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Volume 90, No. 1068 March, 1965

Summaries of Papers in This Issue

The Determination of Zirconium (and Hafnium) in Niobium and Other Metals with Catechol Violet

A procedure, based on the extraction of zirconium ions with a solution of tri-n-octylphosphine oxide in cyclohexane, and the formation of a zirconium - catechol violet complex in the organic extract, has been successfully applied to the determination of zirconium, over the range 20 to about 1000 p.p.m., in niobium. Alloying amounts of chromium, cobalt, molybdenum and tungsten do not interfere.

The procedure has also been satisfactorily applied to the determination of zirconium in complex nickel-base alloys, and tests have shown that the method should be equally applicable to the determination of zirconium in aluminium, copper, iron, stainless steels, magnesium, titanium, tantalum, tungsten and vanadium. In the analysis of tantalum- or tungsten-base materials, a preliminary separation of zirconium from the parent metal is necessary.

Hafnium ions are also extracted into a solution of tri-n-octylphosphine oxide in cyclohexane, and produce a coloured complex with catechol violet in the organic extract; the procedure is, therefore, equally applicable to the determination of hafnium, provided that zirconium is absent.

D. F. WOOD and J. T. JONES

Imperial Metal Industries (Kynoch) Ltd., Kynoch Works, Witton, Birmingham 6.

Analyst, 1965, **90**, 125–133.

Some New Steroid Colour Reactions

A survey has been made of the colour reactions of steroids with perchloric acid and various aromatic aldehydes. More than eighty steroids and thirty aldehydes have been studied. During this work several new steroid colour reactions of potential value in quantitative analysis have been discovered. The nature of these reactions has been investigated, and a possible mechanism is discussed.

J. D. FEW

Department of Chemical Pathology, Charing Cross Hospital Medical School, London, W.C.2.

Analyst, 1965, **90**, 134–146.

Optical Scattering Cross-section of Small Particles and the Design of Photosedimentometers*

A technique is described for measuring variations in the optical scattering cross-sections of small particles. It is shown that absolute variations can be measured for small spheres when an independent measure of the size of the particles is available.

The technique has been used for measuring the scattering cross-sections of small spherical particles in a beam of white light under various conditions.

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* A description of work carried out at Nottingham and District Technical College.

Analyst, 1965, **90**, 147–154.

A Spectrophotometric Method for Determining the Aflatoxins

A method is described for determining the aflatoxins, particularly aflatoxin B_1, based on the intensity of the ultraviolet absorption at 363 mμ (the long-wavelength absorption maximum), after purification by thin-layer chromatography. This procedure has been applied to groundnut meal and crude aflatoxin isolated from cultures of certain strains of *Aspergillus flavus* and *A. parasiticus*. This method has only been applied to meals of high toxicity and is not applicable, in its present form, to meals of low or medium toxicity. The extraction and decomposition of aflatoxin B_1 have also been investigated.

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Analyst, 1965, **90**, 155–160.

The Photometric Determination of Zinc in Nickel Alloys for Use in Electronic Devices

A procedure is described for determining zinc in the range 5 to 400 p.p.m. on samples of nickel of 300 mg or less. After separation from the majority of interfering elements by extraction of chlorozincate ion with dioctylmethylamine, and complexing of co-extracted copper with biscyclohexanone oxalyldihydrazone, zinc is determined photometrically with 1-(2-pyridylazo)-2-naphthol.

Some deliveries of 1-(2-pyridylazo)-2-naphthol have been found unsatisfactory and a suitable test for reagent purity is proposed.

T. R. ANDREW and P. N. R. NICHOLS

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Analyst, 1965, **90**, 161–164.

Determination of Traces of Acrylonitrile Monomer in Liquid Extractants Used in Assessing the Suitability of Styrene - Acrylonitrile Copolymers as Food-packaging Materials

A cathode-ray polarographic procedure is described for determining acrylonitrile monomer in liquids obtained in extractability tests made on styrene - acrylonitrile copolymers. The procedure can be used for determining acrylonitrile in concentrations down to 1 p.p.m. w/v in the standard extraction liquids recommended by the British Plastics Federation and by the Food and Drug Administration (U.S.A.).

T. R. CROMPTON

Carrington Plastics Laboratory, Shell Chemical Company Limited, Carrington, Cheshire.

Analyst, 1965, **90**, 165–172.

The Polarographic Determination of Molybdenum in Niobium-base Alloys

Short Paper

J. B. HEADRIDGE and D. P. HUBBARD

Department of Chemistry, The University, Sheffield, 10.

Analyst, 1965, **90**, 173–175.

A Glass Ring Oven of Simple Design

Short Paper

P. HEATH
West Ham College of Technology, Romford Road, Stratford, London, E.15.

Analyst, 1965, **90**, 175–177.

Photometric Determination of Titanium in Ores, Rocks and Minerals with Diantipyrylmethane

Short Paper

P. G. JEFFERY and G. R. E. C. GREGORY
Warren Spring Laboratory, Department of Scientific and Industrial Research, Stevenage, Herts.

Analyst, 1965, **90**, 177–179.

Determination of Small Amounts of Long-chain Alkylamines in Aqueous Solution

Short Paper

H. M. N. H. IRVING and A. D. DAMODARAN
Department of Inorganic and Structural Chemistry, University of Leeds, Leeds, 2.

Analyst, 1965, **90**, 180–182.

A Rapid Spectrophotometric Method for Determining Parts per Million of Morpholine in Boiler Water

Short Paper

W. H. STEVENS and KIRSTEN SKOV
Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Chalk River, Ontario, Canada.

Analyst, 1965, **90**, 182–183.

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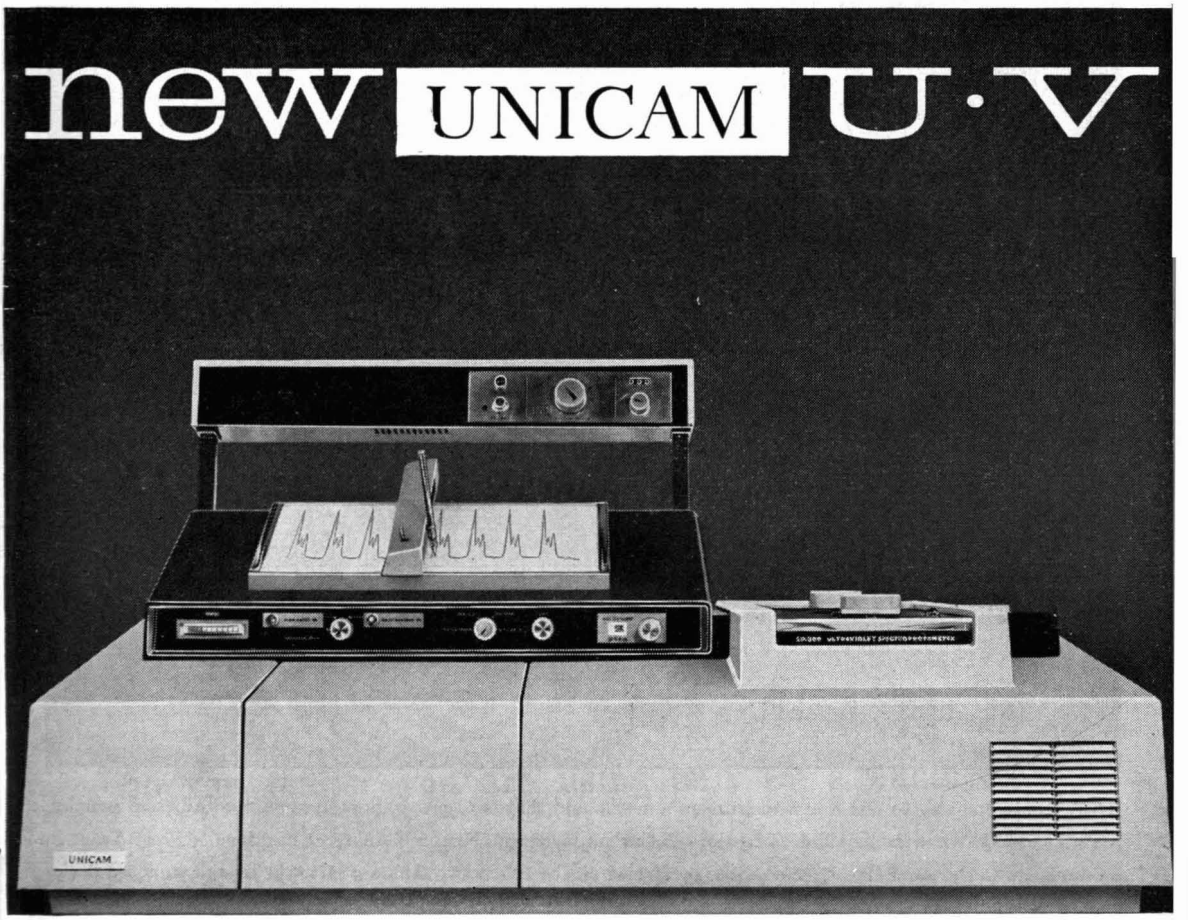

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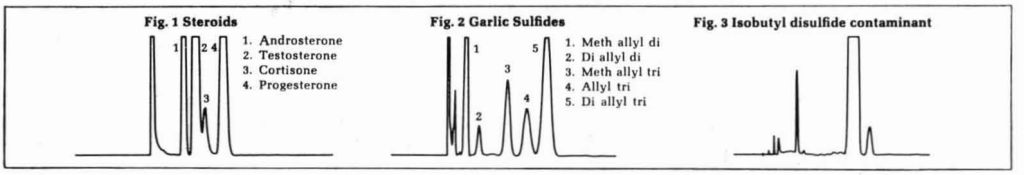

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THE ANALYST

The Determination of Zirconium (and Hafnium) in Niobium and Other Metals with Catechol Violet

By D. F. WOOD and J. T. JONES

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A procedure, based on the extraction of zirconium ions with a solution of tri-n-octylphosphine oxide in cyclohexane, and the formation of a zirconium - catechol violet complex in the organic extract, has been successfully applied to the determination of zirconium, over the range 20 to about 1000 p.p.m., in niobium. Alloying amounts of chromium, cobalt, molybdenum and tungsten do not interere.

The procedure has also been satisfactorily applied to the determination of zirconium in complex nickel-base alloys, and tests have shown that the method should be equally applicable to the determination of zirconium in aluminium, copper, iron, stainless steels, magnesium, titanium, tantalum, tungsten and vanadium. In the analysis of tantalum- or tungsten-base materials, a preliminary separation of zirconium from the parent metal is necessary.

Hafnium ions are also extracted into a solution of tri-n-octylphosphine oxide in cyclohexane, and produce a coloured complex with catechol violet in the organic extract; the procedure is, therefore, equally applicable to the determination of hafnium provided that zirconium is absent.

Specifications for reactor-grade niobium tube limit the zirconium content to a maximum of 500 p.p.m., and this necessitated the provision of a reliable analytical procedure for determining small amounts of zirconium in this material.

Several colorimetric reactions have been described for determining small amounts of zirconium, but none has been applied specifically to the analysis of niobium-base materials.

The most commonly used reagent for the colorimetric determination of zirconium is Alizarin red S.[1,2] This reagent is highly selective, although not as sensitive as *p*-dimethylaminoazophenylarsonic acid,[3] quercetin,[4] 2-*p*-sulphophenylazo-1,8-dihydroxynaphthalene-3,6-disulphonic acid (SPADNS),[5] chloranilic acid,[6] xylenol orange[7] or catechol violet (pyrocatechol violet),[8] which have all been recommended for the same purpose.

From published results, the use of xylenol orange and catechol violet appeared to offer advantages in respect of either sensitivity or selectivity, and these two reagents were chosen for further examination.

The use of either xylenol orange or catechol violet does not afford a direct means of determining small amounts of zirconium in niobium, because niobium salts are hydrolysed, and a precipitate is formed under conditions required for the development of their respective coloured zirconium complexes; further, the complexing agents capable of preventing hydrolysis, *e.g.*, tartrate, citrate, oxalate, fluoride, EDTA or hydrogen peroxide, interfere by inhibiting formation of the coloured zirconium complexes. Hence, a preliminary separation of zirconium is essential.

Of the methods published for isolating relatively small amounts of zirconium from other metals, a procedure involving its extraction into a solution of tri-n-octylphosphine oxide in cyclohexane from a nitric or hydrochloric acid solution, appeared to have the advantage that the zirconium - catechol violet complex could be developed in the organic extract,[9] and these reactions were used as a basis for further experimental work.

EXPERIMENTAL

A Unicam SP600 spectrophotometer was used in this experimental work.

In preliminary tests, a calibration graph was prepared after known amounts of zirconium had been extracted into a solution of tri-n-octylphosphine oxide in cyclohexane from a 7 M nitric acid solution, and the zirconium - catechol violet complex had been developed as described by Young and White[9]; nitric acid was used in preference to hydrochloric acid because fewer elements are extracted under these conditions.

CALIBRATION GRAPH—

The standard solution of zirconium was prepared from high-purity zirconium dissolved in diluted hydrofluoric acid (1 + 3). The solution was evaporated with sulphuric acid, sp.gr. 1·84, to remove fluoride, and the final solution was diluted so that 1 ml of solution contained 20 μg of the metal.

Aliquots of this standard solution were transferred to conical flasks. To each solution, and a blank solution, nitric acid, sp.gr. 1·42, was added, and the solutions were diluted so that the concentration of nitric acid was 7 M. Each solution was then treated as described below—

Zirconium was extracted by shaking the solution with 5·0 ml of a 0·02 M solution of tri-n-octylphosphine oxide in cyclohexane. A 1·0-ml aliquot of the extract was transferred to a *dry* 25-ml calibrated flask, the zirconium - catechol violet complex was developed as described by Young and White,[9] and the solution was diluted to the mark with absolute ethanol. The optical density was measured at 655 mμ in 2-cm cells.

A graph of optical-density values against concentration of zirconium was plotted that curved towards the concentration axis if the amount of zirconium present exceeded 15 μg, indicating that at these higher levels of zirconium, there was insufficient catechol violet for complete development of the characteristic colour. In further tests, the volume of 0·05 per cent. catechol violet solution was increased from 1·0 to 1·5 ml, and all optical-density values were higher. After the optical density of the reagent blank solution (0·03) had been deducted, a graph of the results gave a straight line passing through the origin, with an optical density of 0·62 corresponding to 20 μg of zirconium.

APPLICATION TO SOLUTIONS CONTAINING NIOBIUM—

Niobium is most conveniently dissolved in a mixture of potassium hydrogen sulphate and sulphuric acid, sp.gr. 1·84. Unfortunately, the extraction of zirconium with a 0·02 M solution of tri-n-octylphosphine oxide in cyclohexane, from a 7 M nitric acid solution, is seriously retarded by the presence of sulphate ions; only microgram amounts can be tolerated.[9] An alternative reagent for dissolving niobium is a mixture of concentrated nitric and hydrofluoric acids, but hydrofluoric acid suppresses the extraction of zirconium into the tri-n-octylphosphine oxide solution.

Tests were made to assess the extent to which an increased concentration of tri-n-octylphosphine oxide in cyclohexane would overcome the retarding effect of sulphate. Solutions, each containing 100 μg of zirconium, were made 7 M with respect to nitric acid and M with respect to sulphuric acid, and these were independently extracted with 5·0 ml of a tri-n-octylphosphine oxide - cyclohexane solution in which the amount of tri-n-octylphosphine oxide was varied. When the concentration of tri-n-octylphosphine oxide was increased over the range 0·01 to 0·05 M, there was a gradual increase in the recovery of zirconium, from about 35 to 98 per cent. When the concentration of tri-n-octylphosphine oxide was increased to 0·1 M, the recovery of zirconium was quantitative, and further tests, with the same reagent concentration, showed that quantitative recoveries could be obtained from nitric acid solutions in which the concentration of sulphuric acid was 2 M.

Aliquots of the standard zirconium solution (1 ml of solution ≡ 20 μg of zirconium) ranging from 1·0 to 6·0 ml were then added to 0·1-g samples of high-purity niobium; an additional sample of niobium was carried through the procedure as a blank determination.

Samples were dissolved in a strongly heated mixture of 2 g of potassium hydrogen sulphate and a few drops of sulphuric acid, sp.gr. 1·84, and the cooled melt was dissolved in 25 ml of 7 M nitric acid; at this stage, the sulphate ion concentration is less than M. Hydrolytic precipitation of niobium oxide occurred in the nitric acid solution, but this did not cause interference in the subsequent extraction with 5·0 ml of the 0·1 M tri-n-octylphosphine oxide

reagent; a good separation of the organic and aqueous phases was obtained, and the precipitate settled in the aqueous phase. A 1-ml aliquot of the organic phase was transferred to a 25-ml calibrated flask, and the coloured complex was developed as before with 1·5 ml of 0·05 per cent. catechol violet solution. The solutions were diluted to the mark with absolute ethanol and optical densities were measured as before. After the blank value had been deducted, a linear graph was obtained, identical with that obtained in the absence of niobium, and the graph was suitable for use in the determination of zirconium in niobium in the range 100 to 1000 p.p.m. These recoveries showed that if any zirconium had been adsorbed on to the precipitate of hydrated niobium pentoxide, it was completely extracted into the organic phase.

Further tests showed that the lower limit of zirconium (100 p.p.m.) could be extended to about 20 p.p.m. by taking a 2-ml aliquot of the organic extract and measuring the optical density of the complex in a 4-cm cell, and that higher levels of zirconium could be determined by applying the same procedure to a suitable aliquot of the sample solution.

EFFECT OF VARYING THE CONCENTRATION OF THE REAGENTS—

An increase in the amount of potassium hydrogen sulphate from 2 to 5 g, caused a decrease of about 10 per cent. in the zirconium recoveries. In subsequent tests, therefore, 2 ($\pm$ 0·1) g of potassium hydrogen sulphate were used.

A variation in the concentration of the sulphuric acid, between 0·02 and 2 M had no significant effect on the zirconium recoveries, provided that the nitric acid concentration was adjusted to 7 M before extraction with the tri-n-octylphosphine oxide solution. If the concentration of sulphuric acid was increased beyond 2 M, zirconium recoveries were invariably low; with a 5 M sulphuric acid solution, recoveries were about 75 per cent. In subsequent tests, the sulphuric acid concentration was maintained at about M.

Recoveries of zirconium were complete from solutions containing nitric acid at concentrations between 6 and 10 M; below 6 M recoveries gradually decreased as the nitric acid concentration was decreased. In further tests, a nitric acid concentration of 7 M was used.

By using a 0·1 M solution of tri-n-octylphosphine oxide (in cyclohexane) that had been allowed to stand for about 2 days, low recoveries of zirconium were obtained. ·It is recommended, therefore, that a fresh solution of the reagent should be used with each batch of samples.

Complete extraction of zirconium into 5 ml of the 0·1 M tri-n-octylphosphine oxide reagent was obtained from volumes of the aqueous solution (7 M nitric acid - M sulphuric acid) ranging from 25 to 75 ml, but with larger aliquots there was a gradual decrease in the amount of zirconium extracted. For example, with a 200-ml aliquot, only 80 per cent. of the zirconium was recovered.

A variation in the time of equilibration of the organic and aqueous phases, from 10 minutes up to 30 minutes, had no significant effect on the recovery of zirconium; an arbitrary time of 15 minutes is recommended in the proposed method.

Varying the volume of pyridine, between 2·5 and 10 ml, or the volume of catechol violet solution (0·05 per cent.), between 1·25 and 2 ml, had no effect on the optical density of the complex. However, owing to slow decomposition of catechol violet in ethanol, low recoveries of zirconium were obtained if the reagent solution had not been freshly prepared Further tests showed that widely differing sensitivities could be obtained with different batches of catechol violet. For example, optical densities of 0·64 and 0·85 were obtained (in 2-cm cells) for 20 μg of zirconium, with two different batches of reagent from the same supplier. It was suspected that this difference in sensitivity was due to the presence of an inorganic salt, introduced during manufacture, but the ash content of both reagent batches was less than 1 per cent. Presumably the difference in sensitivity is due to a variation in the amount of reactive constituent in the reagents, but no attempt was made at recrystallisation. It is essential, therefore, that a new calibration graph be prepared for each new batch of reagent.

The zirconium - catechol violet complex was completely developed within 5 minutes of the solution being diluted to 25 ml; thereafter it was stable for at least 2 hours.

EFFECTS OF OTHER METALS—

Tests on solutions containing 0·1 g of niobium in the presence of other metals showed that up to at least 20 per cent. of tungsten, 10 per cent. of molybdenum, or 15 per cent. of

chromium or cobalt, had no significant effect on the determination of zirconium over the range 100 to 1000 p.p.m.

By using solutions containing tungsten, in the absence of niobium, some of the precipitate of hydrated tungstic oxide, which forms in nitric acid solution, was extracted as a suspension into the organic phase, and this made it impossible to remove a clear aliquot for subsequent development of the coloured zirconium complex; when niobium was present, this difficulty did not arise, because the tungstic acid settled in the aqueous phase together with the precipitate of hydrated niobium pentoxide. Subsequent tests showed that the interference by tungsten, in the absence of niobium, could be avoided by separating zirconium as its hydroxide from a sodium hydroxide solution, with a magnesium salt to provide a magnesium hydroxide carrier. The precipitate was calcined, and fused with potassium hydrogen sulphate, before the proposed method was applied. In this way, satisfactory results were obtained in the determination of 200 p.p.m. to 0·5 per cent. of zirconium in solutions containing 0·1 g of tungsten.

Tests showed that titanium, up to about 0·05 mg (equivalent to 0·05 per cent., based on a 0·1-g sample), had no significant effect on the optical density of the zirconium - catechol violt complex. From solutions containing more than 0·05 mg of titanium, a small amount of titanium was extracted into the organic phase, and this subsequently interfered by producing a coloured complex with the catechol violet. Further tests showed that this interference could be avoided by washing the organic extract with 7 M nitric acid before the coloured complex was developed, as recommended by Young and White.[9] By using this modification, solutions containing 0·1 g of titanium were satisfactorily examined.

Iron, above about 0·2 per cent., and vanadium, above about 0·5 per cent., interfered in the same way as titanium, but these interferences could also be eliminated by washing the organic extract with 7 M nitric acid; by using this modified procedure, zirconium over the range 100 to 1000 p.p.m. was satisfactorily determined in solutions containing 0·1 g of iron or vanadium. Satisfactory results were also obtained in the examination of solutions containing 100 to 1000 p.p.m. of zirconium and 0·1 g of a stainless steel (18 per cent. chromium - 12 per cent. nickel - 3 per cent. molybdenum).

The procedure was applied to solutions containing separate 0·1-g additions of aluminium, copper, magnesium, nickel and tin, and satisfactory results were obtained without the need for washing the organic extract with nitric acid; details for preparing solutions of these and other metals, for extending the proposed procedure, are described under "Method II," p. 130.

In the examination of solutions containing 0·1 g of tantalum, low recoveries of zirconium were obtained, presumably owing to retention of zirconium by the precipitate of hydrated tantalum pentoxide that is formed in a nitric acid solution. Tests showed that satisfactory recoveries could be obtained if the zirconium was first separated as its hydroxide. This separation was achieved by fusing the *oxidised* sample with potassium carbonate, cooling and extracting the fused oxides with a potassium hydroxide solution, with a magnesium salt to provide a magnesium hydroxide carrier. The recovered precipitate was calcined, cooled and fused with potassium hydrogen sulphate before the proposed method was applied. In this way, satisfactory results were obtained in the determination of 200 p.p.m. to 0·5 per cent. of zirconium in tantalum.

Hafnium ions are also extracted into the solution of tri-n-octylphosphine oxide in cyclohexane and react in the same way as zirconium to form a coloured complex with catechol violet. In the determination of zirconium, therefore, any hafnium present introduces a positive error.

A series of solutions in 7 M nitric acid containing hafnium in the range 10 to 200 μg was examined by using the procedure applied to the analysis of solutions containing zirconium (see under "Preparation of Calibration Graph," p. 129), and a linear graph was obtained with an optical density of 0·62 (2-cm cells), corresponding to 40 μg of hafnium.

In the examination of solutions containing hafnium, tests showed that the presence of sulphate had a greater inhibiting effect on the extraction than in comparable extractions of zirconium. For example, whereas complete extraction of zirconium was obtained from a 7 M nitric acid solution, made up to 2 M with respect to sulphuric acid, the extraction of hafnium was complete only from solutions that were less than about 0·25 M with respect to sulphuric acid; with M sulphuric acid, the recovery of hafnium was about 80 per cent.

It was shown that maximum optical densities of the zirconium and hafnium complexes both occurred at about 655 mμ, and their molar extinction coefficients were about the same, about 40,000, depending on the quality of the catechol violet. The sensitivity of the hafnium reaction is, therefore, about half that of the zirconium reaction, because of their relative atomic weights.

Method I

This method can be used for determining 20 to 1000 p.p.m. of zirconium, or 40 to 2000 p.p.m. of hafnium in niobium and niobium alloys (see Note 1).

Reagents—

Standard zirconium solution—Transfer 0·1 g of high-purity zirconium to a small platinum dish, add about 5 ml of water, then 40 per cent. hydrofluoric acid, dropwise, until the metal has dissolved. Oxidise the solution with a few drops of nitric acid, sp.gr. 1·42, simmer it gently to expel oxides of nitrogen, then cool it. Add 10 ml of sulphuric acid, sp.gr. 1·84, evaporate the solution to fumes of sulphuric acid, fume it for about 10 minutes, then cool it. Add about 20 ml of water, transfer the solution to a 100-ml calibrated flask, add 20 ml of sulphuric acid, sp.gr. 1·84, cool the solution to room temperature, then dilute it to the mark.

Dilute 5 ml of this solution to 500 ml, then—

1 ml of solution $\equiv$ 10 μg of zirconium.

Standard hafnium solution—Transfer 0·2 g of high-purity hafnium to a small platinum dish and continue as described above for the preparation of the standard zirconium solution.

1 ml of solution $\equiv$ 20 μg of hafnium.

Tri-n-octylphosphine oxide, 0·1 M—Dissolve 1·93 g of tri-n-octylphosphine oxide in 50 ml of cyclohexane. This is sufficient for 10 determinations. This solution must be freshly prepared; it is stable for about 24 hours.

Catechol violet solution, 0·05 *per cent.*—Dissolve 10 mg of catechol violet in 20 ml of absolute ethanol. This is sufficient for about 12 determinations. This solution must be freshly prepared.

Nitric acid, 7 M—Transfer 44 ml of nitric acid, sp.gr. 1·42, to a 100-ml calibrated flask and dilute it to the mark.

Preparation of calibration graph—

Add, separately, 2·0, 4·0, 6·0, 8·0, 10·0 and 12·0 ml of the standard zirconium (or hafnium) solution (see Note 2) to each of six 100-ml conical flasks fitted with B24 sockets.

