm, it was estimated that 15 per cent. of the ^{82}Br disintegrations of a silver bromide precipitate weighing 15 mg per sq. cm were being detected. In these conditions the counting rate was 50 times greater than that observed with a sample having the same activity but assayed in solution in the liquid counter.

SELF-ABSORPTION

Of the many factors that affect the total efficiency of β-assay of solid samples, self-absorption is probably the most important. As it is not convenient to standardise the thickness of the precipitate for all samples, it is essential to construct a self-absorption curve to provide a ready basis for comparing assays made on solid mounts of different thicknesses. Experience at this laboratory has suggested, for general work at least, that corrections based on Libby's equation[3] are quite satisfactory. On the basis of the apparent exponential absorption of β-particles Libby has deduced the expression—

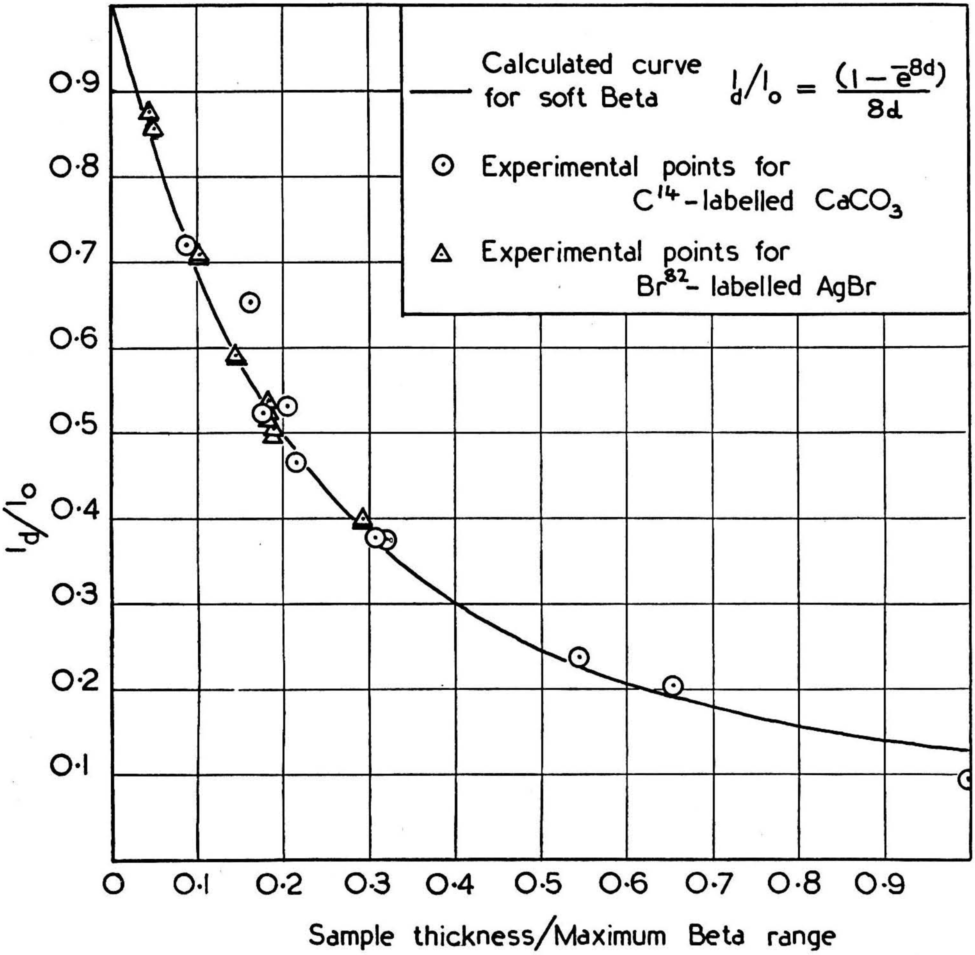

Fig. 6. Theoretical and experimental β-self-absorption curves

$$\frac{I_d}{I_o} = \frac{(1 - e^{-kd})}{kd},$$

where I_d is the observed count, corrected for "dead" time losses, etc., and I_0 is the theoretical count at "infinite thinness," *i.e.*, were there no self-absorption. The value d is the thickness of the sample in terms of the maximum range of the β-particles; this datum is usually more readily available than the absorption coefficient that appeared in earlier equations. The constant k was originally evaluated as 5, but more recent data show that 8 is a better value. The continuous line in Fig. 6 is the curve of Libby's equation. The circles represent the experimental points of a series of ^{14}C assays made by the demountable counter technique as described. The triangles represent the experimental points of a series of ^{82}Br assays made with the alkathene-mounted silver bromide discs to which reference has been made. All points lie reasonably near the theoretical curve, and it is of interest that in the ^{82}Br assays the γ-ray component was of no significance in this method of counting.

The author is indebted to Mr. J. V. P. Long of the Chemical Research Laboratory for making comparative ^{14}C assays by the gas-counting technique. This paper is published by permission of the Department of Scientific and Industrial Research.

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A Study of the Potentiometric Titration of Fluoride with Lead

BY F. R. CROPPER

(*Presented at the meeting of the Microchemistry Group on Wednesday, September 27th,* 1950)

A preliminary study has been made of the conditions suitable for the potentiometric titration of fluoride by means of lead nitrate in presence of ferric and ferrous chloride indicator and of excess of sodium chloride. The fluoride is precipitated as lead chlorofluoride, and as the end-point is approached the ferrifluoride complex ion dissociates and gives a change in the $\mathrm{Fe}^{\cdots}$ - $\mathrm{Fe}^{\cdot\cdot}$ redox potential; the equivalence-point is indicated by maximum change in the potential. Reproducible results can be obtained under specified conditions, and provided that the lead nitrate be standardised against sodium fluoride solution, the method would be accurate to within 1 per cent. With *N* lead nitrate the end-points are reasonably sharp for 40 to 120 mg of fluorine, but on the micro scale they are such that careful working is essential.

The preliminary treatment of organic fluorine compounds, prior to potentiometric titration with lead, is being investigated and will be the subject of a subsequent publication.

A NUMBER of papers have been published during the last two decades on the quantitative precipitation of fluoride as lead chlorofluoride. Since the early work of Hawley[1] on the effects of time of precipitation and of varying amounts of fluoride on the composition of the precipitate, studies have been made with the object of finding the optimum conditions for this method; Hoffmann and Lundell[2] investigated the effects of pH and of chloride concentration for the range 10 to 100 mg of fluorine. Elving and Ligett[3] described the potassium fusion method for decomposing organic fluorine compounds, and the conditions for subsequent precipitation of lead chlorofluoride; they claimed good results for a variety of fluorine compounds. Details have been published for the lead chlorofluoride method as adopted by the Association of Official Agricultural Chemists[4] for the analysis of insecticides.

Although the gravimetric method has received some attention, the usual procedure has involved washing the precipitate with a little saturated lead chlorofluoride solution, re-dissolving the precipitate and determining the chloride content by the Volhard method. Recent evidence of the difficulties still met with in this determination is given by Chapman, Heap and Saunders,[5] who studied the effects of variations in pH, chloride and fluoride ion concentrations and times of stages in the precipitation; these authors recommended application of certain corrections and claimed that for 50 to 60 mg of fluorine the corrected results were accurate to about 1 per cent. of the true value. Since that date, Kaufman[6] has also studied the effect of pH on the precipitation.

Recent work in these laboratories on a procedure similar to that of Chapman, Heap and Saunders, has shown that it is difficult to get reproducible correction curves. A method that would not necessitate filtration and washing, nor be in error even if chloride ions are adsorbed on the precipitate, would probably be more reliable than the existing method. This paper describes a preliminary study of this possibility.

Farkas and Uri[7] describe the determination of lead by potentiometric titration with alkali fluoride in presence of alkali chloride. Excess of sodium chloride is added to a solution containing lead ions so that the lead is partially precipitated as lead chloride; when titrated with standard alkali fluoride, the lead in solution is precipitated as lead chlorofluoride, the precipitated lead chloride then dissolves and is re-precipitated as chlorofluoride, until eventually all the lead has been converted to chlorofluoride; an accuracy of ±0·5 per cent. is claimed. In the present investigation, this titration has been reversed. The test solution containing fluoride is treated with excess of sodium chloride and titrated with standard lead nitrate solution. During the titration small amounts of ferric chloride and ferrous chloride indicator solutions are added, so that most of the ferric ions become $(\mathrm{FeF}_6)'''$; as the end-point is approached, this complex ion dissociates and gives an increase in the $\mathrm{Fe}^{\cdots}/\mathrm{Fe}^{\cdot\cdot}$

ratio, and hence in the potential of a platinum electrode. The end-point is reached when maximum potential change occurs. The advantage of titration in this direction, compared with that of Farkas and Uri, is that lead chloride does not have to redissolve after each addition of titrant, and equilibrium should be attained more quickly.

EXPERIMENTAL

APPARATUS—

Tinsley potentiometer—Graduated in millivolts, 0 to 1·9 volts.

Platinum electrode—About 1 cm square.

