THE ANALYST

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

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No. 20 April, 1954

THE SOCIETY FOR ANALYTICAL CHEMISTRY

BULLETIN

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, May 5th, 1954

AN Ordinary Meeting of the Society will be held at 7 p.m. on Wednesday, May 5th, 1954, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The following papers will be presented and discussed—

"The Determination of Phosphate in the Presence of Soluble Silicates: Application to Basic Slag," by H. N. Wilson, F.R.I.C.

"The Spectrophotometric Estimation of Total Penicillins by Conversion to Penicillenic Acid and the Importance of Copper in Controlling the Reaction," by F. G. Stock, M. Pharm., Ph.C., A.R.I.C.

"Micro Method for the Determination of Bromide in Presence of Chloride," by G. Hunter, M.A., D.Sc., and A. A. Goldspink.

Ordinary Meeting of the Society, July 21st, 1954

AN Ordinary Meeting of the Society will be held on Wednesday, July 21st, 1954, in the Lecture Theatre of the Royal Institution, 21, Albermarle Street, London, W.1.

The meeting will deal with "**The Use of Perchloric Acid in Analytical Chemistry,**" and there will be a preliminary paper by Professor Harold Burton of Queen Elizabeth College, followed by a paper with demonstrations by Professor G. Frederick Smith of the University of Illinois. The demonstrations are believed to be quite striking.

Professor Smith is making a special visit to Europe to give this paper, and it is hoped that a very large number of members will come to the meeting.

Inaugural Meeting of the Western Section, May 8th, 1954

THE Council of the Society has approved the formation of a **Western Section** following an application signed by many members resident in the West.

The Inaugural Meeting will be held at 12 noon on Saturday, May 8th, 1954, in the Lecture Theatre of the Technical College, Newport (Mon). The President, Dr. D. W. Kent-Jones, and other members of Council will attend this inaugural meeting, and it is hoped that as many members as possible will support them.

Following a short morning session, lunch will be taken at the Westgate Hotel, after which the meeting will continue at 2.30 p.m. at the Technical College to complete the business of the day.

After the business session, Dr. D. W. Kent-Jones will address the Section on "Alcohol Determination and its Medico Legal Aspects".

Joint Meeting of the Microchemistry Group with the London Section of the Royal Institute of Chemistry, May 7th, 1954

A JOINT Meeting of the Group with the London Section of the Royal Institute of Chemistry will be held at 6.15 p.m. on Friday, May 7th, 1954, in the Zoological Lecture Theatre, The University, Reading.

The subject of the meeting will be "Microchemical Methods in Biochemistry," and the following papers will be presented and discussed—

"The Determination of Esterases," by W. N. Aldridge.

"The Determination of Sugars," by G. Harris.

"The Measurement of Isotopes of Carbon and Hydrogen," by R. F. Glascock.

During the afternoon visits will be made to the works of Messrs. Huntley and Palmers Ltd., Reading, and to the National Institute for Research in Dairying, Shinfield, nr. Reading. At 5.45 p.m. there will be demonstrations of New Methods in Paper Chromatography and of Continuous Paper Electrophoresis Apparatus in the Agricultural Chemistry Research Laboratory.

Meeting of the Physical Methods Group, May 28th, 1954

AN Ordinary Meeting of the Group will be held at the Harris Institute, Preston, Lancs., at 7.30 p.m. on Friday, May 28th, 1954. The subject of the meeting will be "Fluorimetry," and the following papers will be presented and discussed—

"Quenching Effects in the Fluorimetric Determination of Uranium", by G. N. Walton.

"A Twin Beam Null Point Fluorimeter for Liquid Samples", by J. P. Dowdall, A.R.C.S., D.I.C., and H. Stretch, A.R.I.C.

The meeting will be preceded at 2 p.m. by a visit to Siemens Lamp Works.

PAPERS ACCEPTED FOR PUBLICATION IN *THE ANALYST*

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

"The Colorimetric Estimation of Niobium and Tantalum with Pyrogallol," by E. C. Hunt and R. A. Wells.

> Colorimetric procedures are described for the determination of niobium and tantalum in mixtures of their oxides. The method is applied to the determination of the two metals in mixtures obtained by their chromatographic extraction from minerals and ores. The determinations depend on the formation of a coloured complex between tantalum and pyrogallol in acid solution and between niobium and pyrogallol in alkaline solution. Both systems obey Beer's law, and, with 1-cm cells, the optimum limits of concentration are 0 to 20 p.p.m. for niobium and 0 to 80 p.p.m. for tantalum. The effect of variation in pH and the interference of a number of cations and anions are recorded.

"Inorganic Chromatography on Cellulose. Part XIV. A Shortened Chromatographic Method for the Determination of Niobium and Tantalum in Minerals and Ores," by R. A. Mercer and R. A. Wells.

> A chromatographic procedure is described for the extraction of the mixed oxides of tantalum and niobium from minerals and ores. A solution of the sample in hydrofluoric acid containing ammonium fluoride is absorbed on cellulose and transferred to a 3-inch column of cellulose. The niobium and tantalum are completely extracted on passing 400 ml of ethyl methyl ketone containing 15 per cent. v/v of 40 per cent. w/w hydrofluoric acid through the column. The separation is complete from all metals other than tungsten. The two oxides, recovered from this solvent, are subsequently separated by further chromatography or determined without separation by a suitable colorimetric procedure.

Inorganic Chromatography on Cellulose. Part XV. A Rapid Chromatographic Method for the Determination of Niobium in Low-grade Samples," by E. C. Hunt and R. A. Wells.

A rapid and simple chromatographic method is described for the determination of niobium in a hydrofluoric acid solution of an ore by upward diffusion on a paper strip. The niobium is detected as a yellow band on spraying the strip with aqueous tannic acid. An accurate determination of niobium is permitted by direct visual comparison of the band with standard strips. The chromatographic separation takes 20 minutes and a simple technique is described for carrying out ten separations simultaneously. The accuracy is 10 per cent. on ores containing >0·10 per cent. of niobium pentoxide.

The Determination of Titanium by More Precise Absorptiometry," by W. T. L. Neal.

The titanium content of titanium-base alloys and pure titanium metal can be determined absorptiometrically with an accuracy (coefficient of variation) of 0·03 per cent., with a Unicam SP500 spectrophotometer at a wavelength of 4100 A, use being made of the colour of the titanium - hydrogen peroxide compound in solutions with an optical density of 2·5 to 3·0 in 1-cm cells. In this paper an analysis is made of the effect of factors liable to influence the precision and accuracy of the determination, and the techniques required to secure high precision are described in detail.

The Volumetric Determination of Aluminium in Non-ferrous Alloys," by G. W. C. Milner and J. L. Woodhead.

The determination of aluminium can readily be accomplished by a volumetric procedure involving the addition of an excess of a standard ethylenediaminetetra-acetic acid solution to the aluminium solution followed by the back-titration of the amount of reagent unused in the formation of the aluminium - ethylenediaminetetra-acetic acid complex. A standard iron solution is used for the back-titration and salicyclic acid is a suitable indicator for showing the titration end-point. Under these conditions it is possible to determine up to 60 mg of aluminium with an accuracy of better than ± 1 per cent. This titration has proved advantageous in the rapid analysis of various non-ferrous materials including copper, zinc and magnesium-base alloys after the preliminary separation of the aluminium as its insoluble benzoate.

The Aspecific Detection of Preservatives in Foods by a Simple Fermentation Test, with Special Reference to Cured Meat Products," by D. A. A. Mossel.

The stability of non-sterile canned solid foods, which should not contain preservatives, is occasionally increased by adding low concentrations of highly toxic antimicrobial agents, *e.g.*, derivatives of bromoacetic acid and phenylmercury compounds. Chemical methods for detecting each of the various preservatives are cumbersome and time-consuming. It was therefore of interest to adopt the Kluyver fermentation test as an aspecific reaction for antimicrobial compounds.

For testing the solid food (especially cured meat products), a portion is extracted with 0·5 per cent. tartaric acid solution (pH 3·0 $\pm$ 0·2) and a similar portion is extracted with 0·1 per cent. aqueous sodium hydroxide (pH 8·0 $\pm$ 0·2). The extracts are pasteurised at pH 3, enriched by adding 0·5 per cent. of Difco yeast extract and 2·5 per cent. of dextrose, and the pH is adjusted to 4·0. The extracts are then innoculated with sufficient bakers' yeast to give 10^4 cells per ml of solution and they are placed in Einhorn fermentation tubes. After incubation for 24 to 30 hours at 24° $\pm$ 2° C, the volume of gas formed is measured.

Brominated acetic acid derivatives can be detected when present in meat products at concentrations corresponding to 5 mg of bromine per kg. Sodium chloride, potassium nitrate and sodium nitrite do not interfere at the maximum levels found in meat products, *i.e.*, 5, 0·2 and 0·02 per cent., respectively.

"A Volumetric Method for the Rapid Assay of Palladium Jewellery Alloys," by R. H. Atkinson.

A volumetric method suitable for the assay of palladium has been developed. It is based on the precipitation of palladium as palladous iodide under appropriate conditions, with the precipitate as its own indicator. It has been shown that the presence of 5 per cent. of nickel, iridium, platinum, rhodium, tungsten, molybdenum, copper and tin do not interfere within 5 parts in 1000; interference by gold and silver can be obviated by suitable modifications of procedure. In the analysis of a palladium - ruthenium alloy, 15-mg samples are dissolved in the minimum amount of concentrated aqua regia and the resulting solutions are titrated with 0·01 *N* potassium iodide, after adding hydrochloric acid and ferrous sulphate, the latter to prevent excess of aqua regia from reacting with the potassium iodide. The titration is continued until there is no cloud when a drop of the iodide solution is added; owing to the slowness with which palladous iodide settles, it is necessary to centrifuge a portion of the alloy solution before adding the test drop.

"The Spectrophotometric Determination of Small Amounts of Oxygen in Waters," by T. C. J. Ovenston and J. H. E. Watson.

This paper describes the development of a method for the determination of dissolved oxygen in boiler feed waters applicable to concentrations in the range 0·001 to 0·1 ml of oxygen per litre, with a precision better than 0·001 at the lower concentrations. The method is based on that of Bairstow, Francis and Wyatt, but the precision is improved by avoiding the use of starch and by determining the iodine release by means of the ultra-violet absorption of the tri-iodide ion. The procedure has been simplified by the elimination of the step involving volumetric dilution prior to photometric measurement.

"An Examination of Scottish Heather Honey," by T. J. Mitchell, E. M. Donald and J. R. M. Kelso.

Ling honey from the nectar of *Calluna vulgaris* is unique among European honeys in colour, taste, viscosity and the property of thixotropy. The present work sought to find an explanation of this difference by the determination of nitrogen, mineral matter (percentage of ash) and colloid content in samples of honey drawn from widely scattered districts in Scotland and Northumberland. Forty-two samples were examined, of which 30 were predominantly ling honey.

Correlation was found between the pH and the ash content of the honeys. There was some evidence of relationship between the colloid content and the total nitrogen and the thixotropy. The relation between these properties was not exact, probably because of the variation in floral source of the honeys.

"Design and Operating Technique of a Vacuum Drying Oven. Part II. Solids in Cane Molasses," by S. D. Gardiner and F. J. Farmiloe.

With a new carefully-designed vacuum oven, a drying method at 69° to 70° C was devised for cane molasses. Values for true solids were determined to within ±0·1 per cent. An ash correction was derived to permit solids determined by refractometry with the use of sucrose tables to replace the lengthy and difficult determination of solids by drying. Equations were derived relating true solids to refractometer solids, invert sugar and sulphate ash, and true solids to refractometer solids, invert sugar and sulphate ash minus a correction for sodium and potassium sulphates. This correction involves determinations with a flame photometer and gives more accurate results.

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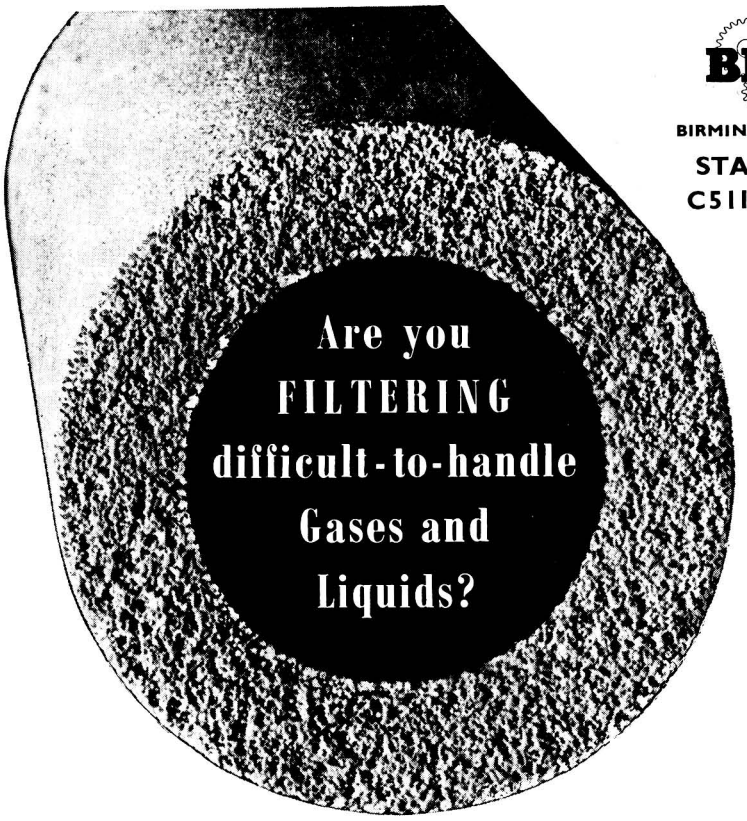

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society, organised by the Microchemistry Group, was held at 7.15 p.m. on Friday, January 29th, 1954, at the Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C.

A film entitled "Old Masters of Microchemistry" was shown and the following papers were presented and discussed: "Organic Ion Exchange," by L. Saunders, B.Sc., Ph.D., F.R.I.C.; "Inorganic Ion Exchange," by G. H. Osborn, A.M.I.M.M., F.R.I.C.

An Exhibition of Microchemical Apparatus was held in the laboratories of the College during the afternoon and evening.

NEW MEMBERS

ORDINARY MEMBERS

Gordon Beswick, B.Sc. (Lond.), A.R.T.C. (Salford); Vincent Binns, M.Sc. (Manc.), F.R.I.C.; Francis William Edward Diggins, Assoc.Inst.Med.Lab.Technology, Dip.Chem.Path.; Charles Oliver Granger, B.Sc. (Lond.), A.R.I.C.; Peter John Jackson, B.Sc. (Lond.), A.R.I.C.; Francis Raban Johnson, M.B.E., M.Sc. (Lond.), F.R.I.C.; Owen Eldred Mott, B.Sc. (Lond.); Edwin Arthur Robinson, B.Sc. (Lond.), A.R.I.C.; Peter Haydock Scholes, A.Met. (Sheff.), L.I.M.; Geoffrey Spencer, M.Sc. (Lond.), A.R.I.C.; Ernest Eric White, F.R.I.C.

JUNIOR MEMBERS

John Buckett, B.Sc. (Lond.); Bernard Collier; James Clinch, B.A. (Cantab.); Alan Roy Gunningham, B.Sc. (Lond.); Ralph Stern, B.Sc. (Lond.), A.R.I.C.

DEATHS

WE regret to record the deaths of

Harold Grovett Colman
Cresacre George Moor.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held at 2 p.m. on Saturday, November 28th, 1953, at the City Laboratories, Mount Pleasant, Liverpool. The Chairman, Mr. T. W. Lovett, F.R.I.C., presided over an attendance of about 100.

At this meeting, Dr. R. L. M. Synge, F.R.I.C., F.R.S., delivered a lecture entitled "Principles of Chromatography" (see summary below).

PRINCIPLES OF CHROMATOGRAPHY

DR. R. L. M. SYNGE defined chromatography as a method for separating substances by the percolation of fluids through porous and powdered media, and explained the counter-current nature of the chromatographic process. Dr. Synge said that he would consider three types of chromatographic operating procedures: (*i*) elution development, (*ii*) frontal analysis and (*iii*) displacement development.

(*i*) In elution development, compact bands resulted only with linear isotherms. Under practical conditions isotherms were often curved, which resulted in diffuse bands. With linear isotherms, bands could sometimes be made sharper by the use of slower rates and finer powders.

(*ii*) In frontal analysis a series of steps was obtained, the original solution finally appearing at the base of the column. A practical example was the stripping of a minor component by ion exchange from a large volume of solution.

(*iii*) In displacement development, components were removed in turn from the adsorbent by the use of a substance that was more strongly held by the adsorbent. There were no gaps between the substances as they appeared at the base of the column, and the technique was useful for preparative work. The technique could be modified by the introduction of a substance of intermediate affinity. Examples were the separation of amino-acids with different alcohols, and the adsorption chromatography of sugars and oligosaccharides.

Dr. Synge considered four different types of equilibria. These were gas - liquid, gas - solid, liquid - liquid and liquid - solid.

Gas - liquid chromatographic techniques were of recent introduction, and an excellent example was furnished by the original work of A. T. James and A. J. P. Martin on the separation of the volatile fatty acids. The relatively non-volatile liquid adsorbent, *e.g.*, high-boiling paraffin, was supported on a solid such as kieselguhr. Special methods of detection, such as those based on thermal conductivity and vapour density, might be required for this type of chromatography. Gas - solid chromatography had so far usually involved a displacement technique, and an example was furnished by S. Claesson's and C. S. G. Phillips' work involving separation of paraffin hydrocarbons on carbon.

The use of liquid - liquid techniques covered a very wide field, including most substances of biochemical interest. As an illustration of the pharmacological applications of this type of system, an interesting study had been made of the changes that took place in the metabolism of an animal when fluoroacetic acid was administered. There were numerous applications to inorganic separations by partition between an aqueous phase held on paper and an organic solvent.

Ion-exchange resins were versatile and were widely used in liquid - solid chromatography. Tswett's work with columns of calcium carbonate and light petroleum solvent furnished an excellent example of a liquid - solid process. Such systems were applied in about 1930 to the carotenoids and vitamin A. By displacement on ion-exchange resins, separations of amino-acids had been made by Partridge at Cambridge on a fairly large scale. Separations of the rare-earth and trans-uranium elements had also been made on ion exchangers.

Dr. Synge emphasised the use of proper detection methods. Fraction cutters were used in conjunction with columns of adsorbent, and there were many different types. Other detection methods involved the use of paper chromatography: radio-autography and bio-autography were important examples.

For compounds of molecular weight below 500, chromatography could give a relatively easy method of separation. There was an enormous choice of methods and it was generally only necessary to find the right solvent. There were difficulties with large molecules—it was difficult to find two phases in which distribution was at all even, and the molecular dimensions of the pores in chromatographic working materials often led to interference.

Finally, Dr. Synge mentioned his latest work on the separation of large molecules on collodion membranes.

THE Twenty-ninth Annual General Meeting of the Section was held at 2.15 p.m. on Saturday, January 30th, 1954, at the Engineers' Club, Albert Square, Manchester, under the Chairmanship of Mr. T. W. Lovett, F.R.I.C. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Mr. T. W. Lovett. *Vice-Chairman*—Mr. J. R. Walmsley. *Hon. Secretary and Treasurer*—Mr. Arnold Lees, 87, Marshside Road, Southport, Lancs. *Elected Committee Members*—Messrs. H. Childs, R. Crosbie-Oates, H. Dedicoat, K. A. Hyde, A. O. Jones and A. N. Leather. *Hon. Auditors*—A. Alcock and C. J. House.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which the President, Dr. D. W. Kent-Jones, F.R.I.C., gave an address on "The Society for Analytical Chemistry."

SCOTTISH SECTION

A JOINT Meeting of the Section was held with the Stirlingshire Sections of the Royal Institute of Chemistry and the Society of Chemical Industry at 7.30 p.m. on Wednesday, December 16th, 1953, at the Lea Park Restaurant, Callendar Road, Falkirk.

At this meeting Dr. R. L. M. Synge repeated his lecture on "Principles of Chromatography" (see summary above).

THE Nineteenth Annual General Meeting of the Section was held in Glasgow on Wednesday, January 20th, 1954, at 12.45 p.m., under the Chairmanship of Mr. R. S. Watson, F.R.I.C. The following office bearers were elected for the forthcoming year:—*Chairman*—Mr. R. S. Watson. *Vice-Chairman*—Dr. F. J. Elliott. *Hon. Secretary and Treasurer*—Mr. J. A. Eggleston, Boot's Pure Drug Co. Ltd., Motherwell Street, Airdrie, Lanarkshire. *Elected Committee Members*—Dr. Christina C. Miller and Messrs. J. M. Malcolm, J. K. McLellan, H. C. Moir, R. T. Potter and A. F. Williams. *Hon. Auditors*—Messrs. J. Andrews and J. W. Gray.

MICROCHEMISTRY GROUP

THE Tenth Annual General Meeting of the Group was held at the Sir John Cass College, Jewry Street, Aldgate, London, E.C.3, on Friday, January 29th, 1954, at 7 p.m., and the following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Dr. A. M. Ward. *Vice-Chairman*—Dr. G. F. Hodsman. *Hon. Secretary*—Mr. A. Bennett, The Brewing Industry Research Foundation, Lyttel Hall, Nutfield, Redhill, Surrey. *Hon. Treasurer*—Mr. G. Ingram. *Elected Committee Members*—Messrs. W. Brown, G. S. Crouch, D. F. Phillips, J. T. Stock, C. L. Wilson and D. W. Wilson. *Hon. Auditors*—Messrs. L. H. N. Cooper and H. Childs.

PHYSICAL METHODS GROUP

THE Ninth Annual General Meeting of the Group was held at 6 p.m. on Tuesday, November 24th, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chairman of the Group, Dr. J. Haslam, presided. The Group Officers and Elected Members of the Committee for the forthcoming year are as follows:—*Chairman*—Mr. A. A. Smales. *Vice-Chairman*—Dr. J. E. Page. *Hon. Secretary and Treasurer*—Mr. R. A. C. Isbell, Hilger & Watts Ltd., Hilger Division, 98, St. Pancras Way, London, N.W.1. *Members of Committee*—Messrs. W. H. Bennett, W. Furness, H. M. N. H. Irving, A. G. Jones, F. C. J. Poulton and R. A. Wells. *Hon. Auditors*—Messrs. C. A. Bassett and D. C. Garratt.

The Annual General Meeting was followed by the Forty-second Ordinary Meeting of the Group, at which the new Chairman, Mr. A. A. Smales, took the Chair. The retiring Chairman, Dr. J. Haslam, gave an address on "Physical Methods in the Analysis of Plastic Materials—Some Observations of an Analytical Chemist" (see summary below).

PHYSICAL METHODS OF ANALYSIS OF PLASTIC MATERIALS—SOME OBSERVATIONS OF AN ANALYTICAL CHEMIST

DR. J. HASLAM, in presenting a survey of the methods used in the examination of plastics of various kinds, said that, although some of them were chemical and some combinations of chemical and physical procedures, many were purely physical. Amongst the most important of the purely physical methods was the determination of relative viscosity; in this different solvents were used for different types of plastic: nylon was dissolved in 90 per cent. formic acid, polythene in tetrahydronaphthalene, polymethyl methacrylate in chloroform, Terylene in *o*-chlorophenol and polyvinyl chloride in ethylene dichloride.

Although the chemist would realise that the only figure determined was the relative viscosity, the results were, except for those found for nylon, usually calculated to arbitrary

values by means of empirical factors. Polythene and polymethyl methacrylate were reported as molecular weight, polyvinyl chloride as the so-called *K* value and Terylene as intrinsic viscosity.

In the analysis of nylon, information on the speed and extent of hydrolysis by hydrochloric acid was desirable. This knowledge, difficult to get by chemical means, was easily obtained by potentiometric titration of the hydrolysis products, a physical method. For nylon 66 the end-points were at pH 2·6 for the free hydrochloric acid (hexamethylenediamine dihydrochloride is neutral) and at pH 8 for the adipic acid. Similarly for nylon 610, the first end-point at pH 3·7 to 3·8 marked the titration of the free hydrochloric acid and the second at pH 8·2 corresponded with that of the sebacic acid. For nylon 6 the end-points were at pH 2·6 for the free hydrochloric acid and pH 8·0 for the hydrochloric acid of the ϵ-aminocaproic acid hydrochloride.

Another physical method, chromatography, has also given valuable results in the examination of nylon; Zahn has described separations on paper that have been useful in dealing with small amounts, and Miss Ayers has shown that nylon polymers can be dealt with by straight chromatography.

In nylon analysis, partition methods have also been useful. From the hydrolysis product of copolymers of hexamethylenediamine with adipic and sebacic acids, the two acids can be isolated, equilibrated between ether and water, and so determined by a procedure that has been described in *The Analyst*.

In the analysis of Perlon U, which is not an ordinary nylon polymer, the acid hydrolysis products are hexamethylenediamine hydrochloride and tetrahydrofuran. The furan derivative reacts with an excess of hydrochloric acid to form 1:4-dichlorobutane, a substance difficult to deal with. The difficulty was overcome by taking advantage of two physical properties of tetrahydrofuran: its low specific gravity and its partial solubility in water. Once separated, the tetrahydrofuran can be identified by conversion to succinic acid.

Of the purely physical methods used in nylon analysis, the examination of the infra-red spectrum of the polymer was the most important of all, for it had reduced considerably the amount of chemical work that would otherwise have been necessary to characterise the polymer.

Again, for polymethyl methacrylate the method of attack was a combination of physical and chemical methods. The plasticiser was first removed from a solution of the plastic in acetone by precipitating the polymer with light petroleum, and the insoluble polymer was then submitted to vacuum distillation to recover the monomer, which can be identified by its saponification value and refractive index. Copolymers of methyl methacrylate with substances such as styrene, *cyclo*hexyl methacrylate and ethyl acrylate are amenable to the same treatment.

Polyvinyl chloride tubing presents a more complicated problem. In addition to the basic polymer or copolymer it could contain a mixed plasticiser, a stabiliser, a pigment, a lubricant and possibly a filler. The plasticiser is removed by extraction with ether; the polymer is then dissolved in tetrahydrofuran, and the fillers and so on are removed by centrifugation. The polymer is recovered by precipitation with ethanol and examined by chemical and physical methods; the polymer itself is examined by infra-red spectroscopy and its chlorine content is determined by fusion with sodium peroxide in a stainless-steel bomb and potentiometric titration of the resulting chloride with standard silver nitrate solution with silver-wire electrodes. The morpholine test is used for polyvinylidene chloride and the methanol - alkali - pyridine test for polyvinyl chloride.

The much used principle of solution of a plastic in a solvent, followed by precipitation with a non-solvent, is not confined to the recovery of the pure polymer; it is frequently used to release from a preparation substances not amenable to direct solvent extraction. For example, after precipitation of polymethyl methacrylate by light petroleum from a solution in acetone, the dibutyl phthalate plasticiser can be recovered from the petroleum solution by evaporation and weighed. Likewise a polythene - poly*iso*butylene mixture can be separated from a solution in toluene by means of light petroleum and the poly*iso*butylene recovered from the filtrate for identification by means of its infra-red absorption spectrum. Elementary analysis of hydrocarbons of this kind is useless, for both polythene and poly*iso*butylene have the same carbon and hydrogen values.

Free phenols are liberated from phenol - formaldehyde and cresol - formaldehyde

resins by solution in alkali and precipitation of the resin at pH 4·5; they can then be identified and determined in the filtrate.

The absorbers of ultra-violet light that are added to Perspex to reduce the amount of yellowing caused by sunlight are also separated by precipitation of polymethyl methacrylate resin with a non-solvent from a solution in acetone, after which they can be identified and determined by methods that have already been published; the results are checked by measuring the optical density of the solution of the original polymer at 308 mμ for methyl salicylate, at 312 mμ for phenyl salicylate and at 289 and 324 mμ for 2:4-dihydroxybenzophenone, the three substances in common use.