When the graph is to be used for determinations of hafnium in the range 40 p.p.m. to 0·2 per cent., it must be prepared from samples containing 0·1 g of high-purity niobium, dissolved as described in "Procedure," below. Conditions for the extraction of zirconium are less critical, and the presence of niobium and potassium hydrogen sulphate is not essential.

Proceed with each solution (and a blank solution), as given below—

Add 11 ml of nitric acid, sp.gr. 1·42, to the solution and dilute it (with water) to 25 ml. Add 5·0 ml of 0·1 M tri-n-octylphosphine oxide, stopper the flask, then shake it well for 15 minutes with a mechanical shaker, to equilibrate the solutions. Transfer the contents of the flask to a 25-ml graduated cylinder and allow the organic and aqueous phases to separate. Transfer 1·0 ml of the organic (upper) layer to a *dry* 25-ml calibrated flask. Add 10 ml of absolute ethanol, 1·5 ml of the catechol violet solution, and 5 ml of pyridine. Dilute the solution to the mark with absolute ethanol, set it aside for 5 minutes, then determine the optical density at a wavelength of 655 mμ, in 2-cm cells.

Procedure—

Determine a reagent blank value with each batch of samples.

Transfer 0·1 g of the sample to a 100-ml conical flask fitted with a B24 socket. Add 2·0 g of potassium hydrogen sulphate, 5 drops (about 0·3 ml) of sulphuric acid, sp.gr. 1·84, heat the mixture gently over a Meker burner until the sample has dissolved, then cool it. Add 11 ml of nitric acid, sp.gr. 1·42, 14 ml of water, heat the mixture gently until the fused mass has dissolved, then cool it; niobic acid precipitates at this stage, but the precipitate does not interfere.

Add 5·0 ml of the 0·1 M tri-n-octylphosphine oxide, stopper the flask, then shake it well for 15 minutes with a mechanical shaker, to equilibrate the solutions.

Transfer the contents of the flask to a 25-ml graduated cylinder and allow the organic and aqueous phases to separate (see Note 3). Transfer 1·0 ml (see Note 4) of the organic (upper) layer to a *dry* 25-ml calibrated flask and continue as described above under "Preparation of Calibration Graph."

Deduct the blank value and calculate the zirconium (or hafnium) content of the sample by using the calibration graph.

NOTES—

1. Up to at least 20 per cent. of tungsten, 15 per cent. of chromium or cobalt, or 10 per cent. of molybdenum, does not interfere. A vanadium content above 0·5 per cent., iron above 0·2 per cent. or titanium above 0·05 per cent. interferes.

2. Prepare a new calibration graph with each new batch of reagent and check at least one point on the graph with each batch of samples.

3. In the examination of samples containing more than about 0·5 per cent. of vanadium, 0·2 per cent. of iron or 0·05 per cent. of titanium, wash the organic extract with 7 M nitric acid, as described below—

Transfer about 4 ml of the organic extract from the 25-ml graduated cylinder into a 100-ml conical flask, fitted with a B24 socket, containing 25 ml of 7 M nitric acid. Stopper the flask and shake it well for about 15 minutes, with a mechanical shaker, to equilibrate the solutions. Transfer the contents of the flask to a 25-ml graduated cylinder and allow the organic and aqueous phases to separate. Transfer 1·0 ml (see Note 4) of the organic (upper) layer to a *dry* 25-ml calibrated flask and continue as above described under "Preparation of Calibration Graph."

4. For zirconium contents in the range 20 to 100 p.p.m., or hafnium contents in the range 40 to 200 p.p.m., use a 2-ml aliquot.

METHOD II

This method may be used for determining zirconium or hafnium in other metals.

REAGENTS—

Prepare, in addition to those detailed under "Method I," p. 129, the reagents given below—

Acidic solvent (for nickel-base alloys)—To 40 ml of water, add 5 ml of nitric acid, sp.gr. 1·42, and 5 ml of sulphuric acid, sp.gr. 1·84, mix well, then cool.

Magnesium sulphate carrier solution (for use in the analysis of tantalum and tungsten)—Dissolve 0·1 g of high-purity magnesium in 25 ml of diluted sulphuric acid (1 + 24).

PREPARATION OF CALIBRATION GRAPH—

Prepare a calibration graph as described under "Method I."

PROCEDURES—

Except in the analysis of tantalum and tungsten, for which special separation procedures are described, transfer the appropriate weight of sample (see under "Method I") to a conical flask fitted with a B24 socket. Dissolve the metal or alloy, as described below, then follow Method I.

In some instances an alternative dissolution procedure, that may permit some alloys to be dissolved more readily, is also described below.

Aluminium and aluminium alloys—(*a*) Dissolve the sample in 10 ml of 10 per cent. sodium hydroxide solution; if necessary, warm the mixture gently to assist dissolution. After the sample has dissolved, add 5 ml of water and 12 ml of nitric acid, sp.gr. 1·42, then cool the mixture.

(*b*) Dissolve the sample in 10 ml of diluted hydrochloric acid (1 + 1); if necessary, warm the mixture gently to assist dissolution. When the sample has dissolved, add 5 ml of diluted sulphuric acid (1 + 4), evaporate the solution to fumes of sulphuric acid, then cool it. Add 11 ml of nitric acid, sp.gr. 1·42, 14 ml of water, heat the mixture to boiling-point to dissolve the residue, then cool the solution.

Copper and copper alloys—Dissolve the sample in 25 ml of diluted nitric acid (1 + 1), boil the solution gently for 2 to 3 minutes to expel oxides of nitrogen, then cool it.

Iron and steels—Dissolve the sample in 5 ml of diluted sulphuric acid (1 + 4); heat the mixture gently to assist dissolution. Oxidise the solution with a few drops of nitric acid, sp.gr. 1·42, heat it to boiling-point, then cool it. Add 11·0 ml of nitric acid, sp.gr. 1·42,

and 9 ml of water. After extraction with the tri-n-octylphosphine oxide reagent, wash the organic extract as described under "Method I."

Magnesium and magnesium alloys—(*a*) Prepare the sample solution for extraction as described above for the analysis of copper and copper alloys.

(*b*) Dissolve the sample in 5 ml of diluted sulphuric acid (1 + 4). Add 11 ml of nitric acid, sp.gr. 1·42, and 9 ml of water, boil the solution gently for 2 to 3 minutes to expel oxides of nitrogen, then cool it.

Nickel and nickel alloys—(*a*) Prepare the solution for extraction as described for copper and copper alloys.

(*b*) The procedure given below is particularly useful for dissolving samples containing alloying amounts of chromium.

Dissolve the sample in 10 ml of the acidic solvent, evaporate the solution to fumes of sulphuric acid, fume the mixture for about 10 minutes, then cool it. Add 11 ml of nitric acid, sp.gr. 1·42, and 14 ml of water, heat the mixture gently to dissolve the residue, then cool the solution.

Tin—Prepare the sample solution for extraction as described for the analysis of iron and steels; in this instance the organic extract need not be washed with 7 M nitric acid.

Titanium and titanium alloys—Dissolve the sample in a heated mixture of 2·0 g of potassium hydrogen sulphate and 5 drops (about 0·3 ml) of sulphuric acid, sp.gr. 1·84, then cool it. Add 11 ml of nitric acid, sp.gr. 1·42, and 14 ml of water, heat the mixture gently until the fused mass has dissolved, then cool it. After extraction with the tri-n-octylphosphine oxide reagent, wash the organic extract as described under "Method I."

Vanadium and vanadium alloys—Dissolve the sample in a mixture of 2 ml of diluted sulphuric acid (1 + 4), 12 ml of water and 11 ml of nitric acid, sp.gr. 1·42; heat the solution gently to assist dissolution, then cool it. After extraction with the tri-n-octylphosphine oxide reagent, wash the organic extract as described under "Method I."

Tantalum—Transfer the appropriate weight (see Note 5) of finely divided sample (see under "Method I") to a small platinum dish, add 2·5 ml of the magnesium sulphate carrier solution, and evaporate the solution to dryness.

Transfer the dish to a muffle-furnace maintained at 700° C, and leave it for about 1 hour, so that the tantalum is oxidised completely, then cool it. Add 2 g of potassium carbonate and heat the dish gently over a Meker burner until the oxides have completely fused, then cool it. Add about 20 ml of water, warm the mixture gently to dissolve soluble salts, add a small amount of paper pulp, then set the solution aside for about 1 hour. Filter the solution through a No. 540 Whatman filter-paper, and wash the precipitate with water. Transfer paper and precipitate to the original platinum dish, dry it, char and ignite it with the usual precautions, then cool it. Add 2·0 g of potassium hydrogen sulphate and heat the mixture gently over a Meker burner until the oxides have completely fused, then cool it. Add 11 ml of nitric acid, sp.gr. 1·42, 4 ml of water, heat gently until the fused mass has dissolved, then cool it. Transfer the solution to a 100-ml conical flask fitted with a B24 socket, rinse the platinum dish with 10 ml of water, and add the washings to the flask.

Tungsten—Transfer the appropriate weight (see Note 6) of finely divided sample (see under "Method I") to a platinum dish (50 ml). Add about 5 ml of water, 5 ml of 40 per cent. hydrofluoric acid and nitric acid, sp.gr. 1·42, dropwise. When the sample has dissolved, cool the solution and add 2 ml of sulphuric acid, sp.gr. 1·84, evaporate to fumes of sulphuric acid, fume for about 10 minutes, then cool the solution. Add 10 ml of water, 2·5 ml of the magnesium sulphate carrier solution, and 10 ml of 50 per cent. sodium hydroxide solution. Stir the solution until the tungstic oxide has dissolved, then add a small amount of paper pulp, and set the mixture aside for 1 hour. Filter the solution through a No. 540 Whatman filter-paper and wash the precipitate with water. Transfer the paper and precipitate to the original platinum dish, dry, char and ignite with the usual precautions, then cool the residue. Add 2·0 g of potassium hydrogen sulphate and heat the mixture over a Meker burner until the oxides have completely fused, then cool it. Add 11 ml of nitric acid, sp.gr. 1·42, and 4 ml of water, heat the solution gently until the fused mass has dissolved, then cool it. Transfer the solution to a 100-ml conical flask fitted with a B24 socket, rinse the platinum dish with 10 ml of water and add the washings to the flask.

NOTES—

5. For zirconium contents less than about 200 p.p.m. (or hafnium contents less than about 400 p.p.m.) use a 0·5-sample, fuse it with 10 g of potassium carbonate and extract the melt with about 100 ml of water.

6. For zirconium contents less than 200 p.p.m. (or hafnium contents less than 400 p.p.m.) use a 0·5-g sample.

EXAMINATION OF TYPICAL SAMPLES

The results of the tests described below are given in Table I.

TABLE I

DETERMINATION OF ZIRCONIUM AND HAFNIUM IN VARIOUS MATERIALS

Sample	Nominal composition	Zirconium		Hafnium	
		Added, p.p.m.*	Determined, p.p.m.	Added, p.p.m.*	Determined, p.p.m.
1	Reactor-grade niobium tube	—	55, 50, 50	—	20, 25†
		100	100, 95	200	195, 190
		200	205, 190	400	390, 410
2	Reactor-grade niobium tube‡	—	275, 275, 290	—	—
		—	300, 285, 275	—	—
3	Reactor-grade niobium tube	—	80, 80, 90	—	40, 35†
		200	190, 200	500	495, 500
		400	410, 385	1000	980, 990
4	Nickel-base alloy	—	130, 130	—	—
5	Nickel-base alloy	—	230, 220	—	—
6	Nickel-base alloy	—	390, 390	—	—
7	Niobium - 12 per cent. titanium alloy	—	60, 70	—	—
		500	495, 480	—	—
8	Tantalum	—	<20, <20	—	—
		1000	950, 970	—	—
9	Vanadium - 12·5 per cent. aluminium - 12·5 per cent. tungsten alloy ..	—	25, 25	—	—
		200	195, 200	—	—
10	Tungsten	—	—	—	<20
		—	—	500	480, 490
11	Tungsten	—	—	—	0·49%, 0·46%

* Zirconium (or hafnium) content of the parent metal has been deducted from the determined values quoted.

† Values obtained by assuming the optical densities obtained are due entirely to hafnium. These two samples are *not* the samples 1 and 3, analysed for zirconium, although the material is essentially the same.

‡ The standard deviation is about 10 at the 300 p.p.m. level.

The proposed method was applied to the analysis of several samples of reactor-grade niobium tube containing about 50 to 300 p.p.m. of zirconium, and reproducible results were obtained. A niobium - 12 per cent. titanium alloy was also examined, and this was shown to contain about 60 p.p.m. of zirconium (interference by titanium was avoided by washing the organic extract with 7 M nitric acid).

The method was also successfully applied to several complex nickel-base alloys containing alloying amounts of aluminium, chromium, cobalt, molybdenum, titanium and small amounts of zirconium (150 to 400 p.p.m.); the organic extracts were washed with 7 M nitric acid to avoid interference by titanium. The zirconium contents of these samples, as determined by the proposed colorimetric procedure, were substantiated by further tests in which the zirconium (and titanium) was first separated from other metals by means of a precipitation with cupferron; the colorimetric procedure was then applied to the precipitate (ignited) containing the zirconium.

A vanadium-base alloy (12·5 per cent. aluminium - 12·5 per cent. tungsten) was also examined, and this was shown to contain about 25 p.p.m. of zirconium, the organic extract was washed with 7 M nitric acid to eliminate interference by vanadium.

A sample of tungsten containing about 0·5 per cent. of hafnium was examined after hafnium had been separated as its hydroxide from a sodium hydroxide solution, and reproducible results were obtained.

Conclusions

Tests have shown that the inhibiting effect of sulphate ions on the extraction of zirconium ions from acidic nitrate solutions, with a solution of tri-n-octylphosphine oxide in cyclohexane, is dependent on the concentration of tri-n-octylphosphine oxide in the organic solvent; an increase in the tri-n-octylphosphine oxide concentration results in an increased tolerance to sulphate ions. If a 0·1 M solution of tri-n-octylphosphine oxide is used, in place of the 0·02 M solution recommended by Young and White,[9] quantitative extraction of zirconium can be achieved from acidic nitrate solutions containing a concentration of sulphuric acid up to as much as 2 M; for quantitative extraction of hafnium, the strength of the sulphuric acid must not exceed about 0·25 M.

The selectivity of the extraction is not impaired by using this higher concentration of tri-n-octylphosphine oxide, and the increased tolerance to sulphate ions permits the extraction to be successfully used in developing procedures for the determination of zirconium (or hafnium) in niobium, tantalum, tungsten and other materials.

The method based on the extraction of zirconium ions from a nitric acid - sulphuric acid solution into a 0·1 M solution of tri-n-octylphosphine oxide in cyclohexane, and the formation of a zirconium - catechol violet complex in the organic extract, is suitable for determining zirconium, over the range 20 to about 1000 p.p.m. in niobium. Modifications to the method permit it to be extended to the determination of zirconium in many other metals and alloys.

Because of the close chemical similarity of zirconium and hafnium, there is no simple chemical procedure available for differentiating between these two elements; it is not surprising that hafnium ions are also extracted into a solution of tri-n-octylphosphine oxide in cyclohexane, and it is somewhat unfortunate that the coloured complex formed between hafnium and catechol violet has a maximum optical absorption at the same wavelength as the zirconium complex. In the determination of zirconium, therefore, any hafnium present in the sample introduces a positive error. When only hafnium is present, the method can be used for the determination of hafnium.

The procedures described have been used regularly, particularly for determining small amounts of zirconium in reactor-grade niobium. Satisfactory results have been obtained in the analysis of this material, and in the less frequent examination of complex nickel-base alloys and other materials of metallurgical interest.

By using the proposed procedure, about 12 determinations of zirconium (or hafnium) in niobium, can be made in a normal working day.

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Some New Steroid Colour Reactions

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A survey has been made of the colour reactions of steroids with perchloric acid and various aromatic aldehydes. More than eighty steroids and thirty aldehydes have been studied. During this work several new steroid colour reactions of potential value in quantitative analysis have been discovered. The nature of these reactions has been investigated, and a possible mechanism is discussed.

Despite the many steroid colour tests that have been described, relatively few have been used for determining urinary steroids, and of these several leave much to be desired in terms of sensitivity, specificity and convenience. This paucity of colour reactions is particularly marked for the non-ketonic steroids, especially the saturated ones. The antimony trichloride reagent of Pincus[1] gives blue colours with androstanediol and pregnanediol, but the reaction is not very sensitive with these compounds. The reagent was used by Pincus for determining total non-ketonic steroids after a Girard separation,[2] but it seems now to have fallen into disuse. The yellow colours given by pregnanediol and pregnanetriol with sulphuric acid are widely used for determining these steroids, but these reactions are extremely susceptible to interference by non-steroidal material.

Indirect methods of colorimetrically determining steroid alcohols based on the individual hydroxyl groups, rather than the molecule as a whole, have been described. Kellie and Wade[3] esterified steroid alcohols with 2,4-dinitrobenzoic acid and determined the resulting steroid 2,4-dinitrobenzoates by a reverse Zimmermann reaction with acetone and potassium hydroxide. Engel and Baggett[4] converted steroid acetates into acetohydroxamic acid, which yielded a coloured complex with ferric ions. The use of these reactions in the steroid field has only rarely been reported.

Of the known colour reactions that had not previously been used in quantitative steroid analysis, two seemed worth investigating for this purpose. Of these, the vanillin - perchloric acid reagent of Godin[5] has already been shown by the author[6] to be useful for locating, on paper chromatograms, pregnanetriol and some related steroids, but not pregnanediol. The other was the Komarowsky reaction,[7,8] which is given by higher alcohols and some other compounds[9] when they are heated with strong mineral acids and an aromatic aldehyde.

A solution to the problem of adapting these two colour reactions to the quantitative determination of steroids was discovered when it was found that pregnanetriol gave intense colours when heated with a solution of vanillin in glacial acetic acid and a concentrated mineral acid; the use of non-hydroxylic solvents was essential, and the best results were obtained by developing the colour in a small volume of liquid and subsequently diluting the solution with glacial acetic acid to a volume convenient for spectrophotometry.

When satisfactory operating conditions for a vanillin - perchloric acid reagent had been established the scope of this type of reaction was investigated with a wide range of steroids and aromatic aldehydes. From this survey, many new colour reactions of potential value in steroid analysis have emerged, and the general principles underlying this type of reaction have been elucidated.

Experimental

Spectrophotometry—

All optical densities of the steroids were read (against reagent blank solutions that had been treated in exactly the same manner as the steroids) in 1-cm glass cells in a Unicam SP600 spectrophotometer.

Reagents—

Vanillin, glacial acetic acid and 72 per cent. perchloric acid were of AnalaR grade and were used as purchased. Most commercial samples of solid aldehydes were purified by passing a solution of the aldehyde in benzene or ether through a short column of alumina, which removed coloured impurities. The eluate was concentrated, and the aldehyde that crystallised out was re-crystallised. Furfural and benzaldehyde were redistilled *in vacuo*.

Many of the steroids used in this study were prepared by the author. The sources of the other steroids are indicated in Tables IV to IX.

COLOUR DEVELOPMENT—

With vanillin—The dry steroid residue was dissolved in 0·1 ml of a 10 per cent. w/v solution of vanillin in glacial acetic acid; 0·2 ml of 72 per cent. perchloric acid was added, the reagents were well mixed and the tubes heated in a water-bath at 70° C for 15 minutes. After the solution had been cooled in cold tap water, 10 ml of glacial acetic acid were added.

With resorcylicaldehyde—As for vanillin except that the tubes were heated at 37° C instead of 70° C.

With p-*hydroxybenzaldehyde*—As for vanillin.

With o-*phthalaldehyde*—The dry steroid residue was dissolved in 0·1 ml of a 5 per cent. solution of *o*-phthalaldehyde in glacial acetic acid; 0·2 ml of a (1 + 1) mixture of glacial acetic acid and 72 per cent. perchloric acid was added. The colour was allowed to develop for 15 minutes at 25° C, and then 10 ml of a (1 + 1) mixture of glacial acetic acid and 60 per cent. perchloric acid were added. The high concentration of perchloric acid in the diluent is necessary to prevent fading of the colours.

RESULTS

REACTION CONDITIONS—

The reaction conditions for the vanillin - perchloric acid reagent described above were chosen as a result of studying the effect of the factors of time, temperature, volume and concentration of vanillin solution, and volume and concentration of perchloric acid on colour

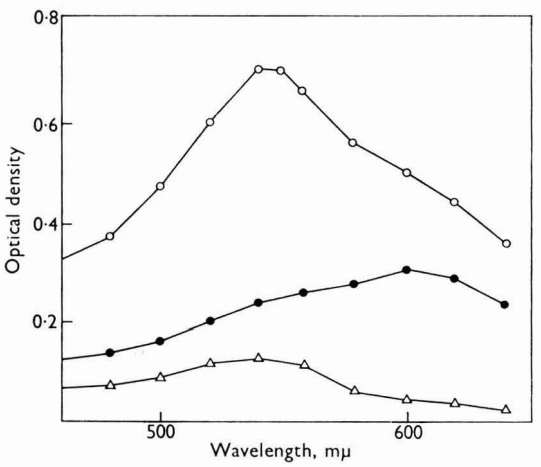

Fig. 1. The effect of temperature on the reaction of pregnanediol with the vanillin - perchloric acid reagent. All the optical densities were measured against water. ○, preganediol heated at 100°C for 5 minutes; △, reagent blank solution heated at 100°C for 5 minutes; ●, pregnanediol heated at 70°C for 15 minutes. (Measurements of the reagent blank solution corresponding to the last mentioned curve are omitted for clarity. At no wavelength does the optical density of the blank solution exceed 0·020)

development. Increasing time, temperature and concentration of vanillin increases the intensity of colour produced with steroids. The conditions chosen were the most energetic commensurate with obtaining blank solutions that were practically colourless. If the colour was developed in the boiling-water bath for 5 minutes, then pregnanetriol and pregnanediol gave a purple colour that was about twice as intense as the blue colour obtained at 70° C. At the same time the optical density of the blank solution, when read against water, increased from 0·020 to 0·120 (see Fig. 1).

Moreover, the reaction becomes relatively less specific for the steroids when the colour is developed at 100° C instead of 70° C, as shown in Table I. As low blank values were considered to be of greater importance than higher sensitivity, colour development at 100° C has not been further investigated.

TABLE I

THE EFFECT OF TEMPERATURE ON THE RELATIVE CHROMOGENICITY OF SOME STEROIDS AND NON-STEROIDS WITH THE VANILLIN - PERCHLORIC ACID REAGENT

	Relative chromogenicity (pregnanediol = 100 per cent.) after heating at—	
	70° C	100° C
Pregnanediol*	100	100
Pregnanetriol	100	93
Dehydroepiandrosterone	88	107
Pentan-1-ol	0	16
Pentene	0	0
Heptanal	26	48
Hexan-1-ol	0	25
Octan-1-ol	0	20
Octan-2-ol	24	93
Oleic acid	18	66
Oleyl alcohol	18	71
Squalene	68	158
Cyclohexene	12	16

* At each temperature the molar extinction coefficient of pregnanediol was given an arbitrary value of 100. The optical density obtained after pregnanediol had been heated at 100° C was approximately twice as high as that obtained after the compound had been heated at 70° C.

The optical density of each solution was measured at the wavelength of maximum absorption.

SOLVENTS—

Apart from acetic acid there are few solvents of suitable boiling-point, miscible with perchloric acid, that are good solvents of vanillin and steroids. During the heating at 70° C, methanol, ethanol and formamide completely inhibit colour formation, whereas formic acid depresses it. Dioxan, propionic acid and acetic anhydride give intense colours in the blank solution. Similar effects are observed when these solvents are partially or wholly substituted for acetic acid as diluents of the coloured solutions. At this stage, ethyl acetate and various concentrations of aqueous acetic acid and aqueous sulphuric acid have been tried, but are all less satisfactory than glacial acetic acid in that the final colour is then less stable. The addition of mineral acids (20 per cent. v/v) to the acetic acid used as a diluent has virtually no effect on the colour given by the sample and blank solutions.

ACIDS OTHER THAN PERCHLORIC ACID—

The effect of substituting other acids for perchloric acid has been studied with a small range of steroids together with cyclohexanone and cyclohexanol. These results are recorded

TABLE II

COMPARISON OF THE EFFECTIVENESS OF VARIOUS ACIDS IN PROMOTING STEROID - VANILLIN COLOUR REACTIONS

	Pregnane-triol	Pregnane-diol	Dehydroepi-androsterone	Cyclo-hexanone	Cyclo-hexanol
Sulphuric acid, concentrated	—	—	++	—	—
Hydrogen bromide, 30 per cent. in glacial acetic acid	—	—	—	++	—
Hydrochloric acid, concentrated	+	—	—	++	—
Boron trifluoride, 20 per cent in glacial acetic acid	—	—	—	—	—
Phosphoric acid, 85 per cent.	+	—	—	—	—
Perchloric acid, 72 per cent.	++	++	++	++	++

in Table II. From this it will be seen that it may be possible to devise colour reactions for limited groups of steroids by using acids other than perchloric acid, but so far this line has not been investigated.