Saturated calomel electrode—Fitted with a saturated potassium chloride bridge.

Tall-form beaker—Fitted with an efficient glass stirrer to give rapid rotation. Capacity 100 ml.

Burette—Graduated in fiftieths of a ml. Capacity 0 to 5 ml.

The platinum electrode was cleaned frequently by immersion in hot concentrated nitric acid and thoroughly washed with distilled water.

STANDARD SOLUTIONS—

Sodium fluoride—Hopkins and Williams, pure, dried at 130° C before use. Tests did not reveal any chloride and since the sulphated ash figure was correct, it was taken as being pure. *N* and 0·10 *N* solutions were used.

Lead nitrate—A.R., standardised gravimetrically as lead sulphate; solutions of approximately *N* and 0·10 *N* concentration were used.

Ferrous chloride—B.D.H. laboratory reagent. Two grams were dissolved in 10 ml of water and 3 ml of concentrated hydrochloric acid; about 0·5 g of granulated zinc was added and the mixture was allowed to stand for several hours, until a test portion showed no reaction for ferric iron on adding ammonium thiocyanate. It was then made up to 100 ml and about 0·5 ml of mercury added to form an amalgam with the residual zinc. This solution is satisfactory for use for about a week.

Ferric chloride—A solution of 0·04 g in 100 ml of distilled water.

Sodium chloride—A solution of 25 g in 100 ml of distilled water.

Buffer solution, pH 3·5—Prepared with 5 ml of *N* sodium acetate and 95 ml of *N* acetic acid; the pH measured was 3·6.

METHOD

The tests were carried out on suitable volumes of *N* or 0·10 *N* sodium fluoride, or on individual weighed portions of dried sodium fluoride, in a 100-ml tall-form beaker, fitted with a rapidly rotating glass stirrer so that a vortex was formed corresponding to about one-third of the height of the liquid at the walls, a saturated potassium chloride bridge, and a platinum electrode; the bridge led to a saturated calomel electrode cell, and the two electrodes were connected in the usual manner to the Tinsley potentiometer.

To the fluoride solution were added 4 ml of 25 per cent. sodium chloride solution and, when required, the 20 ml of buffer solution, 20 ml of ethanol and water to make the total volume about 40 ml. About 90 per cent. of the expected volume of lead nitrate solution was added with vigorous stirring and then the ferrous and ferric chloride solutions. The titration was continued, usually in increments of 0·1 ml at a time, until the end-point had obviously been passed. The exact end-point titre was calculated by means of the second differential.[8]

TITRATIONS WITH *N* LEAD NITRATE (40 TO 120 MG OF FLUORINE)—

Late addition of indicator—In the work of Farkas and Uri, lead solutions were treated with excess of chloride, ferric - ferrous indicator was added, the pH was adjusted to between 4·0 and 4·2 and the mixture titrated with standard sodium fluoride solution, preferably in the presence of ethanol. In the present investigation, the first experiment was simply a reversal of this procedure, *i.e.*, 4 ml of a solution containing 76 mg of fluorine, *i.e.*, *N* NaF, was treated with 1 g of sodium chloride, 1·0 ml of ferric chloride solution, *i.e.*, 0·4 mg of $FeCl_3$, and 1 ml of ferrous chloride solution, *i.e.*, 20 mg of $FeCl_2$, 20 ml of ethanol and 20 ml of *N* buffer of pH 4; the mixture was then titrated with lead nitrate. The result was only 95 per cent. of the theoretical titre. Deliberate addition of the indicator solutions at later stages in the course of the titration showed that higher results were obtained if the indicator was added at about 90 per cent. of the theoretical titre.

TABLE I

EFFECT OF LATE ADDITION OF INDICATOR SOLUTIONS

Theoretical titre,* 4·06 ml

Indicator solutions added at, ml	Observed titre, ml	Percentage of theoretical titre
0	3·85	94·9
3·0	3·85	94·9
3·2	3·93	96·8
3·6	4·05	99·7
3·6	3·95	97·3
3·6	4·0	98·5
3·6	3·85	97·5

* Throughout the paper this figure is calculated for 0·983 *N* or 0·10 *N* lead nitrate solution.

Subsequent work in the absence of alcohol or buffer and in the absence of both alcohol and buffer, confirmed that under these conditions also results rather closer to the theoretical values were obtained when the indicator was added at about 90 per cent. of the theoretical titre; the amount of ferric chloride used (0·4 mg) corresponds to but 0·26 mg of fluorine (*i.e.*, to less than 0·5 per cent.) in the formation of FeF_6''', and it is difficult to see why the late addition should have the observed effect.

Variation in the amount of ferric chloride—The influence of late addition of the indicator solutions suggested that the amount of ferric ion, and hence the ferric - ferrous ratio, might also have an appreciable effect on the level of the results. In the following tests 75 to 80 mg of fluorine (as NaF) were titrated with *N* lead nitrate in presence of 1 g of sodium chloride and 20 ml of ethanol; the indicator solutions comprising 1 ml of ferrous chloride (20 mg $FeCl_2$) and x ml of ferric chloride (0·07 to 5·0 mg of $FeCl_3$) were added at a titre of 3·2 ml. The pH was brought to about 4·0 by adjusting to neutrality against methyl orange after the addition of indicator. The results in Table II show that the amount of ferric ion is not an important factor provided not more than 1 mg of $FeCl_3$ is used; a fixed amount (0·4 mg $FeCl_3$) was therefore used in subsequent work.

TABLE II

EFFECT OF VARIATION IN THE AMOUNT OF FERRIC CHLORIDE

$FeCl_3$ added, mg	Observed titre, ml	Theoretical titre, ml	Percentage of theoretical titre
0·07	3·88	4·09	94·8
0·1	3·82	4·00	95·5
0·1	3·85	3·97	97·0
0·2	3·90	4·06	96·1
0·4	3·93	4·14	94·9
0·4	3·88	4·12	94·2
0·6	3·89	4·09	95·1
1·0	3·90	4·11	95·1
5·0	3·74	4·11	91·2

Variation of pH value—The effect of pH on the precipitation of lead chlorofluoride has been studied extensively[2,5,6]; for amounts exceeding 20 mg of fluorine, a pH between 4·3 and 4·8 is recommended by Kaufman.[6] The stability of the ferrifluoride complex is known to be poor below pH 3, and even above this level the redox potential is influenced considerably by pH variations. Titration of fluoride by lead nitrate in an unbuffered system is accompanied by a significant decrease in pH value, but this effect can be largely counterbalanced by adjusting to neutrality against methyl orange after addition of indicator at about 90 per cent. of the theoretical titre; although this procedure was used in the preceding section a more effective control of pH was desirable. The results given in Table III were carried out with 20 ml of *N* buffer solutions of different pH values added at the commencement of the titration; 1 g of sodium chloride and 20 ml of ethanol were present, and the indicator solutions were added at 3·6 ml.

TABLE III

EFFECT OF VARIATION IN pH

pH of buffer solution	Observed titre, ml	Theoretical titre, ml	Percentage of theoretical titre
5·0	No end-point	4·06	—
4·0	4·00	4·06	98·5
	3·86	3·96	97·5
	3·95	4·06	97·3
	3·93	4·06	96·8
3·5	3·87	4·03	96·0
	3·80	4·03	94·3
	3·85	4·06	94·8

Curves A, B and C of Fig. 1 illustrate the type of titration obtained; the pH of the added buffer must be well below 5; with pH 4 and 3·5 buffers the end-points are satisfactory as regards slope, the former pH giving rather a greater slope but with a smaller over-all e.m.f. change. The pH of the titrated system changes by about 1·5 units from the beginning to the end of the titrations, even in presence of 20 ml of *N* buffer solution; this no doubt in itself causes some decomposition of the ferrifluoride complex.

The effect of ethanol—In all the above tests the e.m.f. tended to drift after each addition of titrant so that sometimes 2 to 3 minutes had to elapse before a steady reading could be obtained. Farkas and Uri found that the equilibrium potential was attained more slowly and greater potential changes were obtained when ethanol was present in the titrated system. Although the delay in reaching equilibrium appeared to be caused solely by the slow rate at which lead chloride dissolved, a study of the titration in absence of ethanol appeared to be desirable. The tests shown in Table IV were carried out on 40 to 120 mg of fluorine in a total volume of 40 ml of water (including 1 g of sodium chloride and 20 ml of *N* buffer pH 3·5), with the ferric - ferrous indicator solutions added at 0·3 to 0·6 ml before the expected end-point.

TABLE IV

TITRATIONS CARRIED OUT IN THE ABSENCE OF ETHANOL

$Fe^{\cdots}$ - $Fe^{\cdot\cdot}$ indicator added at, ml	Observed titre, ml	Theoretical titre, ml	Percentage of theoretical titre
3·5	4·14	4·15	99·8
3·5	4·07	4·13	98·6
3·5	4·06	4·11	98·8
3·5	3·99	4·01	99·4
3·6	4·06	4·06	100·0
3·6	4·07	4·06	100·2
1·7	2·04	2·03	100·5
5·4	6·08	6·09	99·8

The immediate improvement in results, both in accuracy and in freedom from drift of potential after each addition of titrant, was marked; but the end-points were much less sharp and the over-all e.m.f. change was less than in presence of ethanol (see Fig. 1, curve D).