Lauryl mercaptan, added to Perspex to stabilise it towards heat, is determined by amperometric titration with silver nitrate, on the principle of Kolthoff and Harris, by means of a rotating platinum-wire indicator electrode and a reference half-cell containing mercury in contact with a potassium iodide - mercuric iodide and potassium chloride solution connected to the test solution by a salt bridge.

Catechol is released from nylon by hydrolysis with hydrochloric acid in a sealed tube and determined spectroscopically by means of its ultra-violet absorption at 275 mμ.

In the analysis of plasticisers, physical methods are of great importance and are used in conjunction with chemical analysis. For mixed plasticisers, direct infra-red examination is often unsatisfactory, *e.g.*, for mixed butyl, octyl and nonyl esters. These are hydrolysed with potassium hydroxide in ethylene glycol and the resulting alcohols are determined by their infra-red absorption spectra.

Spectroscopic methods are amongst the most important of those used in the plastics industry. They are used in a wide variety of analyses. In the ultra-violet region, acenaphthylene is determined in its copolymers by its absorption at 295 mμ in chloroform solution; the purity of dipotassium phthalate from a plasticiser is checked in approximately 0·1 *N* hydrochloric acid at 276 mμ. The ultra-violet spectrum is also used in many other tests for additives or for stabilisers such as diphenylurea and diphenylthiourea. The visible region is used for dyestuffs, for example, Durindone Pink FF in Terylene, at 555 mμ, after extraction with *o*-chlorophenol. Inorganic fillers are identified by photographing the spectrum of the sample in juxtaposition with the iron spectrum, which serves as wavelength standard. A recently devised piece of apparatus for showing whether or not Perspex is plasticised with dibutyl phthalate consists of a mercury-vapour lamp, a Beck ultra-violet spectroscope and a fluorescent screen. A sheet of ordinary $\frac{1}{8}$-inch Perspex placed in the light beam cuts off the spectrum at 290 mμ; with plasticised material the cut-off is at 295 mμ.

Polarographic methods are used in the analysis of plastics, particularly for zinc, after removal of organic matter, and for the detection of phthalate esters in commercial plasticisers. These sometimes contain castor oil, which causes the usual resorcinol and phenolphthalein tests to fail. In plasticisers of this type, qualitative evidence of the presence of phthalates is given by recording a polarogram on a mixture of the plasticiser with tetramethylammonium iodide in methanol over the range −1·0 to −2·0 volts. If dibutyl phthalate is present, reduction occurs at −1·45 volts *versus* the mercury pool anode. Phosphates, ricinoleates, citrates, sebacates, Cerechlor and Mesamoll do not interfere.

The microscope is used in the examination of fillers. For the determination of coconut-shell flour in a phenol - formaldehyde moulding powder, the lycopodium method of Wallis has been found useful.

Many of the methods mentioned above have been described in detail in *The Analyst* and in *The Journal of Applied Chemistry*.

BIOLOGICAL METHODS GROUP

THE Ninth Annual General Meeting of the Group was held in the Anatomy Lecture Theatre, University College, London, W.C.1, at 4.30 p.m. on December 11th, 1953. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Dr. Leslie J. Harris. *Vice-Chairman*—Mr. K. L. Smith. *Hon. Secretary and Treasurer*—Mr. S. A. Price, Walton Oaks Experimental Station, Vitamins Ltd., Dorking Road, Tadworth, Surrey. *Members of Committee*—Messrs. E. M. Bavin, W. A. Broom, H. O. J. Collier, K. A. Lees, H. Pritchard and G. F. Somers. *Hon. Auditors*—Messrs. D. M. Freeland and J. H.

Hamence. Mr. J. W. Lightbown has consented to attend Committee Meetings in the capacity of Hon. Recorder.

During the afternoon preceding the Annual General Meeting and the evening following the meeting, an Ordinary Meeting of the Group was held jointly with the Pharmaceutical Society on "The Assay and Detection of Pyrogens." About 200 members and visitors were present.

The Afternoon Session was introduced by the Chairman, Professor J. P. Todd, and the following papers were presented and discussed: "The Occurrence and Importance of Pyrogens," by T. D. Whittet, B.Sc., Ph.C., D.B.A., A.R.I.C.; "Routine Pyrogen Testing," by K. L. Smith, M.P.S.; "The Leucocyte Response in the Rabbit to the Pyrogen from *Proteus vulgaris*. Part I. Mononuclear and Temperature Responses," by M. Dawson, Ph.C., and J. P. Todd, Ph.D., Ph.C., F.R.I.C.

Dr. H. O. J. Collier took the Chair for the Evening Session, and the following papers were presented and discussed: "Rabbit Responses to Human Threshold Doses of a Bacterial Pyrogen," by J. G. Dare, Ph.D., Ph.C.; "Standards of Pyrogenic Activity," by W. L. M. Perry, M.D.

The Polarographic Determination of Fluoride

Part I. Basic Principles of the Method: Application to the Cathode-ray Polarograph

BY B. J. MACNULTY, G. F. REYNOLDS AND E. A. TERRY

(*Presented, together with Part II*, at the meeting of the Physical Methods Group on Tuesday, April 14th, 1953*)

Modern interest in traces of fluoride has revealed the need of more sensitive methods of fluoride determination. In this paper a sensitive method for determining fluoride polarographically is described. It is based on the depression by fluoride of the polarographic step given by the reduction of the aluminium - Solochrome Violet R.S. complex. The step depression is shown to be linearly related to the amount of fluoride down to 0·001 μg per ml. The use of this method with the cathode-ray polarograph is described.

DISCOVERIES made in the past twenty years have resulted in a greatly increased use of fluoride and a realisation of the importance of trace quantities of this ion. The researches into dental caries have led to the critical consideration of the safe fluoride concentration in drinking water. The effects of fluoride on animals and human beings have resulted in an increased interest in the fluoride content of fuels because of possible contamination of atmosphere and herbage. Industrially fluoride has found use in catalysts, insecticides and as a food preservative, with the resulting interest in the trace amounts appearing in the final products. The quantity of fluoride in strata and fossils has also been used as an aid to archaeological dating. With the increased interest in fluoride much attention has been paid to its detection and numerous methods of determination have been suggested.

The following is a list of older methods that have some proved usefulness, together with the more promising of the recent methods—

(1) Titration with thorium nitrate.[1,2,3,4,5]
(2) Methods based on the use of alizarin complexes.[6,7,8]
(3) The use of ferric salicylate or sulphosalicylate.[9,10,11]
(4) The use of the thorium - thoron complex.[12]
(5) Fluorescent methods.[13,14]
(6) The use of the aluminium - haematoxylin complex.[15]
(7) The use of the aluminium - Eriochrome cyanine complex.[16,17]

* To be published shortly in *The Analyst*.

Except with samples of potable waters, all methods usually require the prior separation of fluoride by distillation as hydrofluosilicic acid and this usually provides some degree of concentration of the ion.

Although the above methods are adequate for various applications in which very low concentrations of fluoride are encountered and the sample size is limited, even the aluminium - haematoxylin method, which requires 25 ml of sample containing 0·1 μg of fluoride per ml as a minimum, is not wholly satisfactory. An example is the fluoride content of hydrochloric acid, a knowledge of which is required whenever this acid is used as a reagent. It is clear that a more sensitive method of fluoride determination is still required in certain circumstances.

From previous work[13,14,15,16,17] it has been shown that any really sensitive means of determining aluminium would provide a basis for an equally sensitive method for fluoride. The publication by Willard and Dean[18] of a method for the polarographic determination of aluminium down to a concentration of 0·1 μg per ml made it probable that, with this as a basis, a very sensitive method for fluoride could be developed.

Experimental development

Basic principles—

The Willard and Dean method is based on the measurement of the polarographic step produced by the reduction of Solochrome Violet R.S. (the sodium salt of 5-sulpho-2-hydroxy-benzeneazo-2-naphthol), which is known as Pontachrome Violet S.W. in the United States. This compound produces a step with a half-wave potential of about −0·3 volt *versus* the saturated calomel electrode in an acetate buffer solution of pH 4·6. Addition of aluminium to a solution of this dye causes the polarographic step to be reduced in height and a second step to appear at a point about 0·2 volt more negative. Fig. 1 illustrates this effect. The

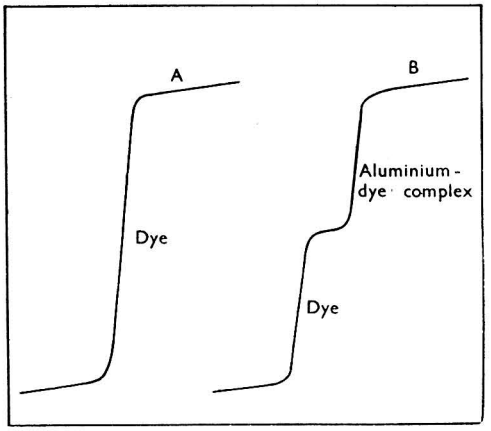

Fig. 1. Curve A: typical step for dye; curve B: typical step for aluminium and dye

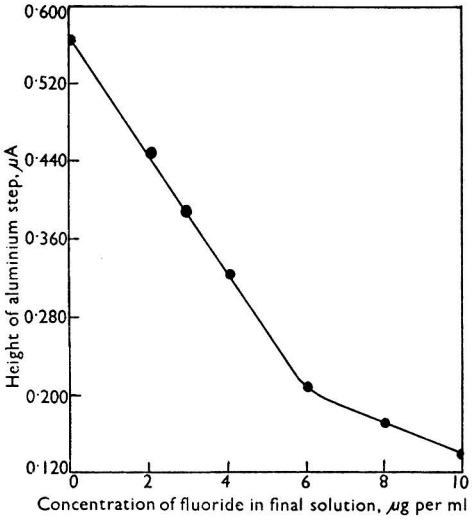

Fig. 2. Relation between height of aluminium step and concentration of fluoride

sum of the height of these two steps is equal to the height of the original step, and the size of the second step is proportional to the concentration of aluminium present.

In their paper Willard and Dean stated that "fluoride forms a more stable complex with aluminium than does the dye" and their figures for fluoride interference indicated an effect of reasonable magnitude.

To decide whether the effect of fluoride was such as to render the development of a method practicable, the following experiment was carried out—

A 2-ml portion of a 0·025-mg per ml solution of aluminium in dilute perchloric acid was neutralised to methyl red with sodium hydroxide and treated with 1 ml of 5 *N* perchloric acid and 5 ml of 2 *N* ammonium acetate. Up to 15 ml of a standard solution (33·3 μg per ml) of fluoride were then added (giving a range of fluoride concentration in the final solution of 0 to 10 μg per ml) followed by 20 ml of a 0·05 per cent. aqueous

solution of dye. The solution was made up to 50 ml, heated at 70° C for 5 minutes, cooled, and an aliquot was deoxygenated and examined polarographically.

The results, which are plotted in Fig. 2, showed a satisfactory straight-line relationship between the height of the aluminium step and the concentration of fluoride up to 6 μg per ml. At this point the gradient changed and another relationship of smaller slope governed the range 6 to 10 μg per ml of fluoride. The significance of this change will be discussed later.

DETERMINATION OF FLUORIDE IN THE RANGE 0·1 TO 0·6 μg PER ml—

First a method was developed for use in the range 0·1 to 0·6 μg per ml, where other methods of determination could be used to check the results. The following experimental technique was used—

To each of a series of 50-ml flasks were added, in solution, 30 μg of aluminium, and then 0, 5, 10, 15, 20 and 25 μg of fluoride to successive flasks. To each flask were added 2·5 ml of 2 *N* ammonium acetate, 0·5 ml of 5 *N* perchloric acid, 2 ml of 0·05 per cent. dye solution and 1·0 ml of proteose peptone (maximum suppressor). The solutions were made up to 50 ml, heated at 70° C for 5 minutes, cooled, and an aliquot taken for polarography. The pH of the solution at the end of this procedure was 4·6.

This technique was the pattern for all succeeding experiments, the concentration of reagents and the pH being altered as desired.

The results were encouraging, but it was considered that the differences between replicates were too great, and it was decided to check whether the pH value of 4·6, at which the experiments were carried out, was the most satisfactory one. Accordingly the depression of the aluminium - dye step resulting from the addition of a standard amount of fluoride was plotted against pH, and the result (Fig. 3) clearly indicated that a pH value of about 3·9 was the most suitable for fluoride determination; all further work was carried out at this value.

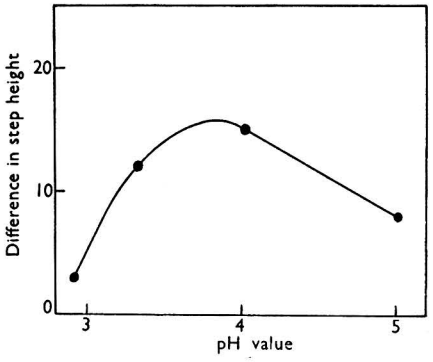

Fig. 3. Effect of change of pH on difference in step height brought about by the addition of 0·2 μg of fluoride per ml

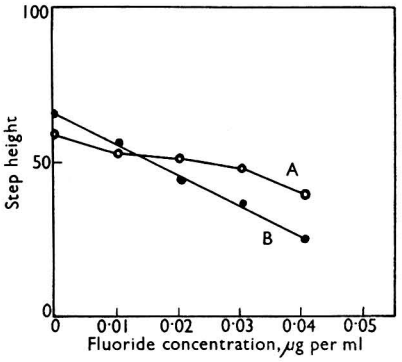

Fig. 4. Effect of time. Curve A: samples examined immediately after heating; curve B: samples examined 20 hours after heating

The experiments at pH 4·6 were now repeated at a pH value of 3·9, and the results in Table I show that the differences between replicates were satisfactorily small.

TABLE I

DEPRESSION OF ALUMINIUM - DYE STEP DUE TO FLUORIDE CONCENTRATIONS OF 0 TO 0·6 μg PER ml AT pH 3·9

Fluoride, μg per 50 ml	Step-height, arbitrary units					
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6
0	72	72	69	72	72	72
5	63	63	65	65	66	65
10	54	56	57	56	56	57
15	50	48	48	50	51	49
20	41	40	39	42	44	42
30	30	29	28	30	32	29

The level of fluoride determination reached at this stage (0·1 to 0·6 μg per ml) was only of the same order as that possible by other methods of analysis, and at the same time the limit set by the use of conventional instruments had almost been reached. The cathode-ray polarograph, an instrument of much greater sensitivity, developed in this department by Davis, Reynolds and Seaborn,[19,20] was therefore used.

THE USE OF THE CATHODE-RAY POLAROGRAPH—

Comparative tests between the cathode-ray polarograph and conventional instruments showed that a similar straight-line relationship between depression of the aluminium - dye step and fluoride concentration (0 to 0·8 μg per ml) existed on both types of instrument.

The method was next tested at a lower level of fluoride concentration, 0·01 to 0·05 μg of fluoride per ml, by the same technique with an approximately tenfold decrease in concentration of reactants. The results obtained by polarography of the solutions immediately showed that, although an effect similar to that expected was produced, the relationship was not strictly linear (Fig. 4). If, however, the solutions were set aside overnight for 16 to 20 hours before polarographic measurement, the relationship became linear and had a slope of the expected order (see Fig. 4). No advantage was gained by allowing the solutions to stand for longer and, in fact, after about 30 hours the magnitude of the fluoride effect began to diminish.

In Table II a number of calibrations on this range of fluoride concentration are shown. As these calibrations were made with different solutions and with material from different sources, and as there were slight variations in the excess of dye present, it is not surprising to find some variation in the recorded step height, although all experiments showed the expected straight-line relationship. Experiments 1 to 6 of Table II were carried out in a water - perchloric acid medium and experiments 7 to 11 in 0·1 *M* perchloric acid made 0·1 *M* with respect to hydrochloric acid.

TABLE II

COLLECTED CALIBRATIONS WITH VARIOUS SOLUTIONS OF REAGENTS AND MATERIALS TO SHOW THE EFFECT OF FLUORIDE ON THE STEP HEIGHT OF A STANDARD ALUMINIUM - DYE SOLUTION

Measurements on cathode-ray polarograph at sensitivity 20 kΩ

	Step height, arbitrary units										
Fluoride, μg per ml	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 8	Expt. 9	Expt. 10	Expt. 11
0	75	65	90	96	84	45	68	62	86	83	77
0·01	—	—	83	84	74	—	61	—	77	74	70
0·02	58	57	75	73	62	37	55	52	73	69	64
0·03	—	—	66	73	—	—	55	—	64	61	58
0·04	47	44	59	68	45	31	47	40	58	55	52
0·05	—	—	51	60	35	—	43	36	52	48	48
0·06	30	36	—	—	—	23	—	—	—	—	—
0·07	—	—	—	—	—	—	—	—	—	—	—
0·08	24	24	—	—	—	16	—	—	—	—	—

Experiments 1 to 6 in water - perchloric acid medium; experiments 7 to 11 in 0·1*M* hydrochloric acid - 0·1*M* perchloric acid medium.

To test the reproducibility, a series of calibrations were carried out by the standardised procedure and with bulked reagents, one or two calibrations being performed daily. The results (Table III) indicate a satisfactory degree of reproducibility. The step heights are measured on a graticule that can be read to half a division, and except at 0·10 μg per ml of fluoride, which is near the limit of this range of concentration, differences do not amount to more than one division (0·02 μg of fluoride).

SUB-MICROGRAM AMOUNTS OF FLUORIDE—

The method was finally extended to deal with sub-microgram amounts of fluoride in the range 0·001 to 0·01 μg per ml.

When the technique previously described was used with a further tenfold decrease in reactants, it was found that, although with bulked reagents satisfactory reproducibility could be obtained, the number of "spoilt" results was too high for the method to be reliable.

It was found that at these low levels of fluoride concentration even AnalaR reagents contained sufficient impurities, such as iron and aluminium, to vitiate the method if they were not removed. A satisfactory method of purifying reagent solutions is by the addition of acetylacetone and extraction of the complexed impurities with chloroform under suitable conditions.

TABLE III

EFFECT OF FLUORIDE ON STEP HEIGHT OF A STANDARD ALUMINIUM - DYE SOLUTION

Measurements on cathode-ray polarograph at sensitivity 20 kΩ: standardised procedure and bulked reagents used

Fluoride, μg per ml	Step height, arbitrary units						
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7
0	52	52	55	51	56	54	53
0·02	49	48	51	47	52	50	49
0·04	45	44	46	43	48	46	45
0·06	41	41	42	39	44	43	42
0·08	37	37	38	35	40	38	38
0·10	33	34	32	33	35	34	34

Difficulties arise from vibration of the mercury pool, when this is used as anode, and it is advisable to substitute a suitably conditioned silver wire. The silver wire is conditioned by applying a potential of 1·5 to 2 volts for 5 minutes between it and a dropping-mercury cathode when both are immersed in chloride solution. This treatment results in the deposition of a thin coating of chloride on the silver wire. In water solutions, when silver wire is used, it is also necessary to add a small amount of a salt (0·12 *N* ammonium chloride) to stabilise the anode potential.

The experimental conditions were also re-examined at this low level of fluoride concentration and it was found that the optimum amounts of aluminium and dye at this level were 6 μg of aluminium and 2 ml of 0·05 per cent. dye solution per 50 ml of stock solution. It was also advisable to keep the amounts of proteose peptone and buffer to a minimum.

A series of calibrations under these new conditions are shown in Table IV. The conditions of the experiments were as follows—

To a series of 25-ml flasks were added respectively 0, 0·1, 0·2, 0·3, 0·4 and 0·5 μg of fluoride and about 10 ml of water. Then 5 ml of aluminium - dye complex solution were added and the volume made up to 25 ml with water. The solutions were heated at 75° ± 5° C for 30 minutes and examined polarographically after 18 to 20 hours.

The complex was made up by taking in a 50-ml flask 6 μg of aluminium (as potassium aluminium sulphate solution) and adding 2 ml of 0·05 per cent. dye solution, 0·25 ml of 0·1 per cent. solution of proteose peptone, 10 ml of buffer (as previously described) and 1 ml of 2 *N* ammonium chloride.

Recoveries of fluoride from water solutions by this technique are included in Table IV.

METHOD

SPECIAL APPARATUS—

A polarograph—The Cambridge photographic instrument is suitable for larger amounts, but a cathode-ray polarograph is required for amounts of fluoride less than 0·2 μg per ml of final solution.

REAGENTS—

The purest obtainable reagents should be used. It is advisable to use redistilled water throughout.

Aluminium solution, 10 μg of aluminium per ml—Dissolve 1·758 g of potassium aluminium sulphate in water, add 10 ml of 60 per cent. perchloric acid, sp.gr. 1·54, and dilute to 1 litre. Take 10 ml of this solution, add 1 ml of 60 per cent. perchloric acid, sp.gr. 1·54, and dilute to 100 ml. It is recommended that this last solution should be freshly prepared at fortnightly intervals.

Ammonium acetate, 2 N.

Aqueous Solochrome Violet R.S. solution, 0·05 per cent.

Sodium fluoride solution, 10 μg of fluoride per ml—Dissolve 0·5525 g of sodium fluoride* in water and dilute to 250 ml. Dilute 1 ml of this solution to 100 ml for use. This solution appears to keep well, but should be freshly prepared at monthly intervals.

Acetylacetone—A 0·1 per cent. v/v solution in water.

Proteose peptone, 0·1 per cent. solution—Dissolve 0·1 g of proteose peptone and 0·2 g of phenol in 100 ml of water.

Buffer solution—To 300 ml of 2 *N* ammonium acetate solution add sufficient 60 per cent. perchloric acid, sp.gr. 1·54, to adjust the pH to 3·66. Add 1 ml of 1 per cent. acetylacetone and extract five times with 25-ml portions of redistilled chloroform, shaking for 2 minutes each time. Discard the used chloroform. Heat the buffer solution so prepared until all smell of chloroform has been removed.

Ammonium chloride, 2 N—Dissolve 32·1 g of ammonium chloride in 300 ml of water. Add 1 ml of 1 per cent. acetylacetone solution and extract with chloroform as described for the buffer solution and heat until all smell of chloroform has been removed.

PROCEDURE FOR RANGE ONE: 5 TO 40 μg OF FLUORIDE IN 50 ml (0·1 TO 0·8 μg PER ml)—

In this range, except for samples such as halogen acids and water, the fluoride is usually separated from the sample by a preliminary distillation as hydrofluosilicic acid by the method of Willard and Winter.

To five 50-ml calibrated flasks add 0, 1·0, 2·0, 3·0 and 4·0 ml of the dilute sodium fluoride solution and sufficient water to bring the volume to just less than 40 ml. In other 50-ml calibrated flasks place 40 ml of the solution or distillate under test, which has been neutralised to methyl red with ammonium hydroxide or acetic acid.

In a 100-ml calibrated flask prepare an aluminium - dye solution as follows—

> To 30 ml of aluminium solution add 25 ml of ammonium acetate solution, 5 ml of proteose peptone solution and 2 ml of ammonium chloride solution. Adjust the pH to 3·9 with 5 *N* perchloric acid against a pH meter, add 20 ml of Solochrome Violet R.S. solution and make up to 100 ml with water.

Add 10 ml of the aluminium - dye solution to each of the 50-ml graduated flasks and make up to the mark with water. Heat the flasks in a bath of water at 70° ± 5° C for 5 minutes and then cool them. (NOTE—It is important that the heating conditions should be the same for all flasks; therefore a bath large enough to take all the flasks at once must be used.)

Place about 4 ml of each solution in polarographic cells, deoxygenate the solutions for 10 minutes and record polarograms at 25° C between −0·2 and −0·9 volt, with the saturated calomel electrode as anode.

A large step should be observed (at about $E_{\frac{1}{2}} = 0{\cdot}4$ volt) followed by a smaller one at approximately 0·1 volt more negative; the first step is due to the reduction of the uncomplexed dye, and the other, which is the step that must be measured, is due to the aluminium - dye complex. Occasionally a further small step occurs after the one due to the aluminium - dye complex. This is caused by impurities such as iron and should be ignored.

Plot a calibration graph of the depression of step height, H, against the fluoride present expressed in micrograms, where $H = H_1–H_2$, $H_1–H_3$, etc., and H_1, H_2, H_3 . . . are the heights of the aluminium - dye step in presence of 0, 1·0, 2·0 . . . micrograms of fluoride. From this calibration graph, which should be linear, the amount of fluoride in the sample can be calculated.

PROCEDURE FOR RANGE TWO: 0·5 TO 2·5 μg OF FLUORIDE IN 25 ml (0·02 TO 0·1 μg PER ml)—

For this and the next range (range three) of fluoride concentration, distillations are carried out with a semi-micro apparatus. The apparatus used is similar to that used by Horton,[12] except that a round-bottomed distillation flask is used and heating is by a small gas flame instead of a heating mantle. It appears that the rate of distillation of fluoride is to some extent governed by the rate at which equilibrium of fluoride compounds between gas and liquid phases in the distillation flask is attained; therefore the largest possible surface area of liquid is desirable. For this reason a round-bottomed flask seems preferable to the inverted-cone type used by Horton. Use of a heating mantle is undesirable, as local hot spots can arise

* The purest available material must be used; that supplied by Baker and Adamson to A.C.S. specification is suitable.

on the sides of the distillation flask above the liquid level and these may result in decomposition of the acid to give products that cause trouble in the subsequent treatment of the distillate. For this reason a small gas burner or an oil-bath is preferred. The use of the semi-micro apparatus permits all the fluoride to be collected in 15 to 20 ml of distillate. This involves some alterations in the total amounts of reagents required and the use of 25-ml in place of 50-ml calibrated flasks. The following modifications of the procedure described for range one are necessary—

(*a*) 25-ml instead of 50-ml calibrated flasks are used.
(*b*) The flasks used for calibration should contain 0·0, 0·5, 1·0, 1·5, 2·0 and 2·5 μg of fluoride. For this, dilute the standard sodium fluoride solution ten times and use 0, 0·5, 1·0, 1·5, 2·0 and 2·5 ml.
(*c*) The aluminium - dye complex solution contains 3 ml of aluminium solution, 2·5 ml of proteose peptone solution, 10 ml of Solochrome Violet R.S. solution, 10 ml of buffer solution (pH 3·66) and 1 ml of 2 *N* ammonium chloride solution. This is made up to 50 ml with water and 5 ml is taken for each flask.
(*d*) The time of heating is made 30 minutes and the solutions must be set aside overnight for 16 to 20 hours before polarograms are recorded.
(*e*) The cathode-ray polarograph must be used, and a silver chloride anode of the straight-wire type is more satisfactory than the saturated calomel electrode.

PROCEDURE FOR RANGE THREE: 0·1 TO 0·5 μg OF FLUORIDE IN 25 ml (0·004 TO 0·02 μg PER ml)—

This range is similar to range two, but the following modifications are necessary—

(*a*) The flasks used for calibration should contain 0·0, 0·1, 0·2, 0·3, 0·4 and 0·5 μg of fluoride.
(*b*) The aluminium - dye complex solution contains 0·6 ml of aluminium solution, 0·25 ml of proteose peptone solution, 2 ml of Solochrome Violet R.S. solution, 10 ml of buffer solution and 1 ml of 2 *N* ammonium chloride solution. This solution is made up to 50 ml and 5 ml is taken for each flask.

POINTS OF SPECIAL IMPORTANCE IN RANGE THREE—

Impurities—One of the chief difficulties encountered at this low level of fluoride concentration is the effect of traces of interfering impurities in reagents. The two reagents used in relatively large amounts, the acetate - perchloric acid buffer and the ammonium chloride, are normally purified sufficiently by extracting the metal acetylacetonates by shaking with chloroform.