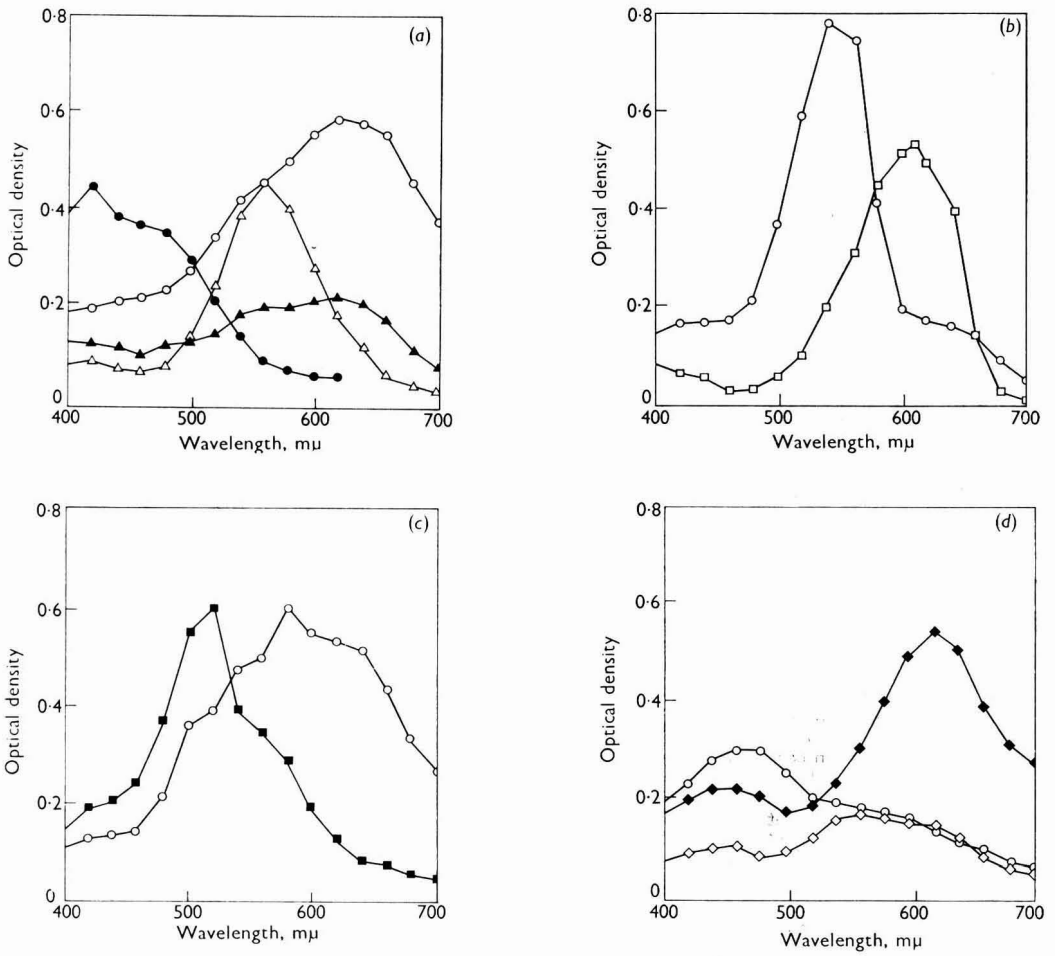

Fig. 2. Representative absorption spectra of steroids with various aromatic aldehyde - perchloric acid reagents; (*a*) vanillin, (*b*) resorcylicaldehyde, (*c*) *p*-hydroxybenzaldehyde, (*d*) *o*-phthalaldehyde. One hundred micrograms of steriod were used for each test

○ = 5β-pregnane-3α,17α,20α-triol
● = 5α-pregnane-3,20-dione
△ = cholesterol
▲ = 3α,17α,21-trihydroxy-5β-pregnan-20-one
□ = 3β,16α-dihydroxy-5α-androstan-17-one
■ = 3α-hydroxy-5β-pregnan-20-one
◇ = 3β-hydroxyandrost-5-en-17-one
◆ = pregn-5-ene-3β,17α,20α-triol

ALDEHYDES OTHER THAN VANILLIN—

Over thirty aromatic aldehydes, in conjunction with perchloric acid, have been tested as possible colour reagents for steroids. A 10 per cent. w/v solution of each aldehyde in glacial acetic acid was first tested with perchloric acid alone, and those that showed little colour in the blank solution were then tested with a small range of steroids at room temperature, 37° C, 70° C and in the boiling-water bath. The steroids used in this initial survey always included pregnanetriol, pregnanediol and dehydroepiandrosterone; usually, cortisol, 11β-hydroxyetiocholanolone and cholesterol were also included. As a result of this survey,

vanillin, resorcylicaldehyde, *p*-hydroxybenzaldehyde and *o*-phthalaldehyde were chosen for testing against a wide range of steroids. The other aldehydes investigated are listed in Table III.

TABLE III

ALDEHYDES TESTED AS POSSIBLE STEROID COLOUR REAGENTS

Group I— Aldehydes giving strong colours with steroids in the presence of perchloric acid, and weak colours in the blank solution—

Vanillin
Resorcylicaldehyde
p-Hydroxybenzaldehyde
Vanillin tri-acetate
Vanillin mono-acetate
Iso-vanillin
Veratraldehyde
3,4-Dihydroxybenzaldehyde
} These compounds behave similarly to vanillin.
Anisaldehyde
o-Phthalaldehyde

Group II— These compounds give acceptable blank solutions and yield colours with many steroids, but the colours are not very strong and, as they did not appear to have any advantages over the aldehydes given in Group I, have not been studied in detail —

Phthalaldehyde acid
Terephthaldialdehyde
2,3-Dimethoxybenzaldehyde
2,5-Dimethoxybenzaldehyde

Group III—Aldehydes that give virtually no colour with the steroids tested—

Benzaldehyde
p-Dimethylaminobenzaldehyde
Pyridine-3-aldehyde
4-Chlorobenzaldehyde
2,4-Dichlorobenzaldehyde
o-Nitrobenzaldehyde
4-Nitro-3-hydroxybenzaldehyde
3-Nitro-4-hydroxybenzaldehyde
6-Nitro-3-hydroxybenzaldehyde

Group IV— Aldehydes that give strong colours with perchloric acid—

m-Hydroxybenzaldehyde
o-Hydroxybenzaldehyde
m-Methoxybenzaldehyde
p-Nitrobenzaldehyde
3,5-Dimethoxybenzaldehyde
2,4,6-Trimethoxybenzaldehyde
3,4,5-Trimethoxybenzaldehyde
2-Hydroxy-1-naphthaldehyde
Piperonaldehyde

COLOURS PRODUCED WITH PURE STEROIDS—

The results obtained by testing pure steroids with vanillin, resorcylicaldehyde, *p*-hydroxybenzaldehyde and, in some instances, *o*-phthalaldehyde, are shown in Table IV to VIII. Some typical absorption spectra are given in Fig. 2. The absorption spectra of many of the weak colours did not show sharp peaks, and the colours had a dull appearance. This was particularly so for vanillin, but was not observed with resorcylicaldehyde.

Of these four aldehydes, *o*-phthalaldehyde is noteworthy, in that it is fairly specific to steroids with the Δ^5-3β-hydroxy grouping. Most of these compounds give purple colours with maximum absorption in the 560 to 600 mμ region, similar to dehydroepiandrosterone, except for Δ^5-pregnanetriol, which gives an intense blue colour of potential value in quantitative analysis because of its high degree of specificity.

The other three aldehydes show a broad similarity in their reactions with steroids. Resorcylicaldehyde fails to react with many of the steroids with which vanillin and *p*-hydroxybenzaldehyde react; it gives weaker colours with the 17-ketosteroids than these other two aldehydes. *p*-Hydroxybenzaldehyde gives stronger colours with some steroids than does vanillin, *e.g.*, the androstanediols (Nos. 1 to 3) and with 20-oxosteroids.

By studying Tables IV to VII it can be seen that the functional groups that are chromogenic are hydroxyl and carbonyl groups at positions 3 and 17 in the androstane series and at positions 3, 20 and 21 in the pregnane series. Confirmation of this has been obtained in the androstane series by studying monosubstituted derivatives (see Fig. 3).

TABLE IV

REACTIONS OF C_{19} STEROIDS

Number	Steroid	Resorcylic-aldehyde		Vanillin		*p*-Hydroxy-benzaldehyde		*o*-Phthal-aldehyde	
		$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ
1	Androst-5-ene-3β,17β-diol	540	7000	600	8500	560	16,000	560	6500
2	5α-Androstane-5β,17β-diol	540	2000	600	10,000	550	13,500	—	—
3	5β-Androstane-3α,17β-diol	540	6000	600	10,000	560	17,500	—	—
4	3α-17β-Dihydroxy-5β-androstan-11-one	540	9000	600	6000	560	11,000	—	—
5*	5α-Hydroxy-5α-androstan-17-one (androsterone)	540	4000	600	11,000	550	18,000	—	—
6	3α-Acetoxy-5α-androstan-17-one	540	4000	600	11,500	580	23,000	—	—
7	5β-Hydroxy-5α-androstan-17-one	540	7000	600	16,000	550	23,000	—	—
8	3β-Hydroxyandrost-5-en-17-one (dehydroepiandrosterone)	540	5000	550	16,000	550	16,000	560	5000
9	3β-Acetoxyandrost-5-en-17-one	540	5000	600	13,500	600	18,000	—	—
10	3β-Propionoxyandrost-5-en-17-one	540	5000	600	12,500	600	17,000	—	—
11	3α,5-Cyclo-6β-hydroxy-5α-androstan-17-one	540	4500	550	16,500	560	12,000	560	5000
12	3β-Hydroxyandrost-5-ene-7,17-dione (7-oxodehydroepiandrosterone)		Nil		Nil	—	—		Nil
13	3α-Hydroxy-5β-androstan-17-one (etiocholanolone)	540	5500	600	14,000	550	19,000		Nil
14	3α-11β-Dihydroxy-5β-androstan-17-one (11β-hydroxyetiocholanolone)	540	9500	550	18,000	550	19,000		Nil
15	3α-Hydroxy-5β-androstane-11,17-dione (11-oxoetiocholanolone)		Nil	600	5000	570	7500	—	—
16	5α-Androst-2-en-17-one	540	3000	600	10,000	580	14,000	—	—
17	5α-Androstane-3,17-dione	540	2500	550	24,000	600	7000	—	—
18	Androst-5-ene-3,17-dione		Nil	550	6000	560	6500	—	—
19	Androst-4-ene-3,17-dione		Nil	550	6500	600	7000	—	—
20†	17β-Hydroxyandrost-4-en-3-one (testosterone)		Nil	600	5000	560	6500	—	—
21	17β-Hydroxy-5β-androstan-3-one	540	1000	600	10,000	550	7500	—	—
22	3β,16α-Dihydroxy-5α-androstan-17-one	600	16,000	580	22,000	550	22,000	—	—

* Obtained from Koch-Light Laboratories Ltd.
† Obtained from The British Drug Houses Ltd.

TABLE V

REACTIONS OF SOME CORTICOSTEROIDS WITH THE VANILLIN - PERCHLORIC ACID REAGENT

Number	Corticosteroid	$\lambda_{max.}$	ϵ
23*	17α,21-Dihydroxypregn-4-ene-3,11,20-trione (cortisone)	Nil	Nil
24*	17α-Hydroxy-21-acetoxypregn-4-ene-3,11,20-trione	Nil	Nil
25†	21-Hydroxypregn-4-ene-3,20-dione (cortexone)	600	10,000
26*	21-Hydroxy-5β-pregnane-3,20-dione (viadril)	620	12,500
27†	17α,21-Dihydroxypregn-4-ene-3,20-dione (cortexolone)	600	5000
28*	11β,17α,21-Dihydroxypregn-4-ene-3,20-dione (cortisol)	600	3000
29	11β,21-Dihydroxypregn-4-ene-3,20-dione (corticosterone)	560	7500
30	11β-Hydroxy-21-acetoxypregn-4-ene-3,20-dione	560	12,000
31*	21-Acetoxypregn-4-ene-3,11,21-trione	560	3000
32*	17α,21-Dihydroxypregn-1,4-diene-3,11,20-trione	Nil	Nil
33	11β,17α,21-Trihydroxy-5α-pregnane-3,20-dione	560	7500
34‡	3α,17α,21-Trihydroxy-5β-pregnane-11,20-dione	600	7000
35	3α,17α,21-Trihydroxy-5β-pregnan-20-one	620	10,000

* Obtained from the Hospital Pharmacy.
† Obtained from Koch-Light Laboratories Ltd.
‡ Obtained from Organon Laboratories.

TABLE VI

REACTIONS OF SOME $C_{21}O_3$ STEROIDS

Number	Steroid	Resorcylic-aldehyde		Vanillin		*p*-Hydroxy-benzaldehyde		*o*-Phthal-aldehyde	
		$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ	λ_{max}	ϵ
36	5β-Pregnane-3α,17α,20α-triol ..	540	26,000	600	18,000	600	19,000	460	10,000
37	5β-Pregnane-3α,17α,20β-triol ..	540	22,000	600	18,000	600	17,000	460	9000
38	5β-Pregnane-3α,16α,20β-triol ..	540	10,000	600	19,000	550	18,000	—	—
39	3α,17α,20β-Trihydroxy-5β-pregnan-11-one	540	1500	600	4500	530	5000	Nil	
40	Pregn-5-ene-3β,16α,20β-triol ..	540	10,000	550	17,000	580	13,000	560	6000
41	Pregn-5-ene-3β,17α,20β-triol ..	540	10,000	600	14,000	580	14,000	620	16,000
42	Pregn-5-ene-3β,17α,20α-triol ..	540	12,000	600	12,000	580	12,000	620	18,000
43	5α-Pregnane-3α,17α,20α-triol ..	540	22,000	600	15,000	600	17,000	460	5500
44	3α,17α-Dihydroxy-5α-pregnan-20-one..	540	2000	560	10,000	500	14,000	Nil	
45	3α,17α-Dihydroxy-5β-pregnan-20-one..	425	6000	560	11,000	500	16,000	Nil	
46*	3α,17α-Dihydroxy-5β-pregnane-11,20-dione	Nil		560	5000	480	6000	Nil	
47	3α,16α-Dihydroxy-5β-pregnan-20-one	Nil		540	2000	510	3500	Nil	
48	3β,16α-Dihydroxypregn-5-en-20-one	540	4000	560	5500	480	7000	540	3000
49†	3β,17α-Dihydroxypregn-5-en-20-one	540	4500	600	7000	580	10,000	580	3000

* Obtained from Organon Laboratories.

† Obtained from Koch-Light Laboratories Ltd.

TABLE VII

REACTIONS OF SOME $C_{21}O_2$ STEROIDS

Number	Steroid	Resorcylic-aldehyde		Vanillin		*p*-Hydroxy-benzaldehyde		*o*-Phthal-aldehyde	
		$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ	$\lambda_{max.}$	ϵ
50	5β-Pregnane-3α,20α-diol	540	24,000	600	18,000	560	19,000	Nil	
51	5β-Pregnane-3α,20β-diol	540	22,000	600	17,000	560	21,000	—	—
52	5α-Pregnane-3α,20α-diol	540	30,000	600	18,000	560	20,000	—	—
53	5α-Pregnane-3α,20β-diol	540	30,000	600	18,000	560	19,000	—	—
54	5α-Pregnane-3β,20β-diol	540	20,000	600	16,000	560	20,000	—	—
55	5α-Pregnane-3β,20α-diol	540	19,000	600	15,000	560	18,000	—	—
56	3α,20α-Diacetoxy-5β-pregnane ..	540	21,000	600	16,000	560	19,000	—	—
57	Pregn-5-ene-3β,20β-diol	540	15,000	600	14,000	560	19,000	560	6000
58*	3β-Hydroxypregn-5-en-20-one ..	540	5000	600	11,000	560	10,000	560	7000
59	3β-Hydroxy-5α-pregnan-20-one ..	Nil		600	14,000	520	18,000	—	—
60	3β-Acetoxy-5α-pregnan-20-one ..	Nil		600	15,000	520	18,000	—	—
61	3β-Acetoxy-5α-pregn-16-en-20-one	Nil		Nil		510	2000	—	—
62	3α-Hydroxy-5α-pregnan-20-one ..	Nil		600	14,000	500	14,000	—	—
63	3α-Hydroxy-5β-pregnan-20-one ..	Nil		600	17,000	520	19,000	Nil	
64	5α-Pregn-2-en-20-one	540	1500	600	13,000	510	15,000	Nil	
65	3α,6α-Dihydroxy-5β-pregnan-20-one	Nil		600	10,000	560	11,000	—	—
66	5α-Pregnane-3,20-dione	Nil		420	12,500	395	12,000	Nil	
67	5β-Pregnane-3,20-dione	Nil		420	4500	—	—	—	—
68	Pregn-4-en-3,20-dione (progesterone)	Nil		Nil		Nil		—	—
69*	17α-Ethynyl-17β-hydroxyandrost-4-en-3-one (ethisterone)	—	—	600	3000	—	—	—	—

* Obtained from The British Drug Houses Ltd.

TABLE VIII

COLOUR REACTIONS OF BILE ACIDS

Number	Bile acid	Vanillin $\lambda_{max.}$	Vanillin ϵ	Resorcylic-aldehyde $\lambda_{max.}$	Resorcylic-aldehyde ϵ	*p*-Hydroxy-benzaldehyde $\lambda_{max.}$	*p*-Hydroxy-benzaldehyde ϵ
70	5β-Cholanoic acid	Nil		Nil		Nil	
71	5β-Cholanoic acid, methyl ester	Nil		Nil		Nil	
72	3-Oxo-5β-cholanoic acid, methyl ester	440*	5000	Nil		Nil	
73	3α-Hydroxy-5β-cholanoic acid (lithocholic acid)	600	11,500	540	3000	530	12,000
74	3α-Hydroxy-5β-cholanoic acid, methyl ester	600	11,500	540	3000	540	15,000
75	3α,12α-Dihydroxy-5β-cholanoic acid	600	12,500	540	12,000	540	13,000
76†	3α,7α,12α-Trihydroxy-5β-cholanoic acid	600	11,000	540	10,000	530	11,000
77	3α,7α,12α-Trihydroxy-5β-cholanoic acid, methyl ester	600	10,000	540	10,000	540	12,000

* Not maximum; no peak in visible region.
† Obtained from The British Drug Houses Ltd.

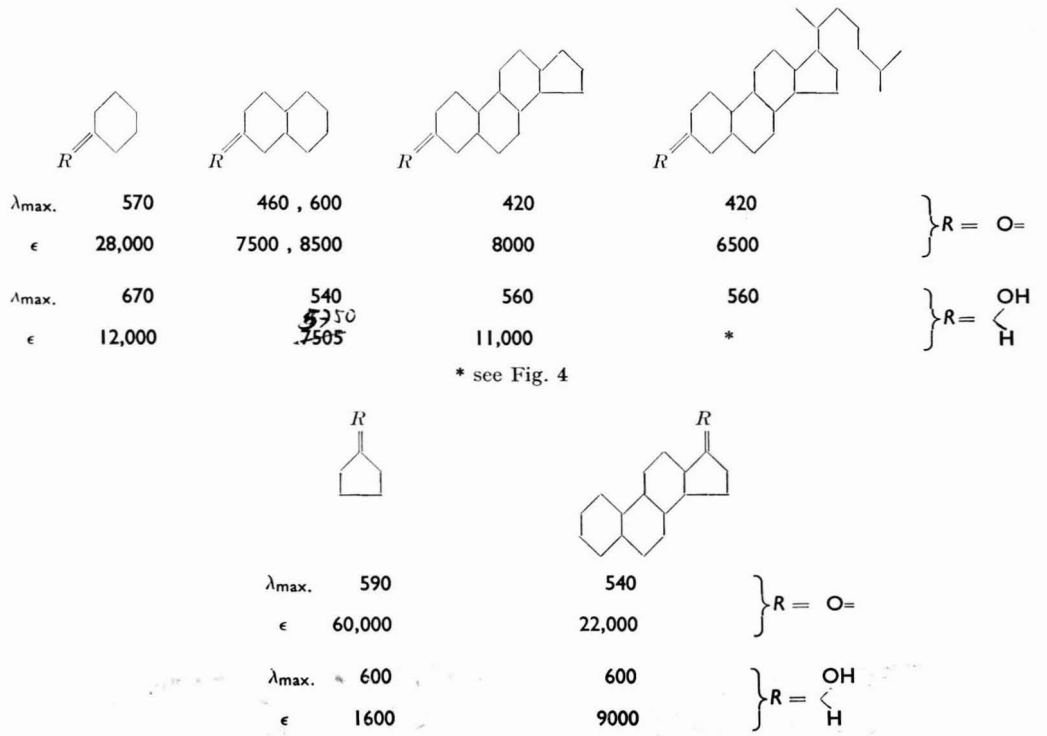

Fig. 3. The variation in the reaction of the vanillin - perchloric acid reagent with monofunctional compounds possessing a different number of rings, giving the wavelength of maximum absorption, $\lambda_{max.}$, and the molar extinction coefficient, ϵ.

All six 11-oxosteroids tested were much less chromogenic (35 per cent. on average) than the corresponding 11-deoxysteroids, whereas an 11β-hydroxyl group was found to have only a small and inconsistent effect. This is in contrast to the Zimmermann and Porter - Silber reactions in which an 11-carbonyl group increases and an 11β-hydroxyl group decreases the chromogenicity.

An olefinic double bond in a steroid has only a small effect on colour development with the reagents described, unless it is in a position of conjugation with a carbonyl group, in which instance chromogencity is strongly diminished relative to the nuclear-saturated analogue. Most of the unsaturated steroids available for study had the double bond in the 5,6 position.

These unsaturated steroids were slightly less chromogenic than their saturated analogues when tested with the vanillin, resorcylicaldehyde and *p*-hydroxybenzaldehyde reagents. However, the unsaturated steroids reacted with the *o*-phthalaldehyde reagent, which gave virtually no colour with the saturated steroids.

It is noteworthy that whereas an α,β-unsaturated carbonyl group strongly diminishes chromogenicity, a conjugated-diene system does not have this effect; both ergosterol and 7-dehydrocholesterol react strongly with the vanillin - perchloric acid reagent.

STEROLS—

Cholesterol reacts with the vanillin - perchloric acid reagent to give an intense purple colour. This reaction is considerably more sensitive than either the Liebermann - Burchardt or the Zak reactions, which are commonly used in the determination of cholesterol (see Table IX). In common with the reagents used in the other two reactions, the vanillin - perchloric acid reagent gives intense colours with the $\Delta^{5,7}$ dienes, ergosterol and 7-dehydrocholesterol, but not with 7-oxocholesterol.

TABLE IX

COMPARISON OF MOLAR EXTINCTION COEFFICIENTS OF STEROLS IN DIFFERENT COLOUR REACTIONS

	Reaction with vanillin - perchloric acid, product measured at 550 mμ	Reaction with ferric chloride - sulphuric acid,* product measured at 560 mμ	Liebermann - Burchardt reaction†, product measured at 620 mμ
Cholesterol‡	18,000	10,000	2500
Cholestan-3β-ol	§	2500¶	Nil
Cholestan-3α-ol	§	2800¶	350
7-Oxocholesterol	Nil	Nil	Nil
Ergosterol‡	22,000	5000¶	4500
Cholesterol benzoate	12,000	10,500	2300
7-Dehydrocholesterol benzoate ..	22,000	4500¶	4400

* The modification described by Abell, Levy, Brodie and Kendall[15] was used.
† As described by Zak, Moss, Boyle, and Zlatkis.[16]
‡ Obtained from The British Drug Houses Ltd.
§ See Fig. 4.
¶ These reactions give a brown, rather than a purple, colour.

When the epimeric cholestan-3-ols were tested with the reagent, it was found that they reacted in a qualitative sense, but that the intensity of the colour developed was virtually independent of the amount of sterol used (see Fig. 4). The same phenomenon was also observed with cholest-2-ene, 3β-hydroxy-5α-cholestan-7-one, 3β-chloro-5α-cholestane and 3β-chlorocholest-5-ene. This is contrary to the findings for all the other steroids tested, which show good conformity to Beer's law.

The vanillin - perchloric acid reagent described in this paper differs from that described by Schaltegger[10], in that the perchloric acid is present in a much higher concentration and does not contain acetic anhydride. The Schaltegger reagent only gave appreciable colour with cholesterol and cholic acid at the 20-mg level, but was extremely sensitive to vitamin D.

STEROID ESTERS—

Only for cholesterol was a comprehensive series of esters available. The chromogenicities of these esters with the vanillin - perchloric acid reagent are shown in Table X. The low

TABLE X

MOLAR CHROMOGENICITY OF CHOLESTERYL ESTERS RELATIVE TO CHOLESTEROL WITH THE VANILLIN - PERCHLORIC ACID REAGENT

Cholesterol	100	Cholesteryl laurate	37
Cholesteryl acetate	111	Cholesteryl stearate	12
Cholesteryl propionate	124	Cholesteryl oleate	80
Cholesteryl butyrate	118	Cholesteryl benzoate	68
Cholesteryl hexanoate (caproate) ..	106	Cholesteryl *p*-toluenesulphonate ..	123
Cholesteryl octanoate (caprylate) ..	61	Cholesteryl *p*-methoxybenzoate ..	35
Cholesteryl decanoate (caprate) ..	47	Cholesteryl methyl ether	87

chromogenicity of the higher fatty esters of cholesterol may be partly owing to their very low solubility in the reaction mixture. In contrast to these findings for the esters of cholesterol, the acetate and propionate of dehydroepiandrosterone were both less chromogenic than the free steroid. The other acetates studied had virtually the same chromogenicity as the parent steroids.

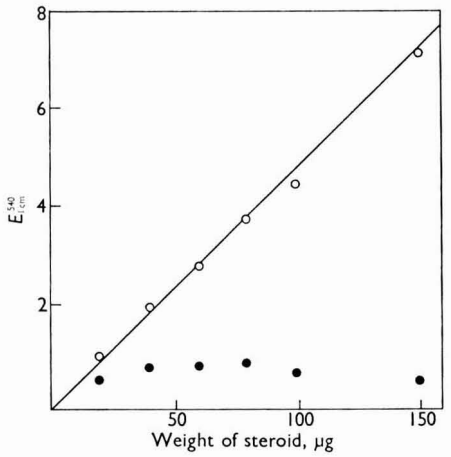

Fig. 4. Calibration curves for cholesterol, ○, and cholestan-3β-ol, ●, with the vanillin - perchloric acid reagent

REACTIONS OF NON-STEROIDS—

The chromogenicity, with the vanillin - perchloric acid reagent, of a few non-steroids is recorded in Table I. Many other non-steroidal compounds were tested with this reagent on a semi-quantitative basis; the results are given in Table XI.

MECHANISM OF THE REACTION

Apart from its intrinsic interest, a knowledge of the mechanism of the reactions described in this paper should be of value in allowing future development to proceed along rational rather than empirical lines.

It was found that the colour resulting from the reaction of steroids with aromatic aldehydes and perchloric acid was discharged on dilution with water or alcohol. If water was used as the diluent then a pale-yellow material could be extracted with ether. When this substance was re-dissolved in glacial acetic acid containing a little perchloric acid, the original colour was restored. (Mylius[11] had found that the coloured product of the Pettenkofer reaction could be extracted into ether, in which solvent it was colourless, and that the residue obtained after evaporation of the ether gave an intense carmine colour with sulphuric acid.) These observations suggest that the coloured product was the halochromic salt of a substance produced by the acid-catalysed reaction of the steroid with the aromatic aldehyde.

In order to study the nature of this reaction, simple derivatives of cyclohexane were used as models and were treated with a veratraldehyde - perchloric acid reagent under the conditions described for the vanillin - perchloric acid reagent. (Veratraldehyde was used rather than vanillin, as the derivatives thus obtained gave more satisfactory paper chromatograms. Also veratraldehyde reacts readily with cyclohexanone in an alkaline medium under the conditions described by Vogel,[12] whereas vanillin does not give a crystalline product under these conditions.) After the colour produced had been observed, the mixtures were diluted with water and extracted with ether. The ethereal extracts were washed with N sodium hydroxide and water, dried over anhydrous sodium sulphate, and evaporated to dryness. Aliquots of the residue were examined chromatographically on paper with the

system iso-octane - toluene - methanol - water in the ratio 120 + 80 + 160 + 40. After development, the papers were dried and examined under ultraviolet light and then sprayed with 10 per cent. aqueous perchloric acid.

TABLE XI

REACTIONS OF NON-STEROIDS WITH THE VANILLIN - PERCHLORIC ACID REAGENT

(*a*) *Comparable with strongly reacting steroids: intense colour given by* 0·1 *mg*—

Indole
α-Naphthol
Resorcinol
Phloroglucinol
Squalene
β-Decalone
Ionone
Cyclopentanone
Cyclohexanone
Terpineol
Tryptophane

(*b*) *Weak colour with* 0·1 *mg: strong colour given by* 1·0 *mg*—

Heptanal
Octan-2-ol
Oleic acid
Oleyl alcohol
Saponin
Glucuronolactone
Glycyrrhetic acid
All common sugars (brown colour)
Cyclohexanol
Cyclohexene
β-Decalol
Homogentisic acid

(*c*) *Faint colour given by* 1 *mg*—

Cyclopentanol
Cyclopentene
Butane-1,3-diol
β-Ethoxyethanol
Selachyl alcohol
Polyethylene glycol
Isobutyl methyl ketone
Naphthalene
Anthracene
Phenol
Fluorene
Menthol

(*d*) *No colour given by* 1 *mg*—

Alanine
Amylene
Ascorbic acid
Barbituric acid
Caffeine
Creatine
Dekalin
Ethylene glycol
Glycine
Hexan-1-ol
Hippuric acid
Inositol
Lauric acid
Malonic acid
Octan-1-ol
Pentan-1-ol
Phenylacetic acid
Phthalic acid
Propylene glycol
Succinic acid
Terephthalic acid

Cyclohexene and cyclohexanol gave identical blue-green colours. In both instances the paper chromatograms of the neutral reaction products showed one main spot that had a yellow fluorescence in ultraviolet light and that gave a blue colour with perchloric acid. The mobilities of the main spots from these two compounds were identical, suggesting that cyclohexanol is first dehydrated to cyclohexene, which then reacts with the veratraldehyde.

In contrast, cyclohexanone gave a purple colour, and the principal spot on the paper chromatogram had a different mobility from those from cyclohexanol and cyclohexene, although it gave the same colours in ultraviolet light and with perchloric acid. Significantly, this product from the acid-catalysed reaction of cyclohexanone and veratraldehyde had the same chromatographic and spectroscopic properties as a compound produced by the base-catalysed condensation of veratraldehyde and cyclohexanone (melting-point, 151° C) and which was assumed to be the bis-(3,4-dimethoxybenzilidene) derivative of cyclohexanone.

These findings are contrary to those of Duke,[13] who investigated the mechanism of the Komarowsky reaction. Duke heated cyclohexanol and benzaldehyde under reflux with 10 N hydrochloric acid, and from the reaction mixture was able to isolate a small amount of the dibenzilidene derivative of cyclohexanone. The colour produced was ascribed to the interaction of this product with the hydrochloric acid.

The higher concentration of acid used in the present work would probably favour dehydration of the cyclohexanol rather than the oxidation by the excess of aldehyde that Duke postulated. Alternatively, veratraldehyde may be a less effective oxidising agent than benzaldehyde.

These experiments show the basis of the colour reactions as applied to simple cyclohexane derivatives, and presumably these principles apply in the steroid field. However, it must be noted that increasing the complexity of a molecule influences the reactivity of a single hydroxyl or carbonyl group, as is shown in Fig. 3.

Discussion

Since 1887, when Mylius[11] showed that furfural was an essential intermediate in the Pettenkofer (1844)[14] reaction for bile acids, aromatic aldehydes, in conjunction with mineral acids, have frequently been described as colour reagents for steroids. Many of these reagents are specific, or relatively specific, to a small group of steroids, whereas the colour reactions described in this paper, with the exception of those obtained with *o*-phthalaldehyde, are given by a very wide range of steroids and with many non-steroids.

The explanation of this contrast between specificity and comprehensiveness lies largely in the difference between the acids used. Evidence has already been given that, at least for cyclohexanol, the first step in the reaction between the vanillin - perchloric acid reagent and a saturated hydroxylic compound is the elimination of water to give an olefin. Consequently in colour reactions with low concentrations of acid only those steroids containing a hydroxyl group that can readily be eliminated, will react.

By using an extremely high concentration of acid and an elevated temperature, as with the vanillin - perchloric acid reagent, even such stable steroids as pregnanediol and androstanediol can be made to react. The extent to which the acid concentration and the temperature can be raised is limited by the fact that under extreme conditions the aromatic aldehydes themselves react with perchloric acid to give colours similar to those given by the steroids. The conditions of acidity and temperature used for the vanillin - perchloric acid reagent are the most drastic possible commensurate with acceptable blank values. It is probable that milder conditions of acidity and temperature would give useful colours with the more reactive steroids such as pregnanetriol, with a corresponding gain in specificity, but loss in sensitivity.

Table XII

Steroids with which aromatic aldehyde - perchloric acid reagents give useful colours

Steroid	Aldehyde	Other reagent
Pregnanetriol	Resorcylicaldehyde	Sulphuric acid
Δ^5-Pregnenetriol	*o*-Phthalaldehyde	Sulphuric acid Sulphuric acid - ethanol
Pregnanediol	Vanillin Resorcylicaldehyde	Sulphuric acid
Androstanediol	Vanillin *p*-Hydroxybenzaldehyde	
Pregnanolone	Vanillin *p*-Hydroxybenzaldehyde	Zimmermann reagent
5β- and Δ^5-pregnane-3,16α,20β-triols	Resorcylicaldehyde Vanillin	Sulphuric acid
Cholesterol	Vanillin	Liebermann - Burchardt and Zak reagents
16α-Hydroxy-17-oxosteroids	Resorcylicaldehyde	Blue tetrazolium
Cholic acid	Vanillin	Pettenkofer reagent
Lithocholic acid Deoxycholic acid	Vanillin	—
17α-Hydroxy-20-oxo-21-methyl steroids	*p*-Hydroxybenzaldehyde Vanillin	Vanillin - phosphoric acid
Squalene	Vanillin	Sulphuric acid - formaldehyde

However, it was not the intention in this paper to describe optimal conditions for developing colours with every steroid available, but to draw attention to the scope of this type of colour reaction. Some potential analytical applications of these colour reactions are summarised in Table XII.

I am grateful to Professor J. Patterson for his continued support of this work and Dr. W. J. Tindall, Organon Laboratories, for generous gifts of steroids.

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Optical Scattering Cross-section of Small Particles and the Design of Photosedimentometers*

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A technique is described for measuring variations in the optical scattering cross-sections of small particles. It is shown that absolute variations can be measured for small spheres when an independent measure of the size of the particles is available.

The technique has been used for measuring the scattering cross-sections of small spherical particles in a beam of white light under various conditions.

In the photosedimentation technique of particle-size analysis, a light beam is used for following concentration changes occurring within a settling suspension. The technique is usually restricted to particles between 100 and 1·0 μ in diameter. Since this size is of the same order as the wavelength of light, the laws of geometric optics can no longer be applied to the system. For particles smaller than about 50 μ, complex diffraction effects have to be taken into account, and under these conditions it is more correct to talk of the scattering cross-section of a particle rather than its hiding power. A coefficient often used as a measure of the scattering power of a small particle is the extinction coefficient, K, which is defined by the equation[1]—

$$K = \frac{\text{Light obscured by particle of effective diameter, } d}{\text{Light that would be obscured by this particle if the laws of geometric optics held}}$$

It can be shown that the extinction coefficient depends on—

(*a*) the refractive indices of the particle and the dispersing medium,

(*b*) the geometry of the optical system used for examining the particle and

(*c*) $\left(\frac{d}{\lambda}\right)$, where d is a measure of particle size, and λ is the wavelength of the incident radiation in the dispersing medium.[2]

It can also be shown that the attenuation of a beam of light traversing the suspension is given by the equation[3]—

$$I = I_0 \exp. \left(-kcl \sum_{r=1}^{r=n} K_r N_r d_r^2\right) \quad .. \quad .. \quad .. \quad (1)$$

where—

kd_r^2 is the average projected surface area of the particles,
I_0 and I are the intensities of the incident and emergent light beam, respectively,
N_r is the number of particles of size d_r per gram of powder,
K_r is the extinction coefficient for particles of size d_r,
c is the concentration, in g per c.c., of particles in suspension,
l is the length of the path of light through the suspension and
d_1 and d_n are the effective diameters of the smallest and largest particles present, respectively.

For an ideal system consisting of a monochromatic, parallel incident beam, a suspension of spherical particles of the same size and a detecting device that does not accept scattered light, K_r may be calculated by using Mie's theory.[4]

Methods of interpreting photosedimentation results can be classified into three main groups. In the first group, the instruments are designed to meet the requirements of the Mie theory, and calculated values of K_r are applied to the results.[5,6] In the second group,

* A description of work carried out at Nottingham and District Technical College.

variations in K_r are ignored, sometimes implicitly[7] and sometimes explicitly.[8] In the third group, empirical calibration is used to allow for the variations.[9,10,11,12]

Variations in the design of particular instruments in the second and third group make it difficult to assess the relative values of the distributions measured. Furthermore, the values of K_r reported are specific to the instrument and to the technique used. For instance, the types and characteristics of the photocells used have not been given, and powder fractions with wide ranges of sizes have been treated as being of the same size.[9,10]

The work reported in this paper was undertaken in order to obtain information on the effect of these variables on the value of K_r.

Theory

Previous attempts to measure K_r experimentally have been based on the study of fractionated powders, and the difficulty has been in obtaining fractions of narrow enough size range to be considered as being of the same size without appreciable error.

However, it can be shown that a sedimenting suspension can be used for measuring changes in K_r with variations in the design of instruments without preparing such closely graded fractions. Furthermore, if an independent assessment of the particle-size distribution of the sample is available, absolute values of K_r can be calculated.

Equation (1) can be written in the form—

$$\int \frac{dI}{I} = kc \int dl \sum_{r=1}^{r=n} K_r N_r d_r^2$$

Integrating for narrow size ranges and subtracting for two close values gives—

$$\log_e I_{x'} - \log_e I_{x''} = kcl \left(\sum_{r=x'}^{r=n} K_r N_r d_r^2 - \sum_{r=x''}^{r=n} K_r N_r d_r^2 \right)$$

giving—

$$\log_e \frac{I_{x'}}{I_{x''}} = kcl \sum_{r=x'}^{r=x''} K_r N_r d_r^2.$$

As $d_{x''} - d_{x'}$ is small, K_r may be considered constant and equal to K_x over the narrow range. Putting $\log_{10} I_x = D_x$ gives—

$$D_{x'} - D_{x''} = (\log_{10} e) kcl\, K_x \sum_{r=x''}^{r=x'} N_r d_r^2 \qquad \ldots \quad \ldots \quad \ldots \quad (2)$$

The dependance of K_x upon instrument variations can be investigated. Since x is a unique function of time and height of fall for a particular sedimenting suspension and K_x is a function of $(D_{x'} - D_{x''})$, by repeated sedimentation of the same system with different instrument designs, the nature of the variations in K_x may be established.

For smooth, dense, spherical particles, $k = \frac{\pi}{4}$, and equation (2) may be written—

$$D_{x'} - D_{x''} = (\log_{10} e)\, \frac{\pi}{4}\, clK_x N_x d_x^2$$

where $d_x = \frac{1}{2}(d_{x'} + d_{x''})$ and N_x is the number of particles within the narrow limits, x' and x''.

Multiplying both sides by d_x and re-arranging—

$$K_x = \frac{4\,(D_{x'} - D_{x''})\, d_x}{\pi\, (\log_{10} e)\, clN_x d_x^3} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

For 1 g of powder of density ρ grams per c.c.—

$$\frac{\pi}{6}\rho \sum_{r=1}^{r=n} N_r d_r^3 = 1$$

Hence—

$$\sum_{r=1}^{r=n} N_r d_r^3 = \frac{6}{\pi\rho} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Let the fractional volume of powder in the range $d_{x'}$ to $d_{x''}$ be $\frac{\Delta v}{v}$. Then—

$$\frac{\Delta v}{v} = \frac{\sum_{r=x'}^{r=x''} N_r d_r^3}{\sum_{r=1}^{r=n} N_r d_r^3}$$

giving in conjunction with equation (4)—

$$\sum_{r=x'}^{r=x''} N_r d_r^3 = \frac{\Delta v}{v} \times \frac{6}{\pi\rho}$$

that is—

$$N_x d_x^3 = \frac{\Delta v}{v} \times \frac{6}{\pi\rho} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

Substituting equation (5) into equation (3) gives—

$$K_x = \frac{2\rho\,(D_{x'} - D_{x''})\,d_x}{3\,(\log_{10} e)\,cl\,\frac{\Delta v}{v}} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

For the type of particle under consideration, $\frac{\Delta v}{v}$ may be determined by a microscope count, hence the absolute value of K_x may be calculated.

Measurements reported in this paper are restricted to this type of particle.

Experimental procedure

The equipment used for these experiments was adapted from the commercially available E.E.L. (Evans Electroselenium Ltd.) photosedimentometer, the photocell being made movable, so that the angle subtended by the photocell at the centre of the suspension, α, could be varied (see Fig. 1). The output of the photocell was found by using Rose's method[9,10] to be a linear function of the incident-light energy. Convection currents within the settling suspension were almost entirely absent on account of the small surface area of the suspension tank, the thermal stability of the wooden container and the use of a microscope slide as a cover for the tanks once the experiment had started. The experiments were carried out

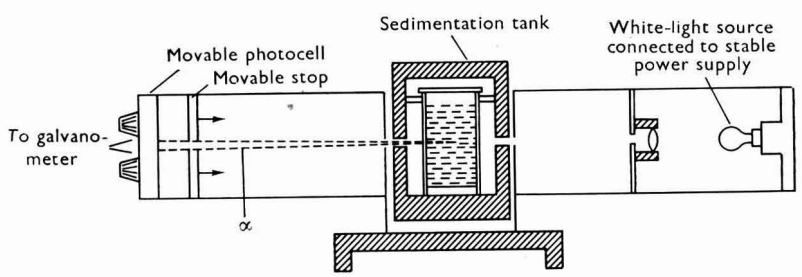

Fig. 1. Diagram of the photosedimentometer

with three closely graded fractions of bronze powder in the theoretical Stokes's size ranges, 5 to 10 μ, 10 to 15 μ and 6 to 13 μ. These were obtained by repeated decantation with an Andreasen bottle in a thermostatically controlled water-bath, the process being repeated 12 to 15 times until the superincumbent liquid was clear. Narrow size ranges were used for reducing the theoretical errors in the evaluation of K_x to less than 5 per cent. and to determine mean values of K_x over size ranges as small as 1 μ.

A sample was taken from such a graded fraction, carefully weighed and, after it had been wetted with a drop of Teepol, dispersed in a known volume of aqueous glycerine solution. After a test run had been completed, the suspension was spun in a centrifuge, the residue dried and then re-weighed in order to determine whether the loss of powder in handling during the experiment was important. The powder was then re-dispersed and allowed to settle on to a microscope slide, a count being made on around 1000 particles.

The solid angle subtended by the photocell at the centre of suspension is defined by the equation—

$$\alpha = \frac{\text{area of stop}}{(\text{distance between stop and tank})^2}$$

where the stop is placed immediately in front of the cell.

Photosedimentation experiments were carried out for ten values of α varying from 0·00025 to 0·009 steradians, first with α increasing, then repeat experiments with α decreasing.

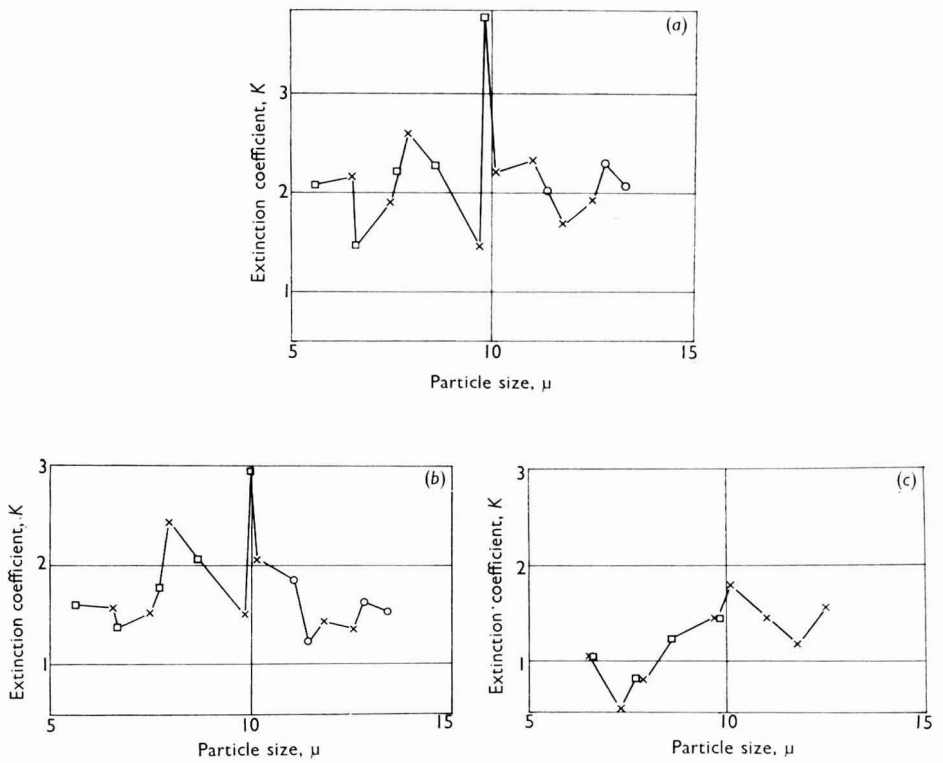

Fig. 2. Graphs showing variation of extinction coefficient with particle size for different values of α; (*a*), α = 0·001 steradians; (*b*), α = 0·005 steradians; (*c*), α = β steradians. The tests were made with bronze spheres and each symbol, *i.e.*, □, ×, ○, refers to a different sedimentation experiment

Measurements were also made for the largest angle possible with the apparatus: this is designated β and is estimated at 3·0 steradians. Three experiments were made each time, giving six experiments for each value of α. Galvanometer readings were taken at intervals, calculated from Stokes's equation, to give 1-μ separation in particle size. From the galvanometer readings, K_x was calculated over narrow size ranges. Table I is a typical set of results.

A cursory glance at Table I is sufficient to show that K fluctuates widely with particle size, the extent of the fluctuations being reduced when K is summed over wider size ranges.

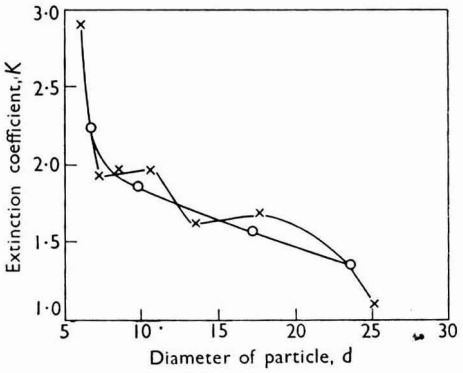

Fig. 3. Graphs showing variation of extinction coefficient with particle size. The tests were made with bronze spheres; ×, mean values of the extinction coefficient over narrow ranges of particle size; ○, mean values of the extinction coefficient over wide ranges of particle size

TABLE I

RESULTS OF EXPERIMENTS TO DETERMINE VALUES OF K_x

Stoke's equation gives—

$$d = 175\sqrt{\frac{\eta h}{(\sigma - \rho)}} \cdot \frac{1}{\sqrt{t}}$$

where d is the effective diameter of the particle, in microns,
σ and ρ are the densities of the powder and fluid, respectively,
η is the viscosity of the suspension, in poises,
h is the sampling height, in cm, and
t is the time of sampling, in minutes.

For the purposes of this experiment—

$\eta = 0{\cdot}01$ poise $\quad \sigma = 8{\cdot}80$
$h = 4{\cdot}00$ cm $\quad \rho = 1{\cdot}00$

Hence—

$$d = \frac{12{\cdot}5}{\sqrt{t}}$$

With a powder-size range of 5 to 12 μ, timed to give 1-μ separation, a concentration of 0·00155 g per c.c., a sampling height of 2·0 cm and a solid angle, α, subtended by the cell of 0·001 steradians, the results given below were determined.

Time, t	Size, $(d_{x''})$ μ	$D_t - D_0$	$D_{x''} - D_{x'}$	$\frac{\Delta v}{v}$	$(D_{x''} - D_{x'})d_x$	K_1	K_2*
0 min. 0 sec. ..	—	0·655	—	—	—	—	—
1 min. 5 sec. ..	12	0·655	0·010	0·010	0·115	—	—
1 min. 18 sec. ..	11	0·645	0·010	0·040	0·105	1·14	—
1 min. 34 sec. ..	10	0·635	0·100	0·110	0·950	3·76	1·92
1 min. 58 sec. ..	9	0·535	0·130	0·210	1·105	2·29	2·85
2 min. 27 sec. ..	8	0·405	0·170	0·250	1·275	2·25	1·82
3 min. 16 sec. ..	7	0·235	0·110	0·210	0·715	1·48	1·61
4 min. 22 sec. ..	6	0·125	0·100	0·115	0·525	2·08	—
6 min. 18 sec. ..	5	0·025	0·055	0·113	—	—	—
9 min. 48 sec. ..	4	0·005	—	—	—	—	—

* K_2 is a mean value over 2-μ size ranges showing how the fluctuations in K diminish with increasing size ranges.

NOTE—The extreme values are omitted in each column since the derived value of K has an error exceeding 5 per cent. in these instances.

Results

In Figs. 2 (*a*), (*b*) and (*c*), the extinction coefficient, K, is plotted against particle size, in microns, for three values of α. It can be seen that the amplitude of the variations of K with particle size decreases with increasing solid angle. If more scattered light were accepted, the fluctuations might well be eliminated and experiments are now being carried out to investigate this.

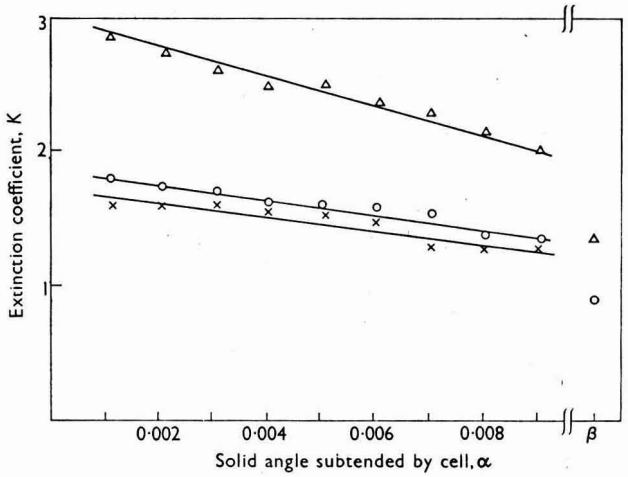

Fig. 4. Graphs showing the variation of the extinction coefficient with the solid angle subtended by the photocell for bronze spheres of different sizes: ×, 4 to 6μ; ○, 6 to 8μ; △, 8 to 10μ

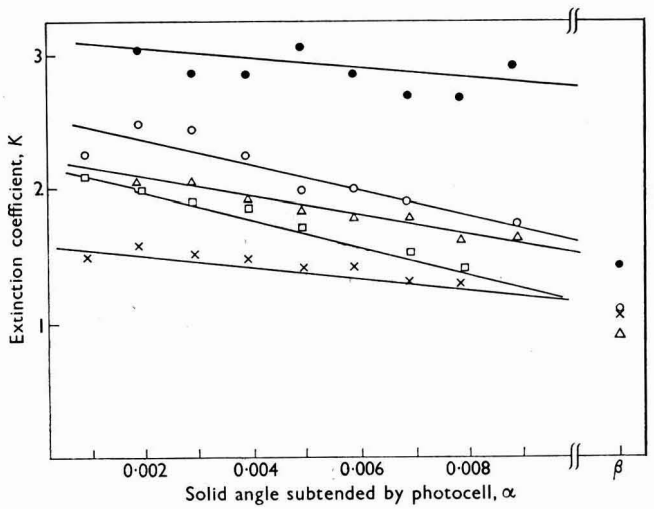

Fig. 5. Graphs showing the variation of the extinction coefficient with the solid angle subtended by the photocell for bronze spheres of different sizes: □, 5 to 6μ; ×, 6 to 7μ; △, 7 to 8μ; ○, 8 to 9μ; ●, 9 to 10μ

It may also be noted (see Fig. 3) that if the experimental size range is increased, a mean value of K is obtained, giving a smoother curve. This curve is in close agreement with earlier work.[11,13] When a detailed size analysis is required, values of K must be found for closely graded size steps. When less detail is required in the analysis, wider size steps may be used.

In Figs. 4 and 5, K is plotted against α; in the former, K is the mean value over a 2-μ

size interval, and in the latter over a 1-μ size interval. From these curves it is seen that there is a nearly linear relationship between the extinction factor and the solid angle subtended by the photocell.

The above experiments have also been made with glass spheres to determine whether their markedly different optical properties would affect the fluctuations of the extinction coefficient with particle size. The results substantiated the above conclusions, although the shape of the K - d curve was entirely different from the curve for the bronze spheres (see Figs. 6 and 7).

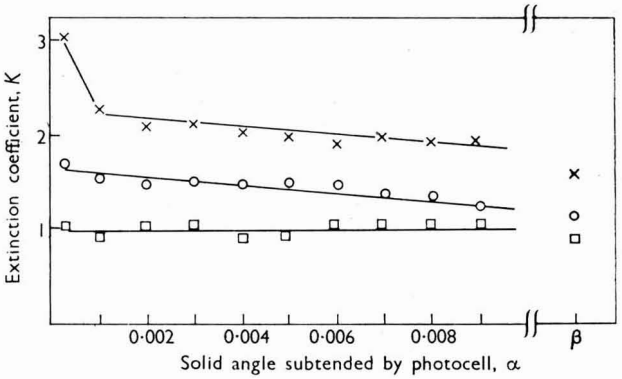

Fig. 6. Graphs showing the variation of extinction coefficient with the solid angle subtended by the photocell for glass spheres of different ranges of size: ×, 25 to 30μ; ○, 35 to 40μ; □, 45 to 64μ

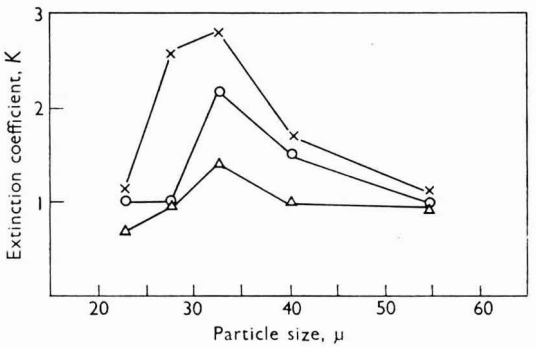

Fig. 7. Graphs showing the variation of extinction coefficient with particle size for glass spheres at differing values of the solid angle subtended by the photocell; ×, α = 0·00025 steradians; ○, α = 0·005 steradians; △, $\alpha = \beta$ steradians

Conclusion

The measurements reported in this paper show that a small angle of acceptance is undesirable in the photosedimentometer because of the large fluctuations of the extinction coefficient under these conditions. It should be noted that this is in direct contradiction to the existing specifications for sedimentometers.[8,9,10] The use of high angles of acceptance has the additional advantages given below—

(*i*) The size of the equipment can be reduced, and this increases the thermal stability by increasing the ease of thermal insulation.

(*ii*) It eliminates the need for optical systems that involve problems of alignment and rigidity.

(*iii*) The cost of the equipment is greatly reduced.

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A Spectrophotometric Method for Determining the Aflatoxins

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A method is described for determining the aflatoxins, particularly aflatoxin B_1, based on the intensity of the ultraviolet absorption at 363 mμ (the long-wavelength absorption maximum), after purification by thin-layer chromatography. This procedure has been applied to groundnut meal and crude aflatoxin isolated from cultures of certain strains of *Aspergillus flavus* and *A. parasiticus*. This method has only been applied to meals of high toxicity and is not applicable, in its present form, to meals of low or medium toxicity. The extraction and decomposition of aflatoxin B_1 have also been investigated.

Recently there has been much interest in the biological action of the aflatoxins,[1,2,3] and an accurate assay for groundnut meal and crude aflatoxin has been required for biological work and for providing a standard for future analytical work.

Previous methods have been based on—

(*a*) bioassay[4,5] and
(*b*) measurement of fluorescence.[6,7,8]

Those based on a bioassay are subject to the normal limitations of this technique, but do, however, provide the ultimate criterion of toxicity.

This paper is confined to the investigation of non-biological methods of assay. Of these, the measurement of fluorescence in solution is an attractive possibility. However, there would appear to be two objections to this method: firstly, it is known that the fluorescence of aflatoxin B_1 in methanolic solution varies with time[9]; secondly, in methanolic solution the fluorescence intensity of aflatoxin B_2 is eight times that of aflatoxin B_1. As aflatoxin B_2 has only one quarter of the toxicity of aflatoxin B_1,[9] if they are not separated, in a sample containing aflatoxin B_2, the contribution made to the total toxicity by aflatoxin B_2 will be over-estimated by a factor of 32. In fact, no method based on the measurement of fluorescence in solution has been reported in the literature. During the present work, an attempt was made to correlate the fluorescence in methanolic solution with the ultraviolet absorption at 363 mμ. This was abandoned because of the much greater spread of results by the fluorescence method.

Previous methods based on the measurement of fluorescence have depended on its being measured on paper[6] or chromatoplates.[7,8] These methods are subject to certain errors, *e.g.*, quenching by impurities and the presence of other fluorescent compounds having a similar R_F value; other difficulties have recently been reported.[8] Variation with time in the fluorescence of aflatoxin B_1 on chromatoplates or paper has not been reported. On chromatoplates of Kieselgel G the fluorescence of aflatoxin B_2, as determined by dilution to extinction, is similar to that of aflatoxin B_1.

All four aflatoxins have an absorption maximum (molar extinction coefficient $\approx$ 20,000) in the ultraviolet region at 363 mμ.[10] The ultraviolet spectrum of aflatoxin B_1 in methanolic solution shows no variation with time in the position, intensity or shape of its peaks. Measurement of the optical density of the peak at 363 mμ was therefore chosen as the basis of an alternative assay procedure.

Method

The method described below is one that has been found to give optimum results. The activity of the plates and the choice of developing solvents appear to be critical for the success of the determination.

Apparatus—

Chromatoplates, 20 $\times$ 10 *cm.*
Ultraviolet lamp, principal wavelength 365 mμ.

REAGENTS—

All solvents should be of analytical-reagent grade.
Light petroleum, free from aromatic hydrocarbons, boiling-range 40° *to* 60° *C.*
Kieselgel G—Obtainable from E. Merck & Co. Inc.

PROCEDURE—

Prepare a slurry by adding two parts by weight of water to one part of dry Kieselgel G and shaking the mixture vigorously in a stoppered flask for 90 seconds. Use this slurry immediately for coating the plates with a layer of Kieselgel G of thickness 750 μ, by using a conventional spreader (the Shandon "Unoplan" is convenient for preparing several uniform plates of the required properties). Allow the plates to stand for 5 minutes and then activate them by drying them in a forced-draught oven for 1 hour at 100° C. Cool the plates to room temperature and store them in a desiccator over "indicating" silica gel for at least 16 hours before use (see Note 1).

Groundnut meal—Extract 40 g of finely ground, homogenised meal with 250 ml of light petroleum (see Note 2), for 6 hours in a Soxhlet extractor (10 to 12 changes per hour). Air dry the Soxhlet thimble and contents thoroughly and re-extract with 250 ml of methanol for 6 hours (10 to 12 changes per hour). Cool, transfer the methanolic extract quantitatively to a flask and dilute it with 125 ml of water. Transfer the aqueous methanolic solution to a downward-displacement, liquid - liquid extractor (1-litre capacity) and extract it with chloroform for 6 hours. Concentrate the chloroform solution under reduced pressure and make it up to 10 ml with chloroform in a calibrated flask.

Apply a 0·2-ml portion of this solution in a line approximately 6 cm wide across a chromatoplate, 3 cm from the bottom (see Note 3). Develop the chromatoplate with diethyl ether until a solvent path-length of 12 cm (from the base-line) has been obtained. Air dry the chromatoplate and examine it briefly in ultraviolet light. Observe that the blue fluorescent band due to the aflatoxins has scarcely moved off the base-line, whereas other blue and green fluorescent bands have moved at R_F 0·8 to 1·0. In visible light, note that a brown material has moved at R_F 0 to 0·3, but does not move further up the plate on subsequent development as described below. Re-develop the dry chromatoplate with a chloroform - methanol (50 + 1) mixture, then dry it and re-develop it with the same chloroform - methanol mixture, both times to a solvent path-length of approximately 12 cm from the base-line. Examine the dry chromatoplate briefly under ultraviolet light. The blue fluorescent bands of aflatoxins B_1 and B_2 should have moved 5 to 7 cm from the base-line (see Note 4) and should have separated cleanly from each other. The faster moving of these bands is that caused by aflatoxin B_1. Under ultraviolet light, mark the position of this band, and then scrape the Kieselgel G containing the aflatoxin B_1 from the plate and extract it with cold methanol for 3 minutes. Filter off the methanol in a 5-ml filter beaker and wash the Kieselgel G three times with methanol, making the combined methanol filtrates up to 5 ml (see Note 5).

Record the ultraviolet absorption spectrum of the methanolic solution in a 2-cm cell (see Note 6) and calculate the amount of aflatoxin B_1 present in the sample.

The optical density at 363 mμ, minus that at 420 mμ, is used for the calculations.

If a 2-cm cell is used, the aflatoxin B_1 concentration is given by—

$$\frac{D \times M \times 10^6}{\epsilon \times 200 \times 2}\ \mu\text{g per 5 ml}$$

where D is the corrected optical density at 363 mμ,
M is the molecular weight of aflatoxin B_1 and
ϵ is the molar extinction coefficient.

Since this is the aflatoxin B_1 content of a 0·2-ml portion of a solution of the extract from 40 g of meal in 10 ml of chloroform, it is equivalent to the aflatoxin B_1 content of 0·8 g of meal.

The aflatoxin B_1 content, in p.p.m., is therefore—

$$= \frac{D \times M \times 10^6}{\epsilon \times 200 \times 2 \times 0{\cdot}8}$$

$$= \frac{D \times M \times 10^5}{\epsilon \times 32}$$

Values of ϵ and M for the 4 aflatoxins are listed below.

	ϵ	M
Aflatoxin B_1	22,000	312
Aflatoxin B_2	23,400	314
Aflatoxin G_1	18,700	328
Aflatoxin G_2	21,000	330

For the determination of aflatoxin B_2, scrape the Kieselgel G containing the slower-moving blue band off 10 plates treated as described before. Combine the Kieselgel G scrapings and extract them with 50 ml of methanol. Filter the extractant, wash the Kieselgel G and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in a small volume of chloroform and spread it in a line across the bottom of a chromatoplate. Develop the chromatoplate twice with chloroform - methanol (50 + 1) mixture. From this point on, carry out the procedure as described previously, in this determination isolating the aflatoxin B_2 band, and calculate the concentration of aflatoxin B_2 from the optical density of the ultraviolet-absorption spectrum at 363 mμ.

Crude aflatoxin—Prepare a chloroform solution containing approximately 100 μg of crude aflatoxin per ml. Apply 0·2 ml of this solution in a line across the bottom of a chromatoplate as before, and develop it with trichloroethylene - chloroform - methanol (8 + 1 + 1) mixture. This solvent system cleanly separates aflatoxins B_1 and B_2 as one band (R_F = 0·5) and aflatoxins G_1 and G_2 as another band (R_F = 0·4). Scrape the Kieselgel G containing each band off the plate separately, and estimate the aflatoxins of the blue band as aflatoxin B_1 and those of the green band as aflatoxin G_1, by using the appropriate molar extinction coefficients (see Note 7).

NOTES—

1. The activity of the chromatoplates should be such that, when they are spotted with an extract of a toxic meal and developed with diethyl ether, the aflatoxins have R_F values of 0·05 or less, and other blue and green fluorescent bands have R_F values of 0·8 to 1·0. Brown material should have an R_F value of 0 to 0·3. When the chromatoplates are re-developed with chloroform - methanol (50 + 1) mixture, aflatoxins B_1 and B_2 (R_F = 0·3 and 0·25) should be cleanly separated. The brown material should not move in this solvent system.

2. **Defatting with diethyl ether[7] is not recommended, as it has been found that this solvent** extracts a portion of the aflatoxin present in the meal.

3. Care should be taken to ensure even distribution and not to damage the surface of the chromatoplate. In practice, a series of spots was put on the plate at approximately 5-mm intervals. The spots were of 3- to 5-mm diameter. A second series of spots was then placed between the first ones, and the process repeated until all the 0·2 ml of solution had been distributed.

4. This carries these bands clear of the brown material and on to a part of the plate that previously showed no fluorescent bands. If necessary, develop the chromatoplate once more in chloroform - methanol (50 + 1) mixture to move the aflatoxin bands to this position. Reject any chromatoplates in which the aflatoxin bands are not clear of the brown material and other fluorescent bands. Reject any chromatoplates in which the bands are not reasonably straight.

5. Filtration is best carried out through a glass sinter, and contact with filter-paper or cotton-wool should be avoided. Transfer the Kieselgel G to the filter beaker and add 2 ml of methanol. After 3 minutes, invert the apparatus with the stem leading into a 5-ml calibrated flask. Filter the mixture under slight air pressure and wash the Kieselgel G with three 0·75-ml portions of methanol in a similar manner. Make the combined methanolic extracts up to 5 ml.

6. Spectra were recorded on an Optica recording spectrophotometer, but the measurement may be made on a manual instrument if desired. In these circumstances it is best to plot the spectrum from 290 to 420 mμ. The spectrum should be similar to that shown in Fig. 1, with a distinct minimum at approximately 310 mμ before the peak at 363 mμ. Reject those that do not conform with this standard. Also shown in this Figure is an absorption spectrum of pure aflatoxin B_1, of the same concentration as that calculated from the spectrum of the material from groundnut meal. The third curve is a curve of the difference between these two. The difference in optical density at 363 mμ of the first two curves is due to the use of the optical density at 420 mμ as a base-line for calculating results.

The difference curve shows a maximum at 280 mμ. There is a slight minimum at 365 mμ and this may be because the impurity present still has some absorption in this region. This would lead to slightly high results being calculated for the aflatoxin B_1 content, but the effect would appear to be small (see results for non-toxic meal, Table I).

7. The results for the amounts of the aflatoxins present can be determined more reproducibly and rapidly by using a solvent system of this type rather than one that separates all four aflatoxins (*e.g.*, chloroform - methanol, 50 + 1), because the separations tend to be incomplete.

If the amounts of aflatoxins B_2 and G_2 present are required, they can be determined as described for aflatoxin B_2 in groundnut extracts. Careful development of the chromatoplates is required to achieve maximum separation of all four components, and normally this is only achieved in about half of the plates developed. A loading of not more than 10 μg of crude aflatoxin per plate is usually required.

Results and discussion

All results quoted are the means of at least four determinations.

Recovery of pure aflatoxin B_1 from chromatoplates—

Solutions containing 5 and 10 μg of pure aflatoxin were spread on chromatoplates and carried through the chromatographic procedure described for aflatoxin B_1. Determination by ultraviolet absorption consistently showed recoveries better than 98 per cent.

Groundnut meals—

Meal A, which had been homogenised and is used in these laboratories as a standard, was examined. As a result of many determinations on several different samples of this meal, a value of 10·2 $\pm$ 0·19 p.p.m. (weighted mean, 95 per cent. confidence limits) was found for the aflatoxin B_1 content. The aflatoxin B_2 content was estimated to be 0·2 p.p.m. Dilution

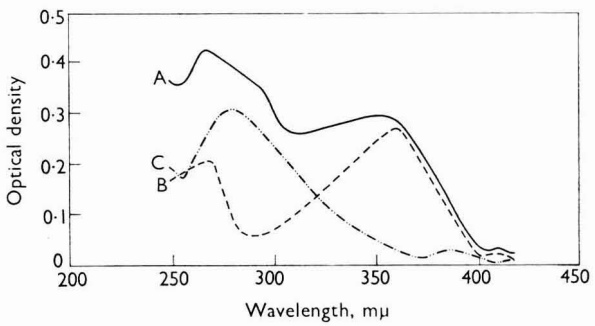

Fig. 1. Ultraviolet-absorption spectra of methanolic solutions of aflatoxin B_1. Curve A, aflatoxin B_1 fraction isolated from a chromatoplate; curve B, pure aflatoxin B_1 of concentration equal to that calculated for A; curve C, difference between curves A and B

to extinction of fluorescence gave a value of 12·5 to 15 p.p.m. of aflatoxin B_1 for this meal.

Meal B, a second standard meal, was found to contain 6·5 $\pm$ 0·2 p.p.m. of aflatoxin B_1 (95 per cent. confidence limits). Dilution to extinction gave a value of 6 to 8 p.p.m. of aflatoxin B_1 for this meal.

To test the accuracy of the spectrophotometric method, suitable amounts of a solution of pure aflatoxin B_1 were added to a methanolic extract of a non-toxic meal prepared from hand-selected peanuts, and the aflatoxin B_1 content determined (see Table I).

Table I

Recovery of aflatoxin B_1 added to methanolic extract of non-toxic meal

Aflatoxin B_1 added, p.p.m.	Aflatoxin B_1 found by ultraviolet absorption, p.p.m.	Difference, p.p.m.
2·5	2·9	+0·4
3·75	4·1	+0·35
5·0	5·0	0
7·5	8·1	+0·6
10·0	11·1	+1·1
12·5	12·6	+0·1

After development on chromatoplates, these extracts appeared to be similar to those from toxic meals, except that the fluorescent band of aflatoxin B_2 and some other fluorescent bands were missing. It will be noted that the results (average + 0·42 p.p.m.) all tended to be high (see Note 6).

Several experiments were carried out on extracts of meal A to investigate possible losses during the analytical procedure and the extraction of aflatoxin B_1 from the meal. These experiments were normally carried out by extracting an 80-g sample of meal with twice the specified volume of methanol and then splitting this extract into two, one half being carried through the normal process as a control sample and the other being treated as described below.

A solution of pure aflatoxin B_1 was added to the chloroform extract to make the equivalent of a meal containing 22·3 p.p.m. aflatoxin B_1. This extract was then concentrated to 10 ml and after normal treatment a value of 21·9 p.p.m. of aflatoxin B_1 was determined.

Additions were also made to the methanolic extract of the meal. This was then carried through the normal procedure to check for any losses subsequent to the methanolic extraction of the meal. A comparison of the calculated and observed concentrations of aflatoxin B_1, in p.p.m., is given below—

Calculated	20·2	16·45
Observed	20·2	16·6

Several experiments were made to investigate the extraction of aflatoxin B_1 from meal A. Attempts were made to find an alternative to the methanolic extraction, by replacing this stage by extraction with either ether, ether followed by chloroform, ether followed by ethyl acetate, or methanol ($\frac{1}{2}$-hour extraction) followed by chloroform (6 hours extraction). None of these proved satisfactory.

To check the completeness of extraction an 8-hour methanolic extraction was carried out. This gave a value of 10·1 p.p.m. for the aflatoxin B_1 content. Re-extraction with methanol of meal that had been extracted for 6 hours with methanol for a further 2, 6 and 18 hours gave no aflatoxin B_1.

To investigate possible destruction of aflatoxin B_1 during the extraction process, the methanol was changed at different stages in the extraction process (see Table II).

TABLE II

EFFECT OF CHANGING THE METHANOL AT DIFFERENT STAGES IN THE EXTRACTION

Duration of methanolic extraction, hours—			Aflatoxin B_1 content, p.p.m.	Total aflatoxin B_1 content, p.p.m.
1st stage	2nd stage	3rd stage		
1	–	–	4·1	10·7
–	5	–	6·6	
2	–	–	5·45	10·7
–	4	–	5·25	
1	–	–	3·9	10·6
–	1	–	2·0	
–	–	4	4·7	

There appears to be some decomposition of aflatoxin B_1 during the extraction process, as the multiple-extraction procedures give values 0·4 to 0·5 p.p.m. higher than those given by the single extraction.

Portions of the methanolic extract obtained from a 6-hour extraction of meal were heated under reflux in methanol and the aflatoxin content determined to verify this; the results are given below—

Duration of reflux heating in methanol, hours	0	3	6	18
Aflatoxin B_1 content, p.p.m.	10·2	9·6	9·1	7·0

Apparently, about 1·5 to 2·0 per cent. of the aflatoxin B_1 is decomposed every hour under the above conditions. When the chloroform-soluble portion of this extract was heated under reflux in methanol or chloroform for periods of up to 18 hours, no change was noted in the aflatoxin B_1 content. It is possible, therefore, that the decomposition of aflatoxin B_1 is caused by some substance present in the methanolic extract, but insoluble in chloroform. From the extraction results it would appear that most of the aflatoxin B_1 is extracted in the first 3 hours, so that, on average, the contact time of the extract with boiling methanol

is probably about 4 hours. Thus about 6 to 8 per cent. of the aflatoxin B_1 may be destroyed during the extraction process. It has already been noted that the results obtained on addition of aflatoxin B_1 to an extract of meal containing no aflatoxin tend to be high by approximately 0·4 p.p.m. If this applies also to meal A then these two errors will tend to cancel. As the exact magnitude of each one is difficult to ascertain, no corrections have been applied to the results quoted here.

An assay of a sample of crude crystalline aflatoxin for aflatoxin B_1 and B_2 and aflatoxin G_1 and G_2 contents was carried out as described. This sample had previously been analysed by separation of the components by column chromatography with subsequent weighing of the fractions, and a comparison of the results is shown in Table III.

TABLE III

RESULTS FOR ASSAY OF CRUDE CRYSTALLINE AFLATOXIN

	Aflatoxin B_1 *plus* B_2 content, per cent.	Aflatoxin G_1 *plus* G_2 content, per cent.	Total aflatoxin content, per cent.
Ultraviolet-absorption method ..	39·5	58·8	98·0
Column-chromatography method ..	42·0	57·0	98·0

Other samples of crude aflatoxin have been analysed and give results that are consistent for each sample. No alternative assays are available for comparison with these samples.

CONCLUSIONS

The method described gives what are believed to be more accurate results than those obtained by previous methods for determining the aflatoxin B_1 content of groundnut meals of high toxicity. It provides a more convenient assay of crude aflatoxin. For meals, the method is lengthier and requires more manipulation than those at present in use, and probably its usefulness will lie in determining samples intended for standards or for biological work.

The separation techniques can also be used to prepare small amounts of pure aflatoxins from crude aflatoxin or relatively pure aflatoxin B_1 from toxic meals.

NOTE—

Since the submission of this paper, a preliminary note has been published on the incorporation of labelled compounds into aflatoxins,[11] in which there is a brief description of a somewhat similar method of determining the aflatoxins in culture broth. This is also based on the measurement of the ultraviolet absorption of a sample purified by thin-layer chromatography.

We thank Miss V. J. Abercrombie, Miss H. Brown and Mr. K. D. Hunt for experimental assistance, Mr. A. B. Wood and Mr. I. Jayaweera for measuring ultraviolet-absorption spectra and Mr. R. D. Coveney for suggesting the trichloroethylene - chloroform - methanol solvent system. The samples of crude aflatoxin were provided by Dr. K. Sargeant of the Microbiological Research Establishment, Porton. We also thank Mr. T. J. Coomes and Mr. B. J. Francis for the quoted results for dilution to extinction, and for a sample of non-toxic meal.

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The Photometric Determination of Zinc in Nickel and Nickel Alloys for Use in Electronic Devices

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A procedure is described for determining zinc in the range 5 to 400 p.p.m. on samples of nickel of 300 mg or less. After separation from the majority of interfering elements by extraction of chlorozincate ion with dioctylmethylamine, and complexing of co-extracted copper with biscyclohexanone oxalyldihydrazone, zinc is determined photometrically with 1-(2-pyridylazo)-2-naphthol.

Some deliveries of 1-(2-pyridylazo)-2-naphthol have been found unsatisfactory and a suitable test for reagent purity is proposed.

The presence of amounts of zinc of the order of 0·005 per cent. in nickel and nickel alloys used in electronic devices can give rise to serious defects in their performance. There is therefore a need for a reliable and sensitive analytical method for its determination.

Commonly available chemical and spectrographic methods[1] are unsuitable for determining zinc in nickel at levels below 0·01 per cent. in samples of 0·5 g or less, although atomic-absorption spectrophotometry or less common spectrographic methods may be used.

No reagent yet proposed is specific for zinc, and a prior separation from, or complexing of, other metals is needed. A chromatographic separation was proposed by Towndrow and Webb,[2] and a procedure relying on disruption of cyano complexes by formaldehyde before photometric determination with 1-(2-pyridylazo)-2-naphthol was put forward by Berger and Elvers.[3]

This latter procedure, being proposed specifically for electronic nickel, was studied by us, but gave occasional wide divergencies from the accepted values on a series of samples of well authenticated zinc content. This was attributed, in part, to the difficulty of assessing the true reagent blank of the method.

Accordingly, work was begun on the development of a more satisfactory method for separating zinc from nickel alloys, which may contain up to 5 per cent. of tungsten, 1 per cent. of cobalt, 0·25 per cent. of each of aluminium, copper, manganese, magnesium and silicon, and 0·05 per cent. of chromium and titanium, before application of the 1-(2-pyridylazo)-2-naphthol photometric procedure.

Experimental

Earlier work in this laboratory[4] based on the published work of Mahlman, Ledicotte and Moore[5] had demonstrated the practicability of extracting zinc from acidic chloride solutions with trichloroethylene solutions of dioctylmethylamine. Since, for the present purpose, it would be convenient to retain the zinc extract in the original separating funnel, a solution of dioctylmethylamine in xylene (less toxic than benzene) was proposed.

A series of experiments, in which zinc was extracted from 3 N hydrochloric acid and the organic extract treated with potassium hydroxide solution to re-extract the zinc before photometric determination, indicated quantitative recovery of zinc, but when this technique was applied to a selected series of nickel alloys having known compositions, results were erroneous. Considerations of the absorption spectra of the 1-(2-pyridylazo)-2-naphthol complex[6] suggested that copper was being co-extracted to a large extent. This was in contrast to published data,[5] but was confirmed by a spectrographic analysis of the extract of the 1-(2-pyridylazo)-2-naphthol complex.

Trials—

As a result of a study of a range of possible copper-suppressing mechanisms, the addition of biscyclohexanone oxalyldihydrazone[7] to the aqueous extract was advocated. Results obtained on a series of commercial samples are given in Table I.

Atomic-absorption measurements were made by using a zinc hollow-cathode lamp and the apparatus described earlier for the determination of magnesium in nickel.[8] Spectrographic

TABLE I

RESULTS FOR DETERMINATIONS OF ZINC MADE WITH COMMERCIAL NICKEL SAMPLES

Sample No.	Zinc, p.p.m., found by— atomic-absorption method	spectrophotometric method
1	15, 18	17, 19, 20
2	22, 22	21, 25, 25
3*	9, 12	8, 9, 11
4	28, 28	32, 33, 33
5†	17, 19	19, 20, 20
6	110, 110	110, 104, 110
7	202, 204	191, 196, 200
8	370, 380	370, 380

* Sample contained 2 per cent. of tungsten.
† Sample contained 4 per cent. of tungsten.

examination of the final chloroform extract from commercial nickel alloys has confirmed the absence of metals other than zinc, indicating the adequacy of the separation.

METHOD

REAGENTS—

Nitric acid, 50 per cent. v/v—Prepare this from nitric acid, sp.gr. 1·42.

Hydrochloric acid, 50 and 25 per cent. v/v—Prepare these from hydrochloric acid, sp.gr. 1·18.

Potassium hydroxide, 4 per cent. w/v, aqueous.

Dioctylmethylamine, 5 per cent. w/v in xylene.

Biscyclohexanone oxalyldihydrazone, 0·1 per cent. w/v ethanolic—Dissolve 0·1 g of biscyclohexanone oxalyldihydrazone in 100 ml of 50 per cent. v/v ethanol.

Chloroform.

Ammonium chloride.

Sodium sulphate, anhydrous.

Ascorbic acid.

1-(2-*Pyridylazo*)-2-*naphthol, 0·1 per cent. w/v, ethanolic*—Dissolve 0·1 g of 1-(2-pyridylazo)-2-naphthol in 100 ml of ethanol. (See Fig. 1 for quality of the solid.)

Standard zinc solution—Dissolve 100 mg of zinc in 10 ml of 50 per cent. v/v hydrochloric acid, and dilute the solution to 1 litre.

For the preparation of the calibration graph, dilute 10 ml of this solution to 100 ml with 25 per cent. v/v hydrochloric acid.

1 ml of solution ≡ 10 μg of zinc.

PROCEDURE—

Weigh up to 300 mg of nickel, containing not more than 15 μg of zinc, into a 100-ml beaker and dissolve the sample in 2 ml of 50 per cent. v/v nitric acid. Add 2 ml of hydrochloric acid, sp.gr. 1·18, evaporate the solution just to dryness and take up the residue in 5 ml of water. (Any precipitate of tungstic acid will not affect the performance of the method, but may be removed, *e.g.*, by centrifugation, if required.) Add 20 mg of ascorbic acid and transfer the solution with the aid of 5 ml of water to a 100-ml cylindrical separating funnel.

Add 10 ml of 50 per cent. v/v hydrochloric acid and 10 ml of 5 per cent. w/v dioctylmethylamine solution. Stopper the funnel, shake it for 1 minute and allow the layers to separate. Run off and reject the lower aqueous layer. Add to the solution remaining in the funnel, 10 ml of 25 per cent. v/v hydrochloric acid, shake the funnel for 1 minute, set it aside and reject the lower layer. Repeat this acid wash.

Add 10 ml of 4 per cent. w/v potassium hydroxide solution to the organic layer, shake the funnel for 1 minute, allow the layers to separate and run off the lower layer into a clean 100-ml separating funnel containing about 0·5 g of ammonium chloride. Reject the organic phase. Dilute the aqueous solution to about 30 ml, add 5 ml of 0·1 per cent. biscyclohexanone oxalyldihydrazone, mix the reagents and set them aside for 3 minutes.

Add, from a pipette, 1 ml of 0·1 per cent. w/v 1-(2-pyridylazo)-2-naphthol solution, mix the solutions, add 10 ml of chloroform, shake the funnel for 1 minute, allow the layers

to separate, and run off the lower layer into a clean, dry 25-ml calibrated flask. Repeat the extraction with two 5-ml portions of chloroform. Combine the extracts and dilute them to the mark with chloroform. Transfer to a dry 50-ml beaker containing about 1 g of anhydrous sodium sulphate, swirl the beaker to remove any entrained water and measure the optical density at 560 mμ in a 1-cm cell against chloroform. A blank sample, containing no nickel, should be carried through the procedure. Values of between 2·9 and 3·1 μg of zinc have been found in practice when analytical-reagent grade chemicals have been used.

A calibration graph is prepared by adding aliquots of a solution of zinc in dilute hydrochloric acid (containing 2, 4, 6, 8, 10 and 15 μg of zinc) to 10 ml of water and carrying through the procedure, commencing at the paragraph that starts "Add 10 ml of 50 per cent. v/v hydrochloric acid. . . ".

Provided that care is taken in obtaining clean separation of the organic and aqueous phases, no "breakthrough" of nickel takes place, and a single washing stage should be adequate, but a second wash with hydrochloric acid provides an added safeguard.

Discussion

Reagents—

In pursuance of a general policy of establishing whether the validity of the method is restricted to any particular supplier or batch of material, samples of 1-(2-pyridylazo)-2-naphthol were purchased in May 1963 from five suppliers. The variation in appearance

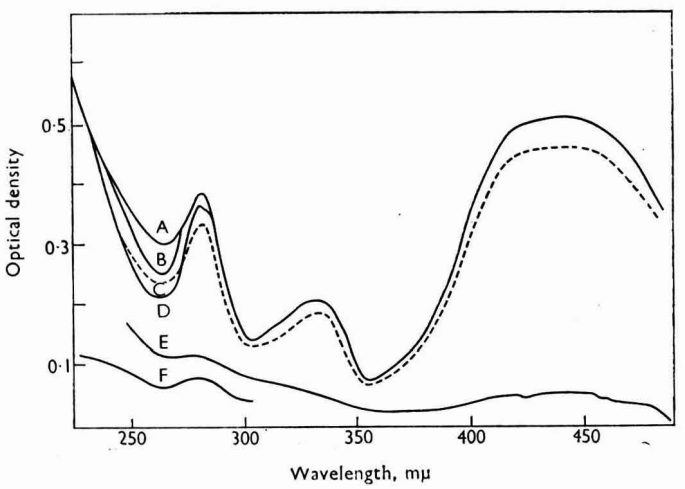

Fig. 1. Absorption spectra of 0·375 mg of 1-(2-pyridylazo)-2-naphthol in 50 ml of 0·1 N hydrochloric acid against water in 1-cm cells. Curve A, supplier A (Hopkin & Williams Ltd., batch 018171 $\frac{14400}{W}$); curve B, supplier B (L. Light & Co. Ltd., of April 1963); curve C, supplier C (Merck & Co., batch 380853); curve D, supplier D (Eastman Kodak Co., of April 1963); curve E, supplier E (first sample); curve F, supplier E (second sample)

of these samples was sufficiently great to cause us to examine their absorption spectra.[6] It is apparent from Fig. 1 that although four of the samples conform to the behaviour reported by Cheng and Bray,[6] one did not. A further sample submitted by the same supplier gave similar results.

When these chemicals were used in the described procedure in the presence and absence of 25 μg of zinc, the absorption curves shown in Fig. 2 were obtained. The presence of a

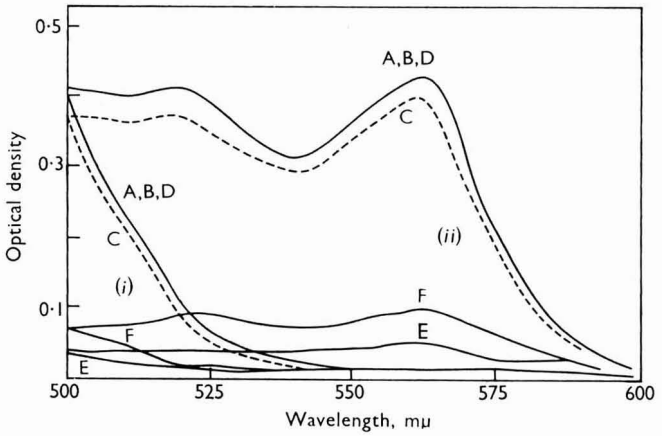

Fig. 2. Absorption spectra of a chloroform extract (50 ml) from a solution at pH 10 containing 0·375 mg of 1-(2-pyridylazo)-2-naphthol. Group (*i*), reagent alone; group (*ii*), reagent *plus* 25 μg of zinc. Curves A, B, D, suppliers A, B, D (Hopkin & Williams Ltd., batch 018171 $\frac{14400}{W}$; L. Light & Co. Ltd., of April 1963; Eastman Kodak Co., of April, 1963; respectively); curve C, supplier C (Merck & Co., batch 380853); curve E, supplier E (first sample); curve F, supplier E (second sample)

significant peak at 280 mμ for a solution of 0·00075 per cent. w/v 1-(2-pyridylazo)-2-naphthol in 0·1 N hydrochloric acid should therefore be a criterion of suitability of any given supply of 1-(2-pyridylazo)-2-naphthol.

NOTE—

During the preparation of this paper, a publication by Ott, MacMillan and Hatch[9] has appeared, based on a similar approach. This method has not been examined by us, but it is felt that the procedure described above is sufficiently different in detail to justify its presentation here.

We thank Mr. C. H. R. Gentry, Head of the Material Research Laboratory, and the Directors of the Mullard Radio Valve Company for permission to publish this work.

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Determination of Traces of Acrylonitrile Monomer in Liquid Extractants Used in Assessing the Suitability of Styrene - Acrylonitrile Copolymers as Food-packaging Materials

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A cathode-ray polarographic procedure is described for determining acrylonitrile monomer in liquids obtained in extractability tests made on styrene - acrylonitrile copolymers. The procedure can be used for determining acrylonitrile in concentrations down to 1 p.p.m. w/v in the standard extraction liquids recommended by the British Plastics Federation and by the Food and Drug Administration (U.S.A.).

Both the British Plastics Federation[1] and the Food and Drug Administration of the United States[2] have described tests for determining the extractability into packaged foodstuffs of polymer additives from a plastic container or wrapping. In these tests the plastic is brought into contact, under specified conditions of time and temperature, with the range of solutions given below, which are chosen to simulate various aqueous, alcoholic and oily foodstuffs and beverages—

Extraction liquids recommended by the British Plastics Federation	Some typical extraction liquids recommended by the Food and Drug Administration
(*i*) distilled water	(*i*) distilled water
(*ii*) 5 per cent. w/v sodium carbonate	(*ii*) 3 per cent. sodium hydrogen carbonate
(*iii*) 6 per cent. w/v hydrochloric acid	(*iii*) 3 per cent. acetic acid
(*iv*) 50 per cent. w/v aqueous ethanol	(*iv*) 8 per cent. aqueous ethanol or 50 per cent. aqueous ethanol
(*v*) light liquid paraffin (or B.P. Lev.) or olive oil B.P.C. containing 2 per cent. w/w of oleic acid	(*v*) n-heptane (*vi*) a food oil, *e.g.*, a vegetable oil or a lard oil

Determination of the additive content of the extractant liquid at the end of the test permits an assessment to be made regarding its extractability from the plastic into various types of foodstuffs. Knowledge of these extractability results and of the toxicity of the neat additive, determined by animal-feeding trials, provides data on which a decision can be reached on whether or not it would be safe to use the additive in plastics manufactured for foodstuff-packaging applications.

Acrylonitrile monomer is usually present in small concentrations in styrene - acrylonitrile copolymers. This monomer is toxic[3] and it should not, therefore, be extracted to any appreciable extent by foodstuffs from packaging grades of styrene - acrylonitrile copolymers. The extractability tests of the British Plastics Federation or the Food and Drug Administration are suitable for determining the degree of extraction of acrylonitrile from polymers into their standard extractants. A method was not available, however, for determining acrylonitrile in all these liquids at the low concentrations that are of interest in toxicity-evaluation studies. Thus, calculations based on data given in the British Plastics Federation Second Toxicity Report[1] show that it is particularly important to have a method for determining acrylonitrile in amounts between 1 and 20 p.p.m. in plastic-extraction liquids.

Bird and Hale[4] have described a method for determining traces of acrylonitrile in water. This method is based on polarography in 0·02 M tetramethylammonium iodide base electrolyte. By using the Southern Analytical K1000 cathode-ray polarograph, the applicability of this procedure to the determination of acrylonitrile in the various aqueous and alcoholic extraction liquids has been examined.

Experimental

Direct polarography of distilled-water extractants—

As in Bird and Hale's procedure,[4] 0·02 M aqueous tetramethylammonium iodide was used as the base electrolyte for the polarographic determination of acrylonitrile. Polarographic cell solutions were prepared by mixing 1 ml of 0·2 M base electrolyte with 9 ml of distilled-water extractant. The results obtained by polarography of 0·02 M aqueous base-electrolyte solutions (start potential of −1·8 volts) showed that it is possible with either the cathodic direct or the cathodic derivative circuits to determine down to 1 p.p.m. of acrylonitrile monomer in the distilled-water extractant. Confirming the conclusions of Bird and Hale[4] and Daues and Hamner[5] it was found that the presence in the cell solution of dissolved oxygen did not interfere in the polarographic determination of acrylonitrile in the range −1·8 to −2·3 volts.

Liquids obtained in extractability tests on styrene - acrylonitrile copolymers also usually contain a small amount of styrene monomer besides acrylonitrile monomer. It has been shown that the presence of up to 500 p.p.m. of styrene monomer in the test solution does not interfere in the determination of acrylonitrile in aqueous solutions.

Determination of acrylonitrile in aqueous-ethanol extractants—

The determination of acrylonitrile in mixtures of water and ethanol containing up to 50 per cent. of the latter was examined. To 9 ml of each ethanol - water mixture containing 50 p.p.m. of added acrylonitrile was added 1 ml of 0·2 M tetramethylammonium iodide base-electrolyte solution. Reagent blank solutions were also prepared by mixing 9 ml of appropriate acrylonitrile-free ethanol - water mixture with 1 ml of 0·2 M base electrolyte. These samples and blank solutions were examined polarographically at a start potential of −1·8 volts and the peak currents occurring at the acrylonitrile maximum were noted. The influence of the ethanol content of the extraction liquid on the peak current obtained with 50 p.p.m. of acrylonitrile (corrected for the peak current of the reagent blank solution) is shown in Fig. 1. It is

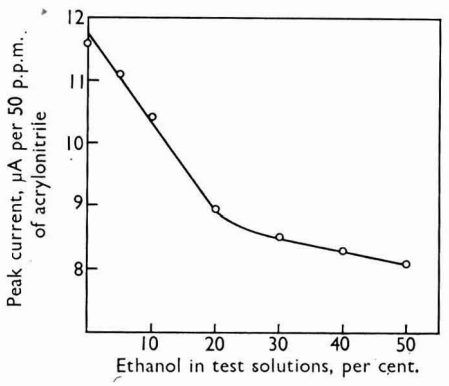

Fig. 1. Graph of peak current *versus* ethanol content of test solution for 50 p.p.m. of acrylonitrile in aqueous ethanolic solutions containing 0·02 M tetramethylammonium iodide base electrolyte

seen that lower peak currents are obtained as the alcohol content of the test solution increases from zero to 50 per cent., *i.e.*, the procedure for determining acrylonitrile becomes rather less sensitive as the alcohol content of the extraction liquid is increased. Acrylonitrile could be reproducibly determined, however, in amounts down to 1 p.p.m. in all the alcohol solutions.

Separation of acrylonitrile from acidic and alkaline extraction liquids by azeotropic distillation with methanol—

Direct polarography of synthetic solutions of acrylonitrile in the 6 per cent. hydrochloric acid and in the 5 per cent. sodium carbonate extractants was not possible as both extractants produced interfering waves in the acrylonitrile polarogram. Also, when tetramethylammonium

iodide was added to the 6 per cent. hydrochloric acid extractant, the quaternary salt decomposed and free iodine was liberated.

The applicability of the azeotropic-distillation procedure described by Daues and Hamner[5] for separating acrylonitrile monomer from interfering impurities before polarography was examined. In this procedure the aqueous sample is distilled in the presence of a mixture of methanol and aqueous sulphuric acid. The methanol - acrylonitrile azeotrope, boiling at 61·4° C, distils first from this mixture and thus the acrylonitrile is recovered in the initial distillate.

The azeotropic-distillation procedure was tested by using a synthetic solution of acrylonitrile in water that had been shown by direct polarographic analysis to contain 0·75 p.p.m. of acrylonitrile. A measured volume (500 ml) of this solution, *i.e.*, 0·375 mg of acrylonitrile, together with 5 ml of concentrated sulphuric acid, 25 ml of methanol and 0·1 g of 2,4-dinitrophenylhydrazine (to destroy carbonyl compounds) was transferred to a round-bottomed flask fitted with a lagged glass column packed with $\frac{1}{8}$-inch helices and a reflux head (as described by Daues and Hamner[5]). This mixture was distilled for 1 hour under total reflux and then three 4-ml portions of the methanol distillate were collected in separate 10-ml calibrated flasks. Then 1 ml of 0·2 M tetramethylammonium iodide and 5 ml of distilled water were added to each flask. The weight of acrylonitrile recovered from the three fractions was determined polarographically by making "standard additions" of a synthetic solution of acrylonitrile in a methanol - water (40 + 60) mixture to the cell solution. It can be seen from Table I (sample A) that approximately 90 per cent. of the 0·375 mg of acrylonitrile monomer present in the original 500 ml of water was recovered in the first two 4-ml methanol distillates. The somewhat low recovery of acrylonitrile might be due to a slight hydrolysis of this substance to ammonium acrylate under the acidic conditions used during azeotropic distillation.

Reagent-grade methanol contains traces of carbonyl impurities that were found to concentrate in the earlier methanol distillates obtained in the azeotropic-distillation procedure. Carbonyl compounds are reduced in a similar voltage range to acrylonitrile when examined polarographically in a 0·02 M tetramethylammonium iodide base electrolyte and interfere, therefore, in the determination of acrylonitrile. A preliminary heating under reflux of the methanol with a mixture of potassium hydroxide and aluminium powder reduced its carbonyl content to an acceptable low level. To remove the last traces of carbonyl impurities, a small amount of 2,4-dinitrophenylhydrazine was present during the distillation.

DETERMINATION OF ACRYLONITRILE IN 6 PER CENT. HYDROCHLORIC ACID EXTRACTANT—

The methanol azeotropic-distillation procedure was also applied to a synthetic solution of acrylonitrile in 6 per cent. hydrochloric acid extractant. Polarographic analysis of the methanol - acrylonitrile azeotrope was not possible, however, owing to the presence of an appreciable amount of free acidity originating from the hydrochloric acid extractant, in the distillates, which interfered in the polarography of acrylonitrile. In a further experiment, a 6 per cent. hydrochloric acid solution of 47·3 p.p.m. of acrylonitrile was neutralised by the addition of a small excess of solid calcium oxide. Methanol and sulphuric acid were added and the azeotropic distillation continued as before. It can be seen from Table I (sample B) that under these conditions more than 90 per cent. of the added amount of acrylonitrile was recovered in the first 8 ml of methanol distillate. A preliminary neutralisation with lime was incorporated, therefore, into the procedure for determining acrylonitrile in 6 per cent. hydrochloric acid extraction liquids. This procedure should also be applicable to the determination of acrylonitrile in the 3 per cent. aqueous acetic acid extractant recommended by the Food and Drug Administration.[2] However, no experiments were carried out with this extractant.

DETERMINATION OF ACRYLONITRILE MONOMER IN 5 PER CENT. SODIUM CARBONATE EXTRACTANT—

The results in Table I (samples C and D) show that above 80 per cent. of the added amount of acrylonitrile was recovered in the methanol distillate when the azeotropic-distillation procedure was applied to 5 per cent. sodium carbonate extractants containing up to 72·4 p.p.m. of acrylonitrile monomer. The recoveries of acrylonitrile in these experiments are lower than those obtained for the distilled-water and 6 per cent. hydrochloric acid extractants. This may be due to an increased amount of hydrolysis of acrylonitrile to ammonium acrylate occurring during reflux in the presence of sodium sulphate, as this salt will elevate

Table I

Determination of acrylonitrile in distilled-water, 6 per cent. hydrochloric acid and 5 per cent. sodium carbonate extractants after azeotropic distillation with methanol

Sample	Composition of synthetic test solution used for azeotropic distillation: Acrylonitrile, p.p.m. w/v	Composition of synthetic test solution used for azeotropic distillation: Solvent	Volume of test solution used for azeotropic distillation, ml	Acrylonitrile added, μg (X)	Weight, in μg, of acrylonitrile in test solution—: Found by direct polarography of sample, μg	Found in distillates obtained by azeotropic distillation with methanol: 1 (1st 4 ml)	2 (2nd 4 ml)	3 (3rd 4 ml)	Total (Y)	Recovery of acrylonitrile in first two fractions obtained by azeotropic distillation $\left(\frac{Y \times 100}{X}\right)$, per cent. w/w
A	0·75	Distilled water	500	376	376	315	21	Nil	336	90
B	47·3	6 per cent. HCl	50 (+ 3·5 g of calcium oxide)	2370	—	2250	93	Nil	2343	99
C	14·4	5 per cent. Na_2CO_3	25	360	—	248	54	Nil	302	84
D	72·4	5 per cent. Na_2CO_3	25	1810	—	1390	83	Nil	1473	82

Table II

Determination of acrylonitrile in light liquid-paraffin extractant

Acrylonitrile content of the synthetic light liquid-paraffin sample solution for extraction with water, p.p.m. w/w	Weight of light liquid-paraffin sample extracted with 2 × 250 ml of water, g	Weight of acrylonitrile present in light liquid-paraffin test solution, μg (X)	Weight, in μg, of acrylonitrile recovered in fractions of azeotropic distillation: 1 (1st 4 ml)	2 (2nd 4 ml)	3 (3rd 4 ml)	Total (Y)	Mean recovery of acrylonitrile in first two fractions obtained by azeotropic distillation $\left(\frac{Y \times 100}{X}\right)$, per cent. w/w
538	10	5380	(*i*) 4940	350	Nil	5290	99
			(*ii*) 4980	370	Nil	5350	
297	20	5940	5160	460	Nil	5620	95
34	200	6800	(*i*) 7220	Nil	Nil	7220	103
			(*ii*) 6840	Nil	Nil	6840	
11·1	9	100	(*i*) 96	2	Nil	98	98
			(*ii*) 96	2	Nil	98	

the temperature at which the mixture boils during the preliminary 1-hour reflux period. The azeotropic-distillation procedure is also applicable to the 3 per cent. sodium hydrogen carbonate extractant recommended by the Food and Drug Administration (U.S.A.).[2]

DETERMINATION OF ACRYLONITRILE IN LIGHT LIQUID-PARAFFIN EXTRACTANT—

Distillation of the liquid-paraffin extractant with methanol and water was tried as a means of isolating an acrylonitrile - methanol azeotrope suitable for polarography. This did not succeed, however, owing to difficulty in establishing steady distillation conditions. The possibility of aqueous extraction of acrylonitrile from the liquid-paraffin extractant was examined. Various synthetic solutions of acrylonitrile in liquid paraffin were extracted with two 250-ml portions of distilled water. The distilled-water extracts were filtered into a 1-litre flask and an azeotropic distillation with methanol made as described previously. The recoveries of acrylonitrile obtained by this procedure for liquid-paraffin extractants containing up to 538 p.p.m. of acrylonitrile are shown in Table II. In all these mixtures, the recovery of acrylonitrile in the first 8 ml of the methanol azeotrope was within ± 5 per cent. of the amount known to be present. Duplicate recoveries obtained in the azeotropic-distillation procedure are reasonably reproducible.

Procedures, based on these principles, involving extraction with water and azeotropic distillation with methanol before polarography should also be applicable to the n-heptane extractant recommended by the Food and Drug Administration[2] and also might be useful for the determination of acrylonitrile in the Food and Drug Administration vegetable-oil extractants, provided that these can be successfully extracted with water. Trials have not been made on these particular extractants.

METHODS

APPARATUS—

Cathode-ray polarograph—K1000. Complete with stand for dropping mercury electrode, polarographic cells (10 ml) and thermostatted (at 25° C) water-bath, supplied by Southern Analytical Ltd., Frimley Road, Camberley, Surrey, England.

Agla micrometer syringe—Capable of delivering 0·01 ml with an accuracy of 0·0002 ml, available from Burroughs Wellcome and Co., London.

Calibrated glassware—Pipettes, measuring cylinders and 10-ml calibrated flasks.

Apparatus for azeotropic distillation with methanol[5]—Connect a 1-litre round-bottomed flask (B24 neck) to a 60 × 1·8-cm column packed with $\frac{1}{8}$-inch glass helices. Connect a reflux head, as described by Daues and Hamner,[5] fitted with side-arm, condenser and stopcock, to the top of the column. Stand the round-bottomed flask, surrounded in aluminium foil, in an electric mantle. Wind an electric heating tape round the column and stand the whole apparatus in a draft-free area to prevent bumping during distillation.

REAGENTS FOR AZEOTROPIC DISTILLATION—

Methanol—Carbonyl-free. Heat 2 litres of methanol under reflux for 3 hours with 20 g of aluminium powder and 50 g of potassium hydroxide. Distil off the methanol into a clean bottle.

Calcium oxide.

2,4-*Dinitrophenylhydrazine*—Pure.

REAGENTS FOR POLAROGRAPHIC ANALYSIS—

Tetramethylammonium iodide base electrolyte, 0·2 M—Dissolve 4·025 g of tetramethylammonium iodide (pure solid available from Kodak Ltd., Kirkby, Liverpool: Code No. 2434, or from The British Drug Houses Ltd., Poole, Dorset) in water and dilute the solution to 100 ml.

Acrylonitrile monomer—Available from Kodak Ltd., Kirkby, Liverpool (Code No. P.5161). Redistil the monomer before use and store it in a refrigerator.

Mercury—For polarographic analysis. Use trebly distilled mercury. Request the manufacturer to supply this mercury in stone containers, as it has been shown that, over a period of time, mercury picks up an impurity that might interfere in polarography, from the polythene storage bottles.[4]

THE EXTRACTABILITY TEST—

Experimental details for carrying out the extractability tests are discussed in the British Plastics Federation's Second Toxicity Report[1] and in the Federal Register, U.S.A.[2]

SEPARATION OF ACRYLONITRILE FROM LIGHT LIQUID-PARAFFIN AND n-HEPTANE EXTRACTANTS—

Weigh an amount of extractant containing between 0·001 and 0·01 g of acrylonitrile into a clean 1-litre separating funnel. Into a further separating funnel, weigh the same amount of the blank extractant that has not been brought into contact with the plastic under test. Into each funnel pour 250 ml of water. Stopper the funnels and shake the contents thoroughly. When the two phases have separated, filter the lower aqueous phase through two layers of Whatman No. 40 filter-paper into a 1-litre round-bottomed flask. Extract the organic phase with a further 250 ml of water and combine the aqueous extracts. As soon as possible after this extraction procedure, carry out an azeotropic distillation of the aqueous extracts with methanol as described below.

DETERMINATION OF ACRYLONITRILE IN EXTRACTANTS—

Distilled water, 6 per cent. hydrochloric acid, 5 per cent. sodium carbonate, 3 per cent. sodium hydrogen carbonate and aqueous extract of light liquid paraffin or n-heptane—Into a 1-litre round-bottomed flask transfer a volume of the plastic-extraction liquid containing between 0·001 and 0·01 g of acrylonitrile. Into a further 1-litre flask transfer the same volume of the blank extraction liquid. Into each flask introduce distilled water to make the volume up to 500 ml. To the 6 per cent. w/v hydrochloric acid extractant only, add a slight excess of calcium oxide (3·5 g of calcium oxide per 50 ml of 6 per cent. w/v hydrochloric acid is sufficient). To the 5 per cent. sodium carbonate and the 3 per cent. sodium hydrogen carbonate extractants only, add sufficient concentrated sulphuric acid to neutralise the alkali present. To all extractants add 5 ml of concentrated sulphuric acid, 25 ml of redistilled carbonyl-free methanol, 0·1 g of 2,4-dinitrophenylhydrazine and a few glass beads. Place the two flasks in 1-litre electric mantles and connect to each flask a 60-mm column packed with $\frac{1}{8}$-inch glass helices and a reflux head. Turn the stopcock on the reflux head to total reflux and turn on the water supply to the condenser on the reflux head. Set the voltage of the heating tape on the column to bring the column to about 10° C above room temperature. Commence heating the flasks and leave them to equilibrate for 1 hour after methanol starts to condense at the reflux head.

After the 1-hour reflux period, transfer by pipette 2 ml of 0·2 M tetramethylammonium iodide base electrolyte and 10 ml of water into each of two dry 25-ml stoppered graduated cylinders. Place one of these cylinders at the outlet of the reflux head and open the stopcock to provide a reflux ratio of approximately 1 to 1. Allow methanol to distil into the cylinder at a rate of approximately 1 ml per minute, until the volume of solution reaches the 20-ml mark. Immediately examine the distillates polarographically as described below. It has been shown that up to 0·02 g of acrylonitrile in the distillation-flask charge is recovered in the first 8 ml of methanol - acrylonitrile azeotrope. Completeness of recovery of acrylonitrile in this distillate can be checked by collecting a further 4 ml of distillate in a 10-ml graduated cylinder (containing 1 ml of 0·2 M tetramethylammonium iodide and 5 ml of distilled water). Polarography of the solution shows whether any acrylonitrile is present in the second distillate. If acrylonitrile is found in this distillate then it should be included in the reported analytical result.

When the K1000 polarograph is used for the analysis, use a dropping mercury electrode and a mercury-pool reference electrode on the cathodic direct circuit. Transfer by pipette 5 ml of sample solution from the 25-ml cylinder into a polarograph cell and immerse the cell in the constant-temperature tank of the cathode-ray polarograph. Lower the dropping mercury electrode system over this cell and insert the anode connection into the side-arm of the polarographic cell. If the approximate concentration of monomer in the polarographic cell solution is known, set the instrument to the appropriate sensitivity setting at a start potential of −1·8 volts. If the composition of the solution is unknown, then adjust the polarograph to its maximum sensitivity setting and move the "X-shift" control and the "Y-shift" control until the light spot on the graticule of the cathode-ray tube commences its horizontal sweep at the origin of axes at the left of the graticule. Repeat this opreation at different sensitivity settings until the polarographic wave is visible on the graticule.

Read off from the graticule the maximum height of the peak, and note the voltage, V, at which this maximum polarographic reading occurs. Transfer by pipette a further 5 ml of solution from the 25-ml graduated cylinder into a dry 25-ml beaker and into this solution deliver a suitable "standard addition" of a solution of acrylonitrile in a methanol - water (40 + 60, v/v) mixture (by using a horizontally held Agla micrometer syringe for delivery). To avoid dilution errors, limit the volume of the "standard addition" added to less than 0·05 ml. Mix the contents of the beaker and pour them into a dry polarographic cell. Note the height of the acrylonitrile wave at V volts. Adjust the instrument to obtain the azeotropic-distillation blank wave on the graticule. Measure the blank peak height corresponding to V volts.

Aqueous ethanol—The azeotropic-distillation procedure cannot be applied to this alcoholic extraction liquid. Transfer 16 ml of the aqueous alcohol plastic-extraction liquid and 16 ml of the blank alcoholic extraction liquid into two 25-ml stoppered graduated cylinders. To each cylinder add 2 ml of 0·2 M tetramethylammonium iodide and 2 ml of distilled water and mix. Examine these solutions polarographically as described above.

Calculation of the acrylonitrile contents of the extractants—

The amount of acrylonitrile in the plastic-extraction liquid, p.p.m. w/v, is given by—

$$\frac{20 \times A \times 10^6}{5 \times S} \times \left[\frac{h_1S_1 - h_3S_3}{h_2S_2 - h_1S_1}\right]$$

(assuming that the methanolic azeotropic distillate is made up to 20 ml and that 5 ml of this solution is used for polarography), where—

S = volume, in ml, of plastic-extraction liquid used in azeotropic distillation,

h_1 = peak height, in cm, of sample solution before the "standard addition,"

h_2 = peak height, in cm, of sample solution after the "standard addition,"

h_3 = peak height, in cm, obtained in the azeotropic-distillation blank determination,

S_1, S_2, S_3 are the corresponding instrument sensitivity settings (the products of h and S are known as peak currents, in μA) and

A = weight, in g, of acrylonitrile present in volume of "standard addition" solution added to the cell solution.

Discussion of results

Provided that a suitable sample size is taken for analysis, the azeotropic distillation - polarographic procedure can be used for determining acrylonitrile in extractants in concentrations down to 1 p.p.m. or a little lower. Thus it is seen in Table I that approximately 90 per cent. of the added amounts of acrylonitrile is recovered when the procedure is applied to 500 ml of a 0·75 p.p.m. solution of acrylonitrile in the distilled-water extractant. The method can be used for achieving a similar level of sensitivity in the determination of acrylonitrile in the other aqueous alcoholic or oily extractants for plastics recommended by the British Plastics Federation and the Food and Drug Administration. This level of sensitivity is quite adequate for the examination of extractants that have been brought into contact with styrene - acrylonitrile copolymers under the British Plastics Federation and Food and Drug Administration extractability-test conditions.

Actual extraction liquids obtained in extractability tests made on various styrene - acrylonitrile copolymers have been found by the described procedures to contain from less than 10 p.p.m. up to 200 p.p.m. of acrylonitrile. The amount of acrylonitrile monomer found in the extraction liquids depends, of course, on the extent to which this monomer is removed from the plastic during the manufacturing process.

It is advisable to analyse an extraction liquid for acrylonitrile as soon as possible after the completion of the British Plastics Federation or Food and Drug Administration extractability tests for plastics. This is because a slow hydrolysis of acrylonitrile to acrylamide or ammonium acrylate occurs on standing, especially in acidic or alkaline extractants. Consequently, low acrylonitrile contents are obtained by the polarographic method. Thus, on analysis immediately after the extraction test, a 6 per cent. hydrochloric acid extractant was

found to contain 110 p.p.m. of acrylonitrile. Analysis of the same sample two months later showed that the acrylonitrile content had decreased to 75 p.p.m. owing to the hydrolysis.

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SHORT PAPERS

The Polarographic Determination of Molybdenum in Niobium-base Alloys

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In a recent paper[1] it was stated that molybdenum^{VI} produces in 0·5 M hydrofluoric acid - 0·5 M sulphuric acid mixture two waves that can be used for analysis. The first wave results from the reduction of molybdenum^{VI} to molybdenum^{V}, $E_{\frac{1}{2}} = -0{\cdot}06$ volt with respect to the saturated calomel electrode ($E_{\frac{1}{2}} - E_{\frac{1}{2}} = 100$ mV), and the second wave from the reduction of molybdenum^{VI} to molybdenum^{III}, $E_{\frac{1}{2}} = -0{\cdot}52$ volt ($E_{\frac{1}{2}} - E_{\frac{1}{2}} = 140$ mV). With this supporting electrolyte, titanium, zirconium, niobium, tantalum and tungsten are held in true solution. For a 10^{-3} M solution of molybdenum^{VI}, 50-fold excesses of zirconium, niobium^{V} and tantalum, and 5-fold excesses of titanium^{IV} and tungsten^{VI} have no interfering effects on the first molybdenum wave.

A method based on these polarographic results has now been applied to the determination of molybdenum in niobium-base alloys.

Method

Apparatus—

Polarograph—A Sargent model XV polarograph was used.

Dropping mercury electrode—This was constructed from Teflon according to the method of Raaen.[2] Polarograms were recorded by using a Teflon segment with an orifice of 60 μ. This segment was fitted to a suitable length of Sargent "2 to 5"-second glass capillary.

Polarographic cell—A 20-ml polythene specimen tube was modified for this purpose. This consisted of sealing a platinum wire into the side of the tube 3 mm from the base to make electrical contact to a mercury-pool anode. A lead-in tube for oxygen-free nitrogen was also sealed into the side of the tube near the base. The lid of the specimen tube was adapted to take a lead-in tube for nitrogen to pass over the surface of the solution in the cell. A hole of suitable diameter was cut from the lid for the dropping mercury electrode. The cell was immersed in a tank thermostatically controlled at 25·0° C.

Reagents—

Hydrofluoric, nitric and sulphuric acids—These were of analytical reagent grade.

Standard molybdenum^{VI} solution—A suitable weight of Specpure molybdenum trioxide, obtained from Johnson, Matthey & Co. Ltd., was dissolved in boiling ammonia solution sp.gr. 0·88, in a polytetrafluoroethylene beaker, the solution was evaporated to dryness, and the residue dissolved in 0·5 M hydrofluoric acid - 0·5 M sulphuric acid. This stock solution contained 0·402 mg of molybdenum trioxide per g of solution. Less concentrated solutions of molybdenum^{VI} in this acid mixture were made by dilution. All solutions containing hydrofluoric acid were stored in polythene bottles.

The constancy of the ratio of diffusion current to concentration—

Six standard molybdenum^{VI} solutions in the concentration range of 0·028 to 0·118 mg per g (approximately 2×10^{-4} to 8×10^{-4} molal) were prepared in 0·5 M hydrofluoric acid - 0·5 M sulphuric acid mixture and polarograms were recorded over the range of applied potential of 0 to -1 volt. The anode was a still mercury pool. The wave heights were measured in each instance from the residual-current line to the plateau of the first molybdenum wave at a potential 0·15 volt more negative than the half-wave potential. This procedure was adopted, since, unlike the saturated calomel electrode, the potential of the mercury pool anode was not constant, but, in our work, varied within the range of $+0{\cdot}27$ to $+0{\cdot}34$ volt *versus* the standard calomel electrode. In all instances the sensitivity setting on the polarograph was 0·02 μA per mm, and by using this conversion factor, wave heights were expressed as diffusion currents in microamps. Maximum current readings rather than average current readings were taken, as recommended in the instrument manual.

A plot of diffusion current *versus* molybdenum concentration produced a straight-line graph that passed through the origin, thus proving that the diffusion current is directly proportional to concentration, as expected. For all polarograms, the head of mercury was 44·5 cm and a 60-μ Teflon segment was used. Under these conditions, m (the flow-rate of mercury) was 1·563 mg per second and t (the drop time) was 4·80 seconds at an applied potential of −0·21 volt with respect to the standard calomel electrode.

The diffusion current constant, $I = i_d/(c\, m^{2/3} t^{1/6})$, calculated from the slope of the graph and the values of m and t, was 2·01, i_d being the maximum and not the average current. The concentration, c, is expressed in millimoles per 1000 g.

In order to assess the precision of a polarographic determination of molybdenum^{VI} in 0·5 M hydrofluoric acid - 0·5 M sulphuric acid mixture, diffusion currents were calculated for each solution from the slope of the graph and compared with the measured diffusion currents as shown in Table I.

TABLE I

STATISTICAL RESULTS FOR THE POLAROGRAPHIC DETERMINATION OF MOLYBDENUM

Solution number	Concentration of molybdenum trioxide, mg per g	i_d calculated, μA	i_d measured, μA	Error, μA
1	0·028	0·68	0·68	0·00
2	0·045	1·09	1·11	+0·02
3	0·065	1·58	1·56	−0·02
4	0·082	1·99	1·97	−0·02
5	0·100	2·43	2·43	0·00
6	0·118	2·88	2·91	+0·03

A mean error of almost zero verifies that the straight line through the origin is, in fact, the best straight line through the six points. The standard deviation from zero for the error is $0{\cdot}02_0$ μA. For an $8{\cdot}2 \times 10^{-4}$ molal solution of molybdenum (number 6), this amounts to a relative standard deviation of 0·7 per cent.

DETERMINATION OF MOLYBDENUM IN ALLOYS—

Dissolve a weighed portion of alloy containing about 5 mg of molybdenum in 4 ml of concentrated hydrofluoric acid and 4 ml of nitric acid, sp.gr. 1·42, in a platinum crucible. Add 1·39 ml of sulphuric acid, sp.gr. 1·84, from a burette and heat the solution to fumes of sulphur trioxide. Cool the crucible, add 1·11 ml of concentrated hydrofluoric acid dropwise from a polythene burette, cool the solution and transfer it to a previously weighed polythene bottle. Dilute the solution to 50 ml with water, and re-weigh the bottle. Calculate the concentration of alloy in the solution in milligrams per gram.

Place approximately 10 ml of solution in the polraographic cell and de-oxygenate by bubbling oxygen-free nitrogen through it for 15 minutes. Add sufficient mercury to cover the platinum wire and record a polarogram from 0 to −1·0 volt with a sensitivity setting on the instrument such that the full length of the current axis corresponds to 5 μA, *i.e.*, 0·02 μA per mm on the Sargent model XV polarograph.

Measure the diffusion current for the first molybdenum wave at an applied potential 0·15 volt more negative than the half-wave potential and read the concentration of molybdenum in the solution from a calibration graph prepared with standard solutions of molybdenum examined polarographically under the same conditions as used for the alloy. Hence calculate the concentration of molybdenum in the alloy. (For alloys, we used the calibration graph prepared to verify that i_d/c is a constant.)

RESULTS

The polarographic method was applied to the determination of molybdenum in three niobium-base alloys that also contained tungsten. The results are given in Table II and are compared with those obtained by a thiocyanate spectrophotometric method.[3,4]

The polarographic result for each sample was obtained from a diffusion current that was the average of two measurements taken from two polarograms of the same solution. The spectrophotometric result for each sample was calculated from an optical-density value that was the average of two optical-density readings for two different aliquots of the same sample solution.

These results are considered to be satisfactory, since the relative standard deviation of each method is about 1 per cent. The polarographic method is straightforward and rapid, and could also be used for determining molybdenum in zirconium- and tantalum-based alloys.

TABLE II

DETERMINATION OF MOLYBDENUM IN ALLOYS

Alloy	Approximate composition	Sample number	Molybdenum found— polarographically	Molybdenum found— spectrophotometrically
A	Niobium base, 15 per cent. tungsten, 5 per cent. molybdenum	1	4·90	4·95
		2	4·90	5·00
B	Niobium base, 15 per cent. tungsten, 5 per cent. molybdenum	1	5·10	5·10
		2	5·10	5·10
C	Niobium base, 15 per cent. tungsten, 5 per cent. molybdenum, 1 per cent. zirconium	1	4·65	4·65
		2	4·75	4·65

No interference is to be expected from elements whose half-wave potentials in acidic solution are more negative than −0·5 volt *versus* the standard calomel electrode, which category includes chromium, magnanese, cobalt and nickel. $Iron^{III}$, if present in more than trace amounts, interferes with the molybdenum determination, since, in 0·5 M hydrofluoric acid - 0·5 M sulphuric acid mixture, the plateau of an irreversible iron wave ($iron^{III} \rightarrow iron^{II}$) is not reached until the potential is −0·2 volt *versus* the standard calomel electrode.

We are indebted to Jessop-Saville for supplying alloys A, B and C. We also gratefully acknowledge the receipt of research grants from the British Iron and Steel Research Association and the Shell Chemical Co. Ltd. to maintain one of us (D.P.H.).

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A Glass Ring Oven of Simple Design

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A RING oven constructed in glass has been described.[1,2] The model described below has simple centring devices for the clamp that holds the paper, and for the pipette. These centring devices are rigidly attached to the body of the apparatus. Another innovation is the use of a pipette of the capillary-feed type.

DESCRIPTION OF THE APPARATUS

Fig. 1 (*a*) shows the complete apparatus. A vessel, V, of about 60-ml capacity, leads to the body of the ring oven, T, and thence to a condenser, C. A clamp holder, H, and a pipette slide, S, are fused on in the positions shown.

The ring-oven body, T, is a hollow toroidal vessel, whose top is ground smooth. The external diameter is 70 mm, the internal diameter is 22 mm and the depth is 20 mm. The central ring should be as sharply defined as possible. It is only this part of the apparatus that requires special equipment and high skill in its manufacture (V and C can each be replaced by commercially available items joined to T by standard ground-glass joints).

The clamp holder, H, is Y-shaped and constructed of 4-mm diameter rod terminated by small glass loops. The arms are of such a length that a piece of spring wire, W, holds the clamp firmly when it is slightly flexed (see Fig. 1*b*). It is fused on parallel to the body, T, and adjusted by playing a flame very carefully on the stem of H and sliding the clamp into position exactly over the hole in T.

The clamp, G (see Figs. 1*b* and 1*d*), is a piece of thick-walled glass tubing 20 mm long, of wall thickness 2 to 3 mm. The internal diameter of this tubing must be between 22 and 24 mm. The axial rod, R, whose diameter is 3 mm, is fused to a point on the edge, and the open ends of G and R are ground flat. (This can easily be done by hand by using a paste of carborundum and paraffin on a flat glass surface.)

The pipette tube, D, is a tube 80 mm long and 10 mm in diameter with a conical bottom, and carries a capillary pipette, P, along its axis. The pipette slide is constructed of 4-mm diameter glass rod (see Fig. 1*a*). It is fused on the body and so adjusted that, with D held in position by a rubber band, P runs exactly along the axis, meeting the centre of the end of R.

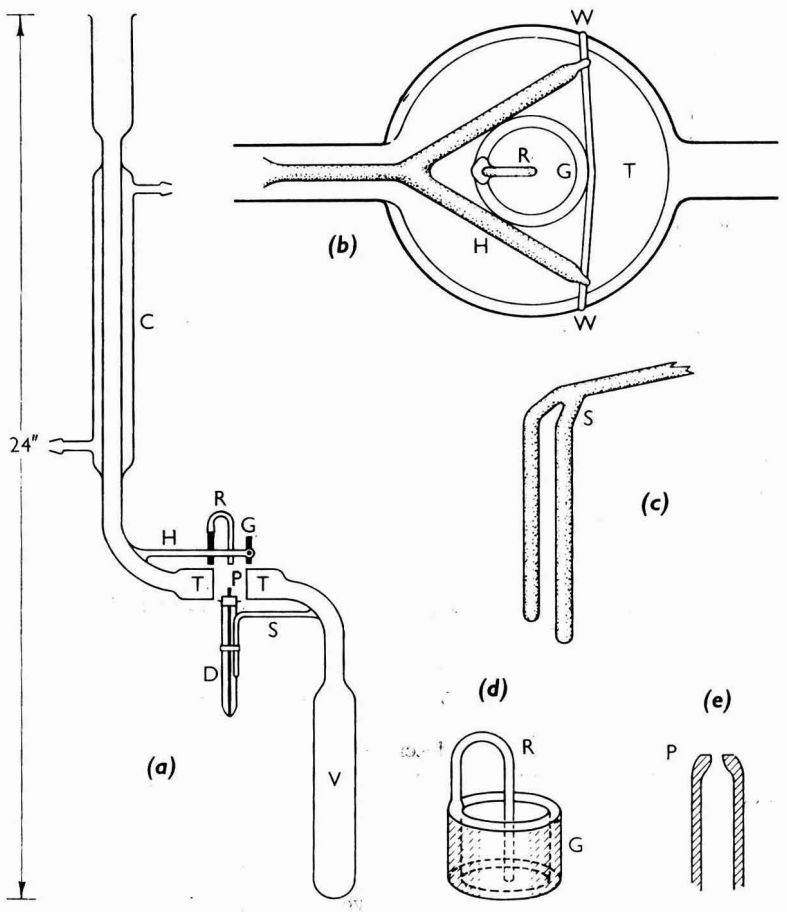

Fig. 1. Diagram of the apparatus: (*a*) assembled; (*b*) the centring device in position; (*c*) pipette slide; (*d*) clamp; (*e*) capillary pipette

The capillary pipette, P, is constructed from a melting-point tube that has been carefully narrowed down in a flame and then ground flat (see Fig. 1*a*). These pipettes can only be tested by trial on the paper to be used, and several can be made to suit different papers. They are readily interchangeable.

Mode of action of the pipette

The tube, D, is filled to such a level that capillary rise will bring the liquid to the tip of P. In operation, D is slid up to make contact with the paper and is kept in contact by R. (In the

absence of R, expansion of the paper on wetting causes problems.) The rate of flow of eluting solvent depends mainly on the nature of the paper and the diameter of the orifice of P. The level of the liquid in D has a small effect. It is sometimes advantageous to incorporate methanol into aqueous eluants. This evaporates before the liquid reaches the hot edge, and the rate of transfer of water to the hot edge is reduced.

SOLVENT—

A suitable solvent is chlorobenzene (b.p. 132° C) for use with aqueous eluants. Since glass is a poor conductor, it is necessary to use a solvent having a fairly high boiling-point.

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Photometric Determination of Titanium in Ores, Rocks and Minerals with Diantipyrylmethane

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THE most widely used reagent for the photometric determination of titanium is hydrogen peroxide. The reaction is, however, of very low sensitivity (molar extinction coefficient, $\epsilon = 740$), and is subject to interference from other metals, such as vanadium, that form similarly coloured compounds with hydrogen peroxide. Many organic reagents have also been used for this determination, notably chromotropic acid[1] ($\epsilon = 17{,}000$) and Tiron (disodium-1,2-dihydroxybenzene-3,5-disulphonate)[2] ($\epsilon = 15{,}000$). Although a little less sentitive than these two organic reagents, diantipyrylmethane ($\epsilon = 13{,}000$: Polyak[3] gives $\epsilon = 18{,}000$) is less subject to interference from other metallic ions and to variation in the acid strength of the solution. This reagent, introduced by Minin,[4] forms a yellow complex with titanium^{IV} in 1 to 4 N hydrochloric acid solution, with a

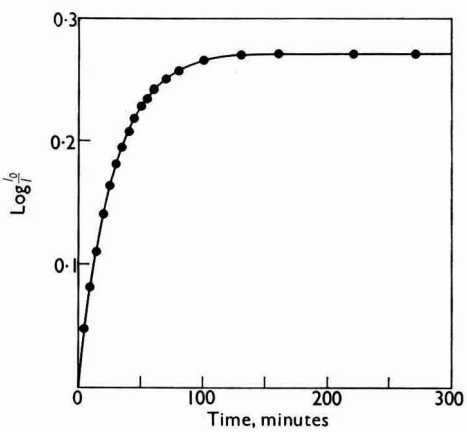

Fig. 1. Graph showing the effect of time on the colour development of a solution of 40μg of titanium dioxide in 100 ml of diantipyrylmethane solution. The measurements were made at 380 mμ in 4-cm cells.

maximum absorption at a wavelength of 380 mμ. The colour develops slowly; Polyak has recommended a period of 45 minutes for colour development, but under the conditions described below 3 hours are required (see Fig. 1). The coloured solutions are stable for several months.

Of the elements commonly present in silicate rocks and minerals, silica can be removed by evaporation with sulphuric and hydrofluoric acids. Perchloric acid should be avoided as this precipitates the reagent. Interference from iron^{III}, molybdenum^{VI} and vanadium^{V} is avoided by prior reduction to lower valency states. None of the remaining elements interferes.

Of the elements present in several other ores and minerals, only niobium, tantalum, tin and uranium have been observed to interfere. Interference by niobium, noted by Polyak, and by tantalum arises from partial precipitation of the titanium with the earth acid, and can be avoided by working in dilute tartaric acid solution. Tin^{II} precipitates the reagent from dilute acid solutions, giving rise to low titanium recoveries. Uranium interferes by forming a similarly coloured complex with the reagent.

Solutions of diantipyrylmethane deteriorate slowly, particularly when exposed to direct sunlight. This deterioration is evidenced by gradual yellowing, and can be considerably reduced by adding ascorbic acid to the solution and by storing it in the dark.

Method

Reagents—

Ascorbic acid solution, 10 per cent. w/v, aqueous.

Diantipyrylmethane solution—Dissolve 5 g of diantipyrylmethane and 5 g of ascorbic acid in 150 ml of 2 N sulphuric acid and dilute the mixture to 500 ml with water. The diantipyrylmethane was obtained from the Aldrich Chemical Co. Inc., U.S.A.

Tartaric acid solution, 10 per cent. w/v, aqueous.

Procedure for silicate materials—

Decompose an aliquot of the finely powdered silicate material by evaporating it with sulphuric and hydrofluoric acids in the usual way, removing excess sulphuric acid by heating on a hot plate. Fuse the dry residue with potassium pyrosulphate, extract the fused melt with dilute hydrochloric acid and dilute the solution to the mark in a calibrated flask. For most silicate samples it is convenient to dilute a 0·1-g sample to 100 ml.

Transfer an aliquot of this solution containing not more than 200 μg of titanium dioxide to a 100-ml calibrated flask, add sufficient hydrochloric acid to bring the final acid concentration to N, and add 5 ml of ascorbic acid solution. Mix the solution by gently swirling the flask, and allow it to stand for 30 minutes. Add 25 ml of diantipyrylmethane solution, dilute to the mark with water and allow to stand for 3 hours, or overnight. Measure the optical density of this solution at a wavelength of 380 mμ against a reference solution prepared in the same way, but without the diantipyrylmethane solution.

Table I

A comparison of results for the titanium content of R117 (granite, Shetland)

	Titanium dioxide, per cent., found by—				
	diantipyrylmethane method				
	A	B	C	hydrogen peroxide method*	Tiron method
	0·061	0·060	0·060	0·05 0·06	0·066*
	0·058	0·059	0·058	0·08 0·10	0·058
	0·059	—	0·062	0·06 0·06	0·059
	0·060	—	0·058	0·06 0·06	—
	0·059	—	0·059	0·05 0·07	—
	0·056	—	0·055	0·06	—
Mean	0·059	0·060	0·059	0·064	—

* Values reported in an interlaboratory comparison of silicate-rock analysis.

Prepare also a calibration curve from aliquots of a standard titanium^{IV} solution, treated in the same way.

Procedure for materials containing niobium or tantalum—

Dissolve a suitable weight of sample material by fusing it with potassium pyrosulphate in the usual way. Dissolve the melt in tartaric acid solution, warming if necessary, and dilute the solution to a suitable volume in a calibrated flask with tartaric acid solution.

Transfer an aliquot of this solution containing not more than 200 μg of titanium dioxide to a 100-ml calibrated flask, add sufficient tartaric acid solution to bring the total volume to 10 ml, 50 ml of 2 N hydrochloric acid and 5 ml of ascorbic acid solution, and allow the flask to stand for 30 minutes. Complete the determination as described above.

Results

This procedure has been used to determine the titanium content of a wide variety of silicate materials and of some other ores and minerals. The results obtained by three analysts for one silicate rock are given in Table I in which they are compared with results obtained by other methods.

A further comparison of results for several ore, rock and mineral samples is given in Table II. In no sample has any serious discrepancy been recorded.

Table II

A comparison of results for some silicate and other materials

Sample No.	Description and locality	Titanium dioxide, per cent., found by— diantipyrylmethane method	hydrogen peroxide method	other methods
(*a*) *Silicates and siliceous materials—*				
BCS 267	Silica brick	0·18	0·17	
BCS 269	Firebrick	1·47	1·48	
BCS 309	Sillimanite	2·08	2·08	
G–1	Granite, Westerly, Rhode Island, U.S.A.	0·26	—	0·26,* 0·24†
R 78	Granite, Kloof Nek, Cape Town, South Africa	0·26	0·25	
	Canadian syenite, rock–1	0·49	—	0·52‡
R 70	Nepheline-syenite, Kokipie, Kenya	1·25	1·26	
R 90	Phonolite, Bandama, Canary Islands	0·95	0·95	
R 77	Dolerite, Kloof Nek, Cape Town, South Africa	2·24	2·20	
R 74	Amphibolite, Entebbe, Uganda	0·90	0·89	
W–1	Diabase, Centerville, Virginia, U.S.A.	1·06	—	1·07,* 1·08†
R 84	Olivine-basalt, St. Helena	2·96	2·98	
S 3	Sandstone, north Yorkshire moors	0·22	0·20	
T–1	Tonalite, Msusule, Tanzania	0·59	—	0·59§
(*b*) *Other ores and minerals—*				
S 186	Pyrochlore soil, Mrima Hill, Kenya	2·88	2·73	
S 188	Columbite, Uganda	0·87	0·85	
S 189	Columbite-tantalite, Canada	1·70	1·65	
S 195	Pyrochlore, Oka, Canada	4·32	4·32	
	Pyrochlore, Søve, Norway	7·15	7·12	
M 41	Pyrochlore, Lokapoi, Karamoja, Uganda	4·24	4·25	
	Tantalite, Canada	0·89	0·88	
No. 57	Magnetite, Nangalwe, Uganda	4·07	4·08	
	Canadian sulphide ore–1	0·91	—	0·76‡

* Stevens and Niles[5] "arithmetic mean; all analyses."
† Filby and Leininger,[6] "recommended values."
‡ Average of reported results.
§ Average of reported results.[8]

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Determination of Small Amounts of Long-chain Alkylamines in Aqueous Solution

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The increasing use of long-chain alkyl tertiary amines and quaternary ammonium salts in the liquid - liquid extraction of inorganic species, as, for example, in the commercial extraction of uranium from its ores, has raised the problem of their determination at low concentration levels in aqueous raffinates and waste-effluent (barren) solutions.

A satisfactory method for use with amounts of tri-iso-octylamine ranging from 0·6 to 5 mg in up to 70 ml of solution involves a preliminary concentration of the amine as its sulphate by extraction into carbon tetrachloride and subsequently by its conversion to its tetrathiocyanato-cobalt^{II} complex by equilibration with an aqueous solution containing cobalt nitrate and ammonium thiocyanate. The optical density of the (separated) organic phase is thus measured in a 1-cm cell at 620 mμ.[1,2]

Ashbrook[1] claims a precision of $\pm$0·00012 per cent. by this method. This is clearly an error, for $\pm$0·00012 is actually the standard deviation for replicates of ten samples containing an average 0·0494 g per litre, *i.e.*, the precision is 0·23 per cent. Indeed, the reproducibility (*cf.* Table III in Ashbrook's paper) was not better than 10 per cent. on duplicate samples of 1·0 mg. Iron interferes by forming a red complex with the thiocyanate. Although this can be masked by adding "sufficient sodium hydrogen phosphate to inhibit the formation of ferric thiocyanate,"[1] the concentration of sulphuric acid, which has no adverse effect on the formation of the cobalt - thiocyanate complex, must now be limited owing to its action in breaking down the iron^{III} - phosphate complex.

This method should be applicable to other long-chain tertiary amines and to suitable quaternary salts, and in studies of the determination of the tricaprylmethylammonium ion, the molecular extinction coefficient of its cobalt^{II} - tetrathiocyanate complex was found to be $1{\cdot}7 \times 10^3$ in benzene.[3] It has been shown recently[4,5] that a strongly coloured liquid anion exchanger can be obtained by equilibrating a solution of tetra-n-hexylammonium iodide in 80 per cent. xylene - 20 per cent. hexone (isobutyl methyl ketone) mixture, by volume, with an aqueous solution of Erdmann's salt $(NH_4)^+[Co(NH_3)_2(NO_2)_4]^-$. Since various long-chain amines and quaternary ammonium ions can be quantitatively extracted into organic solvents in the form $(NR_1R_2R_3R_4)^+[Co(NH_3)_2(NO_2)_4]^-$, where R_4 = H or alkyl, and since these erdmannate solutions all possess a much higher molecular extinction coefficient ($\epsilon_{max.} \sim 1{\cdot}4 \times 10^4$) a method suggested itself that should be some ten times more sensitive than that now in use[1,2,3] and in which interference caused from iron should not arise.

Method

Reagents—

Ethylene dichloride—May and Baker Laboratory Reagent was redistilled.

Anhydrous sodium sulphate—Analytical-reagent grade.

Methanol—Analytical-reagent grade.

Solution of Erdmann's salt, approximately 0·01 M—Dissolve 0·3 g of the salt (prepared by Jørgensen's method[6] and recrystallised from water) in water and dilute the solution to 100 ml.

Tri-n-hexylamine—A specimen from Koch-Light Laboratories Ltd. was purified before use.

Preparation of calibration graph—

Prepare a standard solution in methanol of the tertiary amine containing 0·068 g per litre. Place volumes, x ml (up to 1·0 ml) in 20-ml stoppered glass test-tubes, add $(1 - x)$ ml of water, 4 ml of approximately M hydrochloric acid, and 5 ml of ethylene dichloride to each. Extract the ammonium salt by shaking the stoppered tubes for 3 minutes: vigorous shaking is to be avoided as it leads to emulsification. Allow the layers to separate and with a pipette remove and reject as much of the aqueous phase as possible without disturbing the organic layer. Add 5 ml of water to each tube and repeat the equilibration (15 seconds) to remove any excess of acid. Allow the layers to separate and again remove and reject as much of the aqueous phase as possible. Add 5 ml of approximately 0·01 M aqueous ammonium erdmannate (a factitious excess) and re-equilibrate the solution as previously. When the phases have separated, remove and reject the

excess of aqueous erdmannate solution, and wash the organic phase twice with 5 ml of water. Add approximately 0·5 g of anhydrous sodium sulphate to each tube and filter the organic phases through a dry filter-paper into 1·0-cm cells and measure the optical densities at 353 mμ against ethylene dichloride in the reference cell. The blank value is always found to be negligible.

If desired a calibration graph can be prepared for the range 0 to 0·34 mg by the same procedure starting with a solution of trihexylamine containing 0·34 g per litre by using cells of the appropriate path length, *viz.*, 0·2 and 0·5 cm. In either instance, the graph (not reproduced) of optical density against weight of trihexylamine taken is a straight line passing through the origin.

PROCEDURE—

Unknown samples of the appropriate weight ranging from 7 to 340 μg in 1 ml of solution are treated exactly as described in the method for the instance $x = 1$.

RESULTS AND DISCUSSION

Ethylene dichloride was selected in preference to the xylene - hexone mixture previously studied[4] since it is denser and the separation of phases is speedier. That this caused no substantial change in the molecular extinction coefficient of the erdmannate ion was shown by diluting 0·1 ml of tetra-n-hexylammonium erdmannate solution (approximately 0·01 M in 80 per cent. xylene - 20 per cent. hexone mixture, by volume) with 10 ml of (*a*) the same xylene - hexone mixture, and (*b*) ethylene dichloride. The optical densities at 353 mμ were 0·310 and 0·305, respectively (0·2-mm cells), when measured against the corresponding solvents as blank solutions.

Possible interference from iron^{III} was examined by carrying out the method modified only to the extent of replacing the 4 ml of M hydrochloric acid by x ml of an approximately 0·1 M solution of ferric chloride in M hydrochloric acid and $(4 - x)$ ml of M hydrochloric acid. Typical results are given in Table I.

TABLE I

RECOVERY OF TRI-ISOHEXYLAMINE IN PRESENCE OF IRON

Amine taken, mg	Iron added, mg	Molar ratio, iron - amine	Amine found, mg
0·054	0	—	0·054
0·054	12·5	1130	0·054
0·054	19·0	1710	0·054
0·054	25·0	2250	0·053
0·054	25·0*	2250	0·053

* Sodium fluoride, 0·1 g, added.

TABLE II

RECOVERY OF TRI-n-HEXYLAMINE FROM SYNTHETIC RAFFINATE SOLUTIONS

Sample	Tri-n-hexylamine, mg— taken	found
Amine alone	0·058	0·058, 0·057
Amine in raffinate*	0·058	0·058, 0·058, 0·059

* The procedure was started with a mixture of 1 ml of raffinate solution, 1 ml of tertiary amine (0·0585 g per litre) and 3 ml of approximately 1·3 M hydrochloric acid.

Ashbrook[1] found that 0·5 per cent. w/v of iron could be tolerated in aqueous solutions containing up to 5 per cent. v/v of sulphuric acid if sufficient sodium dihydrogen orthophosphate is present to prevent formation of ferric thiocyanate. The above results indicate that at least 2·5 per cent. w/v of iron can be present without any special precautions being taken. The slightly lower recoveries in the presence of the largest amounts of iron are unexplained. If it is due to oxidation of the amine, it is not prevented by masking the iron with fluoride. The reproducibility of the procedure was never poorer than ± 2 per cent.

By similar experiments, uranium^{VI} was found not to interfere, and considerable amounts of nitrate could be tolerated, although these are generally absent from typical barren solutions.

To confirm the reliability of the above procedure, the determination of tertiary amine in a synthetic raffinate solution[2] of composition—

Species	..	..	SiO_2	SO_4^{2-}	Cl^-	PO_4^{3-}	Cu^{2+}	Fe^{2+}	Fe^{3+}	Al^{3+}	Ni^{2+}	Mn^{2+}
Amount, g per litre	..	..	1·50	50	5	5·0	1·0	1·0	10·0	2·0	1·0	5·0

at pH 1·5 was made, and yielded the results given in Table II.

One of us (A.D.D.) thanks the Colombo Plan Authorities and the Atomic Energy Establishment, Trombay, India, for financial assistance.

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A Rapid Spectrophotometric Method for Determining Parts per Million of Morpholine in Boiler Water

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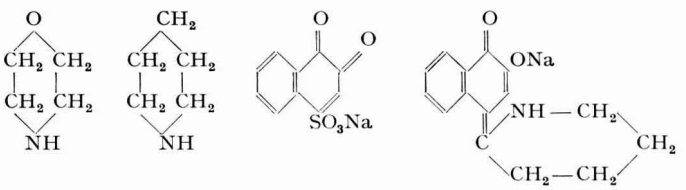
We have developed a spectrophotometric method for readily determining 1 p.p.m. of morpholine in water. It has a precision of about 0·25 p.p.m. in the 1 to 10 p.p.m. range and is simpler, faster and more sensitive than the usual benzoquinone method,[1,2] which was found to be unsatisfactory below 5 p.p.m. The new method has been applied to the determination of the efficiency of ion-exchange columns used for removing morpholine from reactor coolant water before infrared analysis for deuterium oxide, D_2O.

The structure of morpholine (**I**) is comparable to that of piperidine (**II**). According to Feigl,[3] piperidine couples with sodium 1,2-naphthaquinone-4-sulphonate (**III**) to give a scarlet *p*-quinoid condensation product (**IV**) with the elimination of sodium sulphite; the limit of detection for piperidine is 0·6 μg.[3] Morpholine was expected to behave similarly and, indeed, coupling of morpholine with (**III**) in alkaline solution gave a red solution with an absorption maximum at 480 mμ. Since a test for sodium sulphite gave a positive result, it was concluded that an analogous coupling reaction had occurred. Sodium 1,2-naphthaquinone-4-sulphonate gives a red colour on reacting with diethylamine, but no colour with dioxan. It is therefore strongly suspected that, in morpholine, coupling occurs through one of the methylene groups adjacent to the nitrogen atom.



I **II** **III** **IV**

Method

Reagents—

Sodium 1,2-naphthaquinone-4-sulphonate solution—Freshly prepare a 3 per cent. w/v solution of sodium 1,2-naphthaquinone-4-sulphonate in water.

Sodium hydroxide solution, N.

PROCEDURE—

Place a 50-ml sample of the water to be analysed in a 100-ml flask. Add 3 drops of N sodium hydroxide and 200 μl of 1,2-naphthaquinone-4-sulphonate solution. Swirl the flask to mix the solution and allow it to stand for 20 minutes. Measure the optical density at 480 mμ in a 1-cm cell against a reagent blank solution. Determine the morpholine concentration from a calibration curve.

DISCUSSION

The method was investigated for the 1 to 10 p.p.m. range of morpholine in water. Variation in the amount of quinoidal reagent added (from 100 to 400 μl for a 50-ml sample) had little effect on the optical density; a consistent amount within this range should be used, however. The reagent darkens on storage and should not be more than 1 day old, since old reagent will give low results. The coupling reaction requires a slightly basic solution, but the amount of base added is not critical. Colour development requires about 15 minutes, and the colour begins to fade slowly after 30 minutes. A Beckman DU spectrophotometer with a slit width of 0·24 mm was used, and the wavelength, 480 mμ, determined from a spectrum obtained by scanning a sample containing 20 p.p.m. of morpholine with a Beckman DK-2 spectrophotometer. A linear calibration curve was obtained with a slope of 0·057 (optical density *versus* concentration of morpholine, p.p.m.). Typical results for a calibration curve are given below—

Concentration of morpholine in distilled water, μg per ml	1	2	3	4	6	7	8	10
Optical density at 480 mμ in 1-cm cell	0·056	0·113	0·172	0·228	0·348	0·382	0·443	0·568

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Book Reviews

STATISTICAL METHOD IN BIOLOGICAL ASSAY. By D. J. FINNEY, M.A., Sc.D., F.R.S. Second Edition. Pp. xx + 668. London: Charles Griffin & Company Limited. **1964**. Price 105s.

Statistics first intruded into the workaday world of analysts, at least those concerned with the quantitative determination of composition, at the end of the 1930's, when the increasing commercial use of vitamins, hormones and antibiotics, in particular, whose production had not then reached the stage of synthesis, called for the use of living organisms as laboratory reagents. Biochemical individuality as a source of variation overshadowed the variations caused by manipulation, so that the analytical chemist's duplicates had to be replaced by "*n*-tuplicates," *n* generally being a multiple of about 10. This involved an assessment of the reliability of the result, for which the chemist invoked the statistician, since the development of dose-regression methods of assay took him into depths beyond those of straightforward standard deviation and standard error, with which Tocher's Institute of Chemistry lecture in 1931 had made him familiar. Although simple evaluation was made clear by such publications as B.S.I. Specification No. 911 in 1940 for vitamin D assay, the practical assayer found all kinds of problems arising that called for further assistance from the statistician, such as the comparative evaluation of different methods, the combination of a series of results or the significance of the failure of a regression line to pass through the origin. Though many publications had appeared dealing with some statistical aspect of biological assay, from Burns in 1930 to Emmens in 1948, it was not until 1952 that a really comprehensive treatise, written for the assayer rather than for the statistician, appeared. This was Dr., now Professor, Finney's "Statistical Method in Biological Assay." Probably it required some 20 years firstly to apprehend and secondly to solve the various problems that arose. The volume, with its wealth of worked examples taken from practice was invaluable to those laboratories in which biological assay became a regular practice, as, for instance, the industrial laboratories of food and drug

manufacturers. The appearance now of a second edition is evidence of its continued appreciation. Textual changes from the first edition are remarkably few; testimony to the thorough coverage of the original. The chief additions deal with the treatment of dilution assays and the combination of information from different types of assay on the same substance. Subjects dealt with in various aspects in one or more chapters include the design and treatment of parallel-line assays, slope-ratio assays, cross-over assays, incomplete block designs, multiple assays (*i.e.*, simultaneous assays of several samples), quantal assays, choice of metameters, combination of estimates and the particular instances in which time is the response. Though the book deals specially with biological assays, the exposition of principles and methods makes it useful also to the chemical analyst, into whose field there is a growing intrusion of statistical treatment. Those who bought the first edition were lucky or fore-sighted; the price has now risen from 68s. to 105s. J. I. M. JONES

SOLUBILITIES OF INORGANIC AND ORGANIC COMPOUNDS. Volume 2. TERNARY SYSTEMS. Part 1. Edited by PROF. H. STEPHEN, O.B.E., D.Sc., F.R.I.C., and DR. T. STEPHEN, M.Sc., Ph.D. Pp. ii + 944. Oxford, London, New York and Paris: Pergamon Press. 1964. Price £12 10s.

This is the third volume of the series, issued as "Volume 2. Ternary systems. Part 1," and is useful as a compilation of data that will be invaluable on library shelves. It is important to read the introduction that forms a guide to the arrangement of the data tables, since it is necessary for the use of the book. F. H. POLLARD

MICRO-ANALYTICAL ENTOMOLOGY FOR FOOD SANITATION CONTROL. By O'DEAN L. KURTZ and KENTON L. HARRIS. Pp. xvi + 576. Washington: Association of Official Agricultural Chemists. 1964. Price in U.S. $15.00; elsewhere $15.50.

Application of the work of the nineteenth-century microscopists, such as Hassall, led to the virtual stamping out of gross adulteration and falsification of foods. About the time of World War I, attention was turned to "filth," *i.e.*, (natural!) contamination by bacteria and moulds, insects, rodents, etc. Indeed, the first method to be developed was the Howard mould count, which is still a standard procedure. The microbiology of foods developed steadily and could soon be regarded as adequate.

Progress in dealing with larger "filth" was slow, but methods have gradually been evolved, mostly in the U.S.A., for the separation of the larger "filth" and for its measurement. Characterisation of such material has been usually limited to differentiation between mites, rodent and other hairs, and insect fragments. Until recently, little attempt has been made to identify the insects present. One reason for this has been lack of suitable documentation; descriptions of the morphology and structure of whole insects are available, but information on the characteristics of fragments has been sparse and often of restricted circulation. Recent U.S.A. food laws make it necessary that the contaminating insects be identified and it is this pressing need for information that this book satisfies; in other countries, the appearance of this work will be no less welcome.

The first part consists of a brief exposition of entomological principles and includes a clear, useful key to the major groups of common insects and a general description of insect structures. Part II deals with the morphology of the principal orders and covers the general morphology and structure of both the adult insect and the larva. Part III (pp. 119 to 466) deals with stored-product insects. The discussion is arranged according to the insects' entomological position, and the main species are described in terms of their morphology, then their food habits, life-cycle, history and, finally and perhaps most important, the diagnostic features of fragments of the insect. The remainder of the book (Parts IV to VII) deals with, on the same lines, but in less detail, flies and pests of agricultural crops, moth larvae of agricultural pests, cockroaches and miscellaneous insect contaminants.

The book is well produced, is lavishly illustrated with very clear photographs and photomicrographs, and has a good index. Although there is inevitably a large number of entomological terms, these are not unduly proliferated and a great many are clearly defined. The authors and the Association of Official Agricultural Chemists are to be congratulated on producing such a worthwhile book. J. F. HERRINGSHAW

ICUMSA METHODS OF SUGAR ANALYSIS. Edited by H. C. S. DE WHALLEY. Pp. xii + 153. Amsterdam, London and New York: Elsevier Publishing Company. 1964. Price 45s.

This book is a collection of official and tentative methods adopted by the International Commission for Uniform Methods of Sugar Analysis and will be especially welcomed by chemists, in that it brings the majority of the methods of sugar analysis into one volume.

The methods of analysis are in two parts, Part I dealing with general methods and Part II with special methods, and are fully set out and easily followed. Chapter 18, dealing with specifications and tolerances for pure sucrose and reagents, is on a subject of importance, and will be extremely useful in a textbook dealing with sugar analysis.

Praise must be given to the editor, the late H. C. S. de Whalley, and to the Committee for the preparation of this book, which is exceptional value at 45s. J. H. DEFRATES

ANALYTICAL METHODS FOR PESTICIDES, PLANT GROWTH REGULATORS, AND FOOD ADDITIVES. Volume IV. HERBICIDES. Edited by GUNTER ZWEIG. Pp. xvi + 269. New York and London: Academic Press. 1964. Price 86s.; (subscribers to the series) 80s.

This volume completes the series, "Analytical Methods for Pesticides," which touches relatively briefly on food additives also. Volume I dealt with general analytical methods (see *Analyst*, 1964, **89**, 230). The present volume is similar in arrangement to Volumes II and III, which dealt with insecticides, fungicides, etc. (*Ibid.*, 1965, **90**, 63), and deals, in 27 monographs written by a total of 26 authors, with herbicides.

It would be uncharitable not to welcome "unpublished" references, particularly prominent in some of the triazine monographs, where they now become published. Some areas of residue analysis have in the past suffered from this difficulty, now corrected at the expense (in some instances) of over half of the references in a monograph being "unpublished." But it is still not very helpful to state that methods for the formulation analysis of Dacthal have not yet been released by the manufacturer (p. 69); nor are formulation analyses given for dalapon or 2,4-D.

The series as a whole is a welcome consolidation of knowledge in a topical field of analysis. It is written by experts, largely American, and not unnaturally is biased towards North American practice, *e.g.*, to the exclusion from Volume III of the organo-mercurial fungicides. It constitutes a basic work of reference, though at a subscription price of over £20, it will be reserved for the largest laboratories. But bearing in mind that new pesticides are still arriving and new methods are still being developed, a supplementary volume is no doubt not far away. H. EGAN

AN INTRODUCTION TO CHEMICAL NOMENCLATURE. By R. S. CAHN, M.A., Dr.Phil.nat., F.R.I.C. Second Edition. Pp. x + 109. London: Butterworth & Co. (Publishers) Ltd. 1964. Price 13s. 6d.

In his introductory chapter, Dr. Cahn says ". . . chemists should, if they wish to be clearly understood, learn to describe accurately the compounds they are handling or talking about—and a definite act of learning is needed." The book is devoted to explaining the systems for naming inorganic and organic compounds as laid down by I.U.P.A.C. and included in the "Handbook for Chemical Society Authors."

Chapter 2 is concerned with Inorganic nomenclature, and the various types of inorganic compound are concisely and clearly considered. Several interesting points are raised, including the use of lithium tetrahydridoaluminate for lithium aluminium hydride (p. 21), a point also noticed by the reviewer of the First Edition (*Analyst*, 1960, **85**, 693).

The rest of the book, some two thirds, deals with Organic nomenclature, which, Dr. Cahn says, "is an infuriating subject." The author then proceeds to guide the reader expertly through the welter of rules, illustrating them with examples and explanations. He points out, too, where systematic nomenclature may prove unsatisfactory, *e.g.*, in naming diaryl ketones (p. 62).

Finally there are a few exercises to which the reader can apply his acquired knowledge; explanatory examples are given.

The book is well produced, and its lucidity is helped to no little extent by the use of subheadings and thorough cross-referencing. It is readable, despite its meaty content, and well worth the money. S. D. L. KEATING

ELECTRONIC SPECTRA AND QUANTUM CHEMISTRY. By C. SANDORFY. Pp. xiv + 385. Englewood Cliffs, New Jersey: Prentice-Hall Inc. 1964. Price 80s.; $13.35.

This book is a revised and enlarged English-language edition of "Les spectres électroniques en chimie théorique," by Professor Sandorfy, published in 1959. The work is intended to bridge the gap between the undergraduate textbook and the research paper or monograph on the application of quantum mechanics to the interpretation of molecular spectra. After introductory chapters on the wave-particle duality of radiation and matter, the topics dealt with are the molecular-orbital

and valence-bond treatments of π-electron systems, the factors governing the probabilities of electronic transitions, the classification of the electronic states of atoms and molecules, and the uses of symmetry theory in deriving selection rules and molecular orbitals. Thereafter, the main theoretical methods for calculating electronic-transition energies and probabilities are described in order of increasing elaboration, the methods being illustrated by application to two main groups of compounds, the polyenes and aromatic hydrocarbons.

For the graduate student of molecular electronic spectroscopy, the book is an excellent introduction to the theory of π-electron transitions, and it is to be regretted only that the author did not enlarge upon charge-transfer spectra, ligand-field transitions and other topics briefly reviewed in the final chapter. S. F. MASON

EDELMETALL-ANALYSE: PROBIERKUNDE UND NASSANALYTISCHE VERFAHREN. Herausgegeben vom CHEMIKERAUSSCHUSS DER GESELLSCHAFT DEUTSCHER METALLHÜTTEN- UND BERGLEUTE e.V. Pp. xii + 200. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1964. Price DM 39.

Like the three previous volumes of this series dealing with the analysis of metals, this latest publication has the support of the appropriate German chemical authority, with the overall objective of providing an up-to-date record of important industrial aspects of metallurgical analysis in Germany.

In the earlier volumes, particular attention is devoted to such aspects as separation procedures, control analyses, sampling, and metallurgical problems in general, whereas Edelmetall-Analyse deals exclusively with the analysis of the noble metals with no emphasis on sampling.

It is not surprising that the first part of the book, dealing with dry-assaying techniques, is covered in great detail. This section is extremely well written and leaves no room for adverse criticism, and anyone engaged in dry assaying should find it a useful condensation of all that is known on the subject.

In the subsequent analytical sections, emphasis is placed on procedures that are recognised by the panel of 15 experts to have wide application in the analysis of ores, alloys, salts and waste products from industrial processes, including the photographic industry. Details for applying the recommended procedures are clearly stated, and frequently follow a pattern in which the underlying reactions, as applied to relatively simple solutions, are described. After these descriptions are data under such headings as Time, Procedure, Interferences and References. In addition to these references, a list of over 100 references, in chronological order, is given at the end of the book, and these date back to the 16th century. However, this publication is by no means a history book, although some of the earlier references serve to indicate that the practice of dry assaying, in particular, has not changed significantly with time. Credit must be given for including, elsewhere in the book, at least one reference as recent as 1963, but most are much earlier.

On the more up-to-date analytical aspect of the book, comment is made on the use of X-ray fluorescence, and some examples, such as the determination of platinum in alumina-base materials, are given, but it is evident that X-ray fluorescence, as applied to noble-metal analysis in Germany, is still at an early stage.

Many laboratories are called upon to carry out incidental analytical work in the precious-metal field, and on such occasions this book should prove to be a useful source of information, but analysts already engaged exclusively in specialised aspects of the industry may be more critical of some of the recommended procedures. W. T. ELWELL

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Applications are invited for the pensionable post of Senior Scientific Officer in the Industrial Division of the Department of Industrial and Forensic Science, Ministry of Commerce, Belfast. Qualifications: Honours Degree in Chemistry, Dip. Tech. or other appropriate Professional qualification. Experience in analytical methods and techniques is essential, preferably in an industrial organisation or research establishment. Salary scale £1,685—£2,082 entry point according to experience. Preference for ex-Service applicants.

Application forms obtainable from the Secretary, Civil Service Commission, Stormont, Belfast, 4, must be returned by 2 April, 1965.

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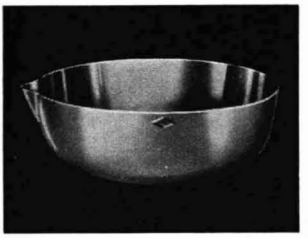

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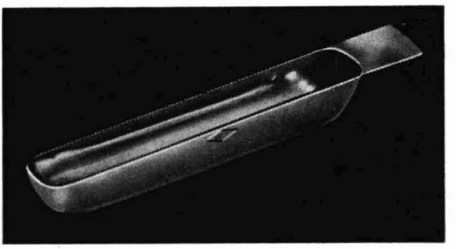

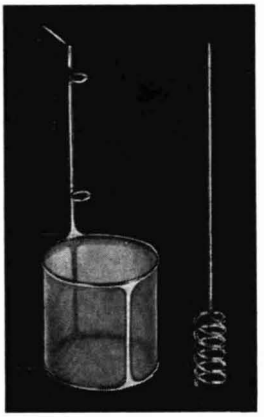

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