Increased concentrations of sodium chloride—As the analysis of organic fluorine compounds in the range of 40 to 120 mg of fluorine would probably necessitate the Parr bomb method for decomposing the sample, the effect of increasing the amount of sodium chloride in the titration system was investigated. The tests shown in Table V were made in presence of 20 ml of *N* buffer, pH 3·5, and 6 g of sodium chloride at a dilution of 40 ml in the absence of ethanol; the indicator solutions were added at 0·4 ml before the expected end-point.

The end-points in these titrations were as sharp as before (see Fig. 1, curve E), but a rather longer time was required to reach equilibrium potential after each addition of lead nitrate, and the end-point occurred rather too early. Nevertheless, the results are reproducible and it is evident that if the lead nitrate solution were standardised against sodium fluoride under these conditions the analysis of unknown fluoride solutions would be at the correct level and would be reproducible to about ±0·7 per cent.

At a later date the effect of variation in the amount of ferrous chloride was investigated; this should have no effect other than a change in the general level of potentials owing to a change in the $Fe^{\cdots} - Fe^{\cdot\cdot}$ ratio. It was found, however, that in the presence of ethanol, 1 g of sodium chloride and buffer of pH 4, correct results (100·0 to 100·5 per cent. of theory) with a sharp end-point could be obtained by reducing the amount of ferrous chloride from 20 to 5 mg, the ferric chloride content of the titration system being kept at 0·4 mg. When the sodium chloride content was increased to 6 g the end-points were very unsatisfactory, the potentials taking a very long time to reach equilibrium, and the results were distinctly low. A reduction in the ferrous chloride content in a titration solution in absence of ethanol, but in presence of 6 g of sodium chloride, gave a low result comparable to those given in Table V, and no advantage was to be gained by reducing the ferrous chloride content under these conditions.

TABLE V

RESULTS IN PRESENCE OF 6 G OF SODIUM CHLORIDE

$Fe^{\cdots} - Fe^{\cdot\cdot}$ indicator added at, ml	Observed titre, ml	Theoretical titre, ml	Percentage of theoretical titre
1·6	1·97	2·03	97·1
2·6	2·98	3·04	98·0
3·6	4·00	4·06	98·5
4·6	4·95	5·07	97·6
		Mean ..	97·8 ± 0·7

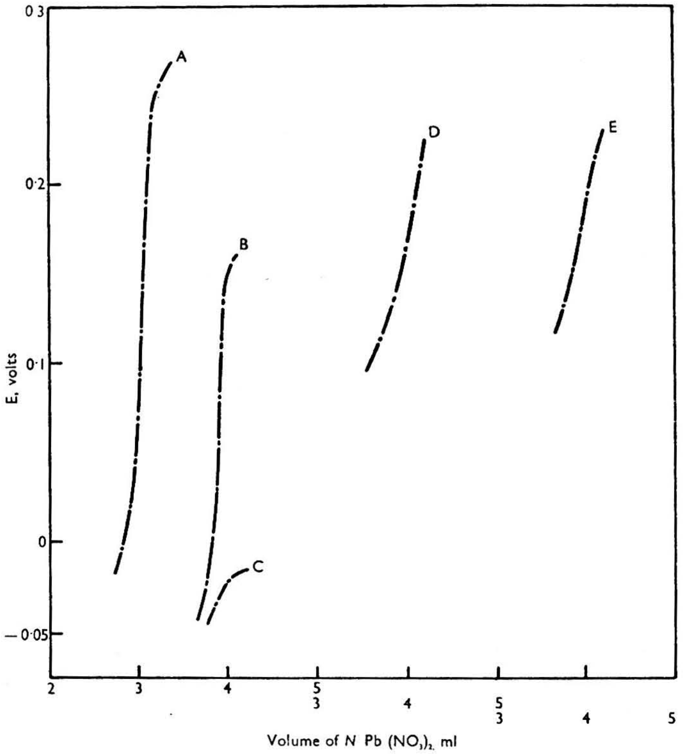

Fig. 1. Titration curves. Curve A, pH 3·5, 1 g of sodium chloride, alcohol present; curve B, pH 4·0, 1 g of sodium chloride, alcohol present; curve C, pH 5·0, 1 g of sodium chloride, alcohol present; curve D, pH 3·5, 1 g of sodium chloride, alcohol absent; curve E, pH 3·5, 6 g of sodium chloride, alcohol absent

TITRATIONS WITH 0·10 *N* LEAD NITRATE (4 TO 16 MG OF FLUORINE)—

The initial titrations of 4 to 16 mg of fluorine were made in presence of 20 ml of *N* buffer, pH 4·0, 0·4 mg of ferric chloride and 20 mg of ferrous chloride, at a total volume of about 40 ml, in the absence of ethanol. The sodium chloride content was reduced to 0·1 g and 0·10 *N* lead nitrate was used as titrant. The titration curves were unsatisfactory; the slope at the end-point being far too small. Reduction of buffer concentration to one-tenth of the previous value, and even in absence of buffer (with methyl orange to indicate adjustment to correct pH after addition of indicator) considerable improvement in the sharpness of the end-point was effected, although it was still not nearly as sharp as in the *N* titrations and the result was less than 95 per cent. of theory. Reduction in the amount of ferric chloride to one-tenth of the original value, *i.e.*, to 0·04 mg, slightly decreased the slope of the titration curve, but raised the level of the results to 98 to 98·5 per cent. of theory; as for the *N* titrations, addition of the ferric - ferrous indicator solutions at the beginning of the titration caused the results to be lower. The tests shown in Table VI were made on 4 to 16 mg of fluorine in presence of 0·1 g of sodium chloride, 20 mg of ferrous chloride and 0·04 mg of ferric chloride, in the absence of ethanol.

TABLE VI

RESULTS OF TITRATIONS WITH 0·10 *N* LEAD NITRATE

$Fe^{\cdots}$ - $Fe^{\cdot\cdot}$ indicator added at, ml	Observed titre, ml	Theoretical titre, ml	Percentage of theoretical titre
1·6	1·98	2·03	97·5
3·6	3·98	4·06	98·0
5·6	6·00	6·09	98·5
7·6	8·00	8·12	98·5
		Mean ..	98·1 ± 0·5

Addition of excess of sodium chloride (1 to 1·5 g) did not alter the level of the result, but slightly decreased the slope of the curve at the end-point. Reduction of ferrous chloride content from 20 to 0·5 mg had little effect in the presence of alcohol, with or without buffer, and in the absence of both alcohol and buffer the slope was poorer, so that the titration as a whole was then considered impracticable. The conclusion reached was that accurate results could be obtained provided the 0·10 *N* lead nitrate solution was standardised against sodium fluoride under controlled conditions (*i.e.*, 0·1 g of sodium chloride, 20 mg of ferrous chloride and 0·4 mg of ferric chloride both added at about 90 per cent. of the expected titre and the system neutralised to methyl orange after the addition of indicator), but the end-point is not by any means as sharp as is desirable, and careful attention to detail is necessary to be sure of obtaining reliable results.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
DYESTUFFS DIVISION
ANALYTICAL LABORATORIES
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MANCHESTER, 9

Symposium on Biological Assay

AT a joint meeting of the Biological Methods Group of the Society and the Biometric Society (British Region) held on March 14th, 1950, the following papers were read and discussed, under the chairmanship of Dr. J. W. Trevan, F.R.S.—

"The Graphical Calculation of the Errors of Biological Assays with Graded Responses," by N. T. Gridgeman.

"The Estimation of Error in Certain Types of Biological Assay," by E. C. Wood.

"The Problem of Combining the Results of Independent Assays," by E. C. Fieller.

Afterwards, under the chairmanship of Mr. N. T. Gridgeman, the meeting devoted its time to an unscripted discussion of a number of questions, raised by previous invitation, on matters of technique and interpretation not normally covered by the standard procedures. A résumé of the discussion follows.

Question—How can non-parallelism of response curves be dealt with?

Answer—The Chairman said he believed that the inescapable inference from non-parallelism of the standard - response and test-material - response curves was that some or all of the active principle in the test material differed from that in the standard not only chemically, but in quantitative action; therefore an assay exhibiting non-parallelism was, normally speaking, biometrically invalid. To this, Mr. M. J. R. Healy added the rider that non-parallelism had in fact been used to detect the presence of foreign substances in pharmacological preparations of uncertain composition. Dr. H. O. Schild feared that this line of argument led to the conclusion that only substances chemically identical with the standard preparation could validly be assayed, yet in practice this was not so; for example, vitamin D_2 could be assayed against vitamin D_3. Mr. A. L. Bacharach drew attention to, and Drs. J. I. M. Jones and E. C. Wood enlarged upon, the modern differentiation of bio-assay into two types whose recognition avoided the difficulties that Dr. Schild had pertinently mentioned. The first type was that in which the assayist knew the chemical composition of the active principle in his test preparation to be identical with that in his standard; the "unknown" was its concentration in the inert diluent, and this the assay was designed to estimate. The second type was that in which the assayist could not assume chemical identity but could assume identity of biological behaviour; here the assay measured relative biological potency. Mr. D. J. Finney, referring to the second type, said that the understanding of the results might sometimes be helped by a study of the relationship between relative potency and level of response.

Question—How can the atypical response curves occasionally obtained in microbiological assays be dealt with?

Answer—This phenomenon, found in some assays of, for instance, pantothenic acid and folic acid, was difficult to handle because the atypical curves often traced no recognisable mathematical function. To evaluate such assays it was usual to draw the best freehand curve for the standard and to read off "equivalent potencies" for each test material response. This was unsatisfactory. Dr. A. F. Parker-Rhodes remarked that abnormal curves could be mathematically linearised—provided that the step was biochemically justifiable. Several speakers agreed with Mr. S. A. Price that progress would be difficult until it could be decided how far the question was statistical and how far biochemical. Dr. E. C. Wood and the Chairman could see no theoretical objection, on biochemical grounds, to linearisation procedures. Dr. Wood added that if abnormality of shape was confined to a falling off of the lower part of the curve, the addition of a trace of the assay factor to the basal medium might produce linearity.

Question—What is the recommended design for an experiment in which the number of dosage groups exceeds the number of animals per litter?

Answer—Complete randomisation of the animals available for test was one suggestion; in this way between-litter and within-litter variances are confounded. The Chairman linked the idea with those presented by Dr. Wood in the paper he had delivered earlier in the day, and pointed out that if only one animal be taken for test from each litter, the assay would not only present no problems of design but would be a more representative assay than one employing litter mates. In practice, however, difficulties of animal economy might arise even if biological work was available to absorb the litter supplies; there was, therefore, much to be said for recourse to the more intricate designs available for multi-assays with litters of smaller membership than the number of dosage groups required. Mr. Finney said that the problem was similar to that faced by agricultural experimentalists wishing to compare more treatments than there were plots per block. The "incomplete block" techniques could therefore be used. Mr. J. V. Smart and Mr. Healy warned of the danger of confounding interactions of importance, and the former held that pilot experiments should be conducted to decide which interactions might safely be confounded in the assays proper.

Question—Is the partial rejection of results justifiable? What is the correct method of dealing with individual animals that have lost weight in assays based on weight gains?

Answer—This query might be said, as Dr. Jones commented, to go beyond the field of statistics into that of ethics, because a decision on a rejection that would affect the assay result was often a matter for the assayist's conscience. Abnormality of unknown cause was the major problem, although animal abnormality of known cause (*e.g.*, injury or misdosage) posed the ancillary question as to whether, in that event, the whole group or litter should be discarded. The Chairman and others thought not. Attempts had been made in the past to set up objective statistical criteria of abnormality and rejection, but none was unexceptionable. It had been suggested that a result was validly rejectable if its withdrawal reduced the fiducial limits, but that was not a reliable test. Mr. Finney said that it was almost impossible to get away from ethical judgments unless one firmly adopted the objective criterion that rejection was permissible only for assignably-caused abnormality. Others concurred.

On the second part of the question, the Chairman said that in growth assays, weight losses were particularly difficult to deal with when there were also deaths. An animal that died must have a lower response than one that lost more weight and yet survived, but simple inclusion of negative growths would not allow for this. The treatment he favoured was to record all weight losses and deaths as "zero growth." Mr. Healy and others thought this solution imperfect, but agreed that a biometrically valid treatment was not obvious.

Question—In the radiographic technique for vitamin-D assay, animals of abnormally low sensitivity to rachitogenesis are not used for test. In the rat-growth assay of vitamin A, animals of abnormally high sensitivity to vitamin-A depletion are also disliked, but are generally included in the test because it is usually only during the test period that this abnormality shows up. Should the results of these animals be rejected? Should all the results of the containing litter be rejected?

Answer—On this question, which is in some respects an extension of the previous one, the concensus of opinion yielded a negative answer. That is to say, results should not be rejected on circumstantial evidence of this kind. It was nevertheless admitted that a litter, most of whose members responded feebly or died during the test period, might justifiably be rejected from the final results.

Question—In some biological phenomena (*e.g.*, the effect of insulin on blood sugar or the effect of vitamin P on capillary resistance) reponse to dose or stimulus is conditioned by the initial status. What are the governing principles? How can the conditioning be allowed for?

Answer—Mr. Finney and Dr. Wood, among others, contributed to the discussion on this question. They agreed that, in general, recordings of the initial status should be made and the responses subsequently adjusted by means of co-variance analysis and the regression equation (response on stimulus). By this means the equivalent responses from animals of uniform initial status could be estimated. The Chairman added that distribution of the animals into the dosage groups in such a way that the average initial status was the same for all dosage groups usually provided a satisfactory solution; if necessary, of course, the co-variance technique could also be applied—it might reduce the limits of error, although it would be unlikely to alter the assay. Mr. C. P. Cox pointed out that the relation between initial status and response could be non-linear, in which event co-variance analysis involving a quadratic, or even high order, equation would be needed. He agreed with Mr. Fieller that the considerable extra computation involved was rarely justified.

Question—Toxicologists in particular, and pharmacologists in general, frequently administer assay doses in proportion to body weight. Is not the correct procedure, unless experiment has shown a direct proportionality between response and body weight, to give equal doses within groups, and to eliminate the effect of body weight (as of any other measurable independent variable) by co-variance analysis?

Answer—The correct procedure, it was agreed, *is* to give equal doses and to use co-variance analysis to allow for size differences. A not unimportant point made incidentally was that co-variance analysis of the results involved less time and trouble than dosage by weight.

Question—Can a statistical basis be given for the choice of levels in techniques involving stepwise dilution? This problem arose in tests designed to compare the efficacy of various sulphonamides as faecal bactericides. Pooled faeces from groups of mice fed on the drugs were suspended in peptone water and serially diluted in steps that each halved the concentration. The tubes were autoclaved and infected, and the degree of bacterial growth after different periods of incubation were recorded, in one experiment, as (−), (− +), (+) and (+ +). In a second experiment, in which the faeces from the individual mice of a group were treated separately, the metameter was the proportion of tubes per group that showed growth. A continuous variate was not possible; turbidimetry, for example, was ruled out by the presence of suspended faecal particles.

Answer—Mr. Healy, referring to the second of the two experiments, suggested the use of probits as in toxicity tests; this would sometimes facilitate the choice of optimum levels. Mr. Finney pointed out that the results of the second trial could be regarded as scores, for which there were standard methods of approach. The same speaker later proposed that the maximal bacteriostatic dilutions (or similar values) of the faeces in that trial be converted to logarithms and that the "*t*" test should be applied to the figures so obtained. It was agreed that the second trial lent itself to statistical treatment more readily than the first.

Notes

NEW SPOT TESTS FOR ZINC

THE addition of zinc ions to a solution containing ferricyanide and ferrocyanide causes an increase in the oxidation potential of the system because of the removal of ferrocyanide as insoluble zinc ferrocyanide. Under these conditions it is possible to oxidise certain organic compounds, which in the presence of ferricyanide alone are oxidised but slowly or not at all, to give highly coloured products. Several tests for zinc based on this reaction have been described, in which organic compounds such as diethylaniline,[1] *p*-phenetidine[2] and diphenylamine[3] are used.

Recently, we have described the use of naphthidine[4] (4 : 4-diamino-1 : 1′-dinaphthyl) as an indicator in the titration of zinc with ferrocyanide in the presence of ferricyanide, the colour change at the end-point depending essentially on the reaction just described. In our opinion, naphthidine is superior to all other indicators hitherto used in this titration.

We have since examined naphthidine and its homologue 3 : 3′-dimethyl naphthidine[5] (3 : 3′-dimethyl-4 : 4′-diamino-1 : 1′-dinaphthyl) as reagents for the detection of zinc. The sensitivity of the test with naphthidine is of the same order as that given by other well-known tests based on the same principle, but 3 : 3′-dimethyl naphthidine provides a much more sensitive test. The limits of identification and the concentration limits for both reagents are shown in Table I.

TABLE I

Reagent	Identification limit	Concentration limit
Naphthidine	1 µg	1 in 50,000
3 : 3′-Dimethylnaphthidine ..	0·1 µg	1 in 500,000

Ions that form coloured precipitates with the ferrocyanide ion will interfere, *i.e.*, ferrous and ferric iron, copper, manganese, cobalt and nickel. Ions that will oxidise the reagents must be absent, *e.g.*, permanganate, dichromate, chromate, vanadate, persulphate and iodate.

With naphthidine, the test is most sensitive under the slightly acid conditions furnished by the solution of the reagent. Under more strongly acid conditions the sensitivity is decreased to a small extent. Hence, using the reagent 0·1 *N* with respect to sulphuric or hydrochloric acids, the identification limit falls to 3 µg.

With 3 : 3′-dimethylnaphthidine, the sensitivity is not affected by a similar increase in acid concentration.

METHOD—

Reagent—Mix 1 part of a 5 per cent. solution of potassium ferricyanide in distilled water with 2 parts of a saturated solution of naphthidine hydrochloride or 3 : 3′-dimethylnaphthidine hydrochloride in distilled water.

Procedure—Place 1 drop of freshly prepared reagent on a spot plate and add 1 drop of zinc test solution. An immediate red-violet colouration denotes the presence of zinc.

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November, 1950

Apparatus

A NEW DESIGN FOR AN ORSAT ABSORPTION PIPETTE

MANY different types of gas absorption pipettes for use on Orsat-type apparatus have been described in the literature, some of which incorporate improvements such as the use of perforated plates or glass sinters that considerably increase the efficiency of absorption. Most of the pipettes require the use of two-way stopcocks, which necessitate a 180° turn for each passage of the gas to and from the gas burette, and an undesirable feature common to most is the U-bend

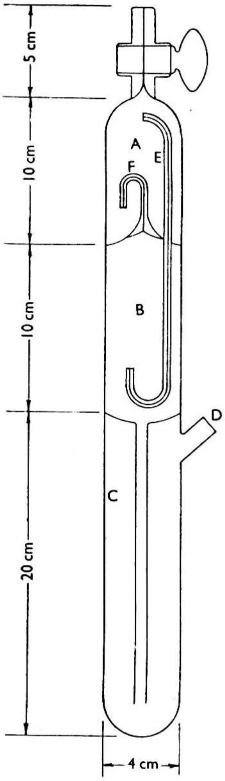

Fig. 1. New design for an Orsat absorption pipette

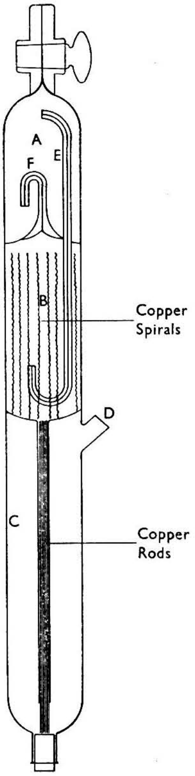

Fig. 2. Modified pipette for use with cuprous chloride in presence of copper

connecting the absorption chamber to the reagent reservoir. This U-bend design is fragile and special clamps or supports are needed to hold the pipette in position on the apparatus.

The pipette described was designed in an attempt to overcome these limitations and to produce a gas absorber that would combine maximum efficiency of absorption with simplicity of attachment, operation and maintenance.

The apparatus, illustrated in Fig. 1, consists of a cylindrical Pyrex glass tube divided into three compartments by means of two internal seals. The compartments A and B are a modification of the Tsikliss pipette,[1] and the lower compartment, C, is the reagent reservoir vented to air by the side-arm D. For concentrated sulphuric acid reagents, the central capillaries are in heavy-walled 2-mm internal diameter tubing, and for aqueous reagents, 1-mm internal diameter tubing.

In operation the gas is passed into the pipette through a single-bore stopcock and bubbles into the compartment, B, through the long capillary tube E, the reagent being displaced into the reservoir. The gas sample collects in compartment B, the liquid level in A being depressed only to the upper end of the long capillary. On withdrawing the sample into the burette, the gas bubbles from the short capillary, F, through the reagent in compartment A.

When the pipette is incorporated in a complete Orsat assembly, absorption of carbon monoxide is normally effected by means of acid cuprous chloride reagent, reduced with stannous chloride. If it is desired to use ammoniacal cuprous chloride, which requires the presence of copper for reduction, the modified design shown in Fig. 2 can be used. A tubulure is fused to the bottom of the pipette through which pulled out spirals of copper wire are dropped into the middle compartment, the spirals being kept in place by copper rods, as shown, and the opening is closed with a rubber bung.

The dimensions given are for a pipette dealing with an initial gas sample volume of 100 ml. The structure of the pipette is robust and compact, and consequently is suitable for inclusion in a multiple absorption apparatus, the connection to, and removal from, the manifold being greatly facilitated without risk of breakage. Attachment to the apparatus frame is made by means of two clips. Cleaning and filling can normally be undertaken through the side-arm without removal of the pipette. The single bore stopcock appreciably reduces the trouble experienced with two-way and double-oblique stopcocks. The double scrubbing action reduces the number of operations and gives increased efficiency of absorption.

Our thanks are due to Mr. E. Kennedy who constructed the apparatus, and we express our appreciation to the Directors of the British Oxygen Company, Ltd., for permission to publish this note.

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ANALYTICAL LABORATORY
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BRITISH OXYGEN COMPANY, LTD.
MORDEN

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November, 1950

A DROPPING ELECTRODE SYSTEM

IN most types of dropping mercury electrode the purified mercury is in contact with rubber or plastic tubing. Attempts to overcome this drawback have resulted in several electrode designs. Kahan[1] has proposed an all-glass apparatus in which mercury is siphoned from the storage vessel to the capillary. Variations in mercury head are made by alteration of the height of the storage vessel with respect to the capillary. The flow may be stopped by the back pressure provided by a test tube, filled with pure mercury, fitting tightly on a ground joint above the capillary.

Other workers adopt the principle of applying gas pressure to the mercury in order to attain the necessary head. Lingane and Kolthoff[2] describe an apparatus in which the pressure is applied to the mercury by two levelling bulbs, the head being measured by a manometer. Muller, Garman, Droz and Petras[3] use a pressure bottle and rubber bulb to attain the mercury head; a similar device is described by McReynolds,[4] who incorporates a three-way stopcock just above the capillary, thus providing a means of reversing the flow of mercury through the capillary. The lubrication of this stopcock is not described, but could be a source of contamination.

The electrode of Kahan is of a fragile nature and the method of stopping the flow could conceivably be a source of hazard. Lingane and Kolthoff's design is cumbersome, and those of Muller and McReynolds are liable to leak.

The apparatus proposed in this note is presented in an attempt to overcome the defects of the assemblies dependent on applied pressure. It is robust, compact, and may be supported by a single clamp. The mercury flow is readily stopped, and the assembly allows for quick dismantling of the capillary attachment and replenishing of mercury.

The apparatus, Fig. 1, is constructed in two parts; the tube A, fabricated as shown, is sealed to the polarographic capillary. The whole is thoroughly cleaned and pure mercury introduced. The ground joint is well greased and the tube inserted into jacket B. The centre tube of the jacket dips into the pure mercury in A.

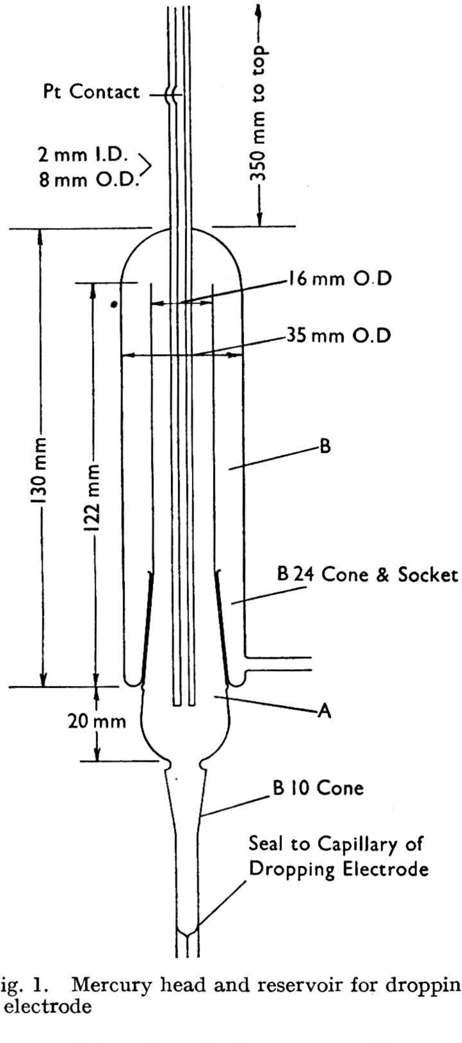

Fig. 1. Mercury head and reservoir for dropping electrode

The side-arm on B is connected by a length of pressure tubing to a levelling bottle. Ordinary mercury is introduced and, by raising the levelling bottle, pressure is applied to the pure mercury in tube A and causes it to rise up the central tube to give the required head of mercury. Electrical contact is made by means of a platinum wire sealed through the wall of the central tube.

This type of dropping-electrode assembly has been found satisfactory in use and no contamination of the mercury occurs over long periods, as the pure mercury is only in contact with glass surfaces. The use of thick-walled capillary tubing is recommended for the central tube; this adds to the robustness of the apparatus and also enables accurate measurement of the mercury head.

Tube A is replenished with pure mercury by lowering the levelling bottle to drain the mercury from the jacket, B, the tube, A, is then withdrawn and more mercury is added.

Our thanks are due to Mr. E. Kennedy who constructed the apparatus; and we express our appreciation to the Directors of the British Oxygen Company, Ltd. for permission to publish this note.

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ANALYTICAL LABORATORY
RESEARCH AND DEVELOPMENT DEPT.
BRITISH OXYGEN COMPANY, LTD.
MORDEN

H. W. HODGSON
J. H. GLOVER
November, 1950

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1951, **76**, 320).

Public Analyst	*Appointments*
DALLEY, Richard Arthur (Deputy)	County Borough of Wakefield.
JAFFÉ, Frederick William Moore	County of West Riding of Yorkshire.
LEWIS, William Maurice..	County of Warwickshire.
LEWIS, William Maurice (Deputy)	County Borough of Walsall.
LEWIS, William Maurice (Deputy)	Borough of Hereford.
TURNER, Mervyn Edward Dennant (Additional)	County Borough of Gloucester.
WOODHEAD, John Ezra	Metropolitan Borough of Lambeth.
WOODHEAD, John Ezra	County of the Parts of Holland, Lincolnshire.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1951, **76**, 320).

Agricultural Analyst	*Appointments*
LEWIS, William Maurice (Deputy)	County of Warwickshire.
LEWIS, William Maurice (Deputy)	County Borough of Walsall.
WOODHEAD, John Ezra	County of the Parts of Holland, Lincolnshire.

Ministry of Food

STATUTORY INSTRUMENT*

1951—No. 668. The Food Standards (Cream) Order, 1951. Price 2d.

This Order, which came into operation on April 30*th*, 1951, *should be read with the Food Standards* (*General Provisions*) *Order*, 1944, *as amended* (*S.R. & O.*, 1944, *Nos.* 42 *and* 654; *Analyst*, 1944, **69**, 49 *and* 247), *and prescribes standards for cream made from cows' milk, as follows*—

STANDARDS FOR CREAM

1. Except as respects clotted cream, cream shall consist of that part of cows' milk rich in fat which has been separated by skimming or otherwise and—
   (*a*) no cream, whether described as cream, single cream, pouring cream, coffee cream, fruit cream or as any other description of cream, shall contain less than 18 per cent. by weight of milk fat;
   (*b*) no sterilised cream shall contain less than 23 per cent. by weight of milk fat;
   (*c*) no double cream or thick cream shall contain less than 48 per cent. by weight of milk fat.

2. Clotted cream shall consist of that part of cows' milk rich in fat which has been produced and separated by the scalding, cooling and skimming of cows' milk or cream and shall contain not less than 48 per cent. by weight of milk fat.

---

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

British Standards Institution

DRAFT SPECIFICATIONS

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

Draft Specification prepared by Technical Committee FCC/4—Solvents and Allied Products.

CN(FCC)710—Draft B.S. for Dimethyl Phthalate.

Book Review

INORGANIC MICRO-ANALYSIS. By H. V. A. BRISCOE, D.Sc., A.R.C.S., F.R.I.C., and P. F. HOLT B.Sc., Ph.D., D.I.C., F.R.I.C. Pp. vii + 171. London: Messrs. Edward Arnold & Co. 1950. Price 12s. 6d.

The techniques of small-scale analysis are not difficult to acquire, nor do they often need, with the exception of the microchemical balance, equipment that the student cannot devise for himself. The best way to acquire such techniques is through the personal guidance of an expert; this book is intended not only to supplement such teaching but also to guide the student who must teach himself.

The qualitative section of the book deals, not strictly with the "micro" scale (no separations on drop quantities are described), but with the more generally useful "semi-micro" scale, involving centrifugal separations in volumes of apparently 0·25 to 2 ml. The techniques described are basic ones and the student may well, with familiarity, improve and simplify some of them. The scheme of cation analysis applies to the academic "common" ones and consists of the usual group separations, followed by identification within a group by spot and drop tests without, in general, further separation. It could be argued that the emphasis on spot tests (a third of the qualitative section deals with them) is not in the best interests of teaching, since a student's chemical knowledge does not benefit by learning many of the reactions involved. More cogently, although such tests have an invaluable and increasing use in analysis, it can be maintained that, without adequate warning, they are often misleading to the student in their excessive sensitivity and their inability to indicate relative quantities.

The quantitative section includes volumetric methods making use of the vertical and the horizontal burette, and there is a useful description of the application of micro-diffusion methods to the determination of ammonia, carbonate, chloride and bromide. Gravimetric methods make use of the microchemical balance, although students might benefit by preliminary experiments on an ordinary analytical balance, using the method of swings. It is not easy to justify the inclusion in such a book of a lengthy description of the photo-electric absorptiometer and experiments with it. Its unquestioned value in analysis is not sufficient reason for singling it out from other instruments of equal importance. Again, the determination of carbon in steels by a micro-combustion method seems rather specialised for the level aimed at.

The text is agreeable, the illustrations clear, the index detailed and the price not unreasonable under present conditions. The score or so references, however, are generally to minor points, and the student would have benefited by a list of more specialised textbooks which would amplify the various sections.

It is not possible in a book of this scope to satisfy all readers, but the student will find it constantly valuable whether he can receive personal tuition or not. DAVID W. WILSON

The Unit of Heat

The Royal Society, in a pamphlet with the above title issued in October, 1950, recommends that quantities of heat and all other dependent concepts such as specific heat, latent heat, heat of combustion, entropy, etc., should be expressed both in joules and in calories. Two conversion tables are given in the Royal Society's pamphlet and are reproduced below.

Conversion Factors for Various Calories

Description of calorie	Value in joules	Reciprocal of value in joules
International steam table calorie (I.T. cal.)	4·1868	0·23885
15° calorie (cal.$_{15^\circ}$)	4·1855	0·23892
4° calorie (cal.$_{4^\circ}$)	4·2045	0·23784
Thermochemical calorie or defined calorie	4·1840	0·23901
Mean 0° to 100° C calorie	4·1897	0·23868

Conversion Factors for Various British Thermal Units

Description of unit	Value in joules	Reciprocal of value in joules
60·5° F British thermal unit	1054·54	0·00094828
Mean 32° to 212° F British thermal unit	1055·79	0·00094716
39° F British thermal unit	1059·52	0·00094383
Steam table British thermal unit (1939)	1055·06	0·00094781

Publications Received

Aids to Clinical Pathology. By D. Haler, M.B., B.S., D.C.P. Second Edition. Pp. viii + 398. London: Ballière, Tindall and Cox. 1951. Price 8s. 6d.

The Technology and Chemistry of Alkaloids. By F. E. Hamerslag. Pp. viii + 319. New York: D. Van Nostrand Company, Inc. London: Macmillan & Co., Ltd. 1950. Price 48s. 6d.

Inorganic Qualitative Analysis: A Concise Scheme. By H. Holness, M.Sc., F.R.I.C. London: Sir Isaac Pitman & Sons, Ltd. 1951. Price 1s. 9d.

A folder, 4-page opening, giving group separation scheme on cloth-backed card suitable for school laboratory use.

Addendum 1951 to the British Pharmacopoeia, 1948. Pp. xviii + 114. London: published for the General Medical Council by Constable & Co., Ltd. 1951. Price 17s. 6d.

Plastics, Scientific and Technological. By H. R. Fleck, M.Sc., F.R.I.C. Third Edition. Pp. xv + 414. London: Temple Press Ltd. 1951. Price 40s.

The New Physics. Talks on Aspects of Science. By Sir C. V. Raman. Pp. vi + 144. New York: Philosophical Library, Inc. 1951. Price $3.75.

Spectrochemical Analysis. By L. H. Ahrens, D.Sc., F.R.I.C. Pp. xxiv + 342. Cambridge, Mass. (U.S.A.): Addison-Wesley Press, Inc. 1950. Price $10.00.

Soybeans and Soybean Products. Volume II. Edited by Klare S. Markley. Pp. xvii + 540–1145. New York: Interscience Publishers, Inc. 1951. Price $11.00.

The Practice and Science of Bread-making. By D. W. Kent-Jones, Ph.D., B.Sc., F.R.I.C., and John Price. Second Edition. Pp. x + 278. Liverpool: The Northern Publishing Co., Ltd. 1951. Price 25s.

Notice to Authors

THE Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro-methods. Papers on these and allied subjects, by members of the Society or by non-members, may be submitted for publication; they may—

(1) record proposals for new methods and the investigations on which the proposals are based;

(2) record the results of original investigations into known methods or improvements therein;

(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;

(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

(5) record minor investigations or kindred matter and descriptions of new apparatus and its applications, which may be accepted for publication under their respective section headings.

Communications—Papers and all communications relating thereto should be sent to the Editor of *The Analyst*, 7–8, Idol Lane, London, E.C.3.

Papers will normally be submitted to at least one referee, by whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication.

Papers accepted by the Publication Committee must not be published elsewhere except by permission of the Committee.

Synopsis—All papers must be accompanied by a short synopsis of about 100 to 250 words indicating the scope and results of the investigation, and an appraisal of the accuracy of any new method proposed. Contributions intended for publication in the "Notes" or "Apparatus" sections do not require synopses.

Proofs—The address to which proofs are to be sent should accompany the paper. Proofs should be carefully checked and returned within 48 hours.

Reprints—Twenty-five reprints, or a maximum of fifty if there is more than one author, are supplied gratis. Additional reprints may be obtained at cost if ordered directly from the printers, W. Heffer & Sons Ltd., Hills Road, Cambridge, at the time of publication. Details are sent to authors with the proofs.

NOTES ON THE WRITING OF PAPERS FOR "THE ANALYST"

Manuscript—Papers and Notes should be typewritten with double spacing and on one side *only* on non-absorbent paper. Two copies should be sent to the Editor, and a further copy retained by the author. A duplicate sketch or tracing of all figures should be included for office use. Manuscripts should be in accordance with the style and usages shown in recent copies of *The Analyst.*

The title should be brief but descriptive.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Descriptions of new methods should be supported by experimental data showing their precision and selectivity.

Generally, the best order of presentation is as indicated below—

(*a*) Synopsis.

(*b*) Statement of object of investigation and, if necessary, historical introduction.

(*c*) Preliminary experimental work.

(*d*) Description of method. Working details of proposed methods are most concisely and clearly given in the imperative mood, and should normally be given in this form, *e.g.*, "Dissolve 1 g of sample in 10 ml of water and add . . ." Well-known procedures must not be described in detail.

(*e*) Presentation of results.

(*f*) Scope and validity of results.

(*g*) Conclusions, and if required, a short summary of the principal results.

Tables, diagrams, etc.—The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns may often be arranged horizontally. No lines should be ruled in tables in the manuscript. Tables should be supplied with titles and be so set out as to be understandable without reference to the text.

Tables or graphs may be used, but not both for the same set of results, unless important additional information is given by so doing.

In general, graphs should have a reasonable number of co-ordinate lines, and not only the two main axes. Graphs consisting of straight lines passing through the origin, such as calibration curves, should not be submitted: instead, an equation should be given in the text. Graphs should be prepared in the same manner as other diagrams.

Diagrams and graphs should be drawn in Indian ink on Bristol board, stout paper or tracing cloth, not larger than foolscap size. The use of squared paper should be avoided if possible, as it often leads to poor reproduction. Red, orange or brown ruled paper must not be used; if it is necessary to use ruled paper, blue or green rulings may be used, as these colours do not reproduce in block-making. All lettering should be inserted lightly in black lead pencil at the appropriate place on the diagram, and will be replaced by type in block-making. All lines in Indian ink should be firmly drawn and sufficiently thick to stand reduction. If the artist is uncertain in his free-hand work on graphs, he should submit a careful pencil drawing that can be traced.

Drawings should be specially prepared for submission to *The Analyst*, as they cannot normally be returned, and may be modified or cut in the course of block-making.

Photographs—Photographs for reproduction on art paper should be submitted in the form of glossy prints made to give the maximum detail.

Abbreviations—Normality and molarity are generally expressed as decimal fractions (*e.g.*, 0·02 *N*, 0·375 *M*). Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, μg, mm) and after ° C, ° F, μ, A and other units represented by symbols; litre and metre, when without prefixes, are printed in full.

Abbreviations of words other than recognised units should be avoided in the text; symbols and formulae are not used instead of the names of elements and compounds in the text, but may be used in addition to names where it is necessary to avoid ambiguity, *e.g.*, to specify crystalline composition, as in $CuSO_4.5H_2O$, to show structure, or in equations.

Percentage concentrations of solutions should be stated as "per cent. w/w" (alternatively "g/100 g"), as "per cent. w/v" (alternatively "g/100 ml"), or as per cent. v/v. Concentrations of solutions of the common acids, however, are often conveniently given as dilutions of the concentrated acids; they should then be given in the form "diluted hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1:4, which might be equivalent to *either* 1 + 4 *or* 1 + 3.

References—References should be numbered serially in the text by means of superscript figures, *e.g.*, Dunn and Bloxam[1] or Allen,[2] and collected in numerical order under "REFERENCES" at the end of the paper. They should be given, with the authors' initials, in the following form—

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, **1933**, **52**, **189T**.
2. Allen, A. H., "Commercial Organic Analysis," Churchill, London, 1882, p. 123.

For books, the publisher, and place and date of publication should be given, followed by volume or page number, or both if required.

Authors are recommended, in their own interests, to check their lists of references against the original papers; second-hand references are a frequent source of error.

Neglect of any of the points mentioned above is likely to cause delay in publication.

Further useful advice to authors is contained in the Royal Society's publication entitled "General Notes on the Preparation of Scientific Papers," published for the Royal Society by the Cambridge University Press, price 2s. 6d.

PETROCHEMICALS LIMITED have the following vacancies in their Control Laboratories:—

(1) Chemist with some years' industrial experience, to be responsible for the Analytical and Products Control Section. The section is also responsible for the development of methods of analysis, with emphasis on physical methods. Salary range £800–£900 p.a. Replies invited from applicants who applied for the vacancy as previously advertised.

(2) Assistant Chemist, preferably a graduate, with some industrial experience, required for the Analytical group. The work includes general organic and inorganic analysis with some research and development of methods. Salary £500–£600 p.a.

Both of these positions carry membership of a generous Non-contributory Staff Assurance Scheme and good opportunity for advancement.

Applications should be made to the Personnel Manager, Petrochemicals Limited, Partington Industrial Estate, Urmston, nr. Manchester.

REQUIRED by the Co-operative Wholesale Society Limited, a well qualified chemist with experience in industry for a progressive post in their Research Department, Manchester. The applicant should be an Honours Graduate, an F.R.I.C., preferably Branch "E," who has had several years' experience in technical research and also have had control of scientific staff. Please forward full details of qualifications and experience to the C.W.S. Ltd., Central Labour Department, 1, Balloon Street, Manchester, 4, endorsed "Research Chemist."

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APPLICATIONS are invited for the above-mentioned appointment from candidates having a University Degree and/or the Associateship of the Royal Institute of Chemistry. The salary scale is £520 per annum, rising by annual increments of £15, £15 and £20 to a maximum of £570 per annum. Further particulars can be obtained from the County Medical Officer of Health, County Buildings, Stafford.

T. H. EVANS,
Clerk of the County Council.

COUNTY BUILDINGS,
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CITY OF LEEDS
PUBLIC HEALTH DEPARTMENT

ASSISTANT ANALYST required in the City Analyst's Laboratory. Applicants should possess either a University Degree or the Associateship of the Royal Institute of Chemistry, and preferably have had some experience in the analysis of food and drugs.

The commencing salary will be in accordance with Grade APT. II of the National Scheme of Conditions of Service, commencing at £420 per annum, with increments of £15, subject to satisfactory service, to a maximum of £465. It is anticipated that revised salary scales will be applied in the near future.

Applications, on a form to be obtained from the undersigned, giving details of age, qualifications and experience, together with the names of three referees and endorsed "ASSISTANT ANALYST," to be delivered without delay at the Health Department, 12, Market Buildings, Vicar Lane, Leeds, 1.

Canvassing in any form, either directly or indirectly, will be a disqualification.

I. G. DAVIES,
Medical Officer of Health.

DISTILLERS CO. LTD. have a vacancy for an Assistant Works Chemist to work on chemical control of raw materials and finished products in the London area. Age up to 26. Applicants should possess a Degree in Chemistry or equivalent, and preferably have 2 to 3 years' experience in industrial laboratories. Salary according to qualifications and experience. Non-contributory pension scheme. Apply Assistant Staff Manager, 21, St. James' Square, London, S.W.1.

DISTILLERS CO. LTD. have a vacancy in one of their factories in the London area for an Assistant Works Manager. Age 28–30. Applicants should possess a Degree in Chemistry or equivalent, and preferably have experience in a factory and of handling process operators. Salary according to qualifications and experience. Non-contributory pension scheme. Apply Assistant Staff Manager, 21, St. James' Square, London, S.W.1.

THE BRITISH DRUG HOUSES LTD. have a vacancy for a chemist, interested in physical chemistry and with some pharmaceutical experience, for work in pharmaceutical development and analysis. Salary according to qualifications and experience. Five-day week. Pension and Profit Sharing Schemes. Apply in writing to RES/GMG, The Staff Manager, Graham Street, London, N.1.

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MINISTRY OF FOOD: PRINCIPAL SCIENTIFIC OFFICERS AND SENIOR SCIENTIFIC OFFICERS.

THE Civil Service Commissioners invite applications for permanent appointments.

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Post (a) in London. To take charge of the section dealing with the technological aspects of food hygiene and welfare foods. Qualifications: a degree in biochemistry with biological subsidiary subjects. Experience in quality control and manufacture of foods essential. Administrative and organising experience is desirable.

Post (b) in Aberdeen. To take charge of the section dealing with vegetable dehydration. Qualifications: a degree in botany or biochemistry. Research experience in plant physiology and plant biochemistry; industrial experience of processing of vegetable products essential.

Post (c) in London. To maintain liaison between the Department's Scientific Adviser, other Government Departments and branches of industry concerned with manufacture and use of foods for emergency purposes. Qualifications: a degree in chemistry or biological sciences. Practical experience of research and development in food production. Administrative experience desirable.

SENIOR SCIENTIFIC OFFICERS

Post (d) in London. To undertake investigations in quality and reduction of wastage in foods. Qualifications: a degree or an equivalent qualification, including chemistry and biological sciences.

Post (e) in London. For advisory and survey work in nutrition. A knowledge of modern theory and practice of nutrition is essential as the officer's duties will include advising and working on dietary surveys, nutritional requirements, composition of foods and nutritional principles underlying rationing schemes (both civilian and service). Some assistance with work on nutritional education and publicity may also be required. The post will include supervision of large and small scale kitchens and a laboratory. Qualifications: a degree in biochemistry, physiology or chemistry. A diploma in dietetics would be an advantage but is not essential. Catering and administrative experience would also be regarded as additional qualifications.

Post (f) in Aberdeen. To undertake research on biochemical problems in the processing of vegetables. Qualifications: a degree in biochemistry or botany. Research experience in plant biochemistry desirable.

Candidates must have been born on or before 1st August, 1920, but the Senior Scientific Officer posts (and similarly graded scientific posts in other Government Departments) are also open to candidates born between 2nd August, 1920, and 1st August, 1925 (inclusive) through the Open Competition under Normal Regulations already announced (No. 3399).

London salary scales are:—

Principal Scientific Officer (men) £1,000–£1,375; (women) £880–£1,200.

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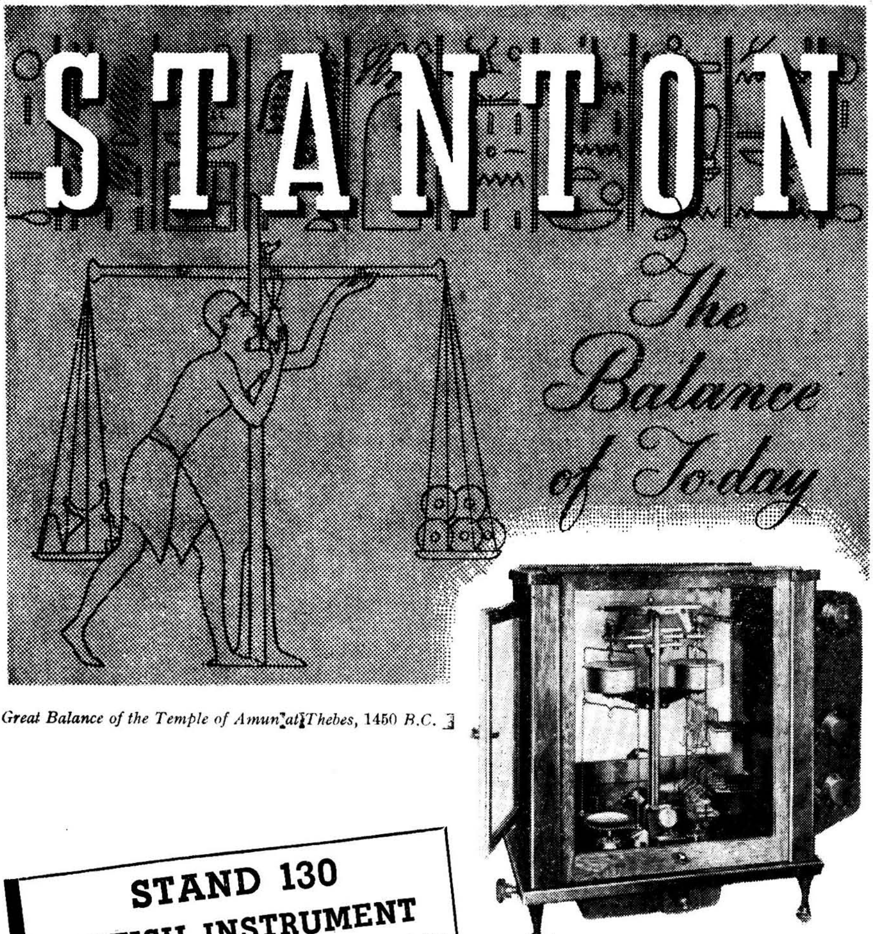

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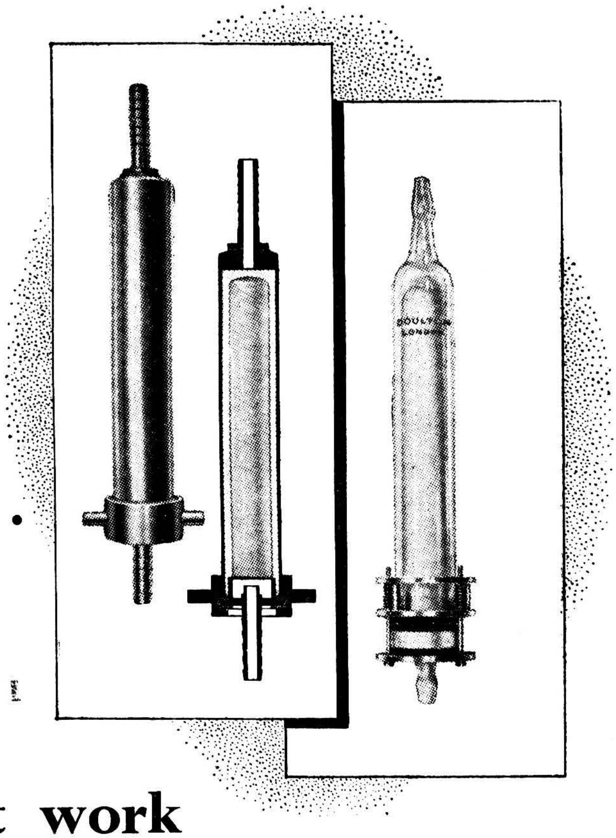

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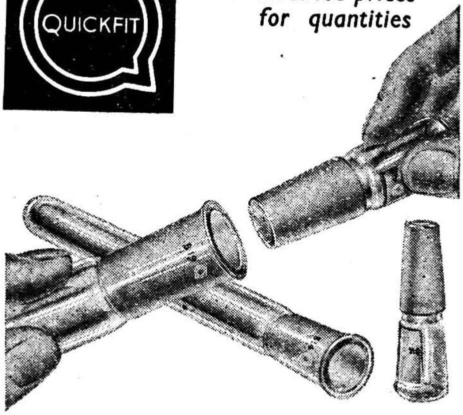

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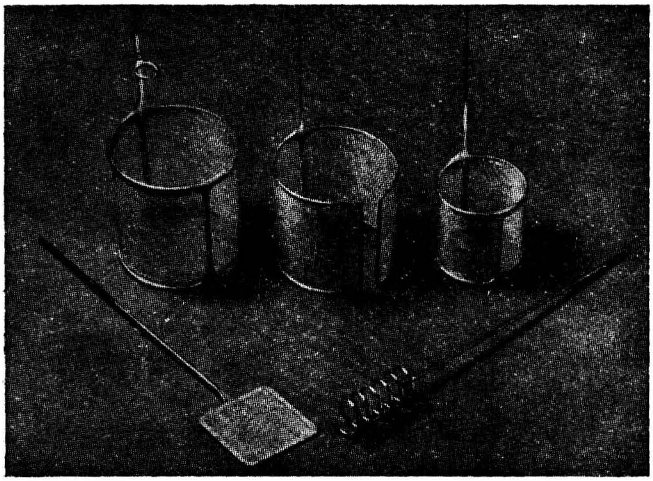
FOR THE LABORATORY



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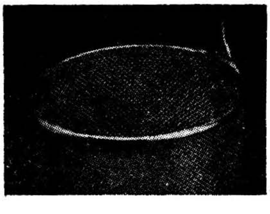

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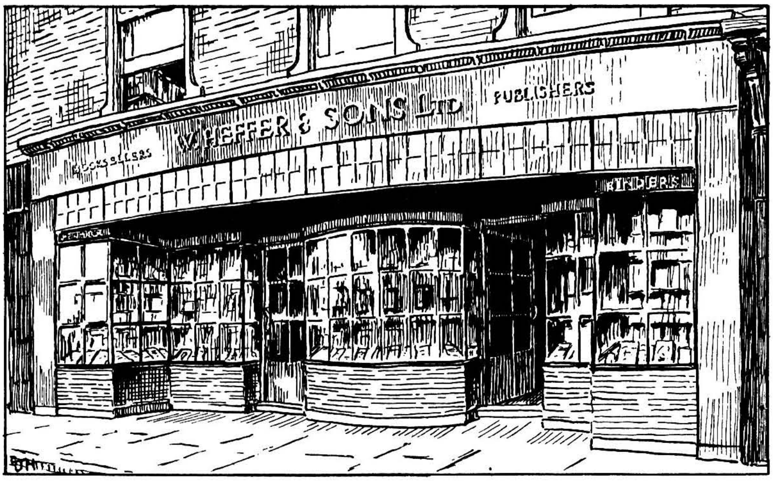
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