If impurities such as iron or aluminium are found to be present in solutions or distillates under examination, they should be removed as follows. To the solution at pH 2 to 4 add 1 ml of 0·1 per cent. v/v acetylacetone in water, neutralise with ammonium hydroxide and extract by shaking with 10 ml of redistilled chloroform. Discard the chloroform layer. Repeat the extraction three more times. Finally, warm the solution to remove traces of chloroform.

Cleaning—The flasks and polarographic cells should be cleaned by rinsing them first with chromic acid, then with tap water, and finally with several changes of distilled water. A better calibration seems to result if, after this initial treatment, the flasks and cells are reserved for use at this range of fluoride concentration only and are cleaned between determinations with hydrochloric acid followed by several rinses with distilled water. Pure acetone can be used for drying purposes or to remove any greasiness.

Ripple—Ripple on the cathode-ray trace diminishes the accuracy with which step heights can be measured and should therefore be kept to a minimum. This can be achieved by using only a very small amount of mercury in the cell, together with a silver chloride anode, and at the same time earthing the water-bath to the cathode-ray polarograph. The best point of attachment of the earth wire varies from day to day and must be determined by trial and error.

Oxygen—The least trace of oxygen upsets the method at this concentration and it is usually necessary to deoxygenate the solutions for 15 to 20 minutes before recording the polarograms.

Results and discussion

The method was tested on solutions of fluoride both directly and after separation of the fluoride by distillation as hydrofluosilicic acid. Results are shown in Table IV.

Table IV

Recovery of fluoride

Direct determination		After separation of fluoride by distillation as H_2SiF_6	
Fluoride added, μg	Fluoride recovered, μg	Fluoride added, μg	Fluoride recovered, μg
5	5·0, 5·5, 5·0	2·2	2·3, 2·3, 2·35
10	10·0, 10·5	6·8	6·7, 6·9, 6·9
15	15·0, 15·5, 15·5	9·6	10·5, 10·5, 10·0, 10·2
20	19·0, 20·0		
0·5	0·5, 0·5	0·7	0·7, 0·8
1·0	1·0, 1·0	1·1	1·0, 1·0, 1·15
1·5	1·4, 1·5	2·2	2·1, 2·1, 2·3
2·0	1·9, 2·0	2·25	2·15, 2·25
2·5	2·3, 2·3		
0·05	0·06	0·1	0·12, 0·12, 0·10, 0·10
0·10	0·10	0·2	0·19, 0·20, 0·16
0·15	0·13		
0·20	0·19, 0·19, 0·22	0·3	0·28, 0·25
0·40	0·39, 0·39, 0·40, 0·38	0·4	0·36

These results are satisfactory, but determinations should always be carried out in duplicate, as spurious results occur fairly frequently.

The method has the advantage of permitting smaller amounts of fluoride to be determined than hitherto; on the lowest range a concentration of 0·004 μg per ml in the final solution gives a step-height depression of the aluminium - dye step of about 7 units and, as the ripple on the cathode-ray trace is of the order of 1 unit under good working conditions, the limit of detection of the present method is of the order of 0·001 μg per ml in the final solution. Nevertheless, the full potentialities of the method have not as yet been realised. The determination depends at present on the measurement of a small depression of a relatively large combined dye and aluminium - dye complex step. In amplifying the step to obtain maximum sensitivity, the limit is set by the size to which the step can be magnified, whereas it is only the step depression that is of importance in the determination of fluoride. The full sensitivity can only be reached when a differential (the term "subtractive" is to be preferred[21]) method of polarography is used. Such a method is now being investigated in the authors' laboratories.

The necessity of allowing the solutions of fluoride and aluminium to stand for a long time (18 to 20 hours) on ranges one and two is another instance of the slowness with which dilute solutions of aluminium and fluoride reach equilibrium, particularly when the amount of aluminium is greater than the amount of fluoride. This phenomenon has been noticed previously by ourselves[15] and by Brosset,[22] and examination of many papers on fluoride determination indicates that this phenomenon is general.

Some applications of this method to the determination of fluoride in specific substances will be given in Part II of this series of papers.

Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper and to Mr. A. S. Nickelson for his interest and encouragement.

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Identification of Hydroxylamine and Hydrazine by Paper Chromatography

BY J. M. BREMNER

A method for the separation and identification of microgram quantities of hydroxylamine and hydrazine is described. It involves paper chromatography with acidic solvents and identification by R_F values and by the colours produced with picryl chloride and other reagents.

ALTHOUGH hydroxylamine has been reported or postulated to be an intermediate in several important biological processes such as the fixation of atmospheric nitrogen by *Azotobacter* and leguminous bacteria and the oxidation of ammonia to nitrite by *Nitrosomonas*, little attention has been given to the development of methods for its identification. The method of detection generally used is that of Blom,[1] which is based on the oxidation of hydroxylamine to nitrite by iodine in acetic acid and detection of the nitrite by means of the colour reaction with sulphanilic acid and 1-naphthylamine. This highly sensitive test has been investigated and improved by Csaky,[2] who showed that interference due to nitrous, hyponitrous and nitrohydroxamic acids, which give the colour reaction without pretreatment with iodine, could be obviated by an acid hydrolysis procedure. He found, however, that although only hydroxylamine gave the test after hydrolysis, it was partly or totally destroyed if any of these interfering substances were present in the test solution during hydrolysis. Apart from this, Blom's test is open to criticism on the grounds that it is an indirect procedure based on the detection of an oxidation product of hydroxylamine and that when used to detect hydroxylamine in complex biological fluids it may be subject to interference from compounds not previously tested. The need for a method of confirming results obtained by Blom's procedure became apparent during the course of an investigation into the reaction between lignin and nitrous acid and in studies of the oxidation of ammonia to nitrite by soil micro-organisms. The object of the work reported in this paper was to develop a method by which microgram quantities of hydroxylamine could be identified after separation from interfering substances. Because a method based on paper chromatography appeared to hold the most promise, the behaviour and detection of hydroxylamine on paper chromatograms run in various solvents have been investigated. Paper chromatography of hydrazine, which gives some of the colour and reducing reactions of hydroxylamine, has also been studied.

Experimental

Apparatus and procedure—

Hydroxylamine was used in the form of a 0·1 *M* aqueous solution of its hydrochloride and hydrazine as a 0·1 *M* aqueous solution of its sulphate. Two to four microlitres of the solutions containing 6 to 13 μg of hydroxylamine or hydrazine were used for paper chromatography, which was carried out by the ascending technique. Whatman No. 4 filter-paper was used throughout. Preliminary runs were made in Pyrex boiling tubes with 2·5-cm × 25-cm strips of paper, the technique being essentially that described by Rockland and Dunn.[3] Further runs with promising solvents were made in glass filtrate jars with cylinders of paper 33 cm high made from sheets by stapling them so that their edges did not overlap (Bray, Thorpe and White[4]). The chromatograms were developed until the solvent front had travelled a distance of 20 to 30 cm. Depending on the solvent used, the time required was 3 to 24 hours. After development, the chromatograms were dried at room temperature and sprayed with the detecting reagents described below. R_F values reported were determined at 18° ± 2° C.

Detecting reagents—

Picryl chloride—After being sprayed with a 1·5 per cent. w/v solution of picryl chloride in ethanol, the chromatograms were allowed to dry and then exposed to ammonia vapour. Hydroxylamine was revealed by a bright orange colour and hydrazine by an intense blue colour.

Ammoniacal diacetylmonoxime - nickel salt reagent—This was prepared according to Feigl.[5] Hydroxylamine on chromatograms sprayed with this reagent was revealed by a red colour; hydrazine gave no colour.

Modified Csaky[2] reagents—For reasons given below the reagents used by Csaky[2] for the detection of hydroxylamine in solution were modified as follows for use as sprays. Reagent (*a*), which was a mixture of 20 ml of a 1·3 per cent. w/v solution of iodine in glacial acetic acid and 20 ml of a 1·0 per cent. w/v solution of sulphanilic acid in 30 per cent. v/v aqueous acetic acid, was prepared immediately before spraying. Reagent (*b*) was a 0·1 per cent. w/v solution of N-(1-naphthyl)-ethylenediamine dihydrochloride in water. The two reagents were used successively. Hydroxylamine on chromatograms sprayed with these reagents was revealed by a red colour; hydrazine gave no colour. Both hydroxylamine and hydrazine could be detected as weak yellow spots on spraying with reagent (*a*), but the yellow spot of hydrazine disappeared and that of hydroxylamine became red on subsequent spraying with reagent (*b*).

Ninhydrin—After being sprayed with a 0·1 per cent. w/v solution of ninhydrin in chloroform, the chromatograms were placed in an oven at 105° C for 5 minutes. Hydrazine was revealed by a yellow colour: hydroxylamine gave no colour.

Solvents—

The solvents used were of the purest quality available. In two-phase systems, the non-aqueous phase was used as solvent and the chromatograms were prepared in an atmosphere provided by the aqueous phase. The *n*-butanol - acetic acid - water mixture (Table I) was of the same composition as that used by Partridge[6] for the separation of sugars. Before use this mixture was shaken thoroughly and allowed to stand for 3 days (Bate-Smith and Westall[7]). The other solvent mixtures were prepared immediately before use. Formic acid was used in the form of the 90 per cent. w/w AnalaR grade solution supplied by Hopkin and Williams Ltd.

Results and discussion

The R_F values of hydroxylamine and hydrazine in various solvents are recorded in Table I. They were obtained with one batch of filter-paper and represent the means of several determinations. The values were found to vary from batch to batch of filter-paper but to be reasonably constant within individual batches.

Neither hydroxylamine nor hydrazine could be detected on chromatograms run in basic solvents and their detection on chromatograms run in neutral solvents, such as water-saturated *n*-butanol, was achieved only when fairly large amounts (20 to 30 μg) of materials were used. Of the acidic solvents tested, only mixtures containing hydrochloric acid were

found to be satisfactory for paper chromatography of both hydrazine and hydroxylamine, the alcohol - hydrochloric acid series of solvents listed in Table I giving small well-defined spots with either compound. Solvents containing acetic or formic acid proved much less useful. Some, *e.g.*, *n*-butanol - formic acid - water, or *tert.*-amyl alcohol - acetic acid - water, gave extensive tailing of both compounds. In others, *e.g.*, benzene - acetic acid - water, or chloroform - formic acid - water, no movement occurred. However, a few gave only slight tailing of hydroxylamine; the R_F values are shown in Table I.

TABLE I

R_F VALUES OF HYDROXYLAMINE AND HYDRAZINE ON WHATMAN NO. 4 FILTER-PAPER AT ROOM TEMPERATURE

Solvent*	Hydroxylamine	Hydrazine
Methanol (70), 6 *N* hydrochloric acid (30)	0·53	0·34
Methanol (80), 90 per cent. w/w formic acid (15), water (5)	0·67	Streak
Ethanol (70), 6 *N* hydrochloric acid (30)	0·41	0·22
Ethanol (70), 90 per cent. w/w formic acid (20), water (10)	0·54	Streak
n-Propanol (70), 6 *N* hydrochloric acid (30)	0·36	0·21
n-Butanol	0·12	0·00
n-Butanol (40), acetic acid (50), water (10)	0·23	0·07
n-Butanol (50), 2 *N* hydrochloric acid (50)	0·18	0·10
n-Butanol (50), 6 *N* hydrochloric acid (50)	0·51	0·42
n-Butanol (70), 6 *N* hydrochloric acid (30)	0·31	0·19
tert.-Butanol (70), 6 *N* hydrochloric acid (30)	0·40	0·20
Diethyl ether (50), 90 per cent. w/w formic acid (50)	0·36	Streak

* The figures indicate the percentage v/v of each component.

Butanol and butanol - acetic acid mixture are unsatisfactory solvents, as it is difficult to detect less than 20 μg of hydrazine or hydroxylamine on their chromatograms. The R_F values in these solvents are presented in Table I only because they show that the weak yellow spots of identical R_F value noted by Bremner and Kenten[8] on chromatograms of hydrazine and hydroxylamine run in butanol (R_F 0·02) and butanol - acetic acid (R_F 0·12) and sprayed with ninhydrin must have been due to some impurity. This is confirmed by the finding that only hydrazine gives a yellow colour reaction with ninhydrin and that its detection by this reagent on chromatograms run in butanol or butanol - acetic acid is possible only if a large amount of hydrazine is present.

It was found that R_F values in the alcohol - hydrochloric acid series of solvents could be varied considerably by altering the proportion of alcohol to acid. With mixtures of *n*-butanol and 6 *N* hydrochloric acid, for example, the R_F value of hydrazine can be increased from 0·15 to 0·42, and that of hydroxylamine from 0·24 to 0·50, by increasing the proportion of 6 *N* hydrochloric acid from 25 to 50 per cent. With mixtures of *n*-butanol and 12 *N* hydrochloric acid, the R_F value of hydroxylamine can be increased from 0·08 to 0·76 by increasing the proportion of 12 *N* hydrochloric acid from 10 to 67 per cent., whereas the R_F value of hydrazine is unaffected by this change, being less than 0·1 in either mixture.

Although complete separation of hydroxylamine and hydrazine can be achieved by the use of any of the alcohol - 6 *N* hydrochloric acid solvent mixtures described in Table I, the time required varies considerably with the alcohol used. With methanol or ethanol 2 to 4 hours is sufficient, but with *tert.*-butanol 20 to 24 hours is required.

No attempt was made to determine the lower limit of sensitivity for the detection of hydroxylamine or hydrazine by the methods described, but 0·5 μg of hydrazine and 3 μg of hydroxylamine can be readily detected by picryl chloride on chromatograms run in the solvents containing hydrochloric acid, and 2 μg of hydroxylamine can be detected on these chromatograms by the ammoniacal diacetylmonoxime - nickel salt reagent or the modified Csaky reagents. According to Kul'berg and Cherkesov,[9] as little as 0·005 μg of hydrazine and 0·2 μg of hydroxylamine can be detected by spot tests on filter-paper by the picryl chloride - ammonia technique. Feigl[5] has reported that the ammoniacal diacetylmonoxime - nickel salt reagent will detect 1 μg of hydroxylamine in spot tests on filter-paper. This method of detecting hydroxylamine would appear to be highly specific, since it requires condensation between hydroxylamine and diacetylmonoxime with formation of diacetyldioxime (dimethylglyoxime) and reaction of this last with ammoniacal nickel salt solution to form red, slightly soluble nickel dimethylglyoxime. The reagents used in Csaky's method

of detecting hydroxylamine,[2] which involves oxidation of hydroxylamine to nitrite by iodine in acetic acid solution in the presence of sulphanilic acid followed by destruction of the excess of iodine with sodium arsenite and coupling of the diazotised sulphanilic acid with 1-naphthylamine, were found to be effective in revealing hydroxylamine when sprayed successively on developed chromatograms. It was found, however, that it was unnecessary to remove the excess of iodine with sodium arsenite when sprays of these reagents were used and that a more intense red colour was produced when N-(1-naphthyl)-ethylenediamine was used as coupling agent in place of 1-naphthylamine (Shinn[10]). The modified technique described above was therefore adopted. As a reagent for the detection of hydrazine, ninhydrin is considerably less sensitive than picryl chloride. Of the four detecting reagents used, only picryl chloride reveals both hydrazine and hydroxylamine. It has also been used for the detection of tertiary pyridine bases on paper chromatograms.[11]

Although it has not been possible to supply direct proof of non-interference from all substances that may be present in biological material, the paper chromatographic method of identifying hydroxylamine described above appears to be highly specific, as it is based not only on R_F values in various solvents, but also on the colours produced with three different chromogenic sprays.

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The Determination of Glucosamine

By R. Belcher, A. J. Nutten and Miss C. M. Sambrook

The colorimetric method for the determination of glucosamine based on the reactions with acetylacetone and *p*-dimethylaminobenzaldehyde has been systematically examined. Several improvements have been made in the method, which has been applied to the determination of glucosamine in N-acetyl-α-methylglucosaminide and heparin. The method has been adapted for use with commercially available instruments.

The colorimetric determination of glucosamine (2-amino-2-deoxyglucose) based on the reactions with acetylacetone and *p*-dimethylaminobenzaldehyde has given erratic results in the hands of several workers. A systematic examination of the method has been made to trace the sources of error, and several improvements have been introduced. The improved procedure has been applied to the determination of glucosamine in N-acetyl-α-methyl-glucosaminide and heparin and has been adapted for use with commercially available instruments.

The determination of glucosamine in heparin is of importance in structural studies; it is one of the few materials available containing glucosamine to which the method described in this paper can be applied. As these materials have to be broken down by acid hydrolysis, a study of the conditions of hydrolysis has been made and the results may be a guide in biochemical work of a similar kind.

The most widely used procedure for the determination of glucosamine is that of Elson and Morgan.[1] After preliminary treatment of the glucosamine test solution with an alkaline

solution of acetylacetone, addition of a reagent containing *p*-dimethylaminobenzaldehyde produces a red colour, which is compared with standards. That the method is not completely satisfactory is evident from the numerous variations of it described in the literature. Nilsson,[2] for example, carried out the reaction with acetylacetone at 95° C instead of at 100° C, while Sørensen,[3] who followed Nilsson's procedure closely, claimed that the optimum pH for the reaction with acetylacetone was 9·5. Sørensen also reported that the intensity of the colour remained constant for a "considerable length of time," although Boyer and Fürth,[4] who used acetic acid as a solvent and heated the solution after the addition of *p*-dimethylaminobenzaldehyde, stated that the colour faded within 20 minutes. Blix[5] claimed that satisfactory results were attained when the concentrations of both acetylacetone and alkali were increased, and when the reaction was carried out at 96° C in a closed vessel. The capriciousness of the method has been further emphasised by Horowitz, Ikawa and Fling,[6] who suggested that the intensity of the colour developed depended not only on the conditions of heating with acetylacetone, but on the size of the vessel in which the reaction was carried out.

After addition of the *p*-dimethylaminobenzaldehyde, Palmer, Smyth and Meyer[7] incubated the solution at 37° C for 30 minutes before measuring the colour. Schloss also favoured incubation, but at 30° C for 24 hours.

There is some difference of opinion about the interferences in the method. Elson and Morgan found that certain sugars and amino-acids caused no appreciable interference, but Boyer and Fürth reported considerable interference from these substances. Sideris, Young and Krauss[9] observed that glycine and lysine in the presence of reducing sugars produced a measurable amount of colour, while Bendich and Chargaff[10] found that glycine in the presence of glucose produced a slight, but not negligible, colour. Blix stated that interference from sugars and amino-acids was appreciable, but claimed to have eliminated the interference by modifying the acetylacetone reagent. Immers and Vasseur[11] also obtained measurable colours from sugars and amino-acids in admixture when they were treated according to the procedure of Elson and Morgan.

In view of the conflicting information about the determination of this biologically important compound, we have carried out a complete systematic examination of the procedure of Elson and Morgan in an attempt to place the method on a sound practical basis. The results of this examination, including the development of a satisfactory method for the determination of glucosamine, are described below.

In order that an accurate and quantitative analysis of glucosamine in mucopolysaccharides can be made, it is essential that the breakdown of the protein - carbohydrate complex with acid be complete. Certain of these mucopolysaccharides, in particular, heparin, are very stable towards acids, and drastic conditions are necessary for the hydrolysis. Little exact information is available about the conditions required for the complete hydrolysis of heparin, and no information is available as to the behaviour of glucosamine itself during a hydrolysis. Accordingly, a lesser, but nevertheless important, consideration during the present investigation was the establishment of conditions that would lead to the complete hydrolysis of heparin without affecting the glucosamine molecule.

The compound N-acetyl-α-methylglucosaminide contains linkages similar to those present in certain materials containing glucosamine, *e.g.*, hyaluronic acid, and the conditions necessary for the hydrolysis of this compound were therefore established.

Experimental

Reaction of glucosamine with acetylacetone—

(*i*) *Effect of temperature*—When the reaction mixture was heated to 100° C, the colours produced were more intense than at lower temperatures, although the temperature coefficient of the reaction was small between 95° and 100° C. A thermostatically controlled heating bath was not necessary to maintain the optimum temperature. The development of the colour was negligible when the reaction mixture was heated to temperatures less than 60° C.

(*ii*) *Effect of heating time*—A heating time of 10 minutes gave almost linear graphs when optical densities were plotted against concentrations of glucosamine. The development of the colour was incomplete when heating times of less than 10 minutes were used, and non-linear curves were given when heating was continued for more than 10 minutes.

(*iii*) *Effect of concentration of acetylacetone*—The intensities of the colours obtained decreased when the amount of acetylacetone added was increased or decreased, but a small amount of latitude was obviously permissible in the preparation of the reagent (see Fig. 1).

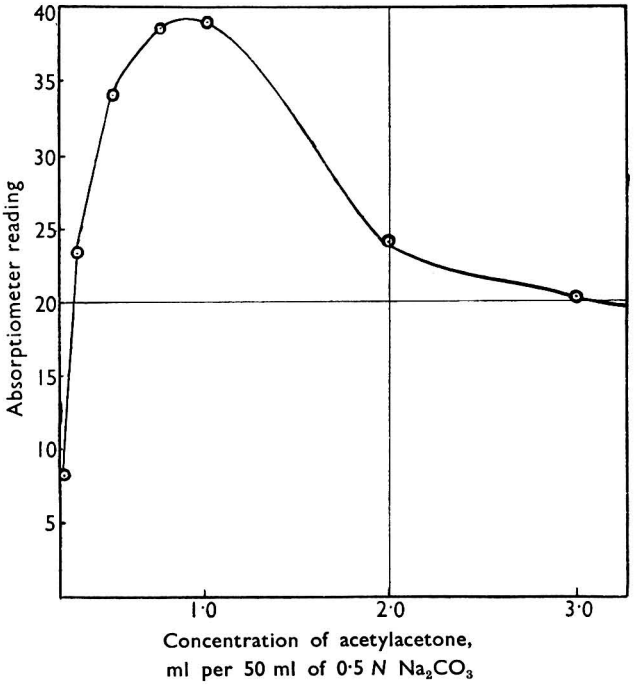

Fig. 1. Effect of concentration of acetylacetone

(*iv*) *Effect of pH*—As the concentration of sodium carbonate in the reagent was increased, the intensities of the colours produced also increased slightly. At concentrations of sodium carbonate greater than *N*, however, some difficulty was encountered in redissolving the precipitate initially formed on adding ethanol to the reaction mixture. The concentration coefficient was small, but by no means negligible. The optimum pH for the reaction was 9·8 (that is, with the reagent *N* with respect to sodium carbonate), but the development of colour was slight in solutions adjusted to this pH with sodium hydroxide.

REACTION WITH *p*-DIMETHYLAMINOBENZALDEHYDE—

(*i*) *Effect of temperature*—When the temperature of the solution was raised after addition of the *p*-dimethylaminobenzaldehyde reagent to the mixture from the reaction between glucosamine and acetylacetone, a considerable increase in the intensity of the colour occurred. The maximum intensity was attained by heating to 75° C. Above this temperature, serious losses of ethanol occurred by evaporation. When the ethanol was replaced by glacial acetic acid and the solution was heated to above 75° C, development of the colour was markedly decreased, probably because of partial destruction of the chromogen.

(*ii*) *Effect of heating time*—The heating time was varied between 5 minutes and 3 hours. Approximately linear graphs were obtained after heating for 10 minutes, but the optical densities were not entirely reproducible. Results were somewhat more reproducible after heating for 20 minutes, and after heating for 30 minutes readings were reproducible and somewhat higher, indicating an increase in sensitivity. This increase in sensitivity, although small, continued up to a heating time of 60 minutes, after which measurements became uncertain and not reproducible. Accordingly, a heating time of 30 minutes was taken as the most suitable.

(*iii*) *Effect of concentration of* p-*dimethylaminobenzaldehyde*—Reagents were prepared in which the concentration of *p*-dimethylaminobenzaldehyde was (*a*) half and (*b*) twice that recommended by Elson and Morgan. When the half-strength reagent was used, the colours

did not develop to the full intensity, and the graphs plotted were neither linear nor reproducible. When the double-strength reagent was used, the development of the colour was considerably enhanced (the reagent itself did not show selective light absorption), but the graphs plotted were again neither linear nor reproducible. Accordingly, the reagent recommended by Elson and Morgan was retained.

(*iv*) *Effect of pH*—The work of other authors appeared to indicate that close control of pH was not essential (the pH of the final solution in the procedure of Elson and Morgan is approximately 0·3). The preparation of the *p*-dimethylaminobenzaldehyde reagent is by no means precise, especially with respect to the concentration of hydrochloric acid, for commercial hydrochloric acid may be anywhere between 10 and 12 *N*. Experiments showed that optimum development of colour occurred when the pH of the final solution was between 0·62 and 0·35 (that is, when the reagent was between 8 and 10·8 *N* with respect to hydrochloric acid). In more weakly acidic solutions, the intensities of the colours decreased with decrease in pH until, at pH 2·9, no development of colour occurred. Considerable latitude was permissible, therefore, in the preparation of the reagent, and it was found convenient to replace the ethanol in the reagent by concentrated hydrochloric acid.

(*v*) *Effect of ethanol*—The amount of ethanol added to the mixture from the reaction between glucosamine and acetylacetone is normally 6 ml. In the present work, 5 ml of ethanol were added, as it had been found convenient to adjust the volume of the mixture of glucosamine and acetylacetone before reaction to 4 ml (Elson and Morgan specify 3 ml) with distilled water. This ensured that the sides of the reaction tube were washed down thoroughly.

When the ethanol was gradually replaced by water, the intensity of the colour developed decreased with increasing amounts of water. The results showed that the amount of ethanol added was not critical, as the graph of optical density against concentration of ethanol reached a maximum after the addition of 4·5 ml of ethanol and remained at this maximum when further amounts of ethanol were added.

Interferences—

Under the conditions of the method developed as a result of the studies described above, the following substances, both alone and in combinations of two and three, caused no interference, even when added in twentyfold excess over the amount of glucosamine present: arabinose, galactose, glucose, mannose, glucuronic acid, alanine, arginine, glycine, lysine, phenylalanine, proline, serine, tryptophan and tyrosine.

The majority of the solutions of glucosamine encountered in practice are produced by acid hydrolysis of naturally occurring materials. However, when solutions of combinations of the above substances with glucosamine were heated under reflux with 4 *N* hydrochloric acid for 24 hours, no interference was found. Even a twentyfold excess of these substances could be tolerated. It was concluded, therefore, that the greatly increased sensitivity of the method, and hence the appreciable dilution of the foreign substances likely to be present, was mainly responsible for eliminating the slight interferences reported by other workers.

Absorption curve—

A 0·05-mg sample of glucosamine was treated according to the method described later and the optical density of the solution was measured over the wavelength range 400 to 700 mμ with a photo-electric quartz spectrophotometer No. SP 500 (Unicam Instruments Ltd., Cambridge). The absorption curve is shown in Fig. 2. The maximum optical density occurs at 512 mμ, in agreement with the findings of Schloss. Only one maximum occurs in the curve, and this would appear to indicate that other chromogens formed in the reaction between acetylacetone and glucosamine have either been destroyed during the "after heat" or have been converted into the chromogen responsible for the colour.

Sensitivity and accuracy—

A measurable absorption was given by 0·5 μg of glucosamine by the method described later.

Colour densities were measured with three instruments, (*a*) an E.E.L. test-tube colorimeter (Evans Electroselenium Ltd.) with No. L 204 tubes and a No. 623 green filter, (*b*) a Spekker photo-electric absorptiometer, model H 760 (Hilger and Watts Ltd.) with a 2-cm

cell and Ilford No. 604 filter, and (*c*) a photo-electric quartz spectrophotometer, No. SP 500 (Unicam Instruments Ltd.) with a 2-mm cell and a wavelength of 512 mμ.

A comparison of the results and the errors incurred with these three instruments is included in Table I. As the colours produced were stable for at least 12 hours, the colour

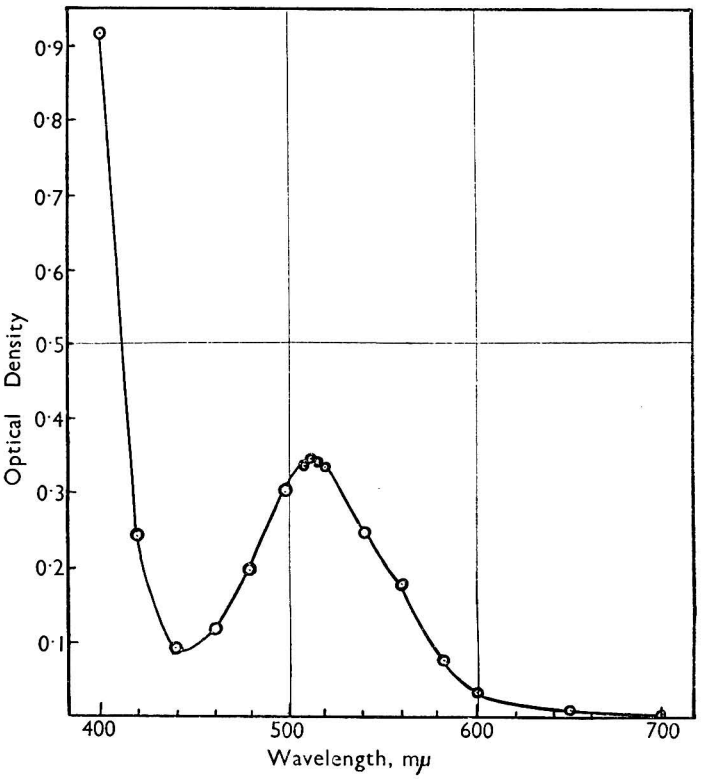

Fig. 2. Absorption curve for 0·05 mg of glucosamine treated by the procedure described

measurements were made successively on the three instruments, and the amounts of glucosamine were read from the standard graphs previously prepared for each instrument.

TABLE I

COMPARISON OF RESULTS WITH DIFFERENT INSTRUMENTS

Glucosamine present, μg	Glucosamine found			Error		
	E.E.L., μg	Spekker, μg	Unicam, μg	E.E.L., μg	Spekker, μg	Unicam, μg
10	10·1	10·0	10·0	+0·1	+0·0	+0·0
20	19·6	20·2	19·9	−0·4	+0·2	−0·1
30	30·8	30·3	30·2	+0·8	+0·3	+0·2
40	40·8	39·7	40·2	+0·8	−0·3	+0·2
50	49·2	50·3	49·8	−0·8	+0·3	−0·2
60	58·6	59·4	60·3	−1·4	−0·6	+0·3
70	71·3	69·5	69·8	+1·3	−0·5	−0·2
80	81·7	80·7	80·4	+1·7	+0·7	+0·4
90	88·1	90·7	90·3	−1·9	+0·7	+0·3
100	101·2	99·1	99·5	+1·2	−0·9	−0·5

BLANKS—

Although the sensitivity of the method had been considerably increased, the blanks were negligible, a measurable absorption not being found.

Applications of the method

Preliminary experiments were designed to determine the effect of heating glucosamine with hydrochloric acid of various concentrations. No visible decomposition of the molecule occurred when glucosamine solutions were heated for 15 hours with N and 2 N hydrochloric acid. When the solution was neutralised with sodium hydroxide and the amount of glucosamine was determined, the recoveries were quantitative.

When 3 N and 4 N solutions of hydrochloric acid were used, the solutions darkened somewhat; when the solution in 4 N acid was cooled, a brown deposit was formed. Quantitative recoveries of glucosamine were, however, obtained at both these concentrations, which indicated that the part of the glucosamine molecule responsible for the colour reaction with *p*-dimethylaminobenzaldehyde was not affected by the hydrochloric acid.

Hydrolysis of N-acetyl-α-methylglucosaminide—

When solutions of N-acetyl-α-methylglucosaminide in 2 N hydrochloric acid were heated under reflux, hydrolysis was complete in 2 hours. The rate of hydrolysis was initially rapid, a 50 per cent. recovery of glucosamine being obtained after 30 minutes. When the hydrolysis was completed, the solution was neutralised with sodium hydroxide and an aliquot was taken for the analysis.

Hydrolysis of heparin—

When solutions of heparin (sodium salt) in 4 N hydrochloric acid were heated under reflux for 12 hours, hydrolysis was not complete, although consistent recoveries of glucosamine were obtained. Hydrolysis was assumed to be complete after 15 hours, as no increase in the amounts of glucosamine recovered occurred after further heating. It is of interest to compare the analytical results for the present sample of heparin with those reported by Wolfrom *et al.*[12] on a different sample (Table II).

Table II

Comparison of results of present work with results by Wolfrom *et al.*[12]

	Sodium heparinate used by Wolfrom *et al.*[12]	Sodium heparinate used in present work	
Nitrogen, per cent. ..	2·6	Dumas: 1·82, 1·86, 1·90, 1·90 Kjeldahl: 1·90, 1·86, 1·85, 1·88	Mean 1·86
Sulphur, per cent. ..	11·66	Parr bomb: 10·87, 10·78, 10·90 Combustion: 10·87, 10·92, 10·94	Mean 10·88
Glucosamine, per cent. ..	25 (±1·7)	18·8, 18·9, 18·7, 18·8 (12 hours) 21·1, 21·1, 21·2 (15 hours) 21·2, 21·3, 21·2 (20 hours)	

The ratios of sulphur to nitrogen for the two samples are in close agreement, and the amounts of glucosamine found agree with the values for nitrogen. It is also of interest to note that the accuracy of the method used by Wolfrom and his co-workers for the determination of glucosamine (the method of Elson and Morgan) is considerably less than the accuracy claimed for the method described in this paper.

Method

Materials—

Acetylacetone—Twice distil commercial acetylacetone and collect the fraction boiling at 139° to 140° C and store it in a dark, well-stoppered bottle.

p-*Dimethylaminobenzaldehyde*—Recrystallise AnalaR *p*-dimethylaminobenzaldehyde three times from ethanol to give pale yellow plates, m.p. 73° C.

Sodium carbonate—Dry AnalaR sodium carbonate at 110° C for 2 hours and store it in well-stoppered bottles.

REAGENTS—

Acetylacetone solution—Dissolve 1 ml of acetylacetone with shaking in 50 ml of *N* sodium carbonate. The reagent must be freshly prepared every 24 hours, and when not in use must be kept in a refrigerator at 0° C.

p-*Dimethylaminobenzaldehyde solution*—Dissolve 0·8 g of *p*-dimethylaminobenzaldehyde in 60 ml of concentrated hydrochloric acid (AsT grade).

PROCEDURE—

Measure 1 ml of a neutral glucosamine solution (containing between 10 and 100 μg of glucosamine hydrochloride) into a 20-ml graduated tube* constricted between the 9 and 10-ml graduation marks, as shown in Fig. 3. Add 1 ml of acetylacetone solution and adjust the volume to 4 ml with a fine jet of distilled water, washing down the sides of the tube. Immerse the tube in a bath of water at 100° C, the level of the water in the bath being just higher than the level of the liquid in the tube. The neck of the tube should project a few

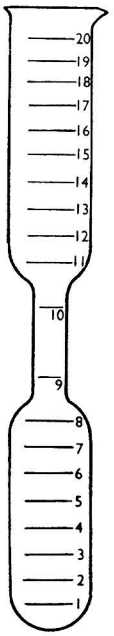

Fig. 3. Special graduated tube

inches above the top of the water-bath. After heating the tube for 10 minutes, remove it from the bath and allow it to cool to room temperature. Adjust the volume of the solution to 9 ml with ethanol and place the tube in a thermostatically controlled bath of water at 75° C for 5 minutes. Then add 1 ml of *p*-dimethylaminobenzaldehyde solution, remove the tube and shake it thoroughly. Replace the tube in the bath so that the water comes just above the level of the liquid in the tube, and heat it at 75° C for 30 minutes with occasional shaking. Remove the tube from the bath and allow it to cool to room temperature. Adjust the volume of the liquid in the tube to 10 ml with ethanol and measure the optical density of the solution. Compare the readings with those for known amounts of glucosamine. Determine the amount of glucosamine from a standard graph prepared for the particular instrument used.

Note—When solutions obtained from the hydrolysis of materials containing glucosamine are neutralised, it is recommended that the solution be made just alkaline with a solution of sodium hydroxide, then just acid with hydrochloric acid and finally neutralised exactly with a solution of sodium carbonate to pH 7, a pH meter being used.

* Supplied by W. J. George and Becker, Birmingham.

SUMMARY

A systematic and critical examination has been made of the method of Elson and Morgan for the determination of glucosamine. A satisfactory method has been described for the determination of glucosamine in the presence of certain sugars and amino-acids; it is based on the experimental observations made during the examination. The optimum conditions have been established for the two main reactions involved; in particular, the effects of varying pH, concentration of the reagents, temperature and time of heating on the reactions have been investigated, and information has been obtained about the latitude permissible in the attainment of the optimum conditions.

Amounts of glucosamine (as hydrochloride) between 10 and 100 μg were determined, but smaller amounts can be determined, if necessary.

The behaviour of glucosamine on heating with hydrochloric acid has been examined, and conditions have been established for the hydrolytic breakdown of N-acetyl-α-methylglucosaminide and heparin.

We wish to thank Professor M. Stacey, F.R.S., and Dr. A. B. Foster, who asked us to examine this problem. One of us (C. M. S.) is grateful to the Colonial Products Research Council for financial assistance.

This paper describes work carried out by Miss C. M. Sambrook in partial fulfilment of the requirements of the degree of M.Sc. in the University of Birmingham.

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The Absorptiometric Determination of Traces of Copper in Highly Purified Water

By E. N. JENKINS

A method is described for the determination of copper in highly purified water at the low levels significant in aluminium corrosion. Traces of copper down to 0·001 p.p.m. can be measured absorptiometrically as cupric diethyldithiocarbamate after a single extraction from a 500-ml sample into 10 ml of chloroform in the presence of a citrate buffer and of disodium ethylenediaminetetra-acetic acid. No interference results from the presence of 1 p.p.m. of common cations or of the sulphide or cyanide anions.

A simple modification is described to obviate the interference of bismuth and antimony.

The biological importance of the presence or absence of traces of copper in natural and treated waters has stimulated the development of sensitive analytical methods for this element. For example, the United States Public Health Service recommends that the copper concentration in drinking waters should not exceed 0·2 parts per million,[1] and reliable methods have long been established for water purity control at this level, for example, the use of the reagent sodium diethyldithiocarbamate to give an intense yellow colloid[2] that can be stabilised by the addition of gum arabic. Such methods reach the limit of their sensitivity at about 0·01 p.p.m. Determination of copper at or below this level becomes necessary in the control of the "pitting" corrosion of aluminium by soft waters, which is greatly influenced by the copper content of the water and is said to persist at copper concentrations as low as 0·02 p.p.m.[3] Analyses at such low levels have normally been preceded by concentration of the sample by evaporation, with the risk of contamination or losses.

Experimental

It was decided to attempt the solvent extraction of copper as a coloured complex from 500-ml water samples containing 0·01 p.p.m. or less of the metal into the minimum volume of immiscible organic solvent, and to measure the optical density of the entire extract in a cell of maximum path length. The basic problems involved are discussed below.

Choice of reagent—

Reagents currently used for the colorimetric analysis of traces of copper include dithizone, sodium diethyldithiocarbamate, $\alpha\alpha'$-diquinolyl, 2:9-dimethylphenanthroline and bis*cyclo*hexanone oxalyldihydrazone. The extinction coefficients of the appropriate copper complexes (that for dithizone being corrected for the extinction loss of the excess of reagent) are, respectively, 24,700 at 5080 A,[4] 14,200 at 4320 A,[5] 5490 at 5470 A,[6,7] 7950 at 4545 A,[8] and 16,500 at 5950 A[9] at a concentration of one gram-atom of combined copper per litre. These reagents are known to form solvent-extractable copper complexes, except the last-named reagent, for which no solvent extraction procedure has been published. Diethyldithiocarbamate was chosen for the present application because of its availability, high sensitivity and freedom from potential difficulties occasioned by the intense and unstable colour of the excess of the dithizone reagent, which would remain in the organic phase under the acid conditions normally used for the cupric dithizonate extraction.

Extraction of copper at low organic - aqueous phase ratios—

Many organic solvents are reported in the literature as being suitable for the extraction of cupric diethyldithiocarbamate. The volume of solvent used is commonly equal to that of the aqueous sample, which contains not less than 0·1 p.p.m. of copper.

Sandell[10] recommends that if it is necessary to determine copper in concentrations below 0·1 p.p.m., the sample should be concentrated by evaporation after acidification. Experiments with radioactive copper have shown, however, that an almost complete extraction of copper by chloroform can still be achieved even at very unfavourable concentrations and phase ratios.

A solid dilution of pure cupric oxide in sucrose was irradiated for 12 hours in the Harwell pile (BEPO) at a slow neutron flux of about 10^{12} neutrons per sq. cm per second, and dissolved in dilute hydrochloric acid to give a stock solution containing 1 μg of copper per ml "labelled" with radioactive ^{64}Cu (12·9-hour half-life, 0·57-MeV beta-particles, 0·60-MeV positrons, 1·35-MeV gamma-radiation). The beta - gamma activity of the stock solution, and also of "labelled" 500-ml water samples before and after solvent extraction of the copper complex, were determined on 10-ml aliquots with a type M6 (liquid sample, skirt type) Geiger tube. Decay measurements indicated a half-life of about 12 hours, and the copper tracer was considered to be of satisfactory radiochemical purity.

Next, 50 ml of 20 per cent. w/v tri-ammonium citrate solution and 0·100 g of sodium diethyldithiocarbamate were added to 500 ml of doubly glass-distilled water, and the mixture was brought to pH 9·3 by dropwise addition of concentrated ammonium hydroxide. Traces of copper present as impurity in the reagent were removed at this stage by an extraction with 25 ml of pure chloroform (the first extraction). An addition of 5 μg of "labelled" copper was made (*i.e.*, the equivalent of a 500-ml sample containing 0·01 p.p.m.), and the aqueous solution was again extracted for 5 minutes with 25 ml of chloroform. Radiochemical assay of the residual aqueous phase demonstrated a satisfactory removal of copper. Further 5-μg additions of copper were made, followed each time by a 5-minute extraction with a small volume of chloroform. The results presented in Table I show conclusively that 0·01 p.p.m. of copper is extracted efficiently at very low organic - aqueous phase ratios (down to 1 to 100). It also appears that the successive extractions did not seriously deplete the reagent.

TABLE I

EXTRACTION OF LABELLED COPPER FROM AMMONIUM CITRATE SOLUTION

Extraction sequence	Volume of chloroform, ml	Beta - gamma assay of aqueous phase (corrected for background)		Extraction, %
		before extraction, counts per minute	after extraction, counts per minute	
Second	25	320	13	96
Third	25	339	12	96·5
Fourth	10	331	11	96·5
Fifth	10	359	13	96
Sixth	5	355	21	94

By way of interest, a further series of extractions was carried out under acid conditions (0·100 g of reagent in 500 ml of 0·1 *N* hydrochloric acid). It was again shown that copper was extracted efficiently (to the extent of 98 per cent.) into 10 ml of chloroform, provided that, in these conditions, fresh reagent was added before each extraction.

The extractions in which labelled copper was used, described above, were not carried out in the presence of ethylenediaminetetra-acetic acid. Subsequently the procedure was modified by the addition of this powerful complexing agent to the sample before the addition of reagent and citrate buffer, in order to mask the interference from cobalt and nickel. Haque, Brown and Bright[5] state that the reaction of copper with diethyldithiocarbamate is a little slower in the presence of the ethylenediaminetetra-acetate ion, but that solvent extraction gives complete recovery of the copper within 1 minute. Jewsbury[11] investigated the time necessary for full colour development from copper with the carbamate reagent, in the aqueous phase, in the presence of the same complexing agent. He reports that the full colour was not developed within 30 minutes at a 25-μg level of copper; the rate of reaction would be expected to decrease still further as the level of copper falls and as the concentration of complexing agent increases. The concentrations of reagent and complexing agent used by Jewsbury are identical with those present in the aqueous phase when the procedure recommended in this paper is used. The following tests were carried out with 10 μg of copper and five times the normal concentration of complexing agent, *i.e.*, in conditions where Jewsbury's results would lead one to expect a prolonged inhibition of full colour development in the aqueous phase. The results show strikingly that, when solvent extraction is used, the copper colour is formed completely within 5 minutes, and that there is no necessity for a preliminary reaction period. Four pairs of samples containing about 0·02 p.p.m. of copper were analysed; the precise copper level varied from one pair to the next. Analysis of one member of each

pair was carried out by the method described in the procedure, but with the increased quantity of complexing agent noted above. Before a solvent extraction was made on the other member of the pair, the aqueous phase containing the reagent and complexing agent was allowed to stand at room temperature for 30 to 45 minutes. The results are shown in Table II.

TABLE II

EXTRACTION OF COPPER IN THE PRESENCE OF COMPLEXING AGENT

	Optical density			
Immediate extraction	0·742	0·753	0·639	0·661
Delayed extraction	0·758	0·780	0·630	0·664

It remained to be shown that the colour intensity per microgram of copper was unchanged in the presence of the complexing agent. This was established as follows. One series of very dilute copper solutions, containing 2 to 6 μg of copper, was extracted in the presence of the same buffer and reagent concentrations as in the tracer experiments described above, but with only 50 ml of aqueous phase (to enhance even further the extraction of copper). The second series, also containing 2 to 6 μg of copper, was extracted from 560 ml of aqueous phase containing 0·50 g of disodium ethylenediaminetetra-acetic acid in addition to the normal citrate buffer and dithiocarbamate reagent. The extractions and subsequent optical density measurements for the first and second series were carried out as detailed in the procedure described below. In the absence of complexing agent, values of 0·065, 0·054, 0·059, 0·062 (mean: 0·060) were obtained for the optical density per microgram of copper. Corresponding values in the presence of complexing agent were 0·0595, 0·061, 0·0605 (mean: 0·060).

It is clear that, under the conditions of reaction and subsequent extraction used in the present work, the ethylenediaminetetra-acetate ion in no way interferes with the extraction of cupric diethyldithiocarbamate.

CHOICE OF ABSORPTION CELL AND FILTER—

When it was established that copper could be extracted efficiently from 500-ml samples into, say, 10 ml of chloroform, it was necessary to measure the colour intensity on a Spekker absorptiometer under optimum conditions. The ratios of path length to working volume of the Hilger cells normally supplied with this instrument are unduly low, while the Hilger micro-cell holds only 0·5 ml. The Ogal type CLZ 3 cell, micro modification, supplied by The Tintometer Ltd., has a path length of 4 cm and a working volume of 8 ml, and has proved suitable for our purpose. The internal width of the cell, 6 mm, allows the passage of a sufficiently wide beam to give ample sensitivity for detecting the balance point. Adapters were specially constructed to hold two such cells on the carriage of a Spekker model H 503 absorptiometer. The Ilford spectrum filter No. 601 (transmission band around 4300 A) is suitable for optical density measurements on the yellow cupric diethyldithiocarbamate (maximum absorption at 4320 A).

MISCELLANEOUS PRELIMINARY TESTS—

Before systematic extractions of known amounts of copper were undertaken to set up a calibration graph, two further possible variables were studied.

Colour fading—Sandell[10] reports quite a rapid fading of the yellow colour of a solution of copper diethyldithiocarbamate in carbon tetrachloride exposed to diffuse daylight. The coloured complex corresponding to 30 μg of copper was extracted and made up to 50 ml with chloroform. The chloroform solution was allowed to stand in a stoppered volumetric flask in diffuse April daylight on our normal working bench. Samples were withdrawn at intervals and centrifuged, and the optical density was measured. The results show the colour to be stable over a period of an hour under conditions of moderate diffuse daylight, the initial optical density reading of 0·381 being unchanged after this time.

Reagent storage—Previous workers have used a 0·1 per cent. aqueous stock solution of the reagent. It was convenient to use a 1·0 per cent. stock solution for the present work, and the efficiency of this reagent after varying periods of storage in the dark in a refrigerator at 0° C was investigated. The colour intensities of extracts containing approximately 10 μg of copper were compared when (*a*) fresh reagent and (*b*) stored reagent was used. A

reagent solution stored for 8 days gave a normal colour intensity (an optical density of 0·65 for 10 μg of copper). After storage for 15 days, the full colour did not develop; the optical density was only 0·60. It was concluded that the reagent solution should be freshly prepared at weekly intervals.

Procedure

Reagents—

Ammonium citrate solution, 20 per cent. w/v—Dissolve 173 g of analytical grade citric acid monohydrate in 800 ml of glass-distilled water and slowly add 165 ml of analytical grade ammonium hydroxide, sp.gr. 0·880. Cool and make up the volume to 1000 ml in a cylinder.

Sodium diethyldithiocarbamate solution, 1 per cent. w/v—Dissolve 1·00 g of pure sodium diethyldithiocarbamate, *e.g.*, the B.D.H. product, in 100 ml of glass-distilled water. Store it in a refrigerator. Prepare freshly each week.

Disodium ethylenediaminetetra-acetic acid (solid)—A pure grade, *e.g.*, the B.D.H. product.

Chloroform—Analytical reagent grade.

Apparatus—

Spekker absorptiometer, model H 503.
Ilford spectrum filters No. 601.
Ogal 4-cm cells No. CLZF 3 (micro), made by The Tintometer Ltd.
Adapter to hold a pair of cells on the Spekker absorptiometer carriage.
Clinical centrifuge with 12-ml centrifuge tubes.

Procedure—

In a 500-ml water sample dissolve with shaking 0·50 g of disodium ethylenediaminetetra-acetic acid and set the solution aside for 30 minutes. The addition of the complexing agent not only prevents interference by cobalt and nickel, but also ensures that the copper is present as a true solution of a stable ionic species.

In the meantime, take 50 ml of buffer solution and 10 ml of carbamate reagent in a 500-ml conical separating funnel, add 10 ml of chloroform, insert the stopper and shake for 2 minutes. Reject the chloroform layer. Repeat the extraction with a further 10 ml of chloroform, again rejecting the lower layer. Extract for a third time with 10 ml of chloroform, shaking for 5 minutes. Allow the layers to settle, dry the stem of the funnel with filter-paper, and deliver the extract into a 12-ml centrifuge tube.

Centrifuge at maximum speed for 2 minutes. Withdraw the chloroform layer with a 25-ml syringe-type pipette with a drawn-out tip. Avoid the inclusion of any droplets of the aqueous phase. Deliver the solution into a clean dry 4-cm absorptiometer cell.

Measure the optical density of this extract without delay, using the Spekker absorptiometer with the tungsten lamp, No. H 503 heat filters and Ilford spectrum filters No. 601. Set the drum at 1·00 with pure chloroform in the reference cell. The blank measured at this stage (normally an optical density of 0·00 to 0·02) allows for the contribution of traces of copper as impurity in the solvent, together with any possible contribution due to extracted dithiocarbamic acid.

Remove any droplets of chloroform from the bottom or stop-cock of the separating funnel. Add the 500-ml sample of water containing the complexing agent. Shake with 10 ml of chloroform for 5 minutes. During the extraction, the chloroform layer decreases in volume to about 8 ml. Allow the chloroform layer to settle, dry the stem with filter-paper, and deliver the extract into a dry 10-ml calibrated flask. Wash out the remaining drops of extract by successive additions of 0·5 ml of chloroform, swirling the funnel. Continue until exactly 10·0 ml has been collected. If the copper content of the sample exceeds 15 μg, make the extract volume up to 25 or 50 ml.

Transfer the extract to a 12-ml centrifuge tube. Centrifuge, separate, and measure the optical density on the Spekker absorptiometer as described above for the solvent blank.

The optical density reading must be corrected for the solvent blank and divided by the calibration factor (an optical density of about 0·065 per microgram of copper) found by experiments with standard copper solutions.

The quotient is further divided by the sample volume in millilitres (normally 500 ml) to find the copper concentration in parts per million.

Calibration results

A bulk stock of nominally high-purity water, produced by a mixed-bed de-ionisation process, was available. Disodium ethylenediaminetetra-acetic acid was added (1·0 g per litre) and well mixed, and 500-ml aliquots were taken. To each aliquot was added an appropriate volume of a standard copper solution freshly prepared from analytical-reagent grade copper sulphate pentahydrate. Each sample was analysed for copper as described above. The results of two calibration series with different bulk samples of de-ionised water are presented in Table III.

Table III

Calibration data

Series	Copper added, μg	Optical density of solvent blank	Optical density of sample	Optical density of sample corrected for blanks	Optical density per microgram
1	0	0·022	0·130	0·000	
	2·50	0·020	0·282	0·154	0·062
	7·50	0·020	0·629	0·501	0·067
	12·5	0·011	0·972	0·853	0·068
	17·5	0·013	1·294*	1·173	0·067
2	0	0·00	0·121	0·000	
	2·50	0·00	0·280	0·159	0·064
	7·50	−0·02	0·570	0·471	0·063
	12·5	−0·02	0·931	0·830	0·066
	17·5	0·00	1·272*	1·151	0·066
				Mean	0·065

* Calculated for an extract volume of 10 ml from optical density measurements made on 25 ml.

The results, corrected for the solvent blanks and for the water blanks, indicate a linear relation between copper content and optical density up to 17·5 μg of copper. Preliminary experiments indicated that linearity persisted up to at least 80 μg. The results also give figures of 0·003 p.p.m. and 0·004 p.p.m. for the copper contents of the two bulk samples of de-ionised water. Doubly glass-distilled water normally had no detectable copper content; this served also to demonstrate the absence of any significant copper impurity in the B.D.H. disodium ethylenediaminetetra-acetic acid used.

The limit of detection of the method is set by the small solvent blank of optical density 0·00 to 0·02, which recurs even on repeated extraction and is variable from day to day. A 500-ml sample containing 0·001 p.p.m. of copper would give a final optical density of 0·033 higher than the blank.

Interferences

Factors likely to interfere with the quantitative extraction of copper as the cupric diethyldithiocarbamate salt include the following.

Metals reacting with the reagent to decrease its effective concentration and in some instances to form extractable salts with appreciable absorptions at 4320 A.

Anions that mask the copper and inhibit the colour reaction.

Oxidising or reducing agents, which oxidise the reagent, *e.g.*, to the disulphide, or which reduce the copper to the cuprous state, which forms a salt of relatively weak colour intensity. These factors are considered below.

Other metals—

Elements reported to form coloured or white insoluble salts with diethyldithiocarbamic acid in alkaline solution[12] include copper (cuprous or cupric), antimony, bismuth, cadmium, cobalt, chromium (as dichromate), gold, indium, iron (ferrous or ferric), lead, manganese, mercury, nickel, platinum metals, silver, tellurium, thallium (thallous or thallic) and zinc. These salts will usually be extractable into chloroform; often the chloroform solutions are coloured and have a certain degree of absorption within the range of the Ilford 601 filter (3800 to 4800 A). Iron, cobalt, nickel and bismuth are usually considered to present the most serious colour interferences to the determination of copper in alkaline solution. The

first element in the ferric state can be masked by the citrate ion. Cobalt and nickel can be formed into complexes with dimethylglyoxime[10] or with ethylenediaminetetra-acetic acid.[5,13]

The present work included tests on a series of 500-ml water samples each containing 5 mg of a potentially interfering element. Each sample was analysed for copper by the method given above, and the results were corrected for the blank obtained on the pure water sample. No significant interfering colour (*i.e.*, the equivalent of less than 1·0 μg of copper) was found in the tests with cadmium, cobalt, chromium (as dichromate), chromium (as the chromic cation), ferric and ferrous iron, lead, manganese (as manganous), manganese (as permanganate), nickel, stannous tin, thallium (as thallous ion) and zinc. It should be noted that the inhibition of cobalt and nickel interferences depends on the addition of complexing agent *before* the reagent. Preliminary experiments in which the nickel or cobalt solutions were added to a mixture of citrate buffer, reagent and complexing agent showed only a partial reduction in the intensity of the extracted colour. Kinetic effects are probably operative, *i.e.*, a cobalt or nickel diethyldithiocarbamate salt, possibly in colloidal suspension, may react only slowly with the complexing agent. In a similar manner, cobalt and nickel sulphides and dithizonates are resistant to decomposition by dilute acid.[14]

Very minor interferences, equivalent to less than 10 μg of copper, were detected with 5 mg of silver or of mercury[11]. For each metal these small interferences were confirmed by duplicate experiments.

For all metals studied and listed above, complete recoveries of 10 μg of added copper were achieved from the residual aqueous phases in a further extraction.

Bismuth and antimony give extractable yellow compounds with the reagent in the procedure described above. The elimination of these interferences is discussed later.

Masking by anions—

We have already established that the strong complexing of traces of copper by 0·003 *M* disodium ethylenediaminetetra-acetic acid at pH 9 does not in any way inhibit the subsequent extraction of this metal as the diethyldithiocarbamate. In view of this fact, it seems extremely unlikely that traces of anions in general will mask the colour reaction, with the possible exceptions of cyanide and sulphide, which form a very stable complex anion and a very insoluble salt, respectively. Amounts of cyanide or sulphide from 1 to 10 p.p.m. were added to a series of 500-ml water samples containing 0·020 p.p.m. of copper. The final mixtures were weakly alkaline, pH 9·5 to 11. Some of the mixtures were allowed time to age before the normal addition of 500 mg of disodium ethylenediaminetetra-acetic acid, followed by addition of the mixture to the copper-free buffer - reagent mixture and final extraction with 10 ml of chloroform. The optical density measured on the centrifuged chloroform extracts (made up to 10·0 ml) are presented in Tables IV and V.

Table IV

Interference by cyanide in copper extraction

Cyanide, p.p.m.	0·0	1·0	5·0	10·0	1·0	5·0
Ageing	None	None	None	None	1 day	1 day
Optical density	0·637	0·634	0·302	0·306	0·632	0·256
		0·622		0·145		

Table V

Interference by sulphide in copper extraction

Sulphide, p.p.m.	0·0	1·0	10·0	5·0	10·0
Ageing	None	None	None	1 day	1 day
Optical density	0·637	0·630	0·631	0·626	0·591

It is clear that up to 5 p.p.m. of sulphide and 1 p.p.m. of cyanide do not inhibit the full extraction of 0·01 p.p.m. of copper. Larger amounts of cyanide produce a marked inhibition.

Oxidising and reducing agents—

Operations in the atomic energy industry may expose water, *e.g.*, cooling water, to heavy ionising radiations, when traces of hydrogen peroxide will be formed. Excessive amounts

of peroxide may be expected to oxidise the diethyldithiocarbamate reagent; interference with the copper determination could then arise either through depletion of the reagent or possibly through the oxidation products. In a series of five experiments, 5 to 26 p.p.m. of hydrogen peroxide were added to 500 ml of pure water containing 10 μg of copper and 0·50 g of complexing agent. After being mixed and set aside for at least 1 hour, the samples were analysed for copper in the standard manner. The results, shown in Table VI, establish that concentrations of peroxide up to 26 p.p.m. do not seriously interfere.

TABLE VI

INFLUENCE OF HYDROGEN PEROXIDE

Hydrogen peroxide, p.p.m. ..	0	5·2	5·2	26	26
Corrected optical density ..	0·636	0·646	0·663	0·650	0·663

The effect of traces of hydroxylamine hydrochloride, which might reduce copper to the cuprous state to give a less intensely coloured diethyldithiocarbamate, was examined in a similar fashion. The results presented in Table VII show that 1 p.p.m. of hydroxylamine hydrochloride does not seriously interfere.

TABLE VII

INFLUENCE OF HYDROXYLAMINE

Hydroxylamine hydrochloride, p.p.m. ..	0·0	1·0	1·0	1·0
Corrected optical density	0·636	0·640	0·630	0·610

REMOVAL OF BISMUTH INTERFERENCE

Bismuth and antimony interference persists even in the presence of the complexing agent. Experiments in which bismuth nitrate solution was added to 560 ml of a copper-free (pre-extracted) solution of reagent, buffer and complexing agent, after which the usual extraction was carried out, gave a consistent ratio of about 20 parts of bismuth equivalent to 1 part of copper. Preliminary experiments with antimony gave a ratio of 120 to 1. Attempts at masking the bismuth with 8-hydroxyquinoline-8-sulphonic acid, or its 7-iodo derivative, were not successful. Though the antimony and bismuth are still extracted into the chloroform, it may yet be possible to separate them from the copper by a suitable back-extraction. Gleu and Schwab[12] infer that macro amounts of antimony and bismuth do not give a precipitate with sodium diethyldithiocarbamate from 1·0 *N* sodium hydroxide solutions. This suggested an attempt to remove these elements from the chloroform extract by back-extraction with an equal volume of 1·0 *N* sodium hydroxide solution.

For these experiments, 500 μg of antimony or bismuth were added to a copper-free solution of the reagent, buffer and ethylenediaminetetra-acetic acid, and extracted into 10 ml of chloroform. The extract was separated and made up to 50·0 ml, 10-ml aliquots from which were then subjected to various back-extraction treatments in a 100-ml separating funnel, and the optical densities of the residual chloroform layers were compared (Table VIII).

TABLE VIII

BACK-EXTRACTION OF BISMUTH FROM CHLOROFORM SOLUTION

Aqueous solution used for back-extraction	None	10 ml of carbonate-free 1·0 *N* sodium hydroxide	25 ml of carbonate-free 1·0 *N* sodium hydroxide	Two extractions with 10 ml of carbonate-free 1·0 *N* sodium hydroxide	10 ml of 1·0 *N* sodium hydroxide (analytical grade)	10 ml of carbonate-free 0·1 *N* sodium hydroxide	10 ml of 0·5 *N* sodium hydroxide + 0·5 *N* sodium carbonate
Optical density of residual chloroform layer, in replicate experiments	0·308 (mean)	0·057 0·059 0·046 0·049	0·039	0·009	0·074 0·138 0·151 0·055	0·242	0·125

A single back-extraction with an equal volume of carbonate-free 1·0 *N* sodium hydroxide removes 80 to 85 per cent. of the bismuth colour, a second extraction bringing the over-all

figure up to 97 per cent. A single preliminary experiment with antimony showed a 90 per cent. removal of the colour. The importance of maintaining a very high pH value during the back-extraction is obvious from the variable results obtained with freshly prepared solution of analytical grade sodium hydroxide and from the poor results with 0·1 *N* sodium hydroxide (even in the absence of carbonate) and with a mixture of 0·5 *N* sodium hydroxide and 0·5 *N* sodium carbonate. The time of shaking during extraction did not appear to be critical; the yellow colour of the chloroform layer disappeared almost immediately and excellent results were achieved after shaking for only 1½ minutes. It may be estimated that 0·5 mg of added bismuth will show only the equivalent of 0·7 μg of copper after a double back-extraction.

The copper diethyldithiocarbamate in chloroform solution completely resists the back-extraction with 1·0 *N* sodium hydroxide, as shown by the following results: 20 μg of copper in an extract made up to 50 ml gave an optical density of 0·258. After back-extraction, the optical density was 0·272 and 0·272 in duplicate experiments.

Modified procedure in the presence of antimony or bismuth

Follow the normal procedure, described above, to the point where the chloroform extract has been made up to exactly 10 ml in a calibrated flask. Shake the extract for 3 minutes in a 100-ml separating funnel with an equal volume of carbonate-free 1·0 *N* sodium hydroxide solution (conveniently prepared by passing a 4 per cent. w/v solution of analytical-reagent grade pellets through a short column of a strong-base anion-exchange resin, such as De-acidite FF, and rejecting the first fraction). Separate the chloroform layer. The efficiency of bismuth (and probably also of antimony) removal can be increased from 80 to 85 per cent. to 97 per cent. by a second treatment of the chloroform solution with an equal volume of alkali.

Centrifuge the residual chloroform extract and measure the optical density as previously described.

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Micro-determination of Acetaldehyde as its 2:4-Dinitrophenylhydrazone

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A colorimetric method for the determination of acetaldehyde is described. Formation of the 2:4-dinitrophenylhydrazone in aqueous solution is followed by quantitative extraction into carbon tetrachloride. It has been found that direct addition of ethanolic sodium hydroxide to the carbon tetrachloride extract produces a strong red colour, which can be measured absorptiometrically. Perchloric acid has been used in the preparation of the 2:4-dinitrophenylhydrazine reagent; this has the advantage that the hydrazine is much more soluble and also that carbon tetrachloride extracts less of the unchanged reagent. Quantities down to 5 μg per 20-ml sample can be estimated with satisfactory precision. Acetaldehyde can be determined in the presence of pyruvic acid.

2:4-Dinitrophenylhydrazine has been extensively used as a reagent for the quantitative determination of carbonyl compounds. Of particular interest from a microchemical point of view are those methods in which a colorimetric technique is used.

The determination of keto-acids with this reagent has been studied by several authors.[1] The keto-acid 2:4-dinitrophenylhydrazone, which is formed in an aqueous system, is extracted with an organic solvent, *e.g.*, ethyl acetate, benzene or chloroform, followed by separation from the 2:4-dinitrophenylhydrazones of aldehydes and ketones by extraction into aqueous sodium carbonate. The red colour produced by addition of sodium hydroxide to the carbonate extract is measured absorptiometrically.

For aldehydes and ketones various procedures have been reported. Greenberg and Lester[2] have determined acetone by measuring the yellow colour of the 2:4-dinitrophenylhydrazone after its extraction into carbon tetrachloride. A method for the determination of formaldehyde, described by Stein and Weiss,[3] involves a further extraction of the 2:4-dinitrophenylhydrazone from the carbon tetrachloride with aqueous sodium hydroxide; the strong red colour of the alkaline layer fades rapidly to a constant yellow, which is then measured.

The red colour formed when acetaldehyde 2:4-dinitrophenylhydrazone is treated with alkali is very intense, and it appeared that, for colorimetric purposes, this would be more sensitive than either the yellow colour of the hydrazone in carbon tetrachloride or the yellow colour produced when the aqueous alkaline solution is allowed to fade. It has been found that if *ethanolic* sodium hydroxide is added to acetaldehyde 2:4-dinitrophenylhydrazone dissolved in carbon tetrachloride the deep red colour produced is relatively stable: this observation has been made the basis of a sensitive method, described below, for the determination of small quantities of acetaldehyde. Recently, Lappin and Clark[4] have reported a method for the determination of various carbonyl compounds that also makes use of the red colour, in this case produced by the addition of methanolic potassium hydroxide to the 2:4-dinitrophenylhydrazones in methanol solution.

Method

Reagents—

All reagents used should be of recognised analytical quality.

2:4-Dinitrophenylhydrazine solution—Dissolve 0·25 g of the base in 100 ml of 30 per cent. v/v perchloric acid.

Ethanolic sodium hydroxide—A 0·1 *N* solution in absolute alcohol.

Carbon tetrachloride—Wash the laboratory grade solvent three times with water. Further purification is unnecessary.

Standard—Use pure acetaldehyde 2:4-dinitrophenylhydrazone.

APPARATUS FOR COLORIMETRY—

The optical densities can be measured in a Spekker photo-electric absorptiometer, a 4-cm cell and Ilford No. 603 blue-green filter being used.

PROCEDURE—

To a 20-ml aliquot, containing between 5 and 35 μg of acetaldehyde, add 5 ml of the 2:4-dinitrophenylhydrazine reagent. Set it aside for 30 minutes at room temperature, and then extract the solution by shaking it vigorously with 20 ml of carbon tetrachloride for 1 minute. Extract the aqueous layer a second time with 5 ml of carbon tetrachloride, shaking for 15 seconds. Transfer the combined tetrachloride extracts to a 50-ml calibrated flask, add 2 ml of ethanolic sodium hydroxide and immediately make up to the mark with ethanol. Measure the optical density of the red solution within 10 minutes after the addition of the alkali. Perform a blank with each determination.

CALIBRATION—

Prepare a standard solution of acetaldehyde 2:4-dinitrophenylhydrazone in carbon tetrachloride. Dilute various amounts of this solution to 25 ml with carbon tetrachloride so that the aliquots each contain between 25 and 180 μg of the hydrazone. Add to each 2 ml of ethanolic sodium hydroxide and make up to 50 ml with ethanol. Measure the colour within 10 minutes.

NOTES ON THE METHOD

THE BLANK—

The value of the blank can be largely attributed to the 2:4-dinitrophenylhydrazine extracted by the carbon tetrachloride. The extent of this extraction has been found to be dependent on the pH of the aqueous phase, the time of shaking, and also on the presence, in either the aqueous solution or the carbon tetrachloride, of certain substances, *e.g.*, alcohols, that are miscible with both water and carbon tetrachloride.

Perchloric acid, rather than hydrochloric acid, has been used in the preparation of the 2:4-dinitrophenylhydrazine reagent. This has the advantage that the hydrazine is much more soluble in the stronger acid[5] and also that carbon tetrachloride extracts less of the unchanged reagent. Preliminary washing of the carbon tetrachloride with water appreciably reduces the blank.

Under the conditions described above, the blank values were kept to a minimum and usually corresponded to drum readings of between 0·05 and 0·1.

FORMATION AND EXTRACTION OF ACETALDEHYDE 2:4-DINITROPHENYLHYDRAZONE—

The formation of the hydrazone at room temperature was found to be complete within 20 minutes. In order to attain complete extraction of the hydrazone in the time stated (1 minute), it is essential to shake sufficiently vigorously to ensure, during this operation, complete emulsification of the aqueous and organic phases.[6]

COLOUR DEVELOPMENT—

Addition of alcohol to the carbon tetrachloride extract gives a homogeneous solution, suitable for colorimetric measurement.

The intensity of the red colour varies both with the strength of the added alkali and also with the ratio of carbon tetrachloride to ethanol in the final solution. The influence of these variables has not been extensively studied; good reproducibility is attained, however, if the standard procedure is adhered to exactly. The colour of the final solution has been found to be quite stable up to a period of 15 minutes, but thereafter it slowly fades to a strong orange-yellow.

RESULTS

The calibration graph constructed from acetaldehyde 2:4-dinitrophenylhydrazone deviates from linearity when the solution contains more than 200 μg per 20-ml sample. The effective working range is from 25 to 180 μg per 20 ml (corresponding to drum readings of up to 0·60).

On application of the recommended method to standard acetaldehyde solutions (the aldehyde used being three times distilled and purified via the ammonia derivative[7]), it has been found that, under the conditions described above, there is complete conversion to the hydrazone and quantitative extraction by the carbon tetrachloride. Some results are presented in Table I. The over-all error involved in the method does not exceed ± 3 per cent.

TABLE I

DETERMINATION OF ACETALDEHYDE IN AQUEOUS SOLUTION BY THE PROPOSED METHOD

Aldehyde added, μg per 20 ml ..	6·5	12·8	13·0	25·1	31·5	32·6
Aldehyde found, μg per 20 ml ..	6·4	12·8	13·2	25·9	32·1	33·0

Calibration can also be effected with acetaldehyde as standard, but the 2:4-dinitrophenylhydrazone, being crystalline and stable, is more suitable.

The method is not specific for acetaldehyde, and could possibly be used also for the determination of other carbonyl compounds.

Acetaldehyde can readily be determined in the presence of pyruvic acid because the interfering keto-acid 2:4-dinitrophenylhydrazone can be removed by extraction with aqueous sodium carbonate. The carbon tetrachloride extracts are shaken with 10 ml of 10 per cent. sodium carbonate, the organic layer is removed and then treated as described in the method. It has been established that no acetaldehyde 2:4-dinitrophenylhydrazone is lost in the sodium carbonate treatment.

The proposed method has been used successfully in a study of the effects of X-rays on aqueous solutions of lactic acid[8] and of ethanol,[9] when small quantities of acetaldehyde are formed, together with other products. Lactic acid (0·2 M), pyruvic acid (10^{-3} M), ethanol (0·2 M) and hydrogen peroxide (10^{-3} M), present in the analysed solutions, did not interfere.

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The Simultaneous Determination of Cadmium and Magnesium with Disodium Ethylenediaminetetra-acetate

By E. G. BROWN and T. J. HAYES

The simultaneous determination of cadmium and magnesium by titration with a solution of disodium dihydrogen ethylenediaminetetra-acetate containing zinc sulphate is described. Selective control of the pH at 6·8 permits cadmium alone to be titrated, and magnesium is subsequently titrated at pH 10 in the same solution. Solochrome Black is used as indicator for both titrations. The molecular ratio of magnesium to cadmium must not be greater than unity for quantitative results, but a large excess of cadmium in the presence of magnesium can be satisfactorily determined. A theory is postulated for the reaction.

The determination of cadmium by titration with disodium dihydrogen ethylenediaminetetra-acetate (Complexone III) and Eriochrome Black T as indicator has been described by Schwarzenbach and Biedermann[1,2] for macro amounts and on the micro scale by Flaschka.[3] The recommended Schwarzenbach procedure has been summarised in a pamphlet.[4] In addition to visual methods, amperometric determinations of cadmium with disodium ethylenediaminetetra-acetate are described by Přibil and Matyska[5] and mentioned by Schwarzenbach.[6] Přibil, Koudela and Matyska[7] also utilised a potentiometric titration method for cadmium by addition of an excess of disodium ethylenediaminetetra-acetate and back-titration with ferric chloride solution.

In all determinations with Eriochrome Black T as an internal indicator, an optimum value of pH 10 is proposed for the titration of cadmium. At this pH many other metals, including magnesium, also react with disodium ethylenediaminetetra-acetate, and the final titration value is equivalent to the sum of all ions complexed at pH 10. For a mixture of cadmium and magnesium, values for both ions can only be found after cadmium has been complexed by potassium cyanide[2] and the residual magnesium titrated, the cadmium content of the solution being calculated by difference from the sum of the two. Investigations by the present authors[8] have shown, however, that the simultaneous titration of zinc and magnesium with disodium ethylenediaminetetra-acetate is possible by a selective pH control method. Zinc has been shown to form a stable coloured complex with Solochrome Black W.D.F.A.* at pH values between 6·3 and 7·0, whilst magnesium only reacts to an appreciable extent in the region of 7·5. This permits zinc to be titrated at pH 6·8 with disodium ethylenediaminetetra-acetate and magnesium is subsequently determined on the same solution at pH 10, Solochrome Black W.D.F.A. (Eriochrome Black T) being used as indicator in both titrations. The limitations of this method and an extension to it are described in a further communication.[9] In view of the close analogy between zinc and cadmium, both chemically and from the aspect of formation of complexes with ethylenediaminetetra-acetate, it was decided to attempt to apply this selective pH procedure to mixtures of cadmium and magnesium.

Preliminary investigations

Initial attempts at the titration of a cadmium sulphate solution at pH 6·8 with disodium ethylenediaminetetra-acetate with Solochrome Black W.D.F.A. as indicator were unsuccessful. Addition of the dyestuff solution to the cadmium gave a deep purplish-red solution, which showed that a complex had actually been formed between the Solochrome Black and cadmium ions at pH 6·8. On addition of disodium ethylenediaminetetra-acetate solution, however, a definite end-point change from purple to pure blue could not be obtained, the solution passing through various intermediate shades of red to purple. This behaviour was observed

* Solochrome Black W.D.F.A., manufactured by Imperial Chemical Industries Limited, is the equivalent of Eriochrome Black T.

in both maleic acid, sodium maleate buffer[8] and ammonium acetate buffer solutions, and is probably due to the cadmium dye chelate at pH 6·8 being relatively unstable. Further titrations at various pH values between 7·5 and 8·0 were also fruitless; only above pH 8 was the titration successful, but magnesium is also titrated at this pH.

We observed in the course of this preliminary work that a mixture of zinc and cadmium sulphates could be quantitatively titrated to a sharp Solochrome Black end-point at pH 6·8, the final titration representing the sum of the two metals. This result was confirmed for several molecular ratios of zinc and cadmium, including one with a very small proportion of zinc to cadmium. This behaviour in effect makes possible the titration of cadmium at pH 6·8 and is best realised in practice by the incorporation of some zinc sulphate into the disodium ethylenediaminetetra-acetate reagent, which is then standardised against zinc sulphate solution as described below. It was also found possible to determine cadmium and magnesium on the same solution by titration first at pH 6·8 (cadmium) and then at pH 10 (magnesium) by the use of this disodium zinc ethylenediaminetetra-acetate reagent, provided that the molecular ratio of magnesium to cadmium was not greater than unity.

Method for determination of cadmium and magnesium

In the following method doubly distilled water should be used throughout. Double distillation is to free ordinary distilled water from traces of metals that would react with disodium ethylenediaminetetra-acetate solution. All reagents should conform to recognised analytical specifications.

Reagents—

Disodium ethylenediaminetetra-acetate solution, 0·01 M—Dissolve 3·72 g of B.D.H. disodium dihydrogen ethylenediaminetetra-acetate dihydrate in water and make up to 1 litre with water.

Disodium zinc ethylenediaminetetra-acetate solution, 0·01 M—Dissolve 4·65 g of disodium dihydrogen ethylenediaminetetra-acetate in about 200 ml of water. Add 0·719 g of zinc sulphate heptahydrate to this solution, shake until dissolved and make up to 1 litre. Adjust to pH 5 with *N* sodium hydroxide solution (about 8 to 9 ml). The solution is approximately 0·01 *M* with respect to free disodium ethylenediaminetetra-acetate and 0·0025 *M* with respect to zinc.

Standard zinc solution, 0·05 M—Prepare this according to the method of Theobald and Stern.[10] Dilute to a 0·01 *M* solution as required.

Magnesium sulphate solution, approximately 0·025 M—Dissolve 6·09 g of magnesium sulphate heptahydrate in water and make up to 1 litre. Dilute to a 0·01 *M* solution as required.

Cadmium sulphate solution, approximately 0·025 M—Dissolve 6·41 g of cadmium sulphate, $3CdSO_4.8H_2O$, in water and make up to 1 litre. Dilute to a 0·01 *M* solution as required.

Sodium maleate - maleic acid buffer solution (*pH* 6·8)—Suspend 6·185 g of B.D.H. maleic acid (98·5 per cent. purity) in 250 ml of water. Add 100 ml of *N* sodium hydroxide solution and dilute to 500 ml with water after dissolution is complete.

Ammonium hydroxide - ammonium chloride buffer solution (*pH* 10)—Dissolve 67·5 g of ammonium chloride in 570 ml of ammonium hydroxide, sp.gr. 0·880. Adjust the pH to 10 to 10·1 with either hydrochloric acid, sp.gr. 1·18, or ammonium hydroxide, sp.gr. 0·880, as required, using a suitable pH meter.

Solochrome Black W.D.F.A. indicator solution—Make a 0·5 per cent. w/v solution in a mixture of equal volumes of the purest available triethanolamine and *iso*propanol.

Standardisation of solutions—

Disodium ethylenediaminetetra-acetate solution, 0·01 M—To 40 ml of 0·01 *M* standard zinc solution add 60 ml of water, 3 ml of ammonium hydroxide - ammonium chloride buffer solution and 5 drops of Solochrome Black indicator. Titrate with disodium ethylenediaminetetra-acetate solution to the very sharp change from reddish-purple through purple to a cornflower blue, free from any traces of violet colour. Each mole of disodium ethylenediaminetetra-acetate ≡ 1 mole of zinc, cadmium, or magnesium.

Magnesium sulphate solution, 0·01 M—Titrate this with the standardised disodium ethylenediaminetetra-acetate solution in a similar manner to that described for zinc.

Cadmium sulphate solution, 0·01 M—Standardise this similarly to the magnesium solution.

Disodium zinc ethylenediaminetetra-acetate solution, 0·01 M—Standardise this against standard zinc solution as described for the pure 0·01 *M* disodium ethylenediaminetetra-acetate solution.

PROCEDURE—

Dilute the neutral sulphate solution to 80 ml with water. Add 20 ml of maleate buffer solution and 5 drops of Solochrome Black. Titrate with the standard disodium zinc ethylenediaminetetra-acetate solution to the change from purple to greyish-blue, titrating very slowly near the end-point and observing very carefully the effect of each addition of titrant. If magnesium is now to be titrated, add 3 ml of ammonium hydroxide - ammonium chloride buffer solution and titrate to the change from purple to greyish-blue, taking the same precautions as prescribed for the cadmium titration.

RESULTS

The results obtained by applying the procedure to various molecular ratios of cadmium and magnesium are shown in Table I.

TABLE I

DETERMINATION OF CADMIUM AT pH 6·8 AND MAGNESIUM AT pH 10

Cadmium		Magnesium		Molecular ratio of cadmium to magnesium
Added, mg	Found, mg	Added, mg	Found, mg	
28·34	28·34	None	None	—
28·34	28·37	None	None	—
27·97	27·95	None	None	—
27·97	27·87	0·12	Not determined	50:1
27·97	27·98	0·60	Not determined	10:1
27·97	27·90	1·20	1·19	5:1
27·97	27·90	1·20	1·19	5:1
27·97	27·99	1·50	1·50	4:1
27·97	27·99	1·50	1·51	4:1
41·95	41·98	2·99	2·96	3:1
41·95	42·07	2·99	2·96	3:1
28·34	28·34	3·02	3·04	2:1
28·34	28·38	3·02	3·04	2:1
27·97	27·92	2·99	3·00	2:1
14·17	14·15	2·99	3·00	1:1
28·34	28·41	5·98	6·00	1:1
17·48	Indistinct end-point	7·48	Not determined	1:2
17·48	17·46	7·48	7·48	1:2
17·48	Indistinct end-point	7·48	Not determined	1:2
27·97	Indistinct end-point	12·97	Not determined	1:2
17·48	17·46	7·48	7·48	1:2
55·93	Indistinct end-point	35·90	Not determined	1:3

DISCUSSION OF RESULTS

SCOPE OF THE PROPOSED PROCEDURE—

An examination of the results detailed in Table I shows that cadmium and magnesium can be satisfactorily determined in admixture as long as the molecular ratio of magnesium to cadmium is not greater than 1 to 1. A molecular ratio of 2 to 1 led to unsatisfactory titrations, in that the cadmium end-point at pH 6·8 was impossible to detect with accuracy in three of the five determinations attempted. A similar effect has been noted in previous work on zinc and magnesium; here the top limit of magnesium to zinc was a 5 to 1 molecular ratio.[9] However, as with zinc and magnesium, the determination of a large excess of cadmium in the presence of magnesium was quantitative in all experiments attempted, with an upper limit of a molecular ratio of 50 to 1. The method is therefore of particular use for the determination of cadmium in the presence of traces of magnesium, where other methods, such as

the use of potassium cyanide, involve a second determination. Any errors in the cadmium titration are, of course, included in the magnesium result.

DETECTION OF THE END-POINT—

At pH 6·8 the cadmium end-point is less sharp than at pH 10, and it is modified to a greyish-blue, as compared with the pure blue at pH 10. A similar phenomenon was noted with zinc.[8] Similarly, in an admixture of cadmium and magnesium, the subsequent end-point of magnesium at pH 10 is modified to greyish-blue. The end-points are easily distinguishable with a little practice, as long as the precaution of slow titration in the vicinity of the indicator change is taken, with observation of the effect of each increment of titrant.

COMPOSITION AND STABILITY OF DISODIUM ZINC ETHYLENEDIAMINETETRA-ACETATE SOLUTION—

A solution of composition 0·01 *M* with respect to free disodium ethylenediaminetetra-acetate and 0·0025 *M* with respect to zinc was found to give satisfactory results. The pH of this freshly prepared solution is 3·5. The solution is unstable at this pH and the free acid tends to be precipitated after about a week. The reagent was accordingly adjusted to pH 5·0 by the addition of *N* sodium hydroxide, and it was then stable for at least three weeks. (Compare Goetz, Loomis and Diehl[11] on the stability of disodium ethylenediamine-tetra-acetate solutions.) Care should be taken in preparing the reagent to dissolve the pure disodium ethylenediaminetetra-acetate initially in water and then to add the requisite amount of zinc sulphate crystals. When an attempt was made to dissolve the two constituents simultaneously in water, dissolution proved very difficult and was never completed.

THEORY OF THE REACTION—

A mechanism for the reaction can be postulated on the assumption that the cadmium chelate is more stable than the zinc chelate, free cadmium ions displacing zinc ions from the zinc chelate. Virtually, therefore, zinc ions (in combination with Solochrome Black) are being used as an indicator for the procedure. Indirect evidence for this theory is given by the general order of the stability constants of the cadmium chelate and the zinc chelate, which are 16·6 and 16·2 (logarithmic values), respectively, as determined by Schwarzenbach and his co-workers.[6,12] These values show the cadmium chelate of ethylenediaminetetra-acetic acid to be slightly more stable than that of the zinc complex, under the particular experimental conditions chosen by Schwarzenbach, which were 20° C in 0·1 *N* potassium chloride solution. The magnitude of the stability constants of the zinc and cadmium complexes with Solochrome Black also enter into the theory of the reaction mechanism, but no figures have been published for these constants. At a pH of 6·8, the cadmium - Solochrome Black complex is obviously less stable than the zinc - Solochrome Black complex, as is shown in the procedure. In general, the reaction probably follows the course shown schematically—

$$
\begin{aligned}
&Cd^{\cdot\cdot} + In \rightleftharpoons CdIn\\
&Cd^{\cdot\cdot} + Na_2H_2Y \rightarrow Na_2CdY + 2H^{\cdot}\\
&Cd^{\cdot\cdot} + Na_2ZnY \rightarrow Na_2CdY + Zn^{\cdot\cdot}\\
&CdIn + Na_2ZnY \rightarrow ZnIn + Na_2CdY\\
&Zn^{\cdot\cdot} + Na_2H_2Y \rightarrow Na_2ZnY + 2H^{\cdot}\\
&ZnIn + Na_2H_2Y \rightarrow Na_2ZnY + In
\end{aligned}
$$

where *Y* is the ethylenediaminetetra-acetate radical and *In* represents the Solochrome Black indicator. The ionisation of the indicator is not shown, as there is some uncertainty as to the reacting species at pH 6·8.

INTERFERING ELEMENTS—

The proposed procedure is subject to interference from several common elements. Iron^{II} reacts at pH 6·8 in the presence of maleate buffer to form an orange-red complex with the indicator, which seriously obscures the end-point. Iron^{III} interferes to a lesser extent. The difficulty can be overcome in the same way as for zinc - magnesium mixtures, *i.e.*, by oxidising iron^{II} to iron^{III} with peroxide at pH 2 to 3 and titrating with disodium ethylene-diaminetetra-acetate with ammonium thiocyanate or sulphosalicylic acid as indicator. The complexed iron^{III} does not then subsequently react with Solochrome Black. If only traces of iron are present, the addition of thiocyanate can be omitted and a slight excess of

disodium ethylenediaminetetra-acetate can be added at pH 2 to 3. The pH is then adjusted to 6·8 and the titration continued, the iron figure being included in that for cadmium.

Calcium is titrated with the magnesium and causes positive errors. There is some evidence that lead is titrated at the lower pH with cadmium; this point, however, requires further investigation. If free zinc ions are present in addition to the cadmium ions, they also titrate at pH 6·8 with the cadmium and the resultant titration gives the sum of the zinc and cadmium present. No method has been found of obviating this interference.

Other interfering elements have not been studied.

INCREASE IN SCOPE OF PROCEDURE—

We have shown[9] that zinc can be satisfactorily determined in the presence of large quantities of magnesium by conversion of zinc ions to the complex cyanide, separation of magnesium on a cation-exchange column, liberation of zinc ions from the complex with formaldehyde and subsequent titration at pH 6·8 with disodium ethylenediaminetetra-acetate solution. Recently Flaschka[13] has shown that potassium cadmicyanide can be decomposed similarly and titrated. Hence an application of this zinc - magnesium procedure seems possible for cadmium - magnesium mixtures, and would increase the scope of the proposed method, assuming that only the cadmium was to be determined.

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RESEARCH AND DEVELOPMENT DEPARTMENT
BRITISH ENKA LIMITED
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The Photometric Determination of Traces of Aluminium in Zinc

By E. F. PELLOWE and F. R. F. HARDY

A rapid method is described for the quantitative determination of from 0·001 to 0·01 per cent. of aluminium by means of aluminon. Thioglycollic acid is used as a complexing agent to suppress interference from iron, copper and tin; the pH at the time of colour development is controlled by the use of a buffer solution. Data pertaining to interference from other metals are given and the method can be modified for the determination of aluminium in certain tin and cadmium-base alloys.

Most methods available for the determination of traces of aluminium are usually of a purely chemical nature and incorporate lengthy separations from other metals. Such methods are slow and do not show a high degree of accuracy. An investigation has therefore been made into the potentialities of absorptiometry, which gives promise of reproducible results with a reasonable degree of accuracy.

Method

As traces of aluminium are to be determined, in the interests of accuracy all glassware should be treated with hot 50 per cent. hydrochloric acid and then rinsed before use.

Reagents—

All reagents must conform to recognised analytical standards of purity.

Aluminon buffer solution—This is a modification of the solution suggested by Luke and Braun.[1]

Place 125 g of ammonium acetate in a 400-ml beaker and add 250 ml of distilled water and, from an automatic pipette, 20 ml of glacial acetic acid. Filter the solution into a 500-ml calibrated flask, washing the beaker several times. Dissolve 0·250 g of aluminon (ammonium aurinetricarboxylate) in 50 ml of water and add this to the acetate solution. Weigh out 0·50 g of benzoic acid and dissolve it in 20 ml of methanol. Add this a few millilitres at a time to the main solution, shaking well after each addition; then make up to 500 ml and mix. Transfer this solution to a 1-litre beaker and add 250 ml of glycerol from a measuring cylinder; allow the cylinder to drain for exactly 2 minutes. Stir the solution and transfer it to a 1-litre amber-glass reagent bottle. (The preparation of this solution is standardised as far as possible so that when further supplies have to be made up a recalibration is unnecessary.) This solution can be used immediately and does not deteriorate over a period of months.

Thioglycollic acid, 10 per cent. solution—Dilute 10 ml of thioglycollic acid to 100 ml in a calibrated flask. Should the solution appear slightly turbid, filter it through a Whatman No. 42 filter-paper. This solution is stable for about 1 month.

Standard aluminium solution—Dissolve 0·020 g of pure aluminium in 10 ml of concentrated hydrochloric acid to which 2 ml of 100-volume hydrogen peroxide has been added; heat at about 70° C until completely dissolved. Boil vigorously for 5 minutes, cool and make up to 2 litres in a calibrated flask; 1 ml of this solution contains 0·00001 g of aluminium.

Standard zinc solution—Dissolve 4·0 g of pure zinc in 25 ml of concentrated hydrochloric acid. Evaporate the solution until the salt crystallises, and about 3 ml of solution is left. Add 50 ml of water, boil, cool and make up to 200 ml in a calibrated flask; 5 ml of this solution contain 0·10 g of zinc.

Standard lead solution—Dissolve 0·20 g of pure lead in 2 ml of 50 per cent. nitric acid by warming gently. When the lead has dissolved, boil the solution to dryness and continue heating for 3 minutes. Add 100 ml of water and 1 ml of concentrated hydrochloric acid, boil to dissolve the salts, cool and dilute to 1 litre in a calibrated flask; 5 ml of this solution contain 0·001 g of lead.

PREPARATION OF CALIBRATION CURVES—

By means of 10-ml burettes, put into each of ten 100-ml calibrated flasks 5 ml of standard zinc solution and 5 ml of standard lead solution; then into the flasks put 0·0, 0·5, 1·0, 2·0, 3·0, 4·0, 5·0, 6·0, 8·0 and 10·0 ml of standard aluminium solution corresponding to 0·0, 0·05, 0·10 . . . 1·0 per cent. of aluminium in the synthetic alloy, and add, from a 10-ml burette, the calculated amount of distilled water to bring the total volume in each flask to 20 ml. Then add to each flask 2 ml of 10 per cent. thioglycollic acid solution, shake the flasks and add 15 ml of aluminon buffer solution. (Extra drainage time should be allowed, as this liquid is rather viscous—1 minute is usually sufficient.) Swirl each flask and place it in a vigorously boiling water-bath, the heat source of which should be capable of bringing it back to boiling in not more than 2 minutes (200 ml of water boiling in a 400-ml squat beaker over a bunsen burner will suffice). Leave for exactly 4 minutes and then place the flask in cold water for the same time. Make each solution up to 100 ml with distilled water and place each flask for 20 minutes in a water-bath maintained at 65° F. Transfer portions of each solution to 1, 2 and 4-cm glass cells and measure the absorptions immediately at approximately 546 mμ, deducting a blank for distilled water from each reading. Plot these differences against the corresponding percentages of aluminium in the synthetic zinc-aluminium alloy; examples of the calibration curves are shown in Fig. 1.

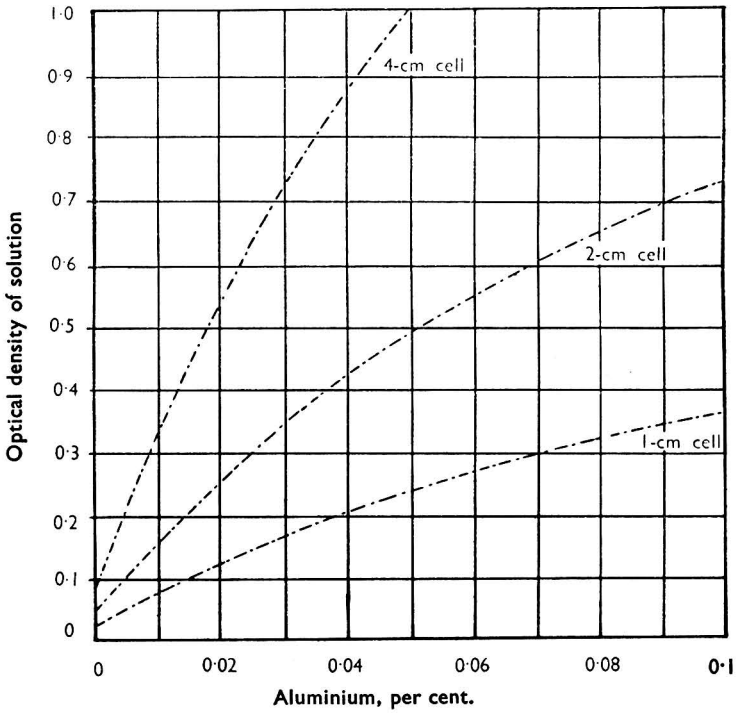

Fig. 1. Calibration curves for 1-cm, 2-cm and 4-cm cells. The percentages refer to the aluminium content of the synthetic alloy

PROCEDURE FOR DETERMINATION—

Place 2·0 g of drillings from which iron has been separated magnetically in a 400-ml conical beaker and add 10 ml of concentrated hydrochloric acid a few millilitres at a time, as the reaction is rather violent. When all the drillings have dissolved, add 1 ml of 100-volume hydrogen peroxide and evaporate the solution to about 2 ml in order to remove most of the excess of acid. Add 50 ml of water, boil, cool and make up the solution to 400 ml in a calibrated flask. Transfer 20 ml of this solution to a 100-ml calibrated flask and add 2 ml of 10 per cent. thioglycollic acid solution; continue as in the calibration procedure. Deduct the blank for distilled water and by means of the calibration curve determine the amount of aluminium present. The time for a determination is about 1 hour.

Results

To test the validity of the curves obtained in the calibration procedure, samples of Prime Western Spelter having the composition: lead, 1·05; iron, 0·015; tin, 0·01; copper, 0·022; cadmium, 0·18 per cent.; remainder zinc, were analysed, standard amounts of aluminium being added. The results are shown in Table I. As the nature of the curves might suggest, the percentage error is approximately the same over the whole range, sensitivity being high for small amounts of aluminium but decreasing as the latter increases.

Table I

Determination of aluminium added to prime western spelter

Aluminium added, on 2-g sample, g	Aluminium added, on 2-g sample, %	Optical density of solution		Aluminium found, %	Error, %
0·0002	0·01	0·343	4-cm cell	0·0103	3·0
0·0006	0·03	0·727		0·0307	2·3
0·0010	0·05	0·508	2-cm cell*	0·051	2·0
0·0014	0·07	0·620		0·072	2·9
0·0018	0·09	0·686		0·089	1·1

* Values on this graph are only accurate to 3 places of decimals.

Effect of time and temperature—

A sample of spelter containing a known amount (0·05 per cent.) of aluminium was dissolved in acid and made up to 400 ml. Three 20-ml aliquots were taken and treated as described in the method with the exception that each was heated for a different length of time on the water-bath. The optical density of the final solutions at 65° F was as follows—

Time of heating, minutes	Optical density (2-cm cell)	Equivalent aluminium, %	Error, %
2	0·486	0·048	−4·0
4	0·495	0·049	−2·0
6	0·515	0·052	+4·0

Whilst the errors show a definite trend, they are not much greater than the over-all error of the method.

To see whether the optical density at the time of measurement was influenced by temperature variations, a similar experiment was performed. Five aliquots were taken from the same sample of spelter solution, heated for the correct time (4 minutes) and the optical density of the final solutions was measured at different temperatures. The results are shown in Table II.

Table II

Effect of temperature on optical density

Temperature, ° F	Optical density (2-cm cell)	Equivalent aluminium, %	Error, %
60	0·490	0·048	−4·0
65	0·500	0·050	—
70	0·513	0·052	+4·0
75	0·528	0·055	+10·0
80	0·545	0·058	+16·0

It will at once be seen that considerable errors occur as the temperature varies, so that if a calibration curve is to be reproducible it is essential that all density readings be made under constant temperature conditions.

Behaviour of other metals with aluminon and thioglycollic acid—

Zinc—In the present investigation aluminium was determined in the presence of up to 10,000 times as much zinc without any interference.

Cadmium—This ion reacts much the same as zinc in that, providing the experimental conditions are observed, it causes no interference even when present in very large amounts.

Iron—Ferric ions, and to a lesser extent, ferrous ions, give a deep purple colour with aluminon; if thioglycollic acid, as suggested by Chenery,[2] is added first, the purple colour disappears in a short time. It will reappear if the solution is diluted or shaken vigorously, a phenomenon probably caused by temporary air oxidation. The action of pouring the liquid into the absorption cells is sufficient to darken the colour; if large amounts of iron are present it will be necessary to allow the solution to stand for a few minutes before the optical density is measured.

Copper—This ion forms a mauve lake with aluminon, and a complex with thioglycollic acid must be formed. If copper is present in amounts much over 1 per cent., a yellow precipitate is formed when the thioglycollic acid is added, but this disappears to give a green coloration when the aluminon buffer solution is added. As absorption readings are made with a green filter, this colour will cause less interference than would the mauve; nevertheless, if large amounts of copper are present some compensation must be made, preferably by adding known amounts of copper when the calibration is made. It is noteworthy that if the solution is heated on the water-bath much longer than 4 minutes a turbidity is formed that does not disappear on cooling.

Lead—If this ion is present in amounts greater than 2 per cent. it will cause interference owing to a turbidity occurring when the solution is heated on the water-bath. This effect can be prevented in two ways, (*a*) by heating for not longer than 3 minutes, as the turbidity forms during the fourth minute, or (*b*) by omitting the addition of thioglycollic acid, when no interference is experienced even with samples containing up to 100 per cent. of lead. The second course would necessitate a new calibration, as thioglycollic acid has a bleaching action and its absence would cause enhancement of all colours; further, the lead must be completely free from copper, iron and tin. Of these two courses, the first is likely to be of more general application because, as has already been shown, the final colour of the aluminium lake is not greatly influenced, between certain limits, by the time of heating on the water-bath.

Tin—The presence of thioglycollic acid is essential if this ion is not to cause interference; if thioglycollic acid is present, however, no marked interference is experienced from tin in concentrations up to at least 10 per cent. (see Table III). After the addition of thioglycollic acid the tin solution remains clear, but when the aluminon buffer solution is added a turbidity forms; this disappears on heating and does not reappear when the solution is cooled. Should the tin hydrolyse after the initial solution in acid and making up to volume, aluminium can still be accurately determined, as this turbidity will disappear on heating.

To investigate the extent to which these metals interfere, determinations were made on synthetic mixtures. Samples of 2 g were dissolved in acid and made up to 300 ml. Two 15-ml aliquots were taken and 5 ml of standard aluminium solution were added to one and 5 ml of distilled water to the other. The results of the analyses are shown in Table III.

TABLE III

DETERMINATION OF ALUMINIUM ADDED TO SYNTHETIC MIXTURES

Aluminium in synthetic alloy: 0·05 per cent.

Synthetic mixture containing 90 per cent. of zinc and 10 per cent. of—	Aluminium found in aliquot to which standard was added, %	Blank found for aliquot with no added aluminium, %	Aluminium found, corrected for blank, %
Cadmium	0·050	0·001	0·049
Iron	0·053	0·002	0·051
Copper	0·051	0·001	0·050
Tin	0·050	0·001	0·049
Lead	0·054	0·005	0·049

These mixtures were analysed exactly as described in the method, so the high figures for the iron mixture could have been reduced by allowing the solutions to stand for some time in the absorption cell; likewise those for the lead mixture could have been reduced by

heating for a shorter time on the water-bath. From these figures it can be seen that this method could be used for the determination of traces of aluminium in a variety of alloys.

OTHER FACTORS—

The lakes formed by aluminium and several other metal ions vary in colour intensity with the pH of the solution in which they are formed, intensity being high for some ions and low for others at certain pH values; hence it is important that strict control of pH at the time of colour development should be maintained. By using a buffer solution the need for a pH meter is avoided, while the addition of small amounts of acid remaining in excess after the sample is dissolved does not materially change the pH of the solution. In the method described the pH at the time of colour development was 5·15, which decreased to 5·07 when the solution was made up to volume.

DISCUSSION OF RESULTS—

All the photometric measurements in this investigation were made with a mercury-vapour-lamp absorptiometer calibrated in true optical density (logarithm of the reciprocal of transmission). An uncalibrated instrument would have served equally well, as the method does not depend upon the measurement of any absolute quantity. If an uncalibrated instrument only is available, it would be in order to construct the calibration curve by plotting percentage of aluminium against the numerical values of the drum readings.

It will be noticed that the gradients of the curves decrease as the aluminium content increases. This tendency is continued when the curves are extended and a resultant loss in sensitivity is experienced. Should it be necessary to determine amounts of aluminium outside the range described, the best course would be to decrease the amount of sample and use a factor.

Different authors have suggested a variety of suspensive agents for the aluminium lake, *e.g.*, gum acacia or gelatin. We were unable to procure satisfactorily pure samples to give a perfectly clear solution when they were introduced into the aluminon buffer solution. Glycerol was finally chosen, as it acted equally well as a suspensive agent and could be obtained in a very pure state. It had the added advantage of not tending to decompose or be deposited on the walls of the calibrated flasks when they were heated in the water-bath. It is possible that the use of this chemical to replace the less pure substances may have something to do with the remarkable absence of interference from other metals.

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BRAINTREE, ESSEX

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Absorptiometric Determination of Iridium and Rhodium

By A. D. MAYNES and W. A. E. McBRYDE

A procedure for the absorptiometric determination of iridium has been developed in which the sulphate is oxidised by ceric sulphate to produce a red solution. The stannous chloride procedure for the absorptiometric determination of rhodium has been examined with special attention to the effect of the presence of iridium. A procedure has been worked out for the application of these methods in sequence to the analysis of solutions containing iridium and rhodium together, without the necessity of a prior separation. Some results are presented to show that the mixed perchloric - phosphoric acid procedure for the absorptiometric determination of iridium may lack precision.

In the systematic analysis of the platinum metals, osmium, ruthenium, platinum and palladium are isolated in turn, and a solution of rhodium and iridium remains for the separation and determination of these constituents. The recommended procedure for this separation is to add titanous chloride, which precipitates metallic rhodium but not iridium.[1] A double precipitation is necessary to purify the rhodium. Before iridium can be determined in the residual solution, titanium must first be removed by the addition of cupferron. This method of separation is laborious, and is said to be unreliable when small amounts of the metals are to be determined.[2]

It should be possible, in principle, to determine these two elements present together in the same solution without recourse to a separation, provided that suitable selective colour-forming reactions are available for each element. The most economical procedure from the standpoint of time and effort is to use a single reagent that generates with each element coloured products that show absorption maxima in different regions of the spectrum. Such a procedure, for instance, is reported for the elements iridium and palladium by Ayres and Quick.[3] No suitable reagent of this sort has been found for iridium and rhodium. A second procedure is to seek two reagents that selectively produce colours, one for each element, and to apply these to aliquot portions of the solution to be analysed or to the whole of the solution in successive operations. We have found that the red colour produced when iridium sulphate solutions are oxidised by ceric sulphate[4] can be used for the determination of iridium without interference by moderate amounts of rhodium. The stannous chloride procedure for rhodium[5] is satisfactory for the colorimetric determination of this element in a wide range of concentrations. Iridium, present with rhodium in this test, increases the optical density of the solution, but we have shown that this increase is a linear function of the iridium concentration. The stannous chloride procedure can successfully be applied to solutions that have been treated by the ceric sulphate procedure, provided that the red colour is first discharged and the sulphates are converted to chlorides. By combining the results from the two colorimetric procedures the amounts of rhodium and iridium present together can be found. The procedure for the colorimetric determination of iridium that involves heating in mixed perchloric - phosphoric acids[3] was rejected for this purpose because of poor precision and because it could not readily be combined with the stannous chloride method for the consecutive analysis of one sample.

Experimental

Apparatus—

Optical density measurements were made with a Beckman model DU spectrophotometer operated at constant sensitivity, matched Corex-glass cells of 1·00-cm light path being used.

As the work progressed, it became apparent that close control of the heating temperature was necessary during the procedures for iridium. This was accomplished by the use of a hot-plate with a self-regulating stepless control (Temco model 1900). With this equipment temperatures could be selected and maintained to within $\pm 1^\circ$ C.

SOLUTIONS OF PLATINUM METALS—

Iridium solution—Ammonium chloro-iridate^{IV} was dissolved in 0·2 *N* hydrochloric acid to give a stock solution containing approximately 2 mg of iridium per ml. This solution was standardised by the procedure of Hill and Beamish.[6]

Rhodium solution—Sodium chlororhodite^{III} was dissolved in 0·2 *N* hydrochloric acid to give a stock solution containing approximately 2 mg of rhodium per ml. This solution was standardised by electro-deposition by a modified procedure of MacNevin and Tuthill.[7]

Platinum solution—A stock solution was prepared by dissolving a known weight of the metal in aqua regia, evaporating repeatedly to dryness to destroy nitric acid, and diluting to a known volume with 0·2 *N* hydrochloric acid.

Palladium solution—Prepared in the same way as the platinum solution.

OTHER REAGENTS—

All reagent solutions were prepared according to the directions in the original publications from analytical-reagent grade chemicals.

STANNOUS CHLORIDE PROCEDURE FOR RHODIUM—

The procedure of Sandell[5] was examined in detail to ascertain the best conditions for producing and measuring the colour, and to observe the effect of the reagent on iridium and on solutions containing rhodium and iridium together. Samples containing the metal in a volume of 5 ml were treated with 10 ml of a 10 per cent. w/v solution of stannous chloride dihydrate in 2 *N* hydrochloric acid, and heated for 0·7 to 1·3 hours in a bath of boiling water. They were then cooled and diluted to a volume of 50 ml with 2 *N* hydrochloric acid, and their optical densities were measured.

A solution containing 996 μg of rhodium in 50 ml gave an absorption spectrum shown as curve A, Fig. 1. Maximum optical density occurred at 470 mμ, and this wavelength was selected for all subsequent measurements.

The effect of varying the heating interval was studied while maintaining a constant concentration of rhodium. When the samples were heated for 40 minutes, the optical densities showed a slow drift during the 24-hour period after removing the samples from the water-bath. When the heating interval was 1 hour the optical densities were stable after 30 minutes for at least 24 hours. Similarly, when the heating interval was 1 hour 20 minutes the optical densities were essentially constant and remained so for more than 24 hours. These effects are illustrated by the results in Table I. Except as noted hereafter, all other samples were heated for 1 hour to generate the colour.

TABLE I

EFFECT OF TIME OF HEATING ON OPTICAL DENSITY AND STABILITY OF RHODIUM SOLUTIONS

Concentration 9·96 p.p.m. of rhodium; wavelength 470 mμ

40 minutes' heating

Time of standing	*D*	Time of standing	*D*
23 minutes	0·375	2 hours 47 minutes	0·379
34 minutes	0·377	3 hours 48 minutes	0·379
50 minutes	0·378	4 hours 43 minutes	0·379
1 hour 5 minutes	0·381	6 hours 50 minutes	0·379
1 hour 36 minutes	0·381	24 hours	0·377
2 hours 11 minutes	0·381	45 hours	0·374

1 hour's heating

Time of standing	*D*
28 minutes	0·376
43 minutes	0·376
1 hour 14 minutes	0·377
3 hours 13 minutes	0·378
6 hours 3 minutes	0·376
9 hours 28 minutes	0·376
24 hours 30 minutes	0·376

1 hour 20 minutes' heating

Time of standing	*D.*
28 minutes	0·375
55 minutes	0·376
1 hour 25 minutes	0·377
3 hours 20 minutes	0·377
6 hours 30 minutes	0·377
25 hours 30 minutes	0·377

The optical densities of samples made up to volume with 6 *N* hydrochloric acid showed the same characteristics on standing as those prepared with 2 *N* acid, but there was a 0·5 per cent. decrease in magnitude.

Agreement with Beer's law was noted for samples containing up to 40 p.p.m. of rhodium when treated by the above procedure. Sixteen samples containing 498 μg of rhodium, or 9·96 p.p.m. in the final solution, gave an average optical density of 0·378, with a relative standard deviation of 0·5 per cent.

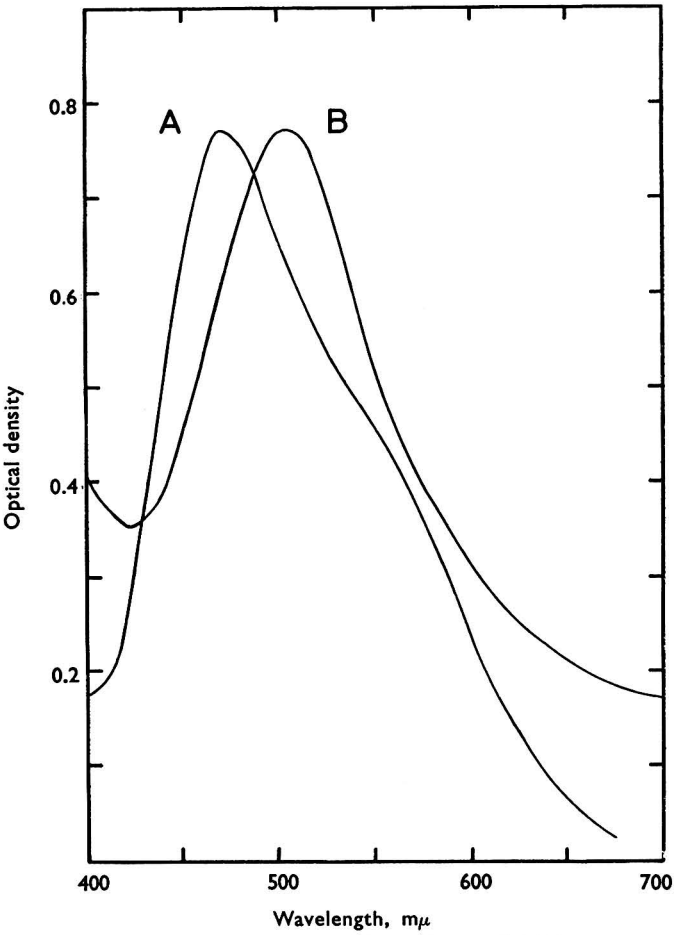

Fig. 1. Absorption spectra of rhodium and iridium complexes.
Curve A: 20 p.p.m. of rhodium; curve B: 90 p.p.m. of iridium

Table II

Effect of time of heating on optical density and stability of iridium solutions

Concentration 180 p.p.m. of iridium; wavelength 470 mμ

1 *hour's heating*		1 *hour* 20 *minutes' heating*	
Time of standing	*D*	Time of standing	*D*
28 minutes	0·250	34 minutes	0·256
43 minutes	0·252	55 minutes	0·258
1 hour 14 minutes	0·252	1 hour 25 minutes	0·258
3 hours 13 minutes	0·244	3 hours 20 minutes	0·248
6 hours 13 minutes	0·233	6 hours 30 minutes	0·242
9 hours 28 minutes	0·218	25 hours 30 minutes	0·212
24 hours 30 minutes	0·182		

Results with iridium—When the same procedure was applied to samples containing 9·02 mg of iridium, the optical density at 470 mμ was observed to be constant between ½ hour and 2½ hours after the heating interval. After this time significant fading occurred. Samples heated for 1 hour 20 minutes gave an optical density approximately 2·5 per cent. higher over the first few hours than those heated for 1 hour only. Typical results are shown in Table II.

Samples containing 498 μg of rhodium and 4·51 mg of iridium were treated by the same procedure. The results confirmed those for rhodium or iridium alone, and indicated an optimum time of standing after heating of between ½ and 2½ hours.

Twenty-two samples containing 498 μg of rhodium with various amounts of iridium up to 9·02 mg were treated by the foregoing procedure. A plot of optical density against concentration of iridium was linear. The regression of optical density on concentration of iridium was calculated and found to be—

$$D = 0{\cdot}376 + 0{\cdot}00150\,C$$

where C = number of p.p.m. of iridium. The standard deviation about the regression line was 0·004 units of optical density; the average deviation about the regression line was 0·6 per cent. In general the relation is of the form—

$$D_{\mathrm{Rh+Ir}} = D_{\mathrm{Rh}} + 0{\cdot}00150\,C$$

MIXED-ACID PROCEDURE FOR IRIDIUM—

Samples of iridium^{IV} chloride were treated rigorously by the procedure of Ayres and Quick.[3] The optical densities observed for a fixed amount of iridium showed a large variation. Efforts were made to assign the cause of this poor precision.

Ayres and Quick state that the following variables affect the intensity of colour developed by a given amount of iridium: (*a*) the ratio of perchloric to phosphoric acid; (*b*) the total amount of mixed acid in the final volume; (*c*) the time of heating after the colour developed (4 minutes at 150° C were required to give full colour; 7 minutes were considered to provide a safe margin); and (*d*) the temperature at which colour developed. Temperatures lower than 150° C necessitated longer heating to give full colour; temperatures above this caused fading. At 150° C a sudden appearance of colour was reported after the sample had been heated for 10 to 30 minutes.

Of these four considerations, the first two can be disregarded as sources of error in a series of replicate determinations. There was some uncertainty in measuring the 7-minute interval after the colour appeared because we found that the colour appeared gradually, rather than suddenly as reported. However, there was sufficient margin allowed to give reasonable assurance of full colour development. During the heating at 150° C the temperature was confined within a range of 2° C to try to avoid errors due to the fourth factor mentioned above. However, we failed to obtain consistent optical densities even for replicate samples treated in a group at the same temperature.

Examination of our results disclosed that a factor not discussed by Ayres and Quick is important in determining the final optical density and its neglect affects the precision of this method. These authors stated that the colour developed after heating for 10 to 30 minutes at 150° C. We found that there was a considerable variation in the time required for the first appearance of the purple colour. Moreover, we observed that there was a relation between this time and the optical density produced. Usually, the shorter the heating time the greater the optical density. This fact is shown in Fig. 2, which is based on a dozen replicate samples treated by the recommended procedure. Even samples heated together in a group showed a wide variation in the heating interval and the final optical density. For instance, the following are observed values for 20·2 p.p.m. of iridium when the three samples were treated in a group—

Heating interval, minutes	16	23	38
Optical density	0·283	0·275	0·254

Of 8 samples containing 20·2 p.p.m. of iridium in which colour appeared within 30 minutes' heating at 150° C, the average optical density was 0·275 with a relative standard deviation of 2·8 per cent.

The successful application of this procedure clearly depends on control of the factor or

factors affecting the interval of heating at 150° C before the development of colour in the sample. As colorimetric results of satisfactory precision and applicability were attained by the procedure to be outlined in the following section, a further study of this method was not made.

CERIC SULPHATE PROCEDURE FOR IRIDIUM—

It has been reported that when solutions of iridium^{III} sulphate are heated with ceric sulphate, a raspberry-red colour develops. The solutions then contain iridium in a net oxidation state of 4·5, from which the element can be reduced by ferrous sulphate, thereby providing a volumetric method for estimating iridium.[4] The procedure for so oxidising iridium involves taking a solution of the chloride to fumes with sulphuric acid, diluting with

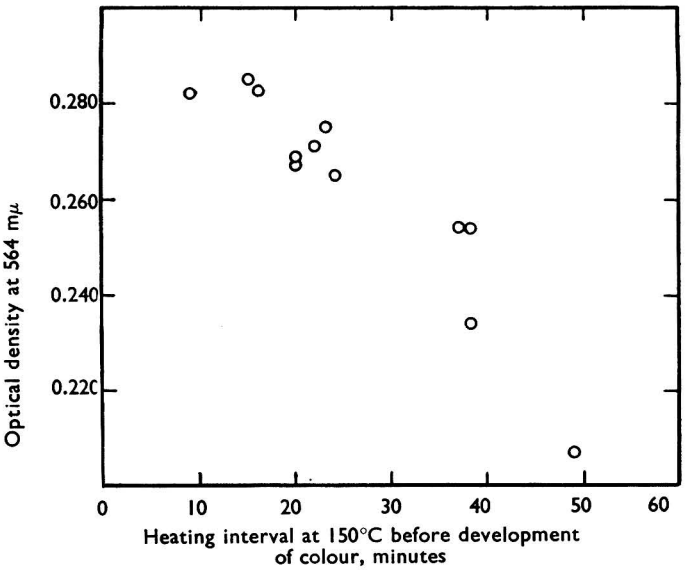

Fig. 2. Effect of heating interval on optical density of iridium solutions

water and adding an excess of ceric sulphate, and heating for at least 10 hours on a steam-bath. A small, but variable, excess of cerium^{IV} remains after this heating. We have shown that this procedure can be carried out in such a manner as to permit a photometric determination of iridium.

By just reducing the excess of cerium^{IV}, the absorption spectrum of the oxidised iridium has been recorded and is reproduced as curve B, Fig. 1. The maximum absorption is seen to occur at 505 mμ. At this wavelength, however, any small amounts of cerium^{IV} remaining in solution also contribute to its absorption of light. It was not particularly easy to remove only the excess of cerium^{IV} without partially reducing the iridium present, and in any event its presence in the solutions greatly increased the stability of the iridium colour. The absorption of light by ceric solutions falls off rapidly at wavelengths greater than 505 mμ, and it was found that by measuring the optical densities of the treated iridium solutions at 515 mμ, or even at 510 mμ, no significant interference by cerium occurred and the results were more precise. The absorption maxima of solutions in which the cerium^{IV} was not reduced was found to vary between 504 and 507 mμ, and this undoubtedly contributed to poorer precision among samples measured at 505 mμ.

As the concentration of sulphuric acid in the samples increased the optical densities decreased, and this effect is shown in Table III. The concentration of acid is expressed in this table as the ratio of volume of concentrated acid to volume of water added after fuming. For some reason the transmissions of samples were found to vary linearly with this acid ratio. It was also found that as the acid ratio decreased the colour developed more rapidly. A third effect of acid concentration was to displace the absorption maximum; the following are

average values for the wavelengths of the peak in the optical density curve at the stated acid ratios—

Acid ratio	0·075	0·15	0·25	0·38
Wavelength at peak, mμ	503	505	510	522

The final optical density for a constant amount of iridium depended on the duration of the heating with ceric sulphate and on the temperature at which this was carried out. The intensity of colour seemed to approach a maximum for each concentration of iridium, and this was independent of the temperature of heating over the range 67° to 92° C. The maximum was achieved more quickly, however, when the heating was carried out at higher temperatures. With an acid ratio of 0·15, heating for 10 to 12 hours at 70° C was required to produce maximum colour intensity, while at 90° to 92° C samples with the same acid ratio required only 8 hours to attain maximum optical density.

TABLE III

EFFECT OF SULPHURIC ACID CONCENTRATION ON OPTICAL DENSITY OF IRIDIUM SOLUTIONS

Concentration 54·1 p.p.m. of iridium; 15½ to 17½ hours' heating

Acid ratio	Optical density
0·075	0·490, 0·516
0·10	0·504
0·15	0·492, 0·490, 0·492
0·20	0·477, 0·492
0·25	0·486, 0·482, 0·470, 0·486
0·30	0·452, 0·462
0·33	0·461, 0·467
0·38	0·452
0·50	0·493, 0·452

The optical densities of these solutions were constant up to 24 hours after preparation. Tests indicated that the stability of colour depended on the amount of cerium^{IV} present. Samples to which 2 ml of 0·1 *N* ceric sulphate solution had been added were stable for up to 2 weeks. Samples that were titrated with ferrous sulphate to just remove the excess of cerium^{IV} faded appreciably in this time.

Recommended procedures—Two procedures were successfully used—

(*a*) The solution containing up to 5 mg of iridium was adjusted to a volume of 10 to 20 ml, 6 ml of concentrated sulphuric acid were added, and the solution was heated to evaporate water and until fumes of sulphuric acid were evolved. Fuming was continued for 15 to 20 minutes after the iridium solution became colourless. The sample was cooled, 35 ml of water were cautiously added and then 1 ml of 0·1 *N* ceric sulphate solution was stirred in. The stirring rod was removed from the solution and rinsed with 5 ml of water. The solution was then covered with a watch-glass and heated for at least 12 hours at 70° ± 2° C, cooled and then made up to 50·0 ml with water. In practice, samples were usually heated overnight. Optical density measurements were made at 510 mμ against a water blank.

(*b*) The solution containing up to 3·75 mg of iridium was adjusted to a volume of 10 to 20 ml, 4 ml of concentrated sulphuric acid were added, and the solution was evaporated to fumes of sulphuric acid. Fuming was continued for 5 to 10 minutes after the iridium solution became colourless. The solution was cooled, 35 ml of water were cautiously added, and the solution was heated at 90° to 92° C for 5 to 10 minutes. One millilitre of 0·1 *N* ceric sulphate solution was stirred in, the stirring rod was rinsed with 5 ml of water, and heating at 90° to 92° C was continued for 6 hours. The solution was made up to 50·0 ml with water and the optical density measured at 510 mμ against a water blank.

Reproducibility—The average optical density of 23 samples containing 54·1 p.p.m. of iridium and treated by procedure (*a*) was 0·497, with a relative standard deviation of 1·1 per cent. For 20 samples containing 50·4 p.p.m. of iridium treated by procedure (*b*) the average optical density was 0·469, with a relative standard deviation of 0·7 per cent. The seemingly better precision of procedure (*b*) may be due only to improved technique as experience accumulated; for example, for the last 9 samples treated by procedure (*a*), the relative standard deviation was only 0·6 per cent.

Beer's law—A linear relation between optical density and concentration was observed. The observations from each procedure were examined by forming the regression of optical density on concentration. The observed regression lines were as follows—

Procedure	(*a*)	(*b*)
Range	20 to 90 p.p.m.	20 to 75 p.p.m.
Equation	$D = 0{\cdot}016 + 0{\cdot}00876\ C$	$D = 0{\cdot}007 + 0{\cdot}00917\ C$
Average deviation about regression, per cent.	1·1	0·6

D represents the optical density and *C* the concentration of iridium in p.p.m.

Samples containing 100 p.p.m. of iridium and treated by procedure (*b*) were found to have an optical density lower than that predicted by the above regression formula. In order to bring the absorption up to the predicted values, more than 9 hours' heating were required. When it is desired to limit the heating to 6 hours, it is necessary to use a "working curve" for converting optical densities to concentrations in excess of 75 p.p.m.

Interferences—The presence of nitric acid in the solutions during oxidation with cerium^{IV}, or even the presence of fumes of nitric acid nearby, caused low and inconsistent results. It is essential that nitric acid be eliminated completely from samples to be analysed by this procedure at the time of fuming with sulphuric acid. The presence of nitric acid is often revealed by the familiar purple colour in the solution at this stage. If water is added and the sample heated again to fumes, this colour can be made to disappear.

When rhodium was present in amounts up to 10 mg it did not interfere. When samples containing rhodium were heated to fuming, a red precipitate was observed, and it required prolonged fuming to effect dissolution. The addition of 1 g of sodium sulphate before fuming was helpful. The formation of this precipitate was not observed in samples that had stood for 1 or 2 days at room temperature after the addition of 20 ml of water and 6 ml of acid, nor in samples containing 5 mg or less of rhodium. In the dissolved form rhodium sulphate had a yellow colour.

At concentrations up to 40 p.p.m., platinum did not interfere. Iridium solutions containing 100 to 200 p.p.m. of platinum gave optical densities that were inconsistent and lower than expected. In concentrations as low as 35 p.p.m. the presence of palladium in samples containing known amounts of iridium caused low and inconsistent results. These observations with platinum and palladium are thought to be the result of catalytic reduction of cerium^{IV} before the iridium was all oxidised. It is known that, under these conditions, iridium or platinum in solution brings about the reduction of large amounts of ceric sulphate.[4]

Consecutive analysis of one sample

Determination of iridium and then rhodium—

Samples containing both elements together were treated by the ceric sulphate procedure (*a*). The optical densities were recorded, and the samples were quantitatively transferred to beakers and heated to evaporate water. When the volume was approximately 10 ml, 0·1 to 0·2 g of sodium sulphite was added. The red colour changed to blue immediately, or sometimes the solution became colourless. The samples always became colourless on heating to fumes of sulphuric acid. The fuming was continued until the volume of the samples had been reduced to 1 to 2 ml. A green colour, probably that recorded by de Boisbaudran,[8] was sometimes observed in the samples after fuming, but it could be destroyed by the addition of a few millilitres of water and re-evaporation.

After fuming, the samples were cooled and 5 ml of water were added. Twenty millilitres of a 20 per cent. w/v ammonium chloride solution were added and the samples were evaporated on a steam-bath overnight to neutralise the sulphuric acid and to convert iridium and rhodium to chlorides. Evaporation to dryness or nearly to dryness was carried out at 100° to 115° C. The samples were transferred to test tubes and the beaker was rinsed twice with 5 to 7 ml of water to complete the transfer. They were then treated by the stannous chloride procedure as described above, except that the initial volume of the samples was 15 ml, not 5 ml, and that optical densities were measured against a water blank. The change in initial volume was proved in separate experiments not to affect the colorimetric results. A white precipitate remained in the test tube during the heating interval in the water-bath, but dissolved when the sample was made up to 50 ml with 2 *N* hydrochloric acid.

The optical densities were consistent, but smaller than predicted by the regression formula given above, *viz.*—

$$D_{Rh+Ir} = D_{Rh} + 0{\cdot}00150\,C$$

The smaller values could not be ascribed to the use of a water blank. Nor did the consistency of the results suggest mechanical losses or failure to convert sulphates completely to the chlorides. However, by increasing the heating interval to 1 hour 20 minutes the optical densities were increased to the predicted values. Thus, for 12 samples containing 9·96 p.p.m. of rhodium and concentrations of iridium between 36·1 and 90·3 p.p.m., the optical densities obtained by the stannous chloride procedure as outlined in this section were compared with the values expected from the regression formula based on other samples that had not previously been treated with ceric sulphate. The average deviations of these 12 samples from the expected values was 0·9 per cent. For 14 samples containing 19·8 p.p.m. and amounts of iridium in the same ranges as above, the average deviation of observed optical densities about the values predicted by the regression formula was 0·5 per cent.

Samples that had been treated by mixed perchloric - phosphoric acid for the colorimetric determination of iridium were treated in the same manner to prepare them for rhodium analysis. During the heating with stannous chloride a heavy white precipitate formed. This may be the well-known material that forms from β-stannic oxide and phosphoric acid. The precipitate was removed by centrifuging, but the optical densities of the solutions were inconsistent and considerably lower than the predicted values.

Determination of rhodium and then iridium—

Attempts were made to perform these determinations consecutively but in the reverse order. These attempts were unsuccessful. Preparation of the samples for analysis by ceric sulphate, following the stannous chloride procedure, required destruction of the coloured compounds of rhodium and iridium and conversion of these elements to the sulphate form. It was not possible to destroy the coloured complexes by fuming with sulphuric acid unless the sample had first been treated with hydrogen peroxide or chlorine gas. Heating with ceric sulphate produced a variety of colours ranging from red to blue. Tin chloride was hydrolysed or was converted after heating for a few hours to give a heavy opalescent precipitate, which made absorption measurements impossible. We were unable to find conditions that would permit the determinations to be carried out successfully in this order.

Discussion of results

By the foregoing procedures we have found it possible to analyse solutions containing rhodium and iridium without a separation. Solutions of these two elements that are substantially free of the other platinum metals are encountered in the customary scheme of separating and determining the precious metals. There is some question about the efficiency of the existing methods of separation of these metals when they are applied to small amounts. For such amounts the colorimetric procedure should prove advantageous.

Some comment should be made about the amounts of the two elements that can be handled by this method. The considerations that follow apply to the procedures as already described; it is recognised that by the use of aliquots, dilution to volumes greater than 50 ml, or by the technique of differential colorimetry, other limits may be reached. The following relations have been demonstrated—

$$D_1 = 0{\cdot}016 + 0{\cdot}00876\,C_{Ir} \quad .. \quad .. \quad .. \quad .. \quad (1a)$$

$$D_1 = 0{\cdot}007 + 0{\cdot}00917\,C_{Ir} \quad .. \quad .. \quad .. \quad .. \quad (1b)$$

$$D_2 = 0{\cdot}00379\,C_{Rh} + 0{\cdot}00150\,C_{Ir} \quad .. \quad .. \quad .. \quad (2)$$

From these we can derive—

Milligrams of iridium, by procedure (*a*) $= 5{\cdot}71\,D_1 - 0{\cdot}09$

by procedure (*b*) $= 5{\cdot}46\,D_1 - 0{\cdot}04$

Milligrams of rhodium, when iridium is determined

by procedure (*a*) $= 1{\cdot}32\,D_2 - 0{\cdot}226\,D_1 + 0{\cdot}004$

by procedure (*b*) $= 1{\cdot}32\,D_2 - 0{\cdot}216\,D_1 + 0{\cdot}002$

The maximum amounts of iridium for which equations (1*a*) and (1*b*) are valid are 5 mg and 3·75 mg, respectively. Various writers have pointed out that the precision with which optical densities can be determined depends upon the region of the transmission scale in which measurements are made. We might, therefore, somewhat arbitrarily decide that

optical density measurements should be confined within the range 0·1 to 1·0 to ensure a precision as good as 1 per cent. In this event 1·3 mg of rhodium will be the maximum amount of rhodium for which the stannous chloride procedure is recommended when iridium is absent. This amount may have to be reduced by as much as 0·2 mg if iridium is present up to 5 mg. By the same token, as the amounts of iridium or rhodium become less than 0·55 mg or 0·13 mg, respectively, it is to be expected that the determinations will be less precise than in the recommended range.

The only previously published method that has been completely worked out for the colorimetric determination of iridium is that of Ayres and Quick.[3] We have experienced difficulty in obtaining precise results by this procedure, and instead prefer to use the new ceric sulphate method, with which we attained satisfactory precision. Unfortunately neither procedure is particularly sensitive, and there remains great need of a sensitive method for the determination of traces of iridium.

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November 25th, 1953

Apparatus

AN APPARATUS FOR DETECTING PLASTICISER IN POLYMETHYL METHACRYLATE SHEET

(*Demonstrated at the meeting of the Physical Methods Group on Tuesday, November 24th*, 1953)

Polymethyl methacrylate as ordinarily manufactured may be plasticised or unplasticised. The plasticised material usually contains about 5 per cent. of dibutyl phthalate plasticiser. The two types of sample are identical in appearance. Although it is possible to obtain evidence of the presence or absence of plasticiser by (*a*) determination of the Vicat softening point, (*b*) actual determination of the plasticiser by the method of Haslam and Soppet[1] or (*c*) a direct spectrophotometric examination of a chloroform solution of the sample, these methods take some time to carry out and are usually costly. A quick method was required that would readily differentiate between plasticised and unplasticised samples. A simple and cheap apparatus has been devised and has been found extremely useful in day-to-day problems connected with the large-scale manufacture of plasticised and unplasticised polymethyl methacrylate sheet; it is probable that the apparatus will have other applications. Further, this method is applicable to polymethyl methacrylate sheet without impairing the sheet in any way. The general portability of the apparatus has been borne in mind in its design.

The principle of the test is that light from a mercury-vapour lamp passes through a condensing lens and filter and thence into a simple ultra-violet spectroscope, where it illuminates a fluorescent screen. Test sheets of polymethyl methacrylate placed in the light path exhibit differences in light absorption according to whether they are plasticised or unplasticised, and these differences are readily seen with the spectroscope. Full details of the apparatus and method are given below.

General description of apparatus

The general assembly of the apparatus is shown in Fig. 1. It is composed of two units, one consisting essentially of an ultra-violet lamp, lens and filter, all suitably mounted, the lamp cooled by an air system and the whole in a housing designed to permit no leakage of light. The other

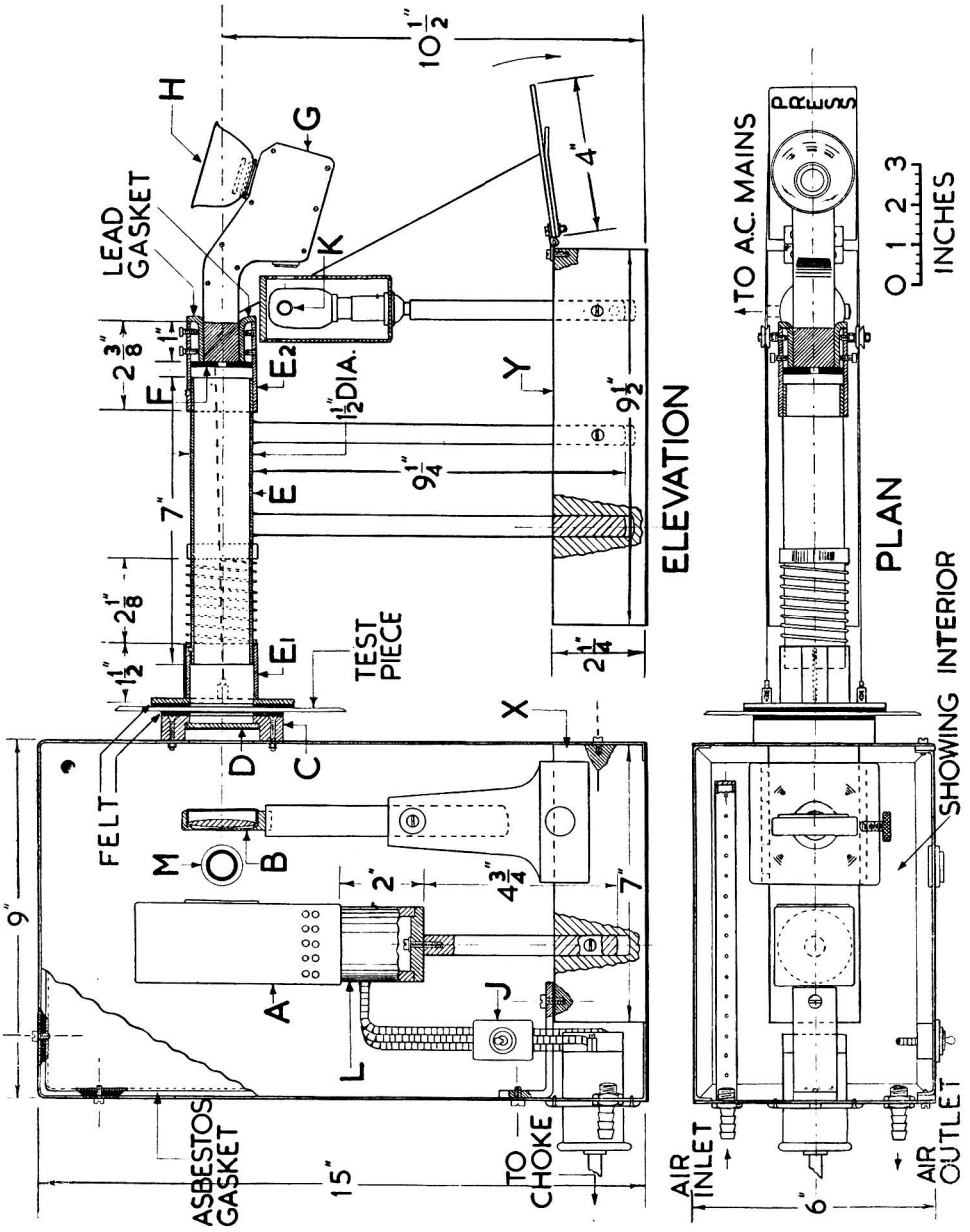

Fig. 1. General assembly of apparatus

unit consists of an optical column, spectroscope and lamp for illuminating the spectroscope scale, all mounted on a solid base and having a lever, pulley and spring system activating a head that clamps the test piece in position.

Fabrication and assembly

Ultra-violet lamp unit—

The ultra-violet lamp, A, is a high-pressure mercury-vapour lamp, box type with quartz window, 250 W, three-pin fitting. This is fitted into a 15-amp. power socket mounted inside a tubular Perspex container, L, having Perspex end-plates; the 15-amp. socket is screwed to the upper end-plate and the lower one is fitted to a brass rod $4\frac{3}{4}$ inches $\times$ $\frac{1}{2}$ inch diameter. The side of the container is drilled for wiring. The brass rod is fitted into a drilled hole in the base block, X, and positioned by a grub screw in the side of the block so that the lamp can be adjusted.

The quartz spherical condenser, B, and stand, Hilger F958, a standard piece of spectrographic equipment, is clamped to the base block $\frac{1}{2}$ inch from the end. The block itself, of solid brass, 7 inches $\times$ $2\frac{1}{4}$ inches $\times$ $2\frac{1}{4}$ inches, is attached to the lamp housing, described below, directly with screws at the right-hand end and indirectly with a strong metal bracket at the other.

The lamp housing is constructed of 18 S.W.G. tinned plate to the following over-all dimensions: length, 9 inches; height, 1 foot 3 inches; width, 6 inches. This is best fabricated by making a strip 3 feet 5 inches $\times$ 6 inches and cutting the following holes for fittings: (*i*) one for the light exit through the filter mounting, C, $1\frac{1}{2}$ inches diameter, on the axis, $11\frac{1}{4}$ inches from one end; (*ii*) two, for air couplings, $\frac{7}{16}$ inch diameter, 1 inch from either side and $1\frac{5}{8}$ inches from the end opposite to hole (*i*); and (*iii*) one for the panel power socket, $1\frac{7}{16}$ inches diameter, on the axis between the air couplings, 2 inches from the end. Right-angled bends are made at either end to give $\frac{1}{2}$-inch flanges and two further inward bends are made at distances of 1 foot 3 inches from these to give the general shape. A back is cut to size with a $\frac{1}{2}$-inch flange all round and soldered in position. The front plate, made to be detachable, is cut and bent with $\frac{1}{2}$-inch inward flanges on all sides to ensure a close fit in the housing, allowance being made for a continuous $\frac{1}{8}$-inch thick gasket on sides and top; the gasket is $\frac{1}{2}$ inch wide and made from "Walkerite" heat-resisting asbestos. Holes are cut in the front plate for the filter, M, 1-inch diameter signal bush, panel mounting, green glass, and on-off switch, J, Bulgin S290. Positions of M and J are shown in Fig. 1 as they would appear on the casing. For final fixing of the front plate, three holes are drilled and tapped on the top and either side, respectively, through the outer casing and asbestos into the flanges.

The mercury lamp generates much heat, but the air cooling system used has proved very successful. Air couplings used are Schrader male couplings No. 6362; to the one acting as the air inlet is attached a brass tube, 8 inches $\times$ $\frac{1}{2}$ inch internal diameter, sealed at the far end and having a series of holes drilled with a No. 4 drill to act as jets; the air outlet coupling is attached directly to the casing. It has been found advisable to tighten the jet tube in position with the jets facing upwards and tending slightly in the direction of the side of the lamp.

The power panel socket, Bulgin P162, housed between the couplings, is wired through the switch, J, to the lamp socket, L, with fish-spine bead insulation. The detachable plug that fits into the panel socket is wired to the a.c. mains through a suitable choke.

The filter housing, C, $\frac{5}{8}$ inch $\times$ 3 inches over-all diameter, with light outlet, $1\frac{1}{2}$ inches diameter, contains a filter, D, $1\frac{3}{4}$ inches diameter, Chance glass OX7, $\frac{3}{32}$ inch thick, held in position by a spring wire retainer, as shown in Fig. 1.

Before assembly, the whole outer casing is given a black crinkle-enamel finish and after assembly the gasket joint is painted over to act as a further seal. The facing of the filter housing, C, and the base of the block and flanges are lined with felt.

Spectroscope unit—

In using the apparatus, the operator stands facing it with the ultra-violet lamp unit on the left-hand side. The dimensions of this unit have been so designed as to suit the focal length of the fixed condensing lens, to allow easy access to the spectroscope, and for simple positioning of the test piece.

The column, E, is a brass tube, 7 inches $\times$ $1\frac{1}{2}$ inches exteral diameter, having a projecting slot pin $\frac{3}{8}$ inch from one end made to slide in a $\frac{1}{8}$-inch slot in E_1 and a collar $1\frac{5}{8}$ inches from the same end to support a spring. Brass supporting rods are $9\frac{1}{4}$ inches $\times$ $\frac{1}{2}$ inch external diameter with concave cut ends silver-soldered to the column.

The column, E_1, is a brass tube, $1\frac{1}{8}$ inches × $1\frac{3}{4}$ inches external diameter, turned down internally to slide fit on column E and having a $\frac{1}{8}$-inch slot cut along its length a distance of $\frac{1}{8}$ inch from either end. A soldered-on face plate, 3 inches diameter, with an aperture $1\frac{1}{2}$ inches diameter, contains two rod sockets, Meccano part No. 179, in a line $1\frac{3}{16}$ inches on either side of the centre; in these are placed two $\frac{1}{2}$-inch lengths of rod having holes bored down their axes with a No. 59 drill and a flattened edge for clamping. Column E_1, with slotted portion uppermost, slides along its pin against a compression spring, 16 S.W.G. × $2\frac{5}{8}$ inches over-all diameter, on E. The slotted portion is covered with a small plate.

The column, E_2, a brass tube $2\frac{3}{8}$ inches × $1\frac{3}{4}$ inches external diameter, is a push fit on column E and is positioned by a grub screw. Disc F, within the column, has a $\frac{3}{16}$-inch diameter aperture and is placed to allow a 1-inch entry of the spectroscope head, fixed by set screws, four each on the horizontal and vertical axes $\frac{1}{4}$ inch and $\frac{3}{4}$ inch, respectively, from the end of the column. All set screws are 4 B.A. Two of these are specially made to act also as pulley bearings, as shown in Fig. 1. The pulleys used are $\frac{1}{2}$-inch diameter ball-race type.

The interior of the optical column is given a dull black finish before assembly.

The spectroscope, G, a Beck ultra-violet spectroscope No. 2438 with adjustable slit, has proved to be a suitable instrument. In this instrument the light passes through the slit to a quartz prism and forms a green coloured spectrum on a fluorescent screen. Adjacent to the spectrum is a wavelength scale illuminated through a window, in this apparatus, by an external light source connected to the a.c. mains. Viewing is carried out through a focussing eyepiece with a rubber eye cup, H. The slit is opened to its maximum width, about 1 mm; under these conditions the spectrum obtained from the mercury source is brightened to give the appearance of a mercury "continuum."

A gasket of $\frac{1}{16}$-inch lead sheet is cut and bent to fit round the head of the spectroscope to serve as a seating for the set screws and also to prevent any leakage of light from the column. The spectroscope with gasket is pushed into column E_2 until the slit is against the aperture of the disc, F, and then adjusted by the set screws.

The lamp housing under column E_2 is essentially a tubular fitting with a cut-out aperture to illuminate the spectroscope scale and a small red coloured glass indicator, K, on the front to show the on - off position of the lamp; the position of K as it would appear on the casing is shown in Fig. 1. The lamp is 15 W, 240 V, mounted in an S.B.C. fitting. The housing has a number of holes drilled at the back for ventilation, spigot-jointed end-plates, and the whole is fixed to a tubular stand, which allows for adjustment and also enables the wiring to pass into the base block.

The base block, Y, of solid brass, $9\frac{1}{2}$ inches × $2\frac{1}{4}$ inches × $2\frac{1}{4}$ inches, is drilled to take the supporting rods of column E and the lampholder; holes are made for positioning grub screws and for an outlet for the lampholder wiring.

A suitable press-lever for activating head E_1 by a cord and pulley system has been made from $\frac{1}{8}$-inch Perspex sheet; the lever is hinged to block Y with a $1\frac{1}{2}$-inch brass butt hinge. Black nylon monofilament, 14 thousanths of an inch thickness, has been found to make a suitable non-stretch pulley cord; this is threaded and knotted through the rods on E_1, over the pulleys, through two small holes, No. 59 drill, near the edges of the lever and given support by a tongue on the underside.

The facing of head E_1 and the base of block Y are lined with felt before assembly. After assembly the whole unit is adjusted and the optical system is suitably aligned.

STANDARD SPECIMENS—

In this laboratory a series of authentic specimens of both plasticised and unplasticised polymethyl methacrylate sheet have been collected in the range of manufactured thicknesses between $\frac{1}{16}$ inch and $\frac{3}{4}$ inch. These have been cut into $3\frac{1}{2}$-inch squares and mounted side by side in line on an arm such that each arm contains a specimen of plasticised and unplasticised material of a given thickness; the arms have been made 1 foot long to give sufficient hand grip, and are engraved according to their nature.

METHOD—

Adjust the compressed-air source to give a reasonably rapid rate of flow and attach it to the air inlet. Switch on the mercury and spectroscope-scale lamps and allow about 5 minutes for the mercury lamp to warm up. Apply gentle finger pressure on the lever to pull back the head, E_1, and place the sheet under test in position, as shown in Fig. 1. Release the lever to clamp

the sheet in place. Read the wavelength of the resulting transmission cut-off through the eyepiece of the spectroscope and remove the sample.

RESULTS—

With this arrangement it is our experience that $\frac{1}{8}$-inch unplasticised polymethyl methacrylate sheet of normal manufacture, when placed in the beam of light from the mercury lamp, shows a sharp cut-off at 290 mμ, whereas with 5 per cent. plasticised sheet of the same thickness the cut-off is at 295 mμ. Although the apparatus has been designed primarily for $\frac{1}{8}$-inch sheet, differences in absorption are also shown by plasticised and unplasticised sheets of other thicknesses in the range $\frac{1}{16}$ to $\frac{3}{4}$ inch; it is always desirable, however, to have available authentic samples of plasticised and unplasticised sheet of the appropriate thickness for purposes of comparison.

NOTES—

The apparatus has also proved to be of great value in the ready detection of ultra-violet light absorbers, which are used to an increasing extent in the polymethyl methacrylate trade.

It may be of interest to note that the system of air cooling enables the mercury lamp to be used continuously for several hours.

The authors wish to thank Mr. L. Bloom for his valuable assistance in the construction of this apparatus.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
PLASTICS DIVISION
WELWYN GARDEN CITY, HERTS.

J. HASLAM
S. GROSSMAN
September 17th, 1953

THE DETERMINATION OF SOIL pH WITH THE GLASS ELECTRODE AND AN IMPROVED TYPE OF MORTON CELL

IT is the practice in our laboratory to determine soil and silage pH values by means of the glass electrode with a direct-reading bench-type Cambridge pH meter. In the past a modified Morton electrode system, as illustrated, for example, in Gallenkamp's "Laboratory Equipment and Scientific Apparatus," Twelfth Edition, Volume I, 1951, p. 226, was used, in which the bridge connection between the two half-cells was formed by a capillary of saturated potassium chloride solution across a three-position ground-in stop-cock. The alternative position of the stop-cock allowed flushing and cleaning of the glass-electrode half-cell. When this was used for soils, however, it was found that the ground-in stop-cock soon wore smooth and that the groove forming the capillary became channelled owing to the action of the soil particles. Consequently, after a comparatively short time (approximately 3000 determinations), the saturated potassium chloride solution began to leak into the glass-electrode cell during each determination, and caused inaccurate results. To obviate this difficulty the Morton cell has been modified, and the purpose of this note is to bring the improved apparatus to the notice of workers determining pH values of liquid suspensions containing abrasive particles. The new electrode system is shown in Fig. 1 (to scale). It consists of the usual calomel half-cell, A, containing a pencil-type calomel electrode immersed in saturated potassium chloride solution and dipping into solid potassium chloride contained in the cup, B. The bridge to the glass-electrode half-cell, C, is effected through a sintered-glass disc, D, of porosity 4. To prevent potassium chloride passing through the disc, hot autoclaved agar in saturated potassium chloride is introduced into the connecting tube, E, to a depth of about $\frac{1}{2}$ inch and allowed to set. The agar solution must be completely liquid when added, the sintered disc and the apparatus must be dry and the apparatus should be stood with the connecting tube, E, upright (sintered disc down) until the agar has set. The agar solution is made by adding 5 g of agar to 100 ml of saturated potassium chloride solution and heating it in an autoclave at 15 lb pressure for 20 minutes. The glass-electrode cell, C, consists of a wide tube at the top to take the glass electrode and having a side tube, F, attached. This wide tube is constricted immediately before the connection with the calomel half-cell, but the constriction is blown out to form a small bulge, G, opposite the sintered-glass disc, D, and the agar plug, H. The bulge is to take the main bulk of the liquid suspension to be tested and to house comfortably the bulb of the glass electrode.

Below the bulge the tube continues to an orifice, I, placed as closely as possible to the bulge, G. The orifice, I, is sealed by a piece of rubber tubing and a strong Mohr spring clip, J. K is a solid glass rod strengthening the structure.

After the apparatus has been standardised against a buffer solution of pH 3·1, the actual determination of soil pH is made by pouring sufficient of the soil suspension (2 parts of soil to 5 parts of water, shaken up and set aside for 30 minutes) down the side tube, F, to fill the bulge, G, above the level of the glass electrode and above the top of the sintered-glass disc (about 7 ml).

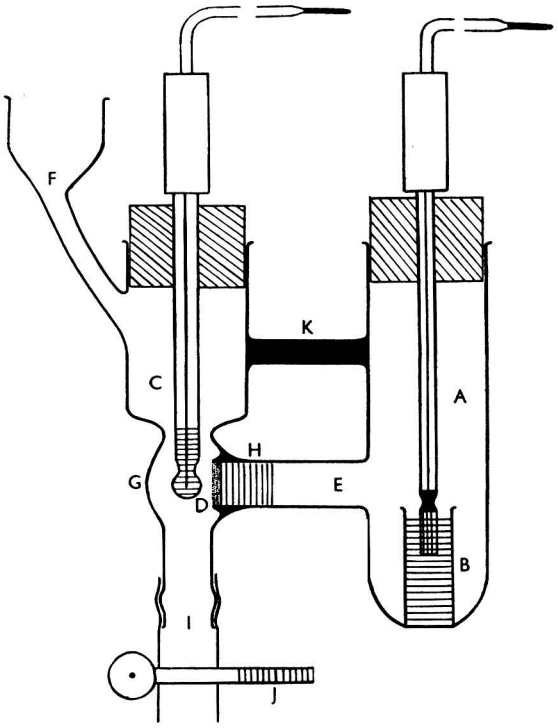

Fig. 1. Modified Morton cell. Half-scale diagram

The reading on the meter is then recorded after 30 seconds, the Mohr spring clip, J, is opened and water is flushed down the side tube from an overhead reservoir. The clip is now closed and another determination is begun. Once the soil suspensions have been prepared, one operator can make about 300 determinations per day. The cell appears to last indefinitely, the limiting factor being the life of the glass electrode. One of these modified cells has been used continuously and satisfactorily for over 30,000 determinations of soil and silage pH. After this time, while still working admirably, the sintered-glass disc was showing a dark patch in the centre. The agar plug was therefore removed and the disc cleaned by immersing the apparatus in dichromate cleaning mixture, followed by repeated sluicing with distilled water. After the glass had been dried and the agar plug replaced, the apparatus was again put into use.

A series of check tests with the old modified Morton cell and the re-designed cell showed excellent agreement.

Elswick Hall
Elswick Park
Newcastle upon Tyne, 4

N. Trinder
F. W. Handley
E. M. Drummond
October 28th, 1953

Ministry of Food

ZINC

THE Minister of Food has approved for publication a Report of the Food Standards Committee's Metallic Contamination Sub-Committee in respect of the limits of zinc in foods. The Report is as follows—

REPORT

"1. Zinc, like copper, has a definite physiological function in the human organism and is an essential constituent of an enzyme, which is found in red blood corpuscles and which plays an important part in the transport of carbon dioxide by the blood. Zinc occurs in the liver and spleen to the extent of 50 to 140 p.p.m. and is found in hair, nails, skin and teeth in amounts up to 200 p.p.m. Small amounts of zinc appear to be necessary for plant growth, but larger concentrations are toxic. Soils may contain from 1 to 5 p.p.m. of zinc and sometimes much more. Sea fish usually have a low zinc content, but very high concentrations (over 1000 p.p.m.) have been found in oysters.

"2. The normal daily intake of an adult has been estimated at 12 mg and the amount required to balance metabolic loss appears to be of the order of 10 mg.

"3. At the other extreme, outbreaks of violent vomiting have occurred from eating food prepared in zinc or galvanised iron containers and showing a zinc content of the order of 800 p.p.m., but there are no records of chronic poisoning. The emetic dose of zinc sulphate is shown in the British Pharmacopoeia at 600 to 2000 mg, but zinc sulphate is rarely, if ever, used for this purpose.

"4. The unsuitability of zinc and galvanised iron containers for storing or cooking food is no doubt widely recognised, but it may be pertinent to point out that outbreaks of poisoning have been attributed to the inadvertent use of galvanised vessels; for example, to drinking rain water collected from galvanised iron roofs or stored in galvanised iron tanks; that acid and saline liquids are particularly corrosive to zinc; and that galvanised iron vessels should not be used in the preparation of foods or for the storage of moist foods. It should also be pointed out that if containers made of sheet zinc are used for the storage of food, a film of zinc oxide may form which is easily detached and may contaminate the food.

"5. We have been furnished with figures of zinc contamination by Port Medical Authorities, Public Analysts and food manufacturing firms and we are satisfied that public health requirements would be met by observing limits consistent with efficient commercial practice as follows—

(*i*)	Beverages ready-to-drink	5 p.p.m.
(*ii*)	Other foods	50 p.p.m.

"For edible gelatin a maximum limit of 100 p.p.m. of zinc has already been prescribed; apart from this and from the reservation in paragraph 6 we do not think it should be necessary to provide limits for zinc contamination in other foods in excess of 50 p.p.m.

"6. In view of the high natural zinc content of certain animal and vegetable products, *e.g.*, herrings, shell-fish and crustacea, cereal offals and animal offals, etc., we consider that no objection should be taken to the sale of such articles containing zinc in excess of 50 p.p.m. if it can be shown that the zinc is of natural occurrence.

"7. In the absence of any new developments we do not consider that statutory effect need be given to the limits recommended."

The members of the Metallic Contamination Sub-Committee are Mr. G. G. Barnes (Chairman), Professor S. J. Cowell, Dr. J. M. Johnston, Dr. W. P. Kennedy, Dr. G. W. Monier-Williams, Dr. J. R. Nicholls, Dr. G. Roche Lynch, Mr. G. Taylor and Mr. B. W. Smith (Secretary).

Any representations that interested parties may wish to make on the Sub-Committee's recommendations should be addressed to the Secretary of the Metallic Contamination Sub-Committee of the Food Standards Committee at the offices of the Ministry of Food, Food Standards Division, Great Westminster House, Horseferry Road, London, S.W.1.

The Report is dated December, 1953.

British Standards Institution

NEW SPECIFICATIONS*

B.S. 2075:1954. Chains and Wheels for Single Bottle Width Slat Conveyors. Price 2s. 6d.

DRAFT SPECIFICATIONS

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

Draft Specification prepared by Sub-Committee C/17/2—Revision of B.S. 188.
CR(C)7651—Draft B.S. for Flow Cups and their Method of Use (for the Consistency Control of such Materials as Paints and Varnishes) (Revision of B.S. 1733).

Draft Specification prepared by Technical Committee CME/6—Inflammability of Films.
CT(CME)206—Draft B.S. Definition of Cinematograph "Safety" Film (Third Draft Revision of B.S. 850).

Book Reviews

PRACTICAL PHARMACOGNOSY. By T. E. WALLIS, D.Sc., F.R.I.C., Ph.C., F.L.S. Sixth Edition. Pp. x + 238. London: J. & A. Churchill Ltd. 1953. Price 18s.

The first edition of this book appeared in 1925, and the fact that a new edition has been called for at five or six-yearly intervals ever since is proof of its worth. The present book contains more than twice the material of the first edition, but differs only in detail from the fifth edition of 1948. The successful application of the principles of Pharmacognosy calls for an intimate knowledge of botanical morphology and histology together with considerable skill in manipulative microscopy. This work, designed primarily as a class book for students, forms a valuable introduction to a difficult subject.

The book is planned in five parts: I, Schedules of Instructions for the Examination of Drugs; II, Drug Description and Habitats; III, Histological Schedules; IV, Quantitative Analysis; V, The Examination of Powdered Drugs. Each of the schedules constitutes a practical lesson for the student, and the whole book is written to that end, the imperative mood being used for the enumerated instructions. There are seventy-two figures distributed through the text, most of these being beautifully executed drawings illustrating anatomical features of vegetable drugs or their tissues. It would have been useful for the ordinary analyst if drawings had been included in every schedule, especially the histological ones in Part III and in the Examination of Powdered Drugs in Part V; but, as the author modestly writes in the Introduction: ". . . illustrative sketches are added in several instances, so as to suggest the kind of drawing which students may make for themselves when writing up the results of their experiments and observations."

The section on Quantitative Analysis contains many interesting examples, most of them being applications of the author's System of Quantitative Microscopy (*Analyst*, 1916, **41**, 357), which is based on the fact that 1 mg of lycopodium always contains just about 94,000 spores. Thus, the method for determining the number of pollen grains in 1 mg of powdered pyrethrum is described and it is stated that good samples should contain between 1000 and 2000 and a specimen exercise with inferior material is quoted to show that it only contained 448 grains per mg. In another exercise it is shown how to determine the epidermal area per gram of powdered senna leaf, whence it is possible to estimate the amount of senna present in compound liquorice powder.

Part V, devoted to the Examination of Powdered Drugs, contains a vast amount of information compressed in tabular form and includes drawings of ten of the more common starches of commerce. An appendix of "Some Numerical Data" gives lists comprising, among other things, vein-islet and stomatal numbers and palisade ratios.

Initially designed as a class book, this work, through successive editions, has inevitably become something much more, and it is to be hoped that in a future edition its utility may be extended by the inclusion of references to the original papers (many of them the author's own) where the methods in Quantitative Analysis were first described. Again, additional illustrations, more particularly of powdered drugs, would greatly enhance the value of an excellent book.

N. L. ALLPORT

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

CHROMATOGRAPHIC METHODS OF INORGANIC ANALYSIS: WITH SPECIAL REFERENCE TO PAPER CHROMATOGRAPHY. By F. A. POLLARD, B.Sc., Ph.D., and J. F. W. MCOMIE, M.A., D.Phil. Pp. vii + 192. London: Butterworths Scientific Publications Ltd. 1953. Price 30s.

The steadily growing interest in the separation of inorganic mixtures by paper chromatography has stimulated the demand for a textbook on the subject. This book, the first by a British author dealing specifically with inorganic paper chromatography, is described by the authors as "an introduction to the use of filter paper and cellulose powder as a means of separation of inorganic compounds."

The first portion of the book is devoted to a simple non-mathematical treatment of the theories of chromatography and is followed by an excellent and comprehensive description of the various techniques and types of apparatus that have been successfully used. A further section deals with the choice of solvent and sets out clearly the various factors affecting inorganic separations. The newcomer to the technique of inorganic chromatography will find extremely useful this enumeration of the variables that he must take into account. A whole chapter is devoted to the location and identification of zones. This is an important part of the subject, but some doubt may be felt as to the value of including details of a long list of spot-test reagents without giving the sensitivity of each reagent. The danger of using an insufficiently sensitive reagent has been demonstrated by the number of separations that have been claimed as complete and that further testing with more sensitive reagents has shown to be only partial. Methods for the quantitative separation and determination of a number of metals are given in some detail, and a chapter on the separation of anions includes a very satisfactory presentation of the separation of condensed phosphates. Details of the separations of various mixtures of cations that have appeared in the literature are listed in tabular form. The list is comprehensive and a good bibliography makes it excellent for reference, but it is to be regretted that no comment is provided by the authors. This, however, in view of the early stage of development of the subject is excusable. A further chapter is devoted to two schemes devised by the authors for the analysis of unknown mixtures of cations. This is an excellent beginning to the attack on a difficult problem, but at the moment the schemes appear to rely as much upon specific spot-tests as upon chromatographic separations for their success.

The inorganic analyst will find this a useful book and it should stimulate his interest sufficiently to persuade him to try out for himself the paper chromatographic method of separation.

R. A. WELLS

Publications Received

TOXIC CHEMICALS IN AGRICULTURE: RESIDUES IN FOOD. Report of the Working Party. Pp. iv + 32. London: H.M. Stationery Office. 1953. Price 1s. 6d.

BIOCHEMICAL PREPARATIONS. Volume 3. Editor-in-Chief, E. E. SNELL. Pp. viii + 128. London: Chapman & Hall Ltd.; New York: John Wiley & Sons Inc. 1953. Price 28s.

HYGIENE IN PICKLE AND SAUCE FACTORIES. Guidance and Recommendations. Pp. 16. London: Food Manufacturers' Federation Inc. 1954. Price 1s.

PORTRAITS OF PAST PRESIDENTS

THE custom of supplying Portraits of Past Presidents to members of the Society and subscribers to *The Analyst* has been restored in a modified form. It is no longer possible to supply these photogravure reproductions with all copies of *The Analyst*, as was done before the war, but a sufficient number will be printed to supply gratis copies to all who make application to the Editor, *The Analyst*, 7 and 8, Idol Lane, London, E.C.3.

Two portraits will be available shortly, those of Dr. G. Roche Lynch (President: 1936–37) and Dr. G. W. Monier-Williams (President: 1945–46). A portrait of Dr. E. B. Hughes (President: 1940–42), who is the Society's Bernard Dyer Memorial Lecturer this year, is in preparation.

Orders for any of these three portraits should be sent in before April 30th, 1954.

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, Å 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 per 100 g"), as "per cent. w/v" (alternatively "g per 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 Mitchell,[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. Mitchell, C. A., *Editor*, "Allen's Commercial Organic Analysis," J. & A. Churchill Ltd., London, 1932, Volume 9, p. 149.

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.

THOMAS TYRER & CO. LTD., Chemical Manufacturers, Stratford, London, E.15, which is associated with Albright & Wilson Ltd., have vacancies in their laboratories for Research and Development Chemists. Duties involve investigational work concerning new products, both organic and inorganic, pilot plant research and technical service on the application of the company's products. Candidates under 30 years of age with Honours degree or A.R.I.C. and some experience of applicational research or analytical control are preferred, but applicants with qualifications of Inter. B.Sc. or National Certificate and some experience would be considered. Salary according to age, qualifications and experience. There is a non-contributory pension scheme. Apply to Appointments Officer, Albright & Wilson Limited, Oldbury, Birmingham.

ASSISTANT ANALYSTS required by London Pharmaceutical Company. Interesting work with reasonable prospects. Good conditions and excellent canteen facilities. Apply with full details of age, experience and salary required to Chief Chemist, Menley & James Limited, 120, Coldharbour Lane, London, S.E.5.

DISTILLERS CO. (BIOCHEMICALS) LTD. have vacancies in their Research Station in Cheshire for male and female analysts. Candidates should be in the age range 25–35 and must have an Honours degree in chemistry or equivalent. Previous analytical experience preferably in the antibiotics or related fields would be an advantage. Duties will relate to the development of new analytical processes rather than routine operations. Apply Staff Dept., Distillers Co. Ltd., 21, St. James's Square, London, S.W.1. Please quote DC(B) 154.

ALBRIGHT & WILSON LIMITED have a further number of vacancies for Junior Shift Chemists at their factory at Barry, Glam., which is engaged in the manufacture of Silicone products. Applications are invited from persons, preferably with Inter. B.Sc. or equivalent, under 30 years of age and who have completed their National Service.

The posts are of two types:—

(*a*) *For Plant Work.* Duties involve assisting in all aspects of plant operation and control.

(*b*) *For Laboratory Work.* Duties consist of quality control and laboratory scale production of new products.

The company possesses a non-contributory pension scheme. Commencing salary according to age, qualifications and experience. Applications to Appointments Officer, Albright & Wilson Limited, Oldbury, Birmingham.

THE UNIVERSITY OF LEEDS,
DEPARTMENT OF AGRICULTURE

THE COUNCIL will shortly appoint an Analyst on the salary scale £500 × £25–£550 a year, according to qualifications and experience. The appointment will be for a period of three years in the first instance. Applications (three copies) stating date of birth, qualifications and experience, together with the names of three referees, should reach the Registrar, The University, Leeds, 2 (from whom further particulars may be obtained), not later than 19th April, 1954.

ASSISTANT WORKS CHEMIST required in connection with the production of industrial alcohol and allied products. The post is tenable near London, and will involve control of raw materials and finished products. Age—up to 26 years. Applicants should have a degree in chemistry or equivalent. Salary will depend on qualifications and experience. Apply, Staff Dept., The Distillers Co. Ltd., 21, St. James's Square, London, S.W.1. Quote 1A.154

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