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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

DEATH

WE regret to record the death of
Sir Jack Cecil Drummond.

The Determination of Small Quantities of Alginates in Rayon Finishes and on Yarn

BY E. G. BROWN AND T. J. HAYES

(*Presented at the meeting of the Society on Wednesday, May 7th, 1952*)

A method is presented for the absorptiometric determination at small concentrations of ammonium di-ethanolamine alginate or ammonium tri-ethanolamine alginate in rayon-finishing liquids and on rayon yarn. The method is also applicable to sodium alginate solutions. It is based on the hydrolysis of alginic acid to furfural and subsequent reaction with Bial's reagent to give a greenish-blue colour, the absorption of which is measured on a suitable instrument, such as the Spekker absorptiometer. Alginic acid at concentrations of up to 0·8 g per litre can be measured with satisfactory precision. A method for the extraction of alginates from rayon yarn is also described.

IN the viscose rayon industry a solution of ammonium di-ethanolamine, or tri-ethanolamine, alginate is used in conjunction with a suitable finishing liquid to impart the requisite degree of stiffness to rayon cakes. The finishing agent is usually some type of lubricating oil, of paraffin origin, emulsified with a suitable agent, often a proprietary mixture of highly sulphated oils. The purpose of the paraffin is to provide lubrication for the yarn and simultaneous stiffening is given by the alginate compound. Ammonium di- or tri-ethanolamine alginate is made by the dissolution of commercial alginic acid in stoicheiometric proportions of di- or tri-ethanolamine and concentrated ammonium hydroxide solution, two molecules of alginic acid combining with one molecule each of ammonia and di- or tertiary base. The problems arose of (*i*) rapidly determining ammonium di- or tri-ethanolamine alginate in finishing liquors for process control purposes where the finishing was part of a continuous process and (*ii*) determining alginate on rayon yarn after drying and conditioning. It was considered that a report of the alginic acid concentration would be adequate and, consequently, a method was developed by one of us (E.G.B.) involving precipitation of the acid by hydrochloric acid, filtration and alcohol washing of the precipitate, and finally dissolution of the acid in calcium acetate solution and titration of the liberated acetic acid.[1] This method, although accurate, was tedious and unsuitable for process control work; it took about 3 hours to perform and required constant attention during filtration. It was inapplicable to alginate on yarn because of the small quantities involved, so a search was made for a more speedy method and, preferably, an absorptiometric one.

The literature on the detection and determination of alginic acid and alginates can be summarised under three main headings. First, methods depending on the hydrolysis of alginic acid by hydrochloric acid under controlled conditions to give carbon dioxide, which is suitably measured gravimetrically or volumetrically.[2,3,4] Secondly, application of the method of Lüdtke[5] and Yackel and Kenyon,[6] entailing the reaction of isolated alginic acid with calcium acetate solution to liberate acetic acid, which is suitably titrated.[1,7,8,9] A modification of this procedure utilises the reaction of alginic acid with an excess of sodium hydroxide solution and titration of the excess with standard acid.[1] Lastly, the colorimetric method of Ross and Percival,[10] requiring carbazole and concentrated sulphuric acid, which is based on the preliminary work of Egami[11] and Dische.[12]

PRELIMINARY INVESTIGATIONS—

It was originally considered that the carbazole method would be the most suitable for the purpose in hand, in spite of the fact that an examination of the calibration data of Ross and Percival shows that the points are only approximately linear. It was found, however, that there was an interference effect in the presence of the finishing liquid and colours either did not develop or had a marked brown tinge. A further disadvantage was that the method required intimate mixing by stirring and cooling of the solution in ice, which makes it not readily usable by unskilled operators.

Mitchell, Shaw and Frary[13] have detected sodium alginate in ice-cream by isolating silver alginate at a pH value of 4·6 by adding silver nitrate and subsequently hydrolysing the precipitate with hydrochloric acid to give furfural, which is qualitatively detected both by aniline and Bial's reagent. This method presented itself as a possible alternative for quantitative use.

Bial's reagent,[14,15] or its various modifications, has been extensively used since the original work on the subject, largely for pentoses, uronic acids, nucleosides and nucleotides. The reagent is composed of a solution of orcinol, hydrochloric acid and ferric alum or ferric chloride, the iron acting as a catalyst. When pentoses are heated with Bial's reagent in a bath of boiling water they yield furfural by hydrolysis. The same reaction probably occurs with heptoses. The furfural then gives a stable bluish-green colour, by complex formation with the orcinol, that can be shaken into amyl alcohol and suitably measured. For example, Scheff[16,17] used the method for a spectrophotometric determination of pentoses and glucuronic acid, whilst Fleury and Poirot[18] utilised the reaction for the determination of furfural. Recently Nordal and Klevstrand[19] adapted a modified reagent to the estimation of sedoheptulose. Many modifications of the composition of the reagent have been proposed, including the use of copper[20,21] instead of iron for catalysis, but a critical evaluation of these reagents has been lacking until recently, when Miller, Golder and Miller,[22] on the basis of a systematic investigation, recommended the reagent proposed by Drury[23] as the most favourable for the determination of pentoses.

EXPERIMENTAL

PREPARATION OF STANDARD SOLUTION—

It was first necessary to prepare a standard alginate solution. It is inconvenient to prepare a standard ammonium di- or tri-ethanolamine alginate solution for several reasons. Commercial di-ethanolamine and tri-ethanolamine are variable mixtures of the mono-, di- and tri- compounds, and it would be necessary to calculate the equivalent weight of the mixture and relate it to commercial alginic acid in this proportion. Secondly, it is difficult to add the calculated amount of concentrated ammonium hydroxide, sp. gr. 0·880, without loss; an excess would almost certainly be required. These effects are minimised under factory conditions by the large volumes used, but they might be undesirable for analytical purposes. Accordingly, it was decided to make up a standard solution of sodium alginate and to test the effect of ammonia and di- and tri-ethanolamine later in the method. Commercial alginic acid (70 to 80 per cent.) was analysed for purity by dissolution in standard sodium hydroxide solution and titration of the excess of alkali. A known amount of alginic acid was then dissolved in the calculated amount of standard sodium hydroxide solution to give a standard solution of sodium alginate. The commercial acid was not purified because it was felt that as any impurities in it would be present in the finishing liquor, these should be retained in the standard solution to compensate for any effect they might have

on colour development. A solution of finish was also made up, of the nominal strength of a factory solution, with the addition of a small amount of sodium bisulphite liquor to keep the pH range between 7 and 8. Suitable aliquots of sodium alginate solution were then placed in a series of standard flasks by means of a pipette and made up to volume with the finishing liquor. This procedure was necessary to simulate factory conditions and to see if the emulsified liquor had any effect on the colour development.

COMPOSITION OF BIAL'S REAGENT—

The composition of the reagent recommended by Nordal[19] was used for a few preliminary experiments but was relinquished subsequently in favour of the reagent given by Drury[23] and quoted by Miller *et al.*,[22] with the exception that alcohol was not used for the solution of the orcinol. The reagent was made up in 100-ml portions and stored in dark bottles in a refrigerator, where it apparently remained stable over several months. If kept in the light at room temperature, some reduction from $iron^{III}$ to $iron^{II}$ may occur. The reagent gives a small constant blank when the reaction is carried out; a blank determination should be made on each fresh bottle of reagent and preferably several times on the same bottle. The blank reading for 4 ml of reagent is in the range 0·05 to 0·08 of a drum reading, as measured on a Spekker absorptiometer.

PERIOD OF BOILING—

The boiling-time for the reaction of Bial's reagent with furfural is critical[16,17]; whilst some investigators[16] recommend keeping the test tube containing the reactants stoppered during the reaction, Nordal and Klevstrand[19] have shown that it is unnecessary under their conditions to use a stopper if the boiling period does not exceed 20 minutes and we have confirmed this; a 15-minute boiling period without stoppering gives reproducible results for alginic acid.

USE OF AMYL ALCOHOL AS SOLVENT—

The addition of 8 ml of amyl alcohol to the mixture was standardised, this volume being suitable with the 1-cm cell of a Spekker absorptiometer. Scheff[16] recommended swirling the mixture and not shaking, and used a correction factor to take into account the increase in volume of the amyl alcohol by solution of some of the acid layer. Nordal and Klevstrand[19] have shown that the increase in volume, and hence the correction factor, can be avoided by adding more water and shaking vigorously; a similar procedure was used in our investigations.

MEASUREMENT AND STABILITY OF COLOUR—

Preliminary tests showed that with a Spekker absorptiometer (Model H 560), Ilford spectrum red filters No. 608 gave the maximum drum reading in conjunction with the smallest blank reading. Previous investigations[19] have shown that there is a maximum absorption peak for the system at about 620 mμ. By the method detailed below, the analysis, as far as taking the extinction reading, can be comfortably completed in 45 minutes; accordingly this was standardised as the reaction time. There was some decrease in drum reading with time; decomposition was at a greater rate than that given by Nordal. There is a fairly rapid drop over the first 2 hours, then a more steady decrease in drum reading (see Table I).

TABLE I

EFFECT OF TIME ON THE DRUM READING

Time, hours	0	0·5	1	1·5	2	4	4·5	5	6	24
Drum reading	0·805	0·798	0·795	0·788	0·786	0·785	0·782	0·782	0·781	0·758

NOTES—1. The solution was kept in the dark in a 1-cm glass cell covered with a microscope slide. The reading is from both the alginic acid and the blank, and is equivalent to 120 μg of alginic acid.
2. The initial reading, 0 hours, is 45 minutes after the start of the analysis.

The colour showed little change of drum reading with change in temperature, a decrease of about 0·0013 of a drum reading per ° C being obtained between the limits 13° and 24° C. Hence the effect of temperature change within normal working limits is negligible.

METHOD OF PREPARING THE CALIBRATION CURVE

The calibration curve prepared from a standard sodium alginate solution is shown in Fig. 1.

APPARATUS—

Use a 1-litre beaker and clamp the Pyrex 2·5 × 15-cm boiling-tube that contains the reactants 2·5 cm from the bottom. Let the initial height of boiling water be 11 cm or alternatively, use the constant-level water-bath of Miller.[24]

REAGENTS—

All reagents should be of recognised analytical purity.

Amyl alcohol.

Bial's reagent—Transfer 0·714 g of orcinol (3:5-dihydroxytoluene) and 0·060 g of crushed ferric ammonium sulphate to a 250-ml beaker and dissolve it in about 75 ml of

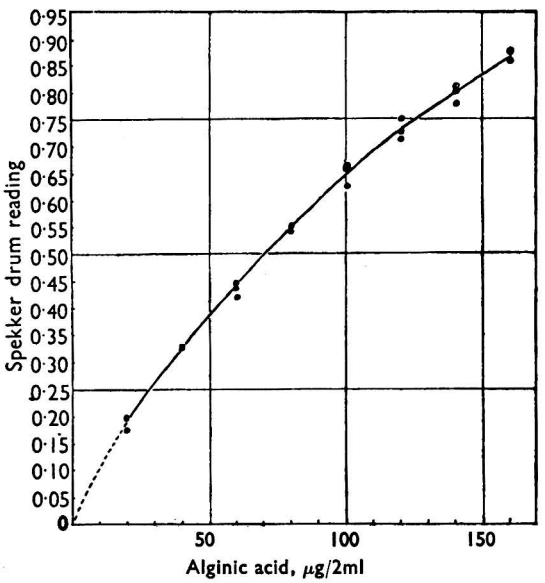

Fig. 1. Calibration curve for alginic acid determination

concentrated hydrochloric acid, sp. gr. 1·18. Transfer it to a 100-ml calibrated flask and make up to the mark with hydrochloric acid, sp.gr. 1·18. Store in a dark tightly-stoppered bottle in a refrigerator.

Sodium alginate solution—Transfer about 0·5 g of commercial alginic acid, accurately weighed, to a 250-ml iodine flask and add 40 ml of 0·1 *N* sodium hydroxide solution. Swirl the flask, stopper it and set it aside for 4 hours. Titrate the excess of alkali with 0·1 *N* hydrochloric acid, using phenolphthalein as indicator. Calculate the percentage of alginic acid in the sample; 1 ml of 0·1 *N* sodium hydroxide solution is equivalent to 0·0176 g of alginic acid ($C_6H_8O_6$).[25]

Weigh out the appropriate amount of crude material so that 1 ml of the final solution is equivalent to 10 mg of alginic acid; dissolve it in the calculated volume of 0·1 *N* sodium hydroxide solution and make up to the requisite volume in a calibrated flask with distilled water. Dilute a suitable volume of this solution ten-fold with distilled water.

1 ml of solution = 1 mg of alginic acid.

Transfer volumes of standard sodium alginate solution, covering the range 1 mg to 8 mg, to 100-ml flasks. Dilute each flask to the mark with a solution containing about 0·4 g per litre of finishing liquid.

PROCEDURE—

Transfer 2 ml of the dilute alginate solution to a boiling-tube and then add from a burette exactly 4 ml of Bial's reagent. Place the tube in a 1-litre beaker containing briskly boiling water maintained at the boil for exactly 15 minutes—timed by a stopwatch. The boiling should be vigorous and even. Remove the tube and cool for exactly 2 minutes in a stream of cold water. Add 8 ml of amyl alcohol from a burette, swirl the resultant mixture and pour into a 100-ml separating funnel. Wash the tube with two separate 5-ml portions of distilled water, using a pipette. Add these washings to the separating funnel and shake vigorously for 10 seconds. Allow the two layers to separate, then run off the lower to waste. Dry the inside of the stem of the funnel with a roll of filter-paper and run off the coloured layer into a clean dry 1-cm Spekker cell, filtering through a small funnel containing a No. 41 Whatman filter-paper to remove any water present. Stir the solution contained in the cell with a fine glass rod. Exactly 45 minutes after the start of the determination read the absorption of this solution on a Spekker absorptiometer, using amyl alcohol or water in the 1-cm reference cell, No. 608 Ilford spectrum red filters and Calorex No. H 503 heat-resisting filters. A tungsten-filament lamp can be used as light source, and the direct method of measurement[26] applied.

Carry out a blank determination on each fresh batch of Bial's reagent, or preferably several times on the same batch, by using 2 ml of finishing solution and 4 ml of Bial's reagent and taking it through the same procedure. Subtract the blank reading from the observed reading and plot the drum reading against concentration to give the calibration curve.

EFFECT OF DI-ETHANOLAMINE, TRI-ETHANOLAMINE AND AMMONIA ON THE PROPOSED PROCEDURE—

The validity of the calibration graph prepared from sodium alginate for use with a liquid containing ammonium di- or tri-ethanolamine alginate was tested. For this purpose a factory solution containing di-ethanolamine alginate was analysed for alginic acid content

TABLE II

EFFECT OF DI-ETHANOLAMINE, TRI-ETHANOLAMINE AND AMMONIA ON THE PROPOSED METHOD

	Solution series	Alginic acid: Added, μg per 2 ml	Alginic acid: Found, μg per 2 ml
1 (*a*)	Ammonium di-ethanolamine alginate	—	90, 91, 93 (Mean, 92)
1 (*b*)	1 ml of 1 (*a*) + 1 ml of standard sodium alginate solution containing 20 μg of alginic acid ..	66	65
2 (*a*)	Ammonium di-ethanolamine alginate	—	91, 93, 93 (Mean, 92)
2 (*b*)	1 ml of 2 (*a*) + 1 ml of standard sodium alginate solution containing 60 μg of alginic acid ..	106	107
3 (*a*)	Ammonium di-ethanolamine alginate	—	91, 93, 93 (Mean, 92)
3 (*b*)	1 ml of 3 (*a*) + 1 ml of standard sodium alginate solution containing 40 μg of alginic acid ..	86	83
4 (*a*)	Ammonium tri-ethanolamine alginate	—	85, 85 (Mean, 85)
4 (*b*)	1 ml of 4 (*a*) + 1 ml of standard sodium alginate solution containing 40 μg of alginic acid ..	83	81

by the proposed method; an initial ten-fold dilution was necessary. Satisfactory precision was attained (see Table II). One millilitre of this solution was then added to 1 ml of standard sodium alginate solution and the resultant mixture was analysed by the proposed method. Satisfactory recovery of sodium alginate related to alginic acid was obtained (see Table II). A solution of ammonium tri-ethanolamine alginate was also made up in the laboratory and treated similarly, with satisfactory results (see Table II). The results show that the presence of tri-ethanolamine hydrochloride, di-ethanolamine hydrochloride and ammonium chloride (formed by the action of concentrated hydrochloric acid on the free bases, which are themselves liberated by hydrolysis) had no effect on the method, and a calibration curve prepared from sodium alginate solution is valid for solutions containing ammonium di-ethanolamine alginate or ammonium tri-ethanolamine alginate.

Effect of different concentrations of finish on the proposed method

In factory conditions, the concentration of finish in a finishing liquid is liable to alter within fairly wide limits, and this work was carried out to see whether or not different concentrations would affect the proposed method. During the heating with hydrochloric acid, the white emulsion initially present became coagulated to some extent and some hydrolysis of the sulphated oils present probably occurred. On extraction with amyl alcohol, however, all turbidity disappeared and two clear layers were formed. Three blank determinations were made (*a*) on pure water, (*b*) on a dilute finish solution containing about 0·4 g per litre and (*c*) on a strong finish solution containing about 4·0 g per litre. The results, in Table III, show no significant difference and prove that large variations in the total oily matter content of a finishing solution do not affect the method.

Table III

Effect of different concentrations of finish on the proposed method

Solution used	Drum reading
Water	0·045
An approximately 0·4 g per litre finish	0·048
An approximately 4·0 g per litre finish	0·053

Method for the determination of ammonium di- or tri-ethanolamine alginate in finish solution

The apparatus and reagents used are the same as those used in the calibration procedure (p. 448).

Procedure—

For concentrations of the order of 0·0 to 0·4 g per litre of alginic acid, dilute the finish solution ten-fold with distilled water. Take 2 ml of this solution and analyse according to the method detailed above.

Calculate the result in terms of grams per litre of alginic acid. The factor for conversion of alginic acid to ammonium di-ethanolamine alginate ($C_5H_7O_4COONH_4 + C_5H_7O_4COOH.NH(CH_2CH_2OH)_2$) is 1·310 and for alginic acid to ammonium tri-ethanolamine alginate ($C_5H_7O_4COONH_4 + C_5H_7O_4COOH.N(CH_2CH_2OH)_3$) is 1·472.

Results and discussion—

The calibration curve is drawn to the best fit as calculated by the method of least squares. On the basis of this calculation the line does not pass through the origin; this portion of the original curve is shown by a dotted line in Fig. 1. The extinction does not obey Beer's law, but this is usual in this type of reaction owing to furfural not being liberated quantitatively, so the reaction conditions must be rigidly standardised. Even under strict control of experimental procedure, there is still some scattering of points for a given concentration of alginic acid; this has been experienced in similar reactions, *e.g.*, by Nordal and Klevstrand.[19] It is considered, however, that the precision of the method on a normal solution of alginic acid of about 0·4 g of litre is about $\pm$ 0·01 g per litre, as the scatter of points for a given concentration does not exceed this value. Table IV shows the precision of typical results as obtained on factory finishing liquors.

Table IV

Precision of results for determination of alginic acid content of finish solutions

Sample	Alginic acid, g per litre		
A	0·45,	0·46,	0·47
B	0·46,	0·47,	0·47
C	0·43,	0·43	

The whole analysis can be carried out in 45 minutes, which is a suitable period for process control work and much quicker than the original gravimetric - volumetric method.

Following the recommendations of Ayres[27] and Ringbom,[28] the percentage absorption

(calculated on the assumption that drum reading on the Spekker is equivalent to extinction) is plotted against the logarithm of concentration as abscissae (see Fig. 2). Ayres[27] states that the conclusions drawn in his article can be applied in a general way to any instrument. From a study of Fig. 2, it is seen that the most accurate range of the determination is

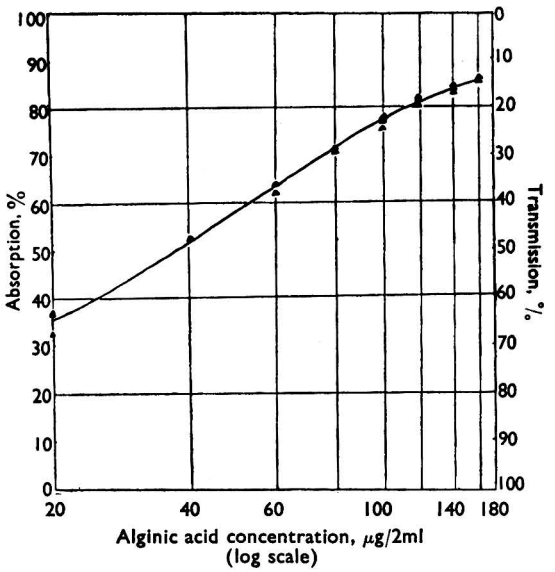

Fig. 2. Range and accuracy

between 40 and 100 μg per 2 ml, corresponding to 0·2 to 0·5 g per litre of alginic acid. Ringbom[28] has shown that the accuracy of a given procedure for this type of plot is greatest where the curve has its steepest slope. The curve is nearly linear within the limits 40 to 100 μg. A relative analysis error of 3·7 per cent. per 1 per cent. absolute photometric error is established by dividing 230 by the slope of the curve over the range 40 to 100 μg of alginic acid.

In the course of the development of the method, it became evident that with Bial's reagent for the visual detection of alginic acid, the lower limit of identification that allows distinction to be made from the reagent colour was about 10 μg per 2 ml. This corresponds to a concentration limit of 1 in 200,000.

Application of the Method to the Determination of the Alginate Concentration on Rayon Yarn

Experimental Work—

The exact state of combination of alginic acid on rayon yarn after it has been dried and conditioned is somewhat doubtful; it was considered, however, that it would be possible to extract the material with boiling water. It would be a difficult matter to prepare a sample of rayon yarn with a known weight of alginate thereon, because of difficulties of drying and decomposition, and so samples with unknown concentrations were used. Preliminary experiments with hot-water treatment showed that alginate was extracted but incompletely in a beaker and by decanting off successive extractions, but with a Soxhlet apparatus, extraction of about 1 g of yarn was complete in 2 to 3 hours, as shown by a second 1-hour extraction and subsequent testing with Bial's reagent. A smaller sample can be taken in semi-micro work. During the treatment with boiling water, some finishing material was also extracted, but this was not detrimental to the determination. Extraction of yarn that had had no finishing or alginate treatment and subsequent treatment of an aliquot of the extract with Bial's reagent showed no matter to be extracted that would interfere in the method.

APPARATUS—

A semi-micro Soxhlet apparatus of capacity 20 ml with a 30-ml flask is required. Otherwise the apparatus is as detailed above.

REAGENTS—

As detailed above (p. 448).

PROCEDURE—

Weigh out accurately 0·1 to 0·15 g of rayon yarn and transfer it to the extraction chamber of the Soxhlet apparatus, using a suitable thimble to retain the yarn. Extract with about 20 ml of distilled water for 2 to 3 hours. Cool the flask and make up to volume in a 25-ml calibrated flask. Take 2 ml of the solution and proceed as in the proposed method. Simultaneously with the initial weighing, take a suitable sample of yarn for moisture determination; determine the moisture by drying to constant weight at 100° to 105° C. Calculate the result in terms of percentage of alginic acid on dry cellulose.

RESULTS AND DISCUSSION—

The precision attained by taking aliquots of a given extract is the same as that on a finishing solution. Some results obtained on different sections of a viscose rayon cake are shown in Table V.

TABLE V

DISTRIBUTION OF ALGINATE ON VISCOSE RAYON CAKE

Sample	Alginic acid, %	
1	0·67,	0·57
2	0·72,	0·79

Hence it is evident that the alginate is distributed unevenly over a given rayon cake, but no study has been made as to the possible relationship between concentration and distribution. Whilst it is impossible to verify that extraction is quantitative by using a known amount of alginate, the negative test given by Bial's reagent on a second extraction after initial boiling for 2 to 3 hours with water makes it extremely probable that all alginate is extracted.

Thanks are due to Mr. P. Morley for help with the preparation of the calibration curve by the method of least squares, and to Mr. E. Stone, Research and Development Department Manager, British Enka Limited, and to the Directors of British Enka Limited for permission to publish this paper.

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DISCUSSION

DR. J. HASLAM congratulated the authors on the excellent way in which they had presented their paper. He drew attention to the fact that the determination of alginates was becoming increasingly important in many industries and for that reason he asked what the authors' experience of interfering substances had been, *viz.*, with spin finishes in day-to-day work. He understood that the constituents of the spin finish used by the authors did not interfere in the test, and he would be pleased if the authors could give more information about the general chemical constitution of such finishes.

MR. BROWN replied that the finish used in the proposed method consisted of commercial liquid paraffin as a lubricating agent emulsified with a proprietary mixture of highly sulphated oils. As the emulsifying agent was a proprietary brand he was not himself sure as to its exact chemical composition, but he understood there were four sulphated oils present. It would be necessary to test each individual finish to see if there were any interference with the method as the authors had not tested the effect of other spin finishes.

DR. J. H. HAMENCE said that the determination of alginates present in small quantities in foodstuffs was always a difficult operation, but he imagined that the method described by the author would not be applicable to foodstuffs containing any carbohydrate that was likely to give rise to the liberation of furfural on treatment with acid.

In spite of this difficulty, the method obviously would be of great assistance to food chemists in the determination of alginates in those instances where it was possible to separate the other carbohydrate materials.

MR. BROWN agreed. He pointed out that a possible method of removing interfering substances might be by isolating alginic acid with acid or as silver alginate with silver nitrate, according to the method of Mitchell, Shaw and Frary[13] for ice-cream. Pectic acid and uronic acids would also interfere with the determination.

DR. H. AMPHLETT WILLIAMS asked if cellulose esters interfered with the determination.

MR. BROWN said he had no specific information on this point, but did not think that cellulose esters would interfere. Cellulose itself did not interfere in the method.

MR. N. L. ALLPORT asked how the authors ensured that the alginic acid used in the preparation of standards was 100 per cent. pure.

MR. BROWN pointed out that before preparation of the standards the crude sample of alginic acid was analysed for purity and the necessary weight of crude material then taken to give the equivalent of 100 per cent. of alginic acid.

Turbidity in Photometry*

Correction for Turbidity in Photometric Methods

By JÖRGEN FOG†

The interfering effects of turbidity in photometric studies are overcome by a technique suitable for use in various photometric determinations.

The spectral absorption curves of various polydispersed sols and suspensions show that extinctions are closely related logarithmically to the wavelengths. Extinctions read at two different wavelengths will then suffice to allow the extinction at a third wavelength to be determined.

If the absorption curve of a clear coloured solution, which shows two troughs (E_1 and E_3) and one peak (E_2) at the wavelengths λ_1, λ_3 and λ_2, respectively, is plotted logarithmically, and a straight line through E_1 and E_3 crosses λ_2 at ΔE_2, then the difference ($E_2 - \Delta E_2$) is linearly related to concentration, provided that Beer's law is valid. When a turbid solution is added, the difference is still closely related to concentration. The error is either negligible or calculable if (*i*) the difference approaches E_2, (*ii*) $E_3 > E_1$ when $\lambda_1 > \lambda_3$, and (*iii*) E_1 and E_3 are chosen at the minima of the absorption curves with foreign coloured substances.

Spectral absorption curves and diagrams demonstrate how the effect of turbidity is eliminated when an artificial turbidity is produced in coloured solutions, even if the degree of turbidity and the amount of the coloured substances are varied within wide limits.

The equation fits well for protein precipitates and dispersed fat, which are the most common causes of turbidity in biological samples.

The accuracy attained in photometry with turbid samples is strictly limited, especially at the shorter wavelengths, and even if the turbidity is almost invisible to the eye.

Morton and Stubbs[1] propose a device for correcting absorption spectra of vitamin A in fish-liver oils, and Lowry and Hastings[2] propose one for determining the quantity of blood retained in animal organs. They assume that within a rather narrow wavelength band the extinction (optical density) is linearly related to the wavelength. This assumption limits the general applicability of their methods, however, because with turbid solutions extinctions and wavelengths are related logarithmically. Zerban, Sattler and Lorge[3] in extensive studies on turbid systems containing coloured matter describe a photometric method for characterising both the absorbing and scattering properties of sugar solutions, but their method is confined to that one particular system only. Snell and Snell[4] consider the use of an integrating-sphere photometer[5] to be the only satisfactory technique for turbid solutions. Fog[6] shows how the error caused by turbidity can be approximately eliminated in photometric determinations of the icterus index, *i.e.*, the yellow colour in blood serum.

This report describes how the effect of turbidity is adequately overcome by a technique well fitted to modification to suit various photometric determinations.

Theoretical considerations

Although the fraction of light that is absorbed in travelling through a colourless monodispersed sol or suspension can be calculated for an ideal turbid medium,[7,8,9] the theoretical treatment only applies when the particles are spherical and of uniform size and when the wavelength of the light is confined to narrow limits. These conditions do not apply to the turbid solutions likely to be met with in problems of practical absorptiometry. A number of experiments with non-coloured turbid samples shows the relationship between extinction and wavelength to be, as shown in Fig. 1, a law of the form $E = k\lambda^y$, where k and y are constants. Extinctions at two different wavelengths then enable the constants to be eliminated and the extinction to be determined at a third wavelength, *e.g.*, the value can be found graphically by plotting log E and log λ (see Fig. 2) assuming that E_1', E_2' and E_3' satisfy the equation $E = k\lambda^y$.

* Read before the Seventh Scandinavian Physiological Congress in Århus, Denmark, 1951.

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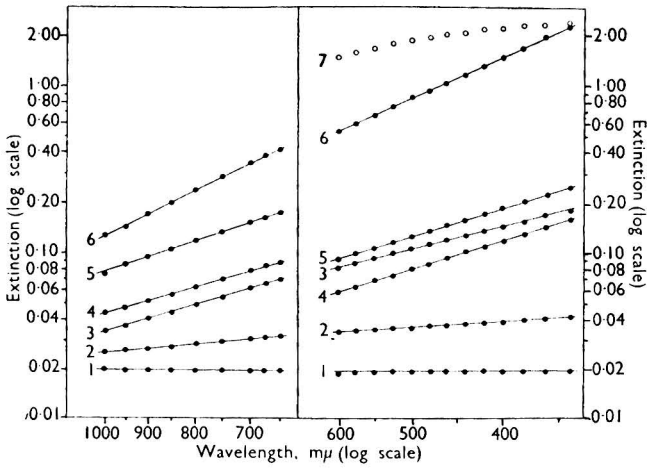

Fig. 1. Extinctions read with different turbid solutions plotted against the wavelengths in logarithmic co-ordinates. Readings with a Beckman Spectrophotometer model B

1. *Emulsio paraffini liquidi* read against the emulsifier system. E × 0·1
2. Suspension of larger particles of barium sulphate in water read against water. E × 0·0455
3. Suspension of smaller particles of barium sulphate in water read against water. E × 0·25
4. Human serum diluted with distilled water read against the same dilution of serum with physiological saline. E × 0·33
5. Suspension of bacteria (*Staphylococcus albus*) in saline read against water. E × 1
6. Mastix-sol in water read against water. E × 2
7. The same suspension as in 5, but with 13 times as many bacteria in the same volume. E × 1·45. Note the decline of the curve with the shorter wavelengths

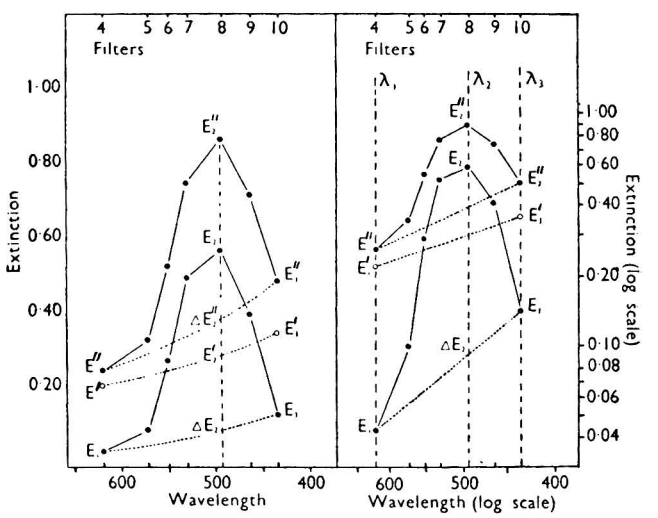

Fig. 2. Cobaltous nitrate. Extinctions read with a Zeiss Pulfrich photometer. The filters Nos. 4, 5, 6, 7, 8, 9 and 10 correspond to s61, s57, s55, s53, s50, s47 and s43, respectively. (On the left are linear and on the right logarithmic co-ordinates)

In Fig. 2 the lower curve with E_1, E_2 and E_3 at λ_1, λ_2 and λ_3, respectively, represents the absorption free from turbidity and the values are therefore identical with those of the pure substance at that concentration. If E_1, E_2 and E_3 are plotted against λ on logarithmic scales the straight line joining the two points E_1 and E_3 gives a value of ΔE_2 at λ_2. $E_2 - \Delta E_2$ is proportional to concentration if Beer's law is valid; if not, a calibration curve can be used (see Figs. 3 and 4).

If the solution is turbid, the observed curve E'' will be the sum of the E and the turbidity E'.

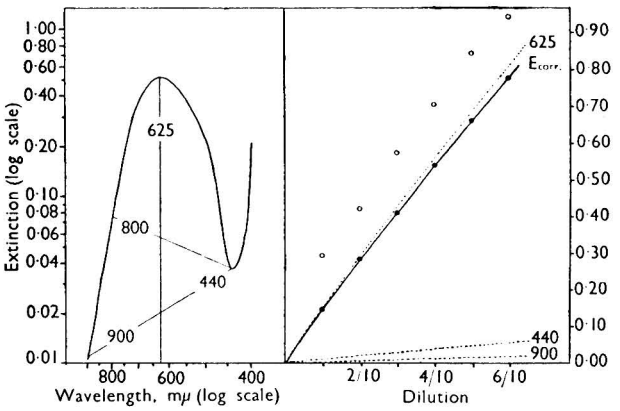

Fig. 3. Alkaline glycerol - copper sulphate. Extinctions read at the wavelength 625 mμ and corrected by means of extinctions read at 900 and 440 mμ

——— E corrected with clear solutions

• • • • E corrected with turbid solutions

o o o o E at 625 mμ with turbid solutions

If $\Delta E_2''$ is calculated (assuming that E_1'', $\Delta E_2''$ and E_3'' satisfy the equation $E = k\lambda^y$), then $E_2'' - \Delta E_2'' + z = E_2 - \Delta E_2$ because $E_2'' = E_2 + E_2'$ and $\Delta E_2'' = \Delta E_2 + E_2' + z$. Here z represents an over-correction because the sum of values obtained from the two expressions $E = k\lambda^y$ and $E' = k'\lambda^{y'}$ do not exactly follow the expression $E'' = k''\lambda^{y''}$. The quantity z will be small (*i*) if ΔE_2 is $\ll E_2$, (*ii*) if $E_3 > E_1$ where $\lambda_1 > \lambda_3$ and (*iii*) if λ_1 and λ_3 are at absorption minima on the curve for any selectively absorbing impurity.

If z should exceed the experimental error of the measurement of E it can be calculated by successive approximations. Thus—

$$E_1 = (E_2'' - \Delta E_2'') \left(\frac{E_1^\circ}{E_2^\circ - \Delta E_2^\circ} \right)$$

$$\text{and } E_3 = (E_2'' - \Delta E_2'') \left(\frac{E_3^\circ}{E_2^\circ - \Delta E_2^\circ} \right)$$

to a first approximation. The quantities designated E° refer to the absorption curve measured with a non-turbid solution of the pure substances. Approximate values for E_1' and E_3' are given by—

$$E_1' = E_1'' - E_1 \text{ (approximately).}$$
$$E_3' = E_3'' - E_3 \text{ (approximately).}$$

Approximate values of ΔE_2 and E_2' can then be obtained graphically, so that $z = \Delta E_2'' - \Delta E_2 - E_2'$ as a first approximation. With this value of z a second approximation can be reached and so on until z is less than the experimental error of E.

Experimental

The experiments were performed with a Beckman spectrophotometer model B and a Zeiss Pulfrich visual filter photometer. In the series with coloured substances, certain

amounts of stock solutions were diluted to the mark in a graduated 10-ml cylinder. The same pipettes and cylinders were used for each member of the series.

Fig. 1 shows how logarithmically expressed extinctions and wavelengths are nearly linearly related in oil-dispersed-in-water emulsions, mastix-sols, precipitated proteins and

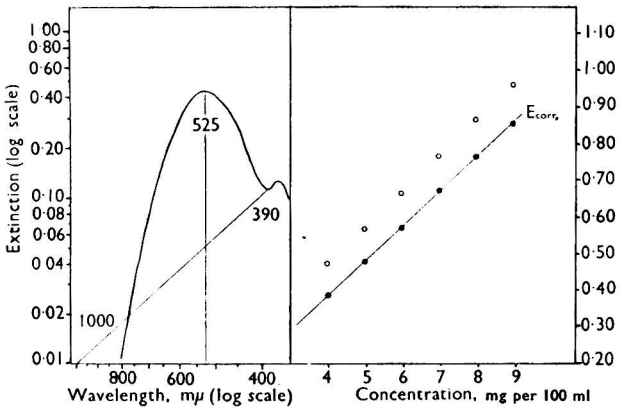

Fig. 4. Sodium salicylate (+ ferric chloride). Extinctions read with the wavelength 525 mμ corrected by means of extinctions read at 1000 and 390 mμ

——— E corrected with clear solutions
• • • • E corrected with turbid solutions
o o o o E at 525 mμ with turbid solutions

suspensions of barium sulphate and bacteria. The figures plotted in Fig. 1 are mean values. The suspensions were shaken, the extinctions read from the red to the blue wavelengths, re-shaken, and then read in the opposite direction.

With heavy turbidities and relatively large particles, the slope of the curves always declines with the shorter wavelengths. Scattered light is probably reflected by the particles and re-scattered to proceed in the direction of the eye or the photo-cell. In the scattered light, the blue fraction predominates and extinctions read at the shorter wavelengths are consequently diminished (Fig. 1, curve number 7). This effect may increase if the bottom

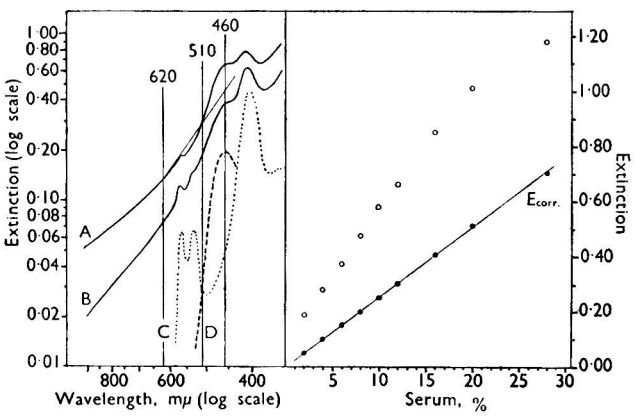

Fig. 5. Bilirubin in human serum. Extinctions read with the wavelength 460 mμ corrected by means of extinctions read at 620 and 510 mμ. The human serum was diluted with distilled water

A, human serum with slight haemolysis
B, human serum with heavier haemolysis
C, oxyhaemoglobin
D, bilirubin in human serum[9]

and the sides of the cells are frosted, but usually, with moderate turbidities, only a decrease in the slope of the whole curve occurs.

Figs. 2, 3 and 4 show the spectral absorption curves for a solution of cobaltous nitrate, an alkaline solution of glycerol - copper sulphate (Haines reagent) read against water, and sodium salicylate dissolved in a dilute acid solution of ferric chloride read against the ferric chloride solution, respectively. A suspension of barium sulphate was added to solutions of the coloured substances at different concentrations. The filters s61, s50 and s43 were used with the cobaltous nitrate, and the wavelengths 900, 625 and 440 mμ and 1000, 525 and 390 mμ with the copper sulphate and the salicylate, respectively. Non-turbid solutions were prepared in triplicate for comparison. The corrected extinctions agree to a great extent independently of turbidity, as shown in Figs. 3 and 4 and Table III. In Table I are shown the readings with different amounts of the turbid solution added to solutions of the coloured substances at the same concentrations. Table II shows corresponding values obtained with cobaltous nitrate solutions.

Blood serum is freed from turbidity and most haemoglobin only with great skill and luck. Spectral absorption curves of undiluted serum plotted in Fig. 5 (A and B) show peaks at 577 and 414 mμ caused by oxyhaemoglobin. The pointed curves show the absorptive properties of pure oxyhaemoglobin (C) and bilirubin (D). Bilirubin in human serum has its maximum absorption at 460 mμ.[10] When serum is added to distilled water, proteins are precipitated to give a turbidity that is dependent on the degree of dilution.[11] In Fig. 5 the wavelengths 620 and 510 mμ are used to correct the extinctions read at 460 mμ (bilirubin). Corrected extinctions for bilirubin are nearly linearly related to the dilution of serum in spite of the variation in turbidity (see Fig. 5).

Discussion of results

The extinctions of polydispersed sols and suspensions can usually be expressed by the formula $E = k\lambda^y$, and it is especially to be noted how well the equation fits for protein

Table I

Effect of different quantities of a turbid barium sulphate suspension added to a constant amount of the coloured substances

Alkaline-glycerol - copper sulphate (900-**625**-440 mμ)*			Sodium salicylate - ferric chloride (1000-**525**-390 mμ)†		
E at 625 mμ	$E_{corr.}$	Difference of $E_{corr.}$ from the mean of $E_{corr.}$	E at 525 mμ	$E_{corr.}$	Difference of $E_{corr.}$ from the mean of $E_{corr.}$
0·670	0·392	0·005	0·793	0·680	0·010
0·632	0·397	0	0·765	0·664	0·006
0·596	0·397	0	0·764	0·670	0
0·569	0·399	0·002	0·750	0·667	0·003
0·527	0·399	0·002	0·726	0·658	0·012
0·488	0·398	0·001	0·694	0·678	0·008
	0·397 (mean)			0·670 (mean)	

* Read against water.
† Read against ferric chloride solution.

precipitates, which, together with finely dispersed fat (oil-dispersed-in-water emulsions), are the common causes of turbidity when photometry is used with biological samples.

Only if $E_3 > E_1$ ($\lambda_1 > \lambda_3$) is z negligible, and ($E_2'' - \Delta E_2''$) should be as great as possible (approaching E_2'') to minimise the influence of errors in the correcting factors, especially as only two significant figures are usually read from the nomogram with a sufficient degree of accuracy. With complicating foreign coloured substances, the wavelengths used to correct for turbidity should be chosen at the minima of their absorption curves (Fig. 5).

The salicylate solution (Fig. 4) did not absorb at 1000 mμ, and a relatively small number (0·001) was therefore added with the clear solutions to simulate a turbidity.

In Table I, for alkaline copper sulphate, the maximum differences between the values obtained for E at 625 mμ and $E_{corr.}$ are 0·182 and 0·007, respectively, and the maximum deviation of $E_{corr.}$ from the mean is only 1·3 per cent. With the salicylate solutions the

TABLE II

EFFECT OF DIFFERENT QUANTITIES OF TURBID BARIUM SULPHATE SUSPENSION ADDED TO A CONSTANT AMOUNT OF COBALTOUS NITRATE SOLUTION

Extinctions read in a Zeiss Pulfrich photometer against water. Filters s61 and s43 were used to correct the extinctions read with the filter s50. The values are means of extinctions read 5 times

Clear solutions*			Turbid solutions*		
E (s50 filter)	$E_{corr.}$	Difference of $E_{corr.}$ from the mean of $E_{corr.}$	E (s50 filter)	$E_{corr.}$	Difference of $E_{corr.}$ from the mean of $E_{corr.}$
0·443	0·367	0·002	0·450	0·361	0·005
0·444	0·370	0·005	0·459	0·351	0·005
0·430	0·358	0·007	0·506	0·364	0·008
0·436	0·359	0·006	0·528	0·357	0·001
0·451	0·374	0·009	0·574	0·353	0·003
0·431	0·357	0·008	0·579	0·348	0·008
0·436	0·364	0·001	0·644	0·352	0·006
0·444	0·364	0·001	0·665	0·355	0·001
0·434	0·356	0·009	0·727	0·358	0·002
0·449	0·377	0·012	0·737	0·359	0·003
	0·365 (mean)			0·356 (mean)	

* The clear and turbid solutions were prepared from different stock solutions.

TABLE III

EXTINCTIONS OF ALKALINE GLYCEROL - COPPER SULPHATE SOLUTIONS

Extinctions read at a wavelength of 625 mμ and corrected for turbidity by means of the wavelengths 900 and 440 mμ ($E'_{corr.}$) and 800 and 440 mμ ($E''_{corr.}$) in a Beckman model B spectrophotometer

Dilution	Type of solution	E at 625 mμ	$E'_{corr.}$	Difference from the mean*	$E''_{corr.1}$	Difference from the mean*	$E''_{corr.2}$	Difference from the mean*
	Clear	0·809	0·781	0·002	0·732	0·004		
6/10	,,	0·804	0·775	0·004	0·726	0·002		
	,,	0·806	0·781	0·002	0·727	0·001		
	Turbid	0·943	0·778	0·001	0·713	0·015	0·730	0·002
	Clear	0·677	0·654	0·001	0·612	0·001		
5/10	,,	0·681	0·656	0·001	0·614	0·001		
	,,	0·681	0·656	0·001	0·613	0		
	Turbid	0·842	0·660	0·005	0·602	0·011	0·619	0·006
	Clear	0·561	0·541	0·005	0·506	0·006		
4/10	,,	0·555	0·534	0·002	0·499	0·001		
	,,	0·552	0·533	0·003	0·496	0·004		
	Turbid	0·702	0·538	0·002	0·490	0·010	0·503	0·003
	Clear	0·427	0·411	0	0·381	0·001		
3/10	,,	0·427	0·412	0·001	0·383	0·001		
	,,	0·424	0·410	0·001	0·381	0·001		
	Turbid	0·569	0·408	0·003	0·370	0·012	0·382	0
	Clear	0·289	0·277	0·002	0·259	0·001		
2/10	,,	0·294	0·282	0·003	0·262	0·002		
	,,	0·289	0·279	0	0·260	0		
	Turbid	0·420	0·278	0·001	0·252	0·008	0·261	0·001
	Clear	0·151	0·146	0·001	0·135	0		
1/10	,,	0·149	0·144	0·001	0·134	0·001		
	,,	0·151	0·146	0·001	0·135	0		
	Turbid	0·293	0·145	0	0·131	0·004	0·137	0·002

* Difference from the mean values of extinctions read with the clear solutions.

maximum deviation from the mean is 1·8 per cent. in spite of the uncertainty attaching to transference of salicylate solutions by means of a pipette (droplets adhere to the glass).

Table II, for cobaltous nitrate, shows that the maximum deviation of $E_{corr.}$ from the mean was 3·3 per cent. with the clear and 2·2 per cent. with the turbid solutions. With the clear solutions, the numerical values of the extinctions read with the filters s61 and s43 are rather small and therefore more liable to error than with the turbid solutions.

Table III demonstrates again how excellently the method works with the alkaline copper sulphate solutions even if E_1 is greater than E_3, but it is then essential that z be calculated and added to the first value of $E_{corr.}$.

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The Determination of Potassium Bromate in Flour

BY A. W. ARMSTRONG

A titrimetric method for the determination of potassium bromate in flour is described. A suspension of flour in zinc sulphate solution is clarified by filtration of precipitated zinc hydroxide. Bromate is estimated iodimetrically in an aliquot of the filtrate by titration of an excess of standard thiosulphate with standard iodate. Results can be corrected by applying a "recovery factor."

OF the methods so far published for the estimation of potassium bromate in flour, those of Geddes and Lehberg[1] and Geddes[2] are too time-consuming for routine use. The colorimetric methods of Hoffer and Alcock[3] and Johnson and Alcock[4] are complicated and require a spectrophotometer. Howe[5] has described the estimation of bromate in zinc filtrates of flour suspensions, but we failed to obtain satisfactory results on applying her method to National flours and have had to modify it.

On treating 40-g samples of National flours with zinc sulphate and sodium hydroxide according to Howe's procedure, filtrates were often obtained that, on acidification and after addition of potassium iodide, gave a precipitate that completely obscured the thiosulphate end-point. Even when clear solutions were obtained the end-point was indefinite, the starch iodide colour changing from purplish blue, through reddish purple and orange, to a yellow that faded slowly. Howe's method of clarification is based on that of Auerbach, Eckert and Angell,[6] which is, itself, an adaptation of Somogyi's[7,8] method of preparing protein-free filtrates from blood and plasma. Auerbach *et al.* found that "with a very few samples, more than the recommended amount of zinc hydroxide was required to bring about a clear zinc filtrate." They prescribe 0·3 *N* zinc sulphate solution when, presumably, 0·3 *M* is

intended, since the "very faintly acid" mixture which they describe can be obtained only with the latter. Howe prescribes 0·18 *N* zinc sulphate solution, containing 51·7 g per litre. With the heptahydrate, 51·7 g of zinc sulphate per litre gives a 0·18 *M* solution. If 0·09 *M*, *i.e.*, 0·18 *N*, zinc sulphate solution is used, the pH value of the filtrate is above 9. When 0·18 *M* zinc sulphate solution is used the filtrate is nearly neutral.

By increasing the zinc concentration in the clarifying medium, we have been able to obtain from 50 g of flour more than 100 ml of filtrate that remains clear throughout the estimation. A 50-ml aliquot of this filtrate is equivalent to 10 g of flour, and can be used for the estimation of additions of bromate of between 1 and 100 parts per million; for the estimation of larger additions 25-ml or smaller aliquots must be used.

The difficulty of judging the thiosulphate end-point is obviated by adding an excess of thiosulphate and titrating the excess with potassium iodate. Thiosulphate in acid solution in presence of an excess of iodide can be titrated accurately with potassium iodate. Standard potassium iodate, which is stable in solution, is therefore used for standardising the thiosulphate and also for titrating the excess of it. A trace of ammonium molybdate is used to catalyse the liberation of iodine from iodide by bromate, so that an excess of thiosulphate can be added immediately.

In the titration of an aliquot of filtrate representing 10 g of flour, 1 ml of 0·00359 *N* thiosulphate solution is equivalent to 10 parts of bromate per million in the flour. In the titration with iodate of the same strength, discrimination at the end-point, in the procedure to be described, is better than 0·05 ml; this corresponds to 0·5 parts of bromate per million in the flour. The precision of the titration is much greater than is necessary for routine work since, in the analysis of commercial flours containing bromate, which may be unevenly distributed and the particles of which may vary greatly in size, the sampling error is often large.

Only about 95 per cent. of the bromate added in solution to flour suspensions can be recovered. Absorption of bromate ion by zinc hydroxide, *per se*, is excluded, since bromate added to solutions of zinc sulphate can be recovered quantitatively from the filtrates after precipitation of the zinc as hydroxide. Reduction of bromate by soluble reducing substances in flour is also excluded; bromate added to flour filtrates can be recovered quantitatively. The low recoveries of bromate added to flour suspensions can be partly accounted for by postulating that dilution of the bromate takes place during the clarifying process. The liquid phase, being hypertonic with respect to wheat endosperm, will extract some moisture from the flour. Further dehydration is almost certainly associated with the actual clarification, which includes at least partial denaturation of the flour proteins. A total extraction of 7·5 g of water from 50 g of flour would, in the procedure to be described, account for an apparent loss of 3 per cent. of the added bromate. Apparent losses of this order are of no importance in ordinary control analyses, but, for more precise work, a "recovery factor" can be determined by recovery of bromate added, in solution, to flour suspensions (Table I).

All the flours examined, including strong and weak, agenised and non-agenised, short extraction and long extraction, have yielded nearly the same recovery factor and, for a given kind of flour, the factor varies around 1 per cent. in replicate determinations at various bromate concentrations. Greater precision is precluded by the limited volumetric accuracy of the procedure. Further, since the discrimination at the end-point of the final iodate titration is about 0·03 ml, recovery factors should not, normally, be calculated from, nor applied to, results obtained when the flour bromate is less than 30 p.p.m.

Howe emphasises that her method is not highly specific and that, if iodate or persulphate are present, they will interfere. She adds, "From a practical standpoint, however, benzoyl peroxide, chlorine, and chlorine dioxide (the bleaching and maturing agents commonly used on flour) do not interfere and, since iodate and persulphate are not permitted in the Standards of Identity of Wheat Flour, bromate normally is the only compound present that would be measurable by the method outlined."

It is, at least, improbable that iodate would be encountered in British flours; if it were, it would react quantitatively with iodide in this modification of Howe's procedure, and the method is, to that extent, non-specific.

Persulphate is commonly added to British flours. There is, however, *a priori*, no reason for expecting that it will interfere in the method described here for the determination of bromate, since (*i*) in acid solution the reaction between persulphate and iodide is extremely slow at low concentrations of the reactants and (*ii*) Auerbach *et al.*[6] find that on adding

water to flour containing persulphate, the persulphate disappears rapidly. At 27° C, a dough prepared by adding 60 parts of water to 100 parts of flour, which contained 200 parts of ammonium persulphate per million, had lost 72 per cent. of the persulphate 8 minutes after addition of the water. Experimentally, it was found that the addition to a suspension of flour (containing bromate) of a freshly prepared persulphate solution, equivalent to 160 parts of ammonium persulphate per million in the flour under test, was without effect upon the recovery of the bromate present.

The addition of 2000 parts of benzoyl peroxide per million to flour did not affect the recovery of added bromate. Neither agene nor chlorine treatments of flour affected the recovery of added bromate. The method has not been applied to flours treated with chlorine dioxide.

Method

Reagents—

Zinc sulphate solution—Dissolve 20 g of zinc sulphate ($ZnSO_4.7H_2O$) in 800 ml of water and dilute to 1 litre.

Sodium hydroxide, 0·4 N—Dissolve 17 g of sodium hydroxide in 1 litre of water. Titrate the solution against standard acid and adjust the strength to 0·4 (± 0·01) *N*.

Sodium hydroxide, 0·5 N—Dissolve 21 g of sodium hydroxide in 1 litre of water. Titrate the solution against standard acid and adjust the strength to 0·5 (± 0·01) *N*.

Sulphuric acid—An approximately 4 *N* solution. Add 112 ml of concentrated sulphuric acid to 800 ml of water. Cool the solution and dilute it to 1 litre.

Potassium iodide solution—Dissolve 25 g of potassium iodide in 30 ml of water and dilute to 50 ml. Store in an amber-coloured bottle in a cool place. Discard any solution that shows a yellow tinge of free iodine.

Ammonium molybdate—Dissolve 3 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in 80 ml of water and dilute to 100 ml.

Starch solution—Pour a suspension of 1 g of soluble starch in 5 ml of water into 100 ml of briskly boiling water. Boil for 2 minutes and cool rapidly. Prepare a fresh solution daily.

Standard potassium bromate solution—Prepare a stock solution by dissolving 5·000 g of potassium bromate (dried for 1 hour at 110° C) in 800 ml of water, and dilute to 1 litre. Prepare a standard potassium bromate solution by diluting 25 ml of the stock solution to 250 ml.

Potassium iodate, 0·0898 N—Dissolve 3·204 g of potassium iodate (dried for 1 hour at 110° C) in 800 ml of water and dilute to 1 litre.

Potassium iodate, 0·00359 N—Dilute 10 ml of the 0·0898 *N* potassium iodate solution to 250 ml. Prepare a fresh dilution daily.

Stock solution of sodium thiosulphate—Dissolve 22·5 g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) and 0·06 g of anhydrous sodium carbonate in 800 ml water, and dilute to 1 litre. Dilute 10 ml to 250 ml. Transfer 5 ml of this diluted solution to a 250-ml conical flask. Add 100 ml of water, 10 ml of the approximately 4 *N* sulphuric acid and 1 ml of potassium iodide solution. Add 5 ml of starch solution and titrate with 0·00359 *N* potassium iodate from a 5-ml burette graduated to 0·01 ml. Adjust the titre of stock sodium thiosulphate so that a 10 to 250 dilution is 0·00359 *N*. Store the stock solution in an amber-coloured bottle in a cool place.

Sodium thiosulphate solution, 0·00359 N—Dilute 10 ml of the stock sodium thiosulphate solution to 250 ml. Prepare a fresh diluted solution daily. Check the titre of the diluted solution as above at least monthly.

Procedure—

Transfer 50 g of flour to a 500-ml conical flask. Add 200 ml of zinc sulphate solution. Close the flask with a rubber bung and shake vigorously at intervals for 10 minutes. While vigorously swirling the contents of the flask, add, from a burette, 50 ml of 0·4 *N* sodium hydroxide. Shake vigorously and allow the mixture to settle for 5 minutes. Filter through a 24-cm Whatman No. 5 (or No. 40) folded filter-paper. Return the first few millilitres of filtrate to the paper. Collect the clear filtrate in a dry vessel. Alternatively, centrifuge the mixture and, if necessary, clarify the supernatant liquid by filtration. Transfer 50 ml of the filtrate to a 250-ml conical flask. If a smaller aliquot is taken, make the volume up to 50 ml with water. Add 10 ml of the approximately 4 *N* sulphuric acid, 1 ml of potassium iodide solution and 1 drop of ammonium molybdate solution. Dilute the mixture with

50 ml of water. With steady mixing, add, from a pipette, an excess of 0·00359 *N* sodium thiosulphate (5 or 10 ml). Add 5 ml of starch solution and titrate the excess of thiosulphate with 0·00359 *N* potassium iodate to the first permanent faint purple tinge of the liquid.

Parts of potassium bromate per million in the flour = x (y — titration)/5,

where x = volume of the aliquot and y = volume of thiosulphate used.

DETERMINATION OF RECOVERY FACTOR—

Dilute a known volume (n ml), greater than 3 ml but less than 10 ml, of standard potassium bromate solution to 250 ml. To 50 ml of this solution add 10 ml of the approximately 4 *N* sulphuric acid, 1 ml of potassium iodide solution and 1 drop of ammonium molybdate solution. Dilute the mixture with 50 ml of water and, with steady mixing, add from a pipette an excess (5 or 10 ml) of 0·00359 *N* sodium thiosulphate solution. Add 5 ml of starch solution and titrate with 0·00359 *N* potassium iodate solution.

Added bromate, p.p.m. = 10 (y — titration), where y = volume of thiosulphate used.

Suspend two 50-g portions of flour in two 200-ml portions of zinc sulphate solution. To one (blank) suspension, add 10 ml water. To the other (recovery) suspension, add n ml of standard potassium bromate solution and (10 — n) ml of water. Shake the mixtures vigorously at intervals for 10 minutes. Vigorously swirling the contents, add to each flask 40 ml of 0·5 *N* sodium hydroxide from a burette. Thereafter proceed as for the estimation of bromate in flour as described above, using 5 ml of thiosulphate for the blank and 10 ml for the recovery estimation. Deduct the blank value, if any, from the value of the bromate found in the recovery estimation to give "Recovered bromate."

$$\text{Recovery factor} = \frac{\text{Added bromate}}{\text{Recovered bromate}}.$$

TABLE I

RECOVERIES OF POTASSIUM BROMATE ADDED IN SOLUTION TO FLOUR SUSPENSIONS

	Recovery Factor = 1·067		
Bromate added, p.p.m.	Bromate recovered, p.p.m.	Recovery × 1·067, p.p.m.	Recovery × 1·067, %
1	1·0		
2	2·0		
5	5·0		
10	9·2	9·8	98·0
15	14·4	15·4	102·7
20	18·3	19·5	97·5
30	28·1	30·0	100·0
40	37·2	39·7	99·3
60	56·4	60·2	100·3
80	75·1	80·1	100·1

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The Determination of Nickel and Manganese in Uranium*

By J. HASLAM,† F. R. RUSSELL and N. T. WILKINSON

Methods have been developed for the determination of 0 to 0·02 per cent. of nickel, and 0 to 1·0 per cent. of manganese in uranium metal.

The method for manganese depends on solution of the sample in nitric acid, and separation of silica by dehydration of the solution in the presence of hydrochloric acid. In the determination of nickel the sample is dissolved in nitric acid, which is subsequently removed by evaporation in the presence of sulphuric acid. In the nickel determination metals that form insoluble sulphides are separated directly in the diluted sulphuric acid solution; and in the manganese determination, after the separation of silica. The filtrate from the sulphide precipitation is boiled until free from hydrogen sulphide, and the nickel and manganese precipitated in the presence of iron by sodium carbonate. Precautions are taken to ensure complete precipitation of the nickel and manganese by decomposition of any bicarbonate by boiling.

The mixed precipitate is filtered and washed with dilute sodium carbonate solution. The precipitate is then dissolved in acid and the nickel determined colorimetrically by the hypobromite - dimethylglyoxime method.

Manganese is determined volumetrically with standard ferrous ammonium sulphate on a solution of the precipitate obtained in the same manner as for nickel after oxidation to permanganate.

Our first experiments were directed to the investigation of the precipitation of nickel in the presence of iron by means of sodium carbonate. Solutions containing appropriate amounts of iron and nickel were precipitated with sodium carbonate, the precipitate dissolved in nitric acid, the solution evaporated to dryness and the residue dissolved in a little hydrochloric acid. The iron in this solution was then precipitated with ammonium hydroxide and the nickel in the filtrate determined by the application of the colorimetric dimethylglyoxime procedure. As was not entirely unexpected, the recoveries of nickel were low, but our experiments suggested that it might be possible to apply the glyoxime reaction directly to the solution containing nickel and iron, interference of iron being prevented by the addition of tartrate ion.

It was found that the amount of iron present in the test solution did not interfere when the colorimetric dimethylglyoxime reaction was applied in the presence of tartrate ion. Experiments on iron - nickel solutions gave satisfactory recoveries of nickel with this direct method.

When carrying out the determination, by preliminary precipitation of iron and nickel with sodium carbonate from an acid solution containing uranium, the recovery of nickel was low, and in general the results were erratic. Modifications of the concentration of the various substances present did not effect much improvement.

It appeared from the experiments that complete precipitation of iron and nickel was not being effected by the sodium carbonate because the application of the test to samples of uranium involved solution of the sample in an excess of acid. On the addition of sodium carbonate to the solution containing the excess of acid, appreciable amounts of bicarbonate would be produced, so that in effect the precipitation of iron and nickel was being carried out with sodium carbonate in the presence of sodium bicarbonate. It appeared, therefore, that for complete precipitation of iron and nickel any sodium bicarbonate that was produced must be decomposed.

* This work was carried out originally in 1945 and 1946, and forms the subject of two reports, BR 623 and BR 687, which were declassified by the Department of Atomic Energy, Ministry of Supply, on February 13th, 1952.

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Efforts to overcome the trouble were made as follows—

(1) By adding an excess of sodium carbonate solution to the cold acid solution of the sample, followed by boiling to effect decomposition of the bicarbonate.
(2) By adding sodium carbonate solution to the boiling solution of the sample. This gave a yellow precipitate.
(3) By adding an excess of sodium carbonate solution to the cold acid solution of the sample, then adding ammonium hydroxide and boiling.
(4) By adding an excess of sodium carbonate solution to the cold solution of the sample, followed by boiling to decompose the bicarbonate. After this operation the solution was diluted and re-boiled.

Of these four procedures, (4) proved to be the most satisfactory, and the method of determination of nickel in samples of uranium is based on this procedure.

The same procedure for the preliminary separation of manganese was applied to the determination of manganese in uranium. The results were satisfactory.

It is necessary to remove silica and metals precipitated by hydrogen sulphide in acid solution before proceeding to the precipitation of the iron, nickel and manganese. For the precipitation of the latter metals a specially purified solution of sodium carbonate is used.

Method for the determination of nickel

Special reagent—

Purified sodium carbonate solution, 14 per cent. w/v—Dissolve 70 g of sodium carbonate in 300 ml of water and add 100 ml of standard ferric chloride solution (1 ml ≡ 0·0001 g of Fe_2O_3). Add a small amount of filter-paper pulp and digest the mixture on a steam-bath for 30 minutes. Cool the solution, filter through a Whatman No. 40 filter-paper and wash the filter with a little water. Dilute the filtrate to 500 ml.

Procedure—

Dissolve about 5 g of the sample (accurately weighed) in 10 ml of concentrated nitric acid by heating the mixture, in a 250-ml beaker, on a water-bath.

Add 9 ml of concentrated sulphuric acid and heat the solution to fuming on a sand-bath. Hold the beaker in a clamp and swirl the mixture continuously during this evaporation.

When white fumes of sulphur trioxide appear, cool the mixture and dilute with water. Usually the amount of insoluble matter is small and filtration is unnecessary. Dilute the solution to about 150 ml, then saturate it with hydrogen sulphide by passing the gas into the solution for about 15 minutes. Heat the solution on the water-bath for about 30 minutes in order to coagulate the precipitate, filter and wash the insoluble precipitate with sulphuric acid solution (2 per cent. v/v) saturated with hydrogen sulphide.

Boil the filtrate and washings until free from hydrogen sulphide, add a few millilitres of bromine water and boil the solution to free it of the excess of bromine. Cool the solution and dilute it to 200 ml with water in a graduated flask.

Take 50 ml of the solution and adjust* the iron content of the solution to 0·0010 g by adding standard iron solution (1 ml = 0·0001 g of Fe_2O_3).

Dilute the solution to 100 ml and add 50 ml of purified sodium carbonate solution to the cold solution. Evaporate the solution to a volume of approximately 80 ml, add 70 ml of hot water and again evaporate to 80 ml. Allow this solution to stand overnight.

Add a small amount of filter-paper pulp, and filter the solution through a 7-cm Whatman No. 42 filter-paper; wash the insoluble matter with sodium carbonate solution (2 per cent. w/v) until free from uranium. Reserve the filtrate and washings.

Return the filter-paper pulp and precipitate to the beaker used for the original precipitation by means of a jet of water (about 10 ml), add 2 ml of concentrated nitric acid and heat the mixture until the precipitate has dissolved; pour this solution through the 7-cm filter-paper and collect the filtrate in a clean 100-ml beaker. Re-treat the filter-paper pulp with a mixture of 10 ml of water and 1 ml of concentrated nitric acid and add the extract through the 7-cm filter-paper to the original extract. Wash the filter-paper pulp well with water.

* When the iron content of the uranium sample is excessively high, it may be desirable to take a smaller aliquot of the test solution.

Evaporate the combined filtrate and washings (in the 100-ml beaker) to dryness by immersing the beaker in a bath of boiling water and bring the residue into solution with 0·5 ml of *N* hydrochloric acid solution by gentle warming.

Wash the solution into a measuring flask of 100-ml capacity, add 5 ml of Rochelle salt solution (30 g of Rochelle salt dissolved in water and diluted to 100 ml and filtered), and then add 2 ml of saturated bromine water and 1 ml of ammonium hydroxide solution (1 volume of ammonium hydroxide, sp.gr. 0·880, to 2 volumes of water).

After setting the solution aside for 5 minutes, add 2 ml of dimethylglyoxime solution (1 g of dimethylglyoxime dissolved in 100 ml of alcohol) and examine the solution in a Spekker photo-electric absorptiometer using, according to the depth of colour, either a 4-cm or a 1-cm cell and No. 5 green filters. Deduce the amount of nickel present in the 100 ml of solution from previously prepared calibration curves that relate the amount of nickel in grams with the Spekker indicator drum readings. These curves are smooth and pass through the following points—

With 4-cm cells and No. 5 green filters		With 1-cm cells and No. 5 green filters	
Nickel per 100 ml of solution, g	Indicator drum reading	Nickel per 100 ml of solution, g	Indicator drum reading
0·000005	0·017	0·00002	0·024
0·000010	0·037	0·00006	0·071
0·000020	0·083	0·00010	0·117
0·000040	0·162	0·00018	0·212
0·000060	0·255	0·00024	0·280
0·000120	0·490		

Where the nickel content of the uranium sample is comparatively high, *viz.*, of the order of 0·003 per cent., a further 0·0010 g of iron (as ferric chloride solution) is added to the previously reserved filtrate and the nickel determined in this filtrate by carrying out the procedure described on p. 465.

The above method has been applied to known uranium - nickel solutions with the results shown in Table I. Each solution used for the precipitation with sodium carbonate contained the equivalent of 1·25 g of uranium. The nickel contents are calculated as percentages of the uranium.

All the results are corrected for a control test on the reagents.

TABLE I

DETERMINATION OF NICKEL IN URANIUM - NICKEL SOLUTIONS

Nickel added, %	Nickel found (one precipitation), %	Nickel found (two precipitations), %
nil	nil	nil
0·00040	0·00032	0·00032
0·00080	0·00072	0·00072
0·00160	0·00152	0·00160
0·00320	0·00304	0·00320
0·00480	0·00456	0·00488
0·00960	0·00896	0·00936
0·00960	0·00872	0·00920
0·01920	0·0189	0·0191
0·01920	0·0186	0·0189

METHOD FOR THE DETERMINATION OF MANGANESE

SPECIAL REAGENT—

Purified sodium carbonate solution, 14 per cent. w/v—Prepare as described on p. 465.

PROCEDURE—

Dissolve about 5 g of the sample (accurately weighed) in 20 ml of concentrated nitric acid by heating on a water-bath; evaporate the acid solution to dryness on a sand-bath. After the removal of the nitric acid, evaporate twice further with hydrochloric acid using

10 ml of the concentrated acid on each occasion. Heat the residue in an oven at 120° C overnight.

Treat the baked residue with 5 ml of concentrated hydrochloric acid and 20 ml of water and heat the mixture on the water-bath to effect complete solution of the uranium salts. Filter off the insoluble silica on a 9-cm Whatman No. 40 filter-paper and wash with 5 per cent. v/v hydrochloric acid solution and with hot water to remove all uranium salts. Ignite the insoluble siliceous residue, weigh it in a platinum crucible and treat it with dilute sulphuric acid and hydrofluoric acid in order to remove the silica. Fuse the residue with a little potassium acid sulphate and incorporate the aqueous extract of the melt in the main uranium solution. Saturate this solution, of volume approximately 200 ml, with hydrogen sulphide. Filter off the precipitated hydrogen sulphide metals, wash with acidulated hydrogen sulphide water and boil the filtrate to free it from hydrogen sulphide. Oxidise the solution by adding bromine water, free it from bromine by boiling, cool and dilute to 250 ml in a measuring flask.

Take an aliquot of this solution equivalent to about 1 g of the original uranium metal for the manganese determination. If from previous iron determinations it is known that the aliquot taken does not contain as much as 0·002 g of iron, then add standard iron solution (1 ml ≡ 0·0001 g of Fe_2O_3) in amount sufficient to give a total of 0·002 g of iron; if the aliquot contains more than 0·002 g of iron, then add no further standard iron solution. Add 50 ml of 14 per cent. w/v sodium carbonate solution and dilute the solution to 150 ml. Boil down the solution to a volume of 80 ml, dilute to 150 ml and again boil down to 80 ml. Add a small amount of paper pulp to the mixture, which is allowed to coagulate on the water-bath for about 10 minutes.

After cooling the precipitate filter it off on a 9-cm Whatman No. 40 filter-paper and wash the residue three times with 2 per cent. w/v sodium carbonate solution. Prepare this sodium carbonate solution by diluting the 14 per cent. w/v sodium carbonate solution.

Place the paper and precipitate in the beaker originally used for the precipitation and heat on the water-bath with a mixture of 5 ml of concentrated nitric acid and 10 ml of water until all the precipitate dissolves and the filter-paper is quite white. Filter the mixture through an 11-cm Whatman No. 40 filter-paper and wash it first with 10 ml of hot 30 per cent. v/v nitric acid solution and then thoroughly with hot water.

Boil the filtrate (volume approximately 150 ml) down to about 50 ml, cool, add 0·5 g of sodium bismuthate and then heat to boiling. Decolorise the clear pink solution with a few drops of sulphur dioxide water and boil off the excess of sulphur dioxide. Again cool the solution, add 10 ml of concentrated nitric acid, followed by 0·5 g of sodium bismuthate and then set it aside in the dark for 1 hour.

Filter the mixture with the aid of suction through a sintered-glass funnel* of porosity 4, and wash the insoluble residue with the minimum amount of 3 per cent. v/v nitric acid solution. Add standard 0·02 *N* ferrous ammonium sulphate to the filtrate (approximate volume 80 ml) until the permanganate colour is destroyed, after which add an excess of 2 to 3 ml. Add 0·02 *N* potassium permanganate fairly quickly at first, then 2 drops at a time, until the solution is just pink. At this stage add 1·00 ml of 0·02 *N* potassium permanganate, then 1·00 ml of 0·02 *N* ferrous ammonium sulphate and finally 0·02 *N* potassium permanganate solution, 2 drops at a time, until the permanent pink end-point appears. Calculate the proportion of manganese in the sample from the amount of ferrous solution consumed by the permanganate produced in the bismuthate oxidation.

The principle of the method has been tested by applying the procedure to known mixtures of thrice crystallised uranium nitrate (equivalent to 1 g of uranium) and reduced standard potassium permanganate solution. To all the mixtures, the equivalent of 0·002 g of iron was added.

The following results were obtained, the manganese added being calculated as a percentage of the uranium present.

Manganese added, % ..	0·11	0·11	0·22	0·22	0·44	0·55	0·55	1·10	1·10	1·10
Manganese found, % ..	0·088	0·090	0·216	0·206	0·444	0·533	0·535	1·095	1·100	1·060

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* Before using the sintered-glass funnel for the final filtering operation, wash it first with potassium permanganate solution, then with 20 per cent. nitric acid and finally with water.

The Colour Reactions of Chloranilic Acid with Particular Reference to the Estimation of Calcium and Zirconium

By R. E. U. FROST-JONES and J. T. YARDLEY

The colour reactions of chloranilic acid are studied in some detail and with particular reference to interference in the colorimetric estimation of calcium and zirconium. A method is described for cancelling interference by sodium, and a detailed procedure is described for estimating zirconium in the presence of a number of other ions. The influence of the perchloric acid concentration on the determination is discussed.

In recent years some interest seems to have been focused on the use of chloranilic acid as a colorimetric reagent for calcium. The demand for analytical grades of the material is fairly substantial and, as the literature is not extensive, it seemed desirable to explore the potentialities of the reagent with a view to providing information beyond that already available.

The reaction with calcium

Chloranilic acid (2:5-dichloro-3:6-dihydroxy-1:4-benzoquinone) is a bright red crystalline powder, which is sparingly soluble in water and forms solutions that are also intensely red. The calcium salt of the acid is almost insoluble in cold water and the colour diminution that accompanies its precipitation has been used as the basis of a colorimetric method for the estimation of this metal. Barretto[1] described both gravimetric and colorimetric methods, but the gravimetric determination shows no advantages over the established oxalate procedure and has received little further attention. The colorimetric method has, however, been the subject of several papers[2,3,4] that are concerned with the estimation of calcium in plant materials and soil extracts.

Chloranilic acid is not a highly selective reagent under the conditions for the determination of calcium, and interference is extensive. Tyner,[2] Gammon and Forbes[3] and Le Peintre[4] have investigated the influence of the ions that are of common occurrence in plant ash and soil extracts, and we have extended the investigation with a view to broader applications. The classification of types of interference given by Tyner is of some general interest. Ions such as $Fe^{\cdots}$ and $Al^{\cdots}$ form soluble complexes that hinder or prevent precipitation of the calcium salt; and barium, strontium, copper and manganese interfere by producing insoluble compounds. Another group of metals, including sodium, potassium and magnesium, were said to interfere because of the occlusion of the metal-chloranilate complexes by the calcium salt. Later work, however, does not support the occlusion mechanism. Gammon and Forbes[3] failed to detect significant amounts of magnesium in calcium precipitates by a spectrographic method, and the present authors observed colour diminution with all three metals in solutions containing no calcium.

The results of our experiments on general interference are summarised in Table I. Where toleration limits are given these are generally in substantial agreement with those previously published,[2] but the magnitude of the interference by sodium proved to be much greater than was expected.

Table I shows the effect of foreign ions present at a concentration of 10 mg per 25 ml of solution containing 5 ml of 0·1 per cent. aqueous chloranilic acid and compared visually with a blank containing chloranilic acid only.

The items marked with an asterisk are discussed more fully in the following section.

It will be seen that some kind of *direct* interference was observed with almost every ion examined, but the amounts concerned were equivalent to about ten times the average amount of calcium that would be present in the same volumes in an analysis. Certain ions are discussed in more detail below.

THE QUANTITATIVE EFFECT OF CERTAIN COMMON IONS—

Aluminium—Although aluminium does not noticeably affect the colour of chloranilic acid solutions, the presence of 10 mg almost completely inhibits the precipitation of calcium at levels of about 0·5 mg. Amounts of aluminium somewhat smaller than that of the calcium present can be tolerated without very serious effects.

TABLE I

INTERFERENCE EFFECT OF FOREIGN IONS

Foreign ion	Effect
Aluminium*	No detectable difference from the blank
Ammonium*	Little or no effect
Antimony (Sb^{***})	Very slight colour change
Arsenic (AsO_4''')	Considerable diminution of intensity
Barium	Precipitate
Beryllium	Deeper red colour
Bismuth	Green colour and precipitate
Cadmium	Precipitate
Chromium (CrO_4'')	Yellow colour
Cobalt	Precipitate
Copper* (Cu^{**})	Some colour diminution and production of violet tint
Iron* (Fe^{***})	Very deep purple - mauve colour
Iron (Fe^{**})	Similar to ($iron^{III}$) but less intense (possibly traces of ferric iron)
Lead	Precipitate
Magnesium*	Considerable colour diminution
Manganese* (Mn^{**})	Precipitate
Mercury (Hg^{*})	Precipitate
Molybdate	Considerable colour diminution
Nickel	Violet colour
Palladium	Deep straw colour
Potassium*	Slight colour diminution
Silver	Precipitate
Sodium*	Slight colour diminution
Strontium	Precipitate
Thallium (Tl^{*})	Slight colour diminution
Thorium	Brilliant violet colour
Tungsten (WO_4'')	Considerable colour diminution
Uranium (UO_2^{**})	Brown colour
Zinc	Pronounced colour diminution with change of tint
Zirconium	Flocculent precipitate
Acid radicles—	
Oxalate	Considerable colour diminution
Tartrate	Slight colour diminution
Citrate	Some colour diminution
Cyanide	Some colour diminution
Sulphate and chloride	No interference apparent

* These are discussed more fully in the text.

Magnesium—Amounts approaching that of the calcium to be determined can be tolerated. The presence of 10 mg of magnesium ion largely decolorises the solution used in the general method for the estimation of calcium (p. 470).

Le Peintre[4] minimised interference by means similar to those adopted by us for cancelling the effect of sodium (see 470).

Iron (Fe^{***})—Up to about 0·01 mg of ferric iron is tolerable in the estimation of amounts of calcium of the order of 1 mg. Amounts of iron comparable with the calcium present vitiate the method chiefly by intensifying the colours considerably. The shade of the colour is also modified.

Gammon and Forbes[3] drew attention to the mutually compensating effect observed when both iron and magnesium are present in soil extracts or plant materials. They recommend the prior determination of these constituents with *o*-phenanthroline and thiazole yellow, respectively, so that additions can be made to the standards. The successful application of this procedure would seem to be limited to cases where the iron level is not high.

Attempts were made to suppress the iron colour by complexing with various reagents, namely citrate, tartrate, cyanide, pyrophosphate and thioglycollic acid. Of these, tartrate, had little effect, citrate and cyanide materially reduced the colour intensity of chloranilic

acid solutions; pyrophosphate suppressed the iron colour but, as anticipated, it also inhibited precipitation of the calcium salt. Thioglycollic acid, in acid solution, was only partially effective in reducing the iron colour unless present in quantities that gave rise to extensive fading of the reagent, presumably through reduction of the chloranilic acid.

Copper—Amounts up to about one-quarter of the calcium present are tolerated, but larger amounts, of the order of ten times the amount of calcium present, cause considerable colour diminution together with change of tint.

Manganese—Amounts up to 0·025 mg have no effect on the determination of calcium at a level of about 0·5 mg.

Sodium—The colour intensity is continuously and rapidly decreased by increasing amounts of sodium up to 10 mg, but beyond this level the rate of decrease falls off rapidly. Thus, the effect of 500 mg of sodium is barely double that of 10 mg. Moreover, in the range of 0 to 10 mg of sodium, the effect on the chloranilic acid colour is independent of the presence of calcium, so the interference is not due to occlusion. These findings led to the supposition that the influence of moderate amounts of sodium could be effectively cancelled by adding a large excess of sodium ions (as chloride) to both test solutions and standards alike. This modification proved satisfactory; some typical results are shown in Table II.

TABLE II

THE DETERMINATION OF CALCIUM AFTER THE ADDITION OF 400 mg OF SODIUM ION TO THE TEST SAMPLE AND STANDARDS

Present in the test sample		
Calcium, mg	Sodium, mg	Calcium found, mg
0·60	0·5	0·57
0·70	10·0	0·70
0·80	1·0	0·81
0·90	5·0	0·92
1·00	20·0	1·03
1·20	10·0	1·20
1·40	1·0	1·38

The average effect of 20 mg of sodium on the recovery of 0·5 mg of calcium, by the general method (see below), was to introduce a positive error of about 25 per cent.

Potassium—In the presence of 100 mg of potassium the colour intensity curve was almost identical with that given by 100 mg of sodium in the same range of calcium concentrations. Although no further quantitative experiments were made, there is every likelihood that the effect of potassium is altogether similar to that of sodium and might be minimised in a similar way.

Ammonium—Large amounts of ammonium ion led to colour diminution, but no quantitative experiments were made, as this ion could normally be removed by ignition or be replaced by sodium.

GENERAL METHOD FOR THE ESTIMATION OF CALCIUM

The following procedure, based on Tyner's method,[2] has proved convenient in practice, and is suggested as a basis for the more general application of the reagent to calcium estimations.

To 10 ml of test solution, made neutral or faintly acid with acetic acid, containing 0·2 to 1·5 mg of calcium, in a 25-ml standard flask, add 10 ml of a 0·1 per cent. aqueous solution of chloranilic acid (filtered if necessary) from a pipette. Shake well and set aside overnight (or for 3 hours), preferably in an ice-chest. Let the solution attain room temperature, dilute to the mark with distilled water and filter through a dry Whatman No. 41 filter-paper. As an alternative to filtration, an aliquot of the solution (after dilution to the mark) may be centrifuged at a relative centrifugal force of about 5000 for 5 minutes. A suitable portion of the supernatant liquor can then be transferred to the absorptiometer cell for measurement of its optical transmittance. Finally, measure the colour intensity of the filtrate at a wavelength of 550 mμ. If a Spekker absorptiometer is used, either the green or yellow-green filters should be used with a 1-cm cell and a water setting of one on the drum. Two standards should be included in each batch of test samples (p. 471).

The transmittance of chloranilic acid solution is said to vary slowly but continuously.[2] Temperature, pH value and other factors also have some effect on the colour intensity. The possibility of minimising calibration variations by rigid control of the conditions of experiment was considered, but as two suitably chosen standards (say 0·3 and 0·9 mg for the range 0 to 1 mg of calcium) are sufficient to define a calibration curve, this further complication of the method appeared to be unnecessary. All the calibration curves prepared were linear and, apart from the waiting period, the method is rapid. A large number of test samples can be handled at a time, so the inclusion of an additional pair of standards is probably less troublesome than the introduction of refinements in general working technique. The solid reagent is stable over a period of years.

Le Peintre[4] recommended working at a pH value between 4 and 5, both in the presence and absence of magnesium, but we did not find it necessary to take any steps beyond ensuring approximate neutrality in the unknown solution.

Although no work was done outside the range of 0·1 to 2 mg of calcium, the use of larger amounts of chloranilic acid would enable larger amounts of calcium to be estimated, but the increased amount of reagent would not be suitable for the lower calcium ranges because of the intense residual colours that would result. The use of smaller amounts of chloranilic acid is advantageous when working towards the lower end of the recommended range, but below this range the usefulness of the reagent is limited by the appreciable solubility of the calcium salt. The effect of variables such as temperature and pH value also becomes more marked at low concentrations of calcium and of the reagent.

Modification of "general method" in the presence of sodium—

When sodium is present in the test solutions, the general method may be followed, except that 400 mg of sodium should be added both to the test solutions and to the standards. This addition may conveniently be made by minimising the volume of the aliquots initially taken and adding 10 ml of a 10 per cent. aqueous solution of sodium chloride before adding the chloranilic acid. Typical results obtained by this modified procedure have been given in Table II.

Le Peintre[4] minimised interference from magnesium in a similar manner by adding a large excess of magnesium sulphate.

The reaction with zirconium

In contrast to its behaviour in the calcium reaction, chloranilic acid yields a relatively selective reaction for zirconium in the presence of perchloric acid. The magenta colour produced was studied by Thamer and Voigt[5] who, in a recent paper devoted largely to physicochemical aspects of the reaction, outlined a method for estimating zirconium at concentrations between 2×10^{-6} and 5×10^{-5} molar. Intensities were measured at 330 mμ and, in 2 *M* perchloric acid, interference from Hf^{++++}, U^{++++}, Th^{++++}, Sn^{++++}, Ti^{++++} and Fe^{+++} was mentioned. No visible reaction was reported with Fe^{++}, Cr^{+++}, Al^{+++}, Cu^{++}, Co^{++}, Mn, Ba or K.

The following summary of our experiments, carried out in the visible waveband, confirms a good degree of selectivity and moderate sensitivity under these conditions.

Procedure—

Transfer a slightly acid aliquot (not more than 25 ml) of the test solution, containing not more than 1·5 mg of zirconium, to a 50-ml calibrated flask, and add precisely 20 ml of 4 *M* perchloric acid. Mix, dilute with water to about 45 ml and add 4 ml of a 0·1 per cent. aqueous chloranilic acid solution. Transfer the flask to a constant temperature bath at 20° C for at least 20 minutes, dilute to the mark and measure the colour intensity at a wavelength of 525 mμ. If the Spekker absorptiometer is used, a 4-cm cell, tungsten lamp and Ilford No. 604 green filter and a water setting of 1 are convenient.

When a large number of readings are to be taken, the above method may be somewhat simplified by preparing a composite reagent from the 4 *M* perchloric acid by diluting 500 ml with 100 ml of 0·1 per cent. chloranilic acid solution and 25 ml of water. Twenty-five-millilitre portions of this solution can then be added instead of the two separate additions, but the solution does not keep well.

A calibration curve should be constructed with known amounts of zirconium. For this purpose "pure" zirconium nitrate can be used. This salt is usually basic (more basic than

would correspond to the zirconyl salt) but the zirconium content can be readily estimated by ignition to ZrO_2.

It is possible to estimate rather larger amounts of zirconium; but beyond the limit given above, calibration curves were not always satisfactory. Precipitation never occurred within a reasonable time, but the coloured solutions were not always clear and intensities were often irregular within the range 1·5 to 2·0 mg of zirconium.

INTERFERENCES—

The colour intensities of a number of solutions containing 1 mg of zirconium plus 1 mg of foreign ions were measured and compared with the intensities of solutions containing 1 mg of zirconium alone.

With water settings of 1 drum unit, solutions containing the following ions gave Spekker readings within 0·01 unit of those given by the pure zirconium solutions—NH_4, Na, K, Mg, Ca, Sr, Ba, $Cu^{\cdot\cdot}$, Pb, Zn, $Tl^{\cdot}$, $As^{\cdot\cdot\cdot}$, $Hg^{\cdot}$, Al, Co, $Cr^{\cdot\cdot\cdot}$ and $Mn^{\cdot\cdot}$.

Slightly greater differences were given by Bi, Ni, Pd, Th, $UO_2^{\cdot\cdot}$ and tartrate, whilst $Sb^{\cdot\cdot\cdot}$, WO_4'', MoO_4'', $Fe^{\cdot\cdot\cdot}$, $Sn^{\cdot\cdot}$, $Sn^{\cdot\cdot\cdot\cdot}$, Cd and Ag gave rise to serious interference of varying degrees. The effect of chromate, oxalate and quadrivalent titanium was considerable.

The concentration of perchloric acid has a marked effect on the colour intensity of zirconium chloranilate solutions; this intensity decreases quite sharply over the concentration range 0 to about 3·5 molar perchloric acid (higher acid concentrations were not examined). Unfortunately, interference effects are much more pronounced at low perchloric acid concentrations. Iron, for example, when present in quantity equal to that of the zirconium to be determined, has no effect at perchloric acid concentrations of 3·5 molar, but its effect soon becomes apparent as the perchloric acid concentration is diminished. The iron colour completely obscures the reaction as the perchlorate concentration approaches zero. Conditions then become virtually identical with those prevailing in the calcium determination and interference generally becomes widespread.

The acidity level finally chosen is merely a working compromise and individual circumstances, such as those considered above, might demand higher acid concentrations. Lower acid concentrations, with consequent gain in sensitivity, might equally be desirable in the absence of interfering ions.

Thanks are due to the Directors of Hopkin & Williams Ltd. for permission to publish this paper.

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ANALYTICAL LABORATORY
HOPKIN AND WILLIAMS LTD.
CHADWELL HEATH

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The Polarography of *cyclo*Octatetra-ene and Vinyl *cyclo*Octatetra-ene

By J. H. GLOVER and H. W. HODGSON

The polarography of *cyclo*octatetra-ene and of a new derivative, vinyl *cyclo*octatetra-ene, has been examined. Previously published work by Elofsen on *cyclo*octatetra-ene has been confirmed, and it has been shown that the recently isolated vinyl derivative is almost identical in polarographic behaviour with the parent compound. Both compounds give waves at the same half-wave potential, which is independent of the pH of the solution. It has also been found that *cis*-1-phenyl-1:3-butadiene is polarographically inert.

*cyclo*OCTATETRA-ENE is a highly reactive hydrocarbon that has potential uses in organic synthesis. Its reduction at the dropping-mercury electrode has been examined by Elofsen,[1] who showed that polarographic waves, suitable for analytical use, are obtained in base solutions containing tetramethylammonium ions. The half-wave potential was — 1·51 volt, measured against the saturated calomel anode, and it was found to be independent of the pH of the base solution.

On the basis of an analysis of the *cyclo*octatetra-ene wave, Elofsen concluded that two electrons are involved in the electrode reaction. The formation of negatively charged *cyclo*octatetra-ene ions was suggested as the primary electrode process, these ions being stabilised by the tetramethylammonium ion, which appears to be essential for the reduction.

During experimental work involving the use of Elofsen's method, it was found that a polarographic wave was obtained from an unidentified fraction containing $C_{10}H_{10}$ homologues of *cyclo*octatetra-ene. The wave was in all respects identical to the *cyclo*octatetra-ene wave, but evidence from other sources indicated that there was no *cyclo*octatetra-ene present. Recently, Craig and Larrabie[2] and Withey[3] have identified the constituents of this $C_{10}H_{10}$ fraction as vinyl *cyclo*octatetra-ene and *cis*-1-phenyl-1:3-butadiene. The polarographic behaviour of these two compounds has been examined, and it has been shown that vinyl *cyclo*octatetra-ene is polarographically reducible, and would therefore constitute a source of interference in Elofsen's method.

The calibration was carried out with purified *cyclo*octatetra-ene and vinyl *cyclo*octatetra-ene, and the accuracy is consistent with that of normal direct polarographic methods.

Experimental

*cyclo*OCTATETRA-ENE

Effect of pH—The polarographic behaviour of *cyclo*octatetra-ene was examined in a series of buffer solutions covering the pH range 5 to 13. The buffer solutions were prepared by adding a dilute solution of citric or boric acid in 50 per cent. alcohol to 0·2 *M* tetramethylammonium hydroxide solution.

An examination of the graphs of pH value plotted against neutralisation of tetramethylammonium hydroxide shows that neutralisation with citric acid gives rise to a well-buffered solution over the range pH 4·0 to pH 7·5, and neutralisation with boric acid covers the range 9·5 to 11·0. Tetramethylammonium hydroxide is buffered at pH 13·0. In practice, the buffer solutions were prepared by titrating tetramethylammonium hydroxide with the acid until the desired pH was reached, the glass-electrode system being used as a continuous pH indicator. The pH of each solution was checked after mixing with the *cyclo*octatetra-ene solution.

The effect of pH on the half-wave potential of the *cyclo*octatetra-ene wave confirms Elofsen's findings, in that no variation is obtained from pH 7·5 to 13·0. The actual values are shown in Table I.

No well-defined diffusion current was obtained at pH 5·3 with either compound; it is probable that the hydrogen wave interferes at this pH. The slope of the limiting current increases with decreasing pH, and definition of the wave was best at a pH of 13·0. Calibration experiments showed that the diffusion current bore a linear relationship to the concentration of *cyclo*octatetra-ene, and details of the calibration constants are shown in Table II.

VINYL *cyclo*OCTATETRA-ENE—

This compound was examined in a similar series of base solutions to that used for *cyclo*-octatetra-ene, in an attempt to separate the waves due to the two compounds. It was found, however, that no separation was possible, since the half-wave potential of vinyl *cyclo*octatetra-ene was also independent of pH and was within 0·05 volt of the *cyclo*octatetra-ene wave over the pH range 7·5 to 13·0. At pH 5·3, no well defined diffusion zone was obtained, and the same variation in the slope of the limiting current was given as with *cyclo*octatetra-ene. Half-wave potentials of these two substances at different pH values are presented in Table I. A linear relationship between diffusion current and concentration for vinyl *cyclo*octatetra-ene

TABLE I

EFFECT OF pH ON HALF-WAVE POTENTIALS

pH	Half-wave potential *vs.* S.C.E.	
	*cyclo*Octatetra-ene, volts	Vinyl *cyclo*octatetra-ene, volts
5·3	—	—
7·5	− 1·53	− 1·49
10·0	− 1·53	− 1·49
11·0	− 1·52	− 1·49
13·0	− 1·50	− 1·49

was found in calibration experiments; details of this calibration are compared with those of *cyclo*octatetra-ene in Table II.

An attempt was made to estimate vinyl *cyclo*octatetra-ene in the presence of *cyclo*-octatetra-ene by acid hydrolysis of the vinyl group to acetaldehyde. Previous experiments on mixtures of vinyl *cyclo*octatetra-ene and acetaldehyde showed that it is possible to estimate the two compounds polarographically in tetramethylammonium hydroxide base solutions, at a pH value of 13. Well-defined acetaldehyde waves were obtained (Fig. 1). Hydrolysis

TABLE II

CALIBRATION DATA FOR *cyclo*OCTATETRA-ENE AND VINYL *cyclo*OCTATETRA-ENE

*cyclo*Octatetra-ene $m^{\frac{2}{3}}t^{\frac{1}{6}}$, 2·01 $mg^{\frac{2}{3}}$ $sec.^{-\frac{1}{2}}$ at 20° C		Vinyl *cyclo*octatetra-ene $m^{\frac{2}{3}}t^{\frac{1}{6}}$, 2·35 $mg^{\frac{2}{3}}$ $sec.^{-\frac{1}{2}}$ at 20° C	
Concentration, mg per 100 ml	Diffusion current, μa	Concentration, mg per 100 ml	Diffusion current, μa
2·0	0·80	2·0	1·00
4·0	1·56	4·0	2·04
6·0	2·36	6·0	3·16
8·0	3·14	8·0	4·12
10·0	3·92	10·0	5·16
Diffusion current constant[4]—2·54		2·28	

with hydrochloric acid, for times varying from five minutes to two hours, failed to produce any acetaldehyde; there was a variable reduction of the vinyl *cyclo*octatetra-ene wave after this treatment owing to polymerisation to the dimer. No basis for a separation of the two compounds appears to be possible on these lines.

cis-1-PHENYL-1:3-BUTADIENE—

This compound, which occurs in the $C_{10}H_{10}$ fraction from *cyclo*octatetra-ene, was found to be non-reducible at the dropping-mercury electrode over the whole of the pH ranges tried. Tetrahydrofurfuryl alcohol, di*iso*amyl acetal, and diethylcarbitol, which are solvents for *cyclo*octatetra-ene, were also examined and found to be non-reducible.

The criteria of purity of the compounds examined are—

cyclo*Octatetra-ene*—Redistilled; $n_D^{25} = 1{\cdot}5347$. (Elofsen;[1] $n_D^{35} = 1{\cdot}5342$; Benson and Cairns,[5] $n_D^{25} = 1{\cdot}5350$.)

Vinyl cyclo*octatetra-ene*—Freshly distilled material was used for all experiments, as the pure material polymerises slowly on standing, to form an orange glass; $n_D^{25} = 1{\cdot}5695$. B.p. 83° C at 21 mm of mercury (Craig and Larrabee;[2] $n_D^{25} = 1{\cdot}5682$, b.p. 83·3° C at 20 mm of mercury).

METHOD

The following method is proposed for the estimation of small concentrations of *cyclo*-octatetra-ene or vinyl *cyclo*octatetra-ene.

REAGENT—

Base solution—Dilute 9·1 g of tetramethylammonium hydroxide solution (10 per cent. in water as obtained from the suppliers) to 100 ml with 50 per cent. v/v of aldehyde-free ethanol.

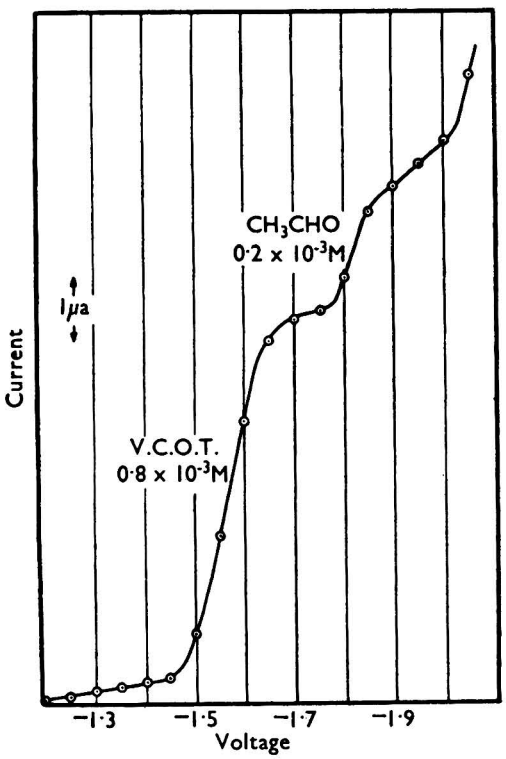

Fig. 1. Polarogram of vinyl *cyclo*octatetra-ene and acetaldehyde in tetramethylammonium hydroxide base solution

PROCEDURE—

Prepare a solution of the sample in aldehyde-free ethanol so that 5 ml contains up to 2 mg of *cyclo*octatetra-ene. Place exactly 5 ml of the base solution into a dry container, by means of a pipette, and then 5 ml of the sample solution. Mix and transfer to the polarographic cell and remove oxygen by passing a stream of nitrogen for 5 minutes. No significant loss of *cyclo*octatetra-ene occurs during this period. Polarograph over the range − 1·2 to − 1·9 volts, measured against the saturated calomel electrode, and calculate the *cyclo*octatetra-ene content by reference to the calibration curve. This method will give a measure of the combined *cyclo*octatetra-ene and vinyl *cyclo*octatetra-ene contents of the samples, if both compounds are present.

DISCUSSION

Vinyl *cyclo*octatetra-ene and the parent compound *cyclo*octatetra-ene showed almost identical behaviour at the dropping-mercury electrode. The electrode reaction appears to be reversible with both compounds, and a linear relation exists between cathode potential and $\log i/(i_d - i)$. By applying values obtained from this relation, the number of electrons

involved in the reduction has been shown to be two with both compounds; the relevant values for n fall between 1·9 and 2·1 in all the experiments.

Elofsen's findings with respect to the independence of half-wave potential and pH have been confirmed for *cyclo*octatetra-ene, and support has been gained for his theory of the electrode reaction.

All the compounds examined were prepared in the Research Laboratory. The authors are indebted to the Directors of the British Oxygen Company for permission to publish their findings.

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The Absorptiometric Determination of Zirconium by means of Alizarin-S with Special Reference to Magnesium Alloys

BY A. MAYER AND G. BRADSHAW

It is shown that the coloured reaction product of zirconyl ions and alizarin sulphonate is of definite composition and stable in acid solution. The reaction is almost specific for zirconium and can be used for its determination in magnesium alloys, iron and steel, and minerals. A rapid method for determining the approximate composition of hafnium - zirconium salt mixtures is also indicated.

The method compares favourably in accuracy, speed and cost with the standard gravimetric procedure.

THE determination of zirconium in magnesium alloys assumed major importance when the remarkable mechanical properties of these alloys was realised. They are to-day one of the major groups of magnesium-base alloys widely used in aircraft construction. The composition generally varies within fairly narrow limits, and the alloys may contain up to 5 per cent. of zinc, 3 per cent. of rare earths (of the Mischmetall type), and not more than about 1 per cent. of zirconium. A small proportion of the alloys contains thorium. The zirconium is present to a large extent in solid solution, 1 per cent. representing its solid solubility. A small fraction of the zirconium is present as intermetallic compounds with iron, manganese, aluminium and silicon; these are insoluble in magnesium and only sparingly soluble in mineral acids, but are soluble in hydrofluoric acid. In general, the insoluble portion is of little interest and, for the purpose of analysis, the only account taken of it is in specifying the concentration and amount of acid used for solution.

The method of analysis laid down in D.T.D. specifications involves solution in acid, separation of zirconium with ammonium hydroxide, separation from rare earths by hydrolysis with hexamine, precipitation of zirconium arsenate and ignition, removing arsenic by treatment with sugar charcoal during a further ignition and weighing as the oxide. Although accuracy can be attained by this procedure it is tedious, lengthy and expensive; silica crucibles used for the determination rarely outlast four ignitions without seriously flaking. Furthermore, the conversion factor is rather large (0·7403).

The same criticisms apply to the many organic precipitants of the arsonic acid type. Precipitation as phosphate necessitates preliminary separations, and the ignited precipitate

of pyrophosphate is frequently grey in spite of prolonged ignition, and sometimes contains slightly less zirconium than would correspond to ZrP_2O_7.

A great deal of literature has appeared recently recommending various organic precipitants for separating zirconium from rare earths and thorium; these include mandelic acid,[1] *p*-chloro- and *p*-bromo-mandelic acid and other glycollic acid derivatives,[2,3] phthalic acid,[4] sodium flavianate,[5] *m*-cresoxyacetic acid,[6] hydrazine sulphate,[7] tannin,[8,9] aneurine and adenylpyrophosphoric acid,[10] and cupferron.[11] With the exception of *p*-bromo-mandelic acid all these reagents finally yield the oxide, so that comparatively large weights of sample are necessary to give a reasonable degree of accuracy. The bromo-mandelic acid - zirconium compound can be dried and weighed, and it is probably the most promising of the gravimetric reagents, but so far supplies of the reagent have not been readily available.

After a careful review of the many colorimetric reagents it was found that alizarin was most promising since it was almost specific for zirconium. Alizarin, purpurin and quinalizarin

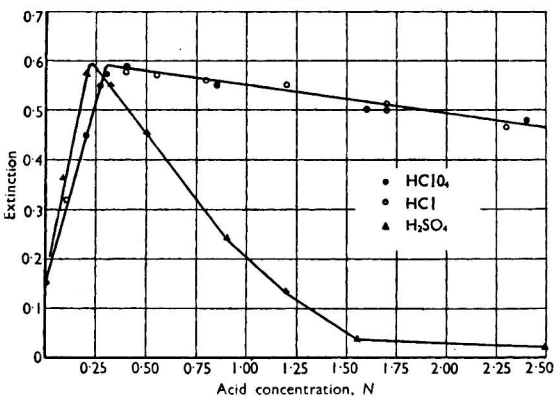

Fig. 1. Effect of acid on a partly hydrolysed zirconium solution

form strongly coloured lakes with a number of cations, of which the zirconium and hafnium lakes are the only ones that persist in acid solution.

Experimental

Zirconyl chloride octahydrate ($ZrOCl_2.8H_2O$) was purified by recrystallisation from concentrated hydrochloric acid, washed with acetone and dried in air. It was found to correspond to the formula within the error of the assay; iron and titanium were just detectable spectrographically. No allowance was made for the 0·4 per cent. of hafnium present and all subsequent reference to zirconium will mean zirconium plus residual hafnium. A Spekker absorptiometer fitted with a tungsten-filament lamp was used for the determination.

Choice of colour filters—

Green[12] and Flagg, Liebhafsky and Winslow[13] state that the absorption maximum of the complex is at 520 mμ, corresponding to the Ilford green No. 604 filter; this was found to be true, but, since the alizarin-S has an extinction of 0·10 for a 2-cm cell at this wavelength, it is preferable to measure at 560 mμ with yellow-green Ilford No. 605 filters, the extinction for a 2-cm cell then being 0·02.

Effect of temperature and acid on colour development—

It is well known that zirconium solutions hydrolyse rapidly and that precipitation of hydroxide occurs at a pH of 2·8. Even before precipitation occurs hydrolysis takes place. When alizarin-S solution is added to a freshly prepared zirconium solution a red colour develops. In the cold this reaction is slow; Green[12] allows the colour to develop for 20 hours and Sandell[14] recommends 1 hour. Green recommends 0·1 *N* and Sandell a 0·2 *N* hydrochloric acid solution; we believe that this acidity is not great enough to overcome hydrolysis of zirconium solutions. In one method in which the colour was developed at room temperature for 1 hour in *N* hydrochloric acid solution fairly good results were given, but occasionally

low results were obtained, which we ascribe to hydrolysis. All these difficulties can be overcome by developing the colour above 85° C for 2 minutes in 1·5 *N* acid. This increases the rate of reaction with alizarin-S and also converts hydrolysed zirconium compounds to zirconyl ions.

The effect of acid on a partly hydrolysed zirconium solution is illustrated in Fig. 1. It can be seen that hydrochloric and perchloric acids act in the same way, and even sulphuric acid causes a similar increase in extinction owing to the arrest of hydrolysis. However, an excess of sulphuric acid rapidly reduces the extinction owing to formation of complex zirconium ions, whilst the extinction decreases only slowly with increase in hydrochloric or perchloric acid concentrations.

There are two basic preliminary reactions that determine the combination of zirconium cations with alizarin-S anions—

$$(1)\quad ZrO^{\cdot\cdot} + H_2O \rightleftharpoons ZrO(OH)^{\cdot} + H^{\cdot}$$

or

$$Zr^{\cdot\cdot\cdot\cdot} + H_2O \rightleftharpoons Zr(OH)^{\cdot\cdot\cdot} + H^{\cdot};$$

and

$$ZrO(OH)^{\cdot} + H_2O \rightleftharpoons ZrO(OH)_2 + H^{\cdot}$$

or

$$Zr(OH)^{\cdot\cdot\cdot} + H_2O \rightleftharpoons Zr(OH)_2^{\cdot\cdot} + H^{\cdot}.$$

The question of whether $ZrO^{\cdot\cdot}$ or $Zr^{\cdot\cdot\cdot\cdot}$ ions react has not been settled, but it does not affect the issue under discussion.

$$(2)\quad HA \rightleftharpoons A' + H^{\cdot},$$

where A = alizarin-S.

Therefore, enough hydrogen ions are needed to counteract hydrolysis of the zirconyl ions, but a large excess must be avoided or the dissociation of HA will be largely prevented, and not enough alizarin-S ions will be available to form the zirconium - alizarin-S complex.

From experimental evidence the optimum conditions for the development of the zirconium - alizarin-S compound were found to be as follows. Exactly 10 ml of standard zirconium solution were added to a 100-ml graduated flask, and subsequently 3 ml of 11·3 *N* hydrochloric acid and 10 ml of 0·15 per cent. w/v alizarin-S solution in water. The colour was developed by heating in a water-bath at 100° C for 2½ minutes; after it had been cooled, the solution was diluted to 100 ml and its absorption measured. Under these conditions hydrolysed zirconium compounds are converted to $ZrO^{\cdot\cdot}$ or $Zr^{\cdot\cdot\cdot\cdot}$ ions; with very old zirconium solutions irreversible hydrolysis may occur and the less acid present the more pronounced will be this effect. This is illustrated in Table I.

In these tests 3 ml of hydrochloric acid were present, but the solutions were allowed to stand for various times and at various dilutions before the alizarin-S was added. All solutions were heated to 85° C to develop the colour.

TABLE I

EFFECT OF ACID CONCENTRATION ON HYDROLYSIS OF ZIRCONIUM SOLUTIONS

Normality of hydrochloric acid solutions during colour development	Zirconium found after setting the solutions aside before developing the colour for		
	1 hour	70 hours	266 hours
	%	%	%
1·5	0·625	0·625	0·625
1·15	0·64	0·625	0·615
0·7	0·635	0·615	0·55
0·5	0·63	0·60	0·505

It follows that the volumes of the solutions during colour development are not critical, provided that permanent hydrolysis has not taken place. Hydrolysis can also occur when zirconium solutions containing a small excess of acid are boiled, and in general it is not safe to boil a solution with an acid concentration of less than 0·5 *N*. The acidity of the solution of the zinc - zirconium magnesium alloy used in the tests in Table I was 0·5 *N* and the figures indicate that under the recommended conditions for colour development no permanent hydrolysis had taken place.

From equations (1) and (2) it follows that when an excess of acid is present the maximum colour will not be developed. Since colour development is carried out in a volume of 22·5 ml and solutions are later diluted to 100 ml, excess of acid prevents the formation of the full colour in the more concentrated solution, but on dilution the colour increases slowly with

time. Hence, a solution containing 5 ml of acid instead of 3 ml gave an extinction of 0·78 after 10 minutes and this increased to 0·855 after 20 hours. The extinction for this solution should have been 0·865. Once the colour has been developed and the solution has been cooled, further addition of acid makes little difference. For example, after colour development and cooling an extra 2 ml of acid were added to a similar solution and the extinction was found to be 0·855; it increased to 0·865 after 4 hours.

AMOUNT OF ALIZARIN-S AND RANGE—

The addition of various amounts of alizarin-S solution to zirconium solutions showed that at least 6 ml of aqueous alizarin-S solution (0·15 per cent. w/v) were required for maximum development of colour when 0·74 mg of zirconium is present per 100 ml. An addition of 10 ml of alizarin-S was found to be adequate for the range 0 to 0·8 mg of zirconium per 100 ml. Up to 1 mg of zirconium can be tolerated in 100 ml of solution, but with larger concentrations precipitation occurs.

When a calibration graph was prepared for the range 0 to 0·15 mg of zirconium, only 2 ml of alizarin-S solution were added for use in a 10-cm cell to reduce the optical density of the blank. The volume during colour development was adjusted to 23 ml by the addition of water. Under these conditions a satisfactory graph was obtained, which was linear for between 0·04 and 0·15 mg of zirconium per 100 ml and had a slightly smaller slope than that obtained with 10 ml of alizarin-S present. For 0 to 0·04 mg of zirconium per 100 ml the graph curves.

This method can be used as a semi-micro method for zirconium, the range 0 to 1·5 per cent. of zirconium being covered with a 10-mg sample.

EFFECT OF TEMPERATURE—

After development of the colour and cooling, change in temperature makes little difference. The extinction changed from 0·30 to 0·315 for an increase in temperature from 13·5° to 28° C.

EFFECT OF CATIONS—

The following amounts of various cations did not interfere: 100 mg of Mg^{++}, 50 mg of rare earths, 50 mg of Zn^{++}, 100 mg of Na^{+}, K^{+}, Cd^{++} or Mn^{++}, 50 mg of Li^{+}, Cu^{++}, Ag^{+} (in perchloric acid), Ca^{++}, Sr^{++}, Ba^{++}, Pb^{++}, Tl^{+++}, Bi^{+++}, 100 mg of Fe^{++}, 11 mg of In and 2·2 mg of Ni^{++}. A slight colour, which can be allowed for by means of a correction graph, was given by Al^{+++} and Sn^{++}; 100 mg of $Al^{+++} \equiv 0{\cdot}0075$ mg of zirconium, and 100 mg of Sn^{++} or $Sn^{++++} \equiv 0{\cdot}03$ mg of zirconium. Oxidising agents, such as Ce^{++++}, bleach the colour. Under the recommended conditions any cerium will be present in the cerous state. If for some reason ceric ions are present, their interference can be overcome readily by reducing them with hydroxylamine hydrochloride.

$Iron^{III}$ interferes, and Sandell[14] suggests the use of thioglycollic acid to reduce the iron to the ferrous state. We have confirmed Sandell's observations that a large amount of thioglycollic acid interferes, as is shown in Table II. Hence, we consider it safer to use stannous chloride for the reduction of ferric iron. This gives rise to a very slight colour, which can be allowed for easily by adding a similar amount of stannous chloride to the blank. In the recommended procedure for magnesium alloys the amount of ferric iron that can be formed is much too small to make any difference to the results.

TABLE II

INTERFERENCE BY THIOGLYCOLLIC ACID

Zirconium taken, mg	Thioglycollic acid (96%) added, ml	Zirconium found, mg
0·21	0·25	0·21, 0·20
0·74	0·25	0·735, 0·725
0·74	0·5	0·71
0·74	1·0	0·67

EFFECT OF HAFNIUM—

All the work described was carried out on solutions containing residual amounts of hafnium. The effect of hafnium was tested separately with a solution prepared from 97 per cent. hafnium dioxide. Exactly the same procedure was followed as described for zirconium

for the construction of a calibration graph. This graph was linear over the range 0·2 to 0·8 mg of hafnium per 100 ml. For less than 0·2 mg the graph curved to zero. The molar extinction was found to be almost exactly one-quarter of that of zirconium. The large difference in extinction makes it possible to use this procedure for determining the hafnium content of, say, a sample of mixed oxides. It is only necessary to weigh the mixed oxides accurately and to determine the extinction by the method described. This method is accurate to about ±2 per cent. of hafnium dioxide in zirconium dioxide and so is suitable for rapidly grading such samples.

EFFECT OF THORIUM—

When the proposed procedure is applied to samples containing zirconium and thorium high results are obtained. With large amounts of thorium precipitation occurs. Thorium alone gives a slight colour with alizarin-S under these conditions. The absorption of a solution containing thorium and zirconium is greater than the sum of the absorptions measured separately. This interference can be overcome by adding 2 ml of hydrochloric acid after the usual development of the colour and cooling. Results in Table III indicate that up to 10 mg of thorium per 100 ml can be tolerated. There is a slight tendency for the extinction to increase with time, and it is recommended that the measurement should be carried out within 1 hour of colour development. Ten milligrams of thorium per 100 ml is equivalent to an alloy containing 10 per cent. of thorium, which is a percentage well above that likely to be present in magnesium alloys.

TABLE III

EFFECT OF ADDITION OF THORIUM TO 0·1-g SAMPLES OF MAGNESIUM - ZIRCONIUM ALLOYS

Thorium added, %	Zirconium present, %	Zirconium found after colour development for ½ hour	1 hour	2 hours	3 hours
10	0·21	0·215, 0·215	0·22, 0·22	0·23, 0·23	0·23, 0·23
10	0·74	0·74, 0·75	0·74, 0·75	0·75, 0·76	0·745, 0·755
20	0·74	0·77			0·815
40	0·74	Precipitate present, not measured			

The same calibration graph can be used as in the normal determination, since the extra amount of hydrochloric acid makes no difference to the zirconium colour.

EFFECT OF ANIONS—

Chloride or perchlorate does not interfere; nitrate bleaches the colour; fluoride, oxalate and organic hydroxy acids prevent the formation of the colour; orthophosphate in small amounts can be tolerated and with a solution containing 0·74 mg of zirconium the values shown in Table IV were obtained after adding diammonium hydrogen phosphate.

TABLE IV

INTERFERENCE BY ORTHOPHOSPHATE IN A SOLUTION CONTAINING 0·74 mg OF ZIRCONIUM

Orthophosphate (PO_4''') added, mg..	0·03	0·06	0·12	0·15	0·3
Zirconium found, mg	0·74	0·735	0·73	0·72, 0·73	0·71, 0·69

Results are low when more than 0·1 mg of orthophosphate is added and this effect is even more pronounced when less zirconium is present. Magnesium - zirconium alloys generally contain not more than 0·001 per cent. of phosphorus. Furthermore, on solution in hydrochloric or dilute perchloric acid most of this phosphorus is lost by evolution as phosphine.

Up to 10 mg of sulphate per 100 ml cause no interference, larger amounts reduce the absorption. With solutions containing 0·74 mg and 0·21 mg of zirconium per 100 ml, the results shown in Table V were obtained.

TABLE V

INTERFERENCE BY SULPHATE IN 100 ml OF SOLUTION

Sulphate added, mg ..	0	0	10	10	20	20	40	60	100
Zirconium added, mg ..	0·21	0·74	0·21	0·74	0·21	0·74	0·74	0·74	0·74
Zirconium found, mg ..	0·21	0·74	0·21	0·74	0·20	0·73	0·72	0·695	0·645

At least 3·5 mg of silicon per 100 ml can be tolerated in solution. The effect of silicon was tested by adding sodium silicate to a solution containing 0·74 mg of zirconium and results are shown in Table VI.

TABLE VI

INTERFERENCE BY SODIUM SILICATE IN A SOLUTION CONTAINING 0·74 mg OF ZIRCONIUM

Silicon added, mg	3·5	7·0	14·0
Zirconium found, mg	0·74, 0·75	0·73, 0·73	0·71, 0·69

COMPOSITION OF THE ZIRCONIUM - ALIZARIN-S COMPOUND—

Various amounts of alizarin-S solution were added to 0·25, 0·5 and 0·74 mg of zirconium and the colours measured on an absorptiometer at 560 mμ; as the absorption of the excess of dye is negligible at this wavelength, the values obtained represent the true absorption of the zirconium - alizarin-S compound. The absorption was then measured at 430 mμ, when the dye showed greater absorption than the compound. There was a change in slope at a ratio of about 1·0 mole of alizarin per mole of zirconium.

It is assumed that below this value the absorption was due to the pure compound and above this value it was due to compound plus excess of dye. From these results the excess of alizarin-S at all the points was calculated and gave the results shown in Table VII.

TABLE VII

COMPOSITION OF THE ZIRCONIUM - ALIZARIN-S COMPOUND

Ratio of moles of alizarin to zirconium added	Total alizarin, moles × 10^{-6}	Free alizarin, moles × 10^{-6}	Total zirconium, moles × 10^{-6}	Combined alizarin, moles × 10^{-6}	Combined zirconium, moles × 10^{-6}	Ratio of combined moles of alizarin to zirconium
0·27	2·19	0	8·16	2·19	1·85	1·17
0·54	4·38	0	8·16	4·38	3·6	1·22
0·81	6·58	0	8·16	6·58	5·38	1·22
1·08	8·76	0·66	8·16	8·10	6·75	1·20
1·35	11·0	1·75	8·16	9·25	7·35	1·26
1·89	15·3	5·3	8·16	10·0	7·8	1·27
2·43	19·7	9·2	8·16	10·5	8·1	1·28
3·24	26·3	15·5	8·16	10·8	8·2	1·30

From the results of 15 such calculations the average was found to be 1·26 ± 0·05 moles of alizarin-S per mole of zirconium.

These results appear to be in line with those obtained by other workers. Flagg, Liebhafsky and Winslow[13] found the ratio to be between 1·0 and 1·4 with alcoholic alizarin (not alizarin-S) solution. From the data given by Green[12] it can be calculated that about 0·95 mg of alizarin-S is required to combine with 0·275 mg of zirconium, and this gives a molar ratio of 1·24. These last figures are open to doubt, since Green gives no indication of the purity of his alizarin-S.

All our work, except that on the composition of the compound and on the effect of sulphate, was carried out with the commercially available dye. This was found to contain on the average about 30 per cent. of sodium sulphate. It cannot be purified by precipitating the sulphate with barium salts because the alizarin appears to prevent the precipitation of barium sulphate. For the study of the complex, the commercial dye was purified by extraction with aqueous alcohol and dried at 110° C; it assayed better than 99 per cent. purity.

In a recent study of the zirconium - chloranilate complexes by Thamer and Voigt[15] it was found that two complexes existed in M and 2 M perchloric acid: $ZrCh_2$ which is not very stable and $ZrCh^{\cdot\cdot}$ which is stable.

No average value was given but it seems reasonable that this would be slightly greater than unity. A precipitate of zirconium and chloranilic acid was found to be of composition $ZrCh_{1\cdot24\pm0\cdot09}$. Chloranilic acid, like alizarin-S, has a quinone structure and it seems reasonable to assume that two complexes are also formed with alizarin-S. Whatever the explanation for the observed ratio of 1·26:1, it seems clear that, under the specified conditions, the complex is of constant average composition.

METHOD

REAGENTS—

Concentrated hydrochloric acid, 11·3 N.

Sodium alizarin sulphonate solution—Dissolve 1·5 g in about 300 ml of hot water, filter through a pulp pad and dilute to 1 litre.

PROCEDURE—

Weigh out accurately 3·5 to 4·5 g of sample (see Note 1). Transfer it to a 250-ml beaker and add 20 ml of water plus 10 ml of hydrochloric acid per gram of sample. When solution is complete boil for 5 minutes, filter through a pulp pad and wash with hot water containing a few drops of hydrochloric acid.

Cool the filtrate, transfer it to a 500-ml graduated flask, dilute to the mark and mix well. Transfer exactly 10 ml to a 100-ml graduated flask, add 2·5 ml of 11·3 *N* hydrochloric acid from a burette and 10 ml of alizarin sulphonate solution and heat on a water-bath at 100° C for not less than 2½ and not more than 3½ minutes. Cool (Note 2) and dilute to 100 ml (Note 3). Measure the extinction at 560 mμ. If a Spekker absorptiometer is used, use 2-cm cells, Ilford yellow-green filters No. 605 and H.503 heat filters, and a water - water setting of 1·00. Carry out a blank determination on the reagents and deduct the extinction; the blank should not exceed 0·03.

PREPARATION OF THE CALIBRATION GRAPH—

Dissolve 8·85 g of pure zirconyl chloride octahydrate in 250 ml of water and 50 ml of 11·3 *N* hydrochloric acid. Filter if necessary and dilute to 500 ml. Standardise this solution by precipitating zirconium as hydroxide with ammonium hydroxide, ignite and weigh as zirconium dioxide. One millilitre of the solution should contain approximately 5 mg of zirconium. Dilute this solution so that 1 ml ≡ 0·1 mg of zirconium.

Add various amounts of this solution to 100-ml graduated flasks containing 0·1 g of magnesium as neutral magnesium chloride. Make each solution up to 10 ml with water. Add 3 ml of 11·3 *N* hydrochloric acid from a burette and 10 ml of alizarin-S solution. Heat to develop the colour, and continue as in the recommended procedure.

NOTES ON THE PROCEDURE—

1. For alloys containing less than 0·3 per cent. of zirconium use about 6 g of sample.
2. If thorium is present add 2 ml of 11·3 *N* hydrochloric acid before diluting to 100 ml, and measure within 1 hour.
3. When a large amount of zirconium is present a precipitate may appear on cooling, but this will redissolve on diluting to 100 ml.

CONCLUSIONS

OTHER APPLICATIONS—

The method has been used to determine the composition of segregates in metallurgical specimens on a semi-micro scale with 10-mg samples. It can be applied to materials other than magnesium alloys, for instance, clays or rocks, but a correction should be made if large amounts of tin or aluminium are present, and ferric iron should be reduced by adding stannous chloride. Another possible application is in the determination of zirconium in ferrous materials. It would then be necessary to ensure that all the iron was present in the ferrous state by adding stannous chloride to the solution and a similar amount to the blank. Difficulty would only be experienced if phosphorus was present in these materials, as not all the phosphorus is removed as phosphine during solution, and small amounts of phosphate in solution would cause the precipitation of zirconium phosphate. The insoluble residue would have to be examined for zirconium in the usual manner.

As already pointed out the procedure can be used for determining the hafnium and zirconium contents of binary mixtures of these elements.

TYPICAL RESULTS—

To check the accuracy of the method, 35 determinations, spread over a fortnight, were made by two analysts using a sample free from zirconium to which the equivalent of 0·50 per cent. of zirconium was added. The mean of all the results gave 0·503 per cent. of zirconium and the greatest differences from the mean were +0·02 and −0·015 per cent. of zirconium. The standard deviation was 0·008.

An unknown sample was then checked by thirteen analysts. The mean of 52 results was found to be 0·675 per cent. of zirconium and the greatest differences from the mean were ±0·02 per cent. of zirconium, whilst the standard deviation was 0·009. The method was compared with the gravimetric procedure described in the specifications D.T.D. 711 and 721 (5 per cent. zinc - zirconium magnesium alloy), and with a modified gravimetric method applicable to alloys containing 3 per cent. of rare earths. Typical results are shown in Table VIII.

TABLE VIII

COMPARISON OF THE RESULTS BY GRAVIMETRIC AND ABSORPTIOMETRIC METHODS

Zirconium found in a zirconium - magnesium alloy containing 5 per cent. of zinc—						
Gravimetrically, %	0·61	0·29	0·75	0·69	0·43
Absorptiometrically, % ..	0·62	0·31	0·73	0·68	0·43
Zirconium found in a zirconium - magnesium alloy containing 3 per cent. of rare earths and 3 per cent. of zinc—					
Gravimetrically, %	0·59	0·64	0·68		
Absorptiometrically, % ..	0·59	0·66	0·67		

The authors wish to express their thanks to the Chairman and Board of Directors of Magnesium Elektron Limited for permission to publish this report.

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The Volumetric Determination of Calcium and Magnesium by the Ethylenediamine Tetra-Acetate Method

By J. BANKS

A description is given of work carried out to investigate the determination of calcium and magnesium in siliceous materials by titration with di-sodium ethylenediamine tetra-acetate. The significance and removal of interfering ions is considered and the results of analyses of typical solutions presented.

Much attention has been paid to the work of Schwarzenbach, Biedermann and Bangerter[1] and of Biedermann and Schwarzenbach[2] on the determination of hardness in water with di-sodium ethylenediamine tetra-acetate as titrant. Investigations have also been made by American workers[3,4,5,6,7] and by the "Alfloc" division of Imperial Chemical Industries Ltd.[8] on the application of this reagent, and methods have been published for the separate determination of calcium hardness and magnesium hardness. In view of this, the determination of calcium and magnesium in other materials, particularly in coal ash and refractory materials, appeared to be possible by applying the same methods, or a modification of them.

As a general application of the method would be dependent upon its capacity for dealing with calcium and magnesium in any proportions, the following experimental work was undertaken to test its capabilities.

Experimental

Reagents—

*Di-sodium ethylenediamine tetra-acetate**—A 0·02 *N* solution, 1 ml = 0·56 mg of calcium oxide, or 0·40 mg of magnesium oxide.

*Indicators**—Eriochrome Black T (for total hardness) and Murexide (for calcium hardness).

Standard calcium solution—A stock solution was prepared by dissolving pure calcium carbonate in the minimum volume of dilute hydrochloric acid and diluting with distilled water. It contained approximately the equivalent of 1·0 g per litre of calcium oxide. This stock solution was diluted ten-fold to provide the test solution, of which 1 ml contained approximately 0·1 mg of calcium oxide.

Standard magnesium solution—A stock solution was prepared by dissolving pure magnesium sulphate ($MgSO_4.7H_2O$) in distilled water. It contained the equivalent of 1·0 g of magnesium, as MgO, per litre. This stock solution was diluted ten-fold to provide the test solution, of which 1 ml contained 0·1 mg of magnesium, as MgO.

The concentrations of the stock solutions of calcium and magnesium were verified by the conventional gravimetric methods.

From the dilute test solutions, mixtures were made as follows—

Calcium solution, ml—100, 90, 80 . . . 10, 0

Magnesium solution, ml—0, 10, 20 . . . 90, 100

Each mixture was prepared in quadruplicate and each had a volume of 100 ml.[8] With one pair of solutions duplicate determinations of the combined titre for both metals were made, and with the other, determinations of the titre for calcium alone. The titre due to magnesium was found by difference.

Buffer solutions—

*Ammonia - ammonium chloride buffer solution.**

Sodium hydroxide—A 4 *N* solution.

* The di-sodium ethylenediamine tetra-acetate solution, the indicators and the ammonia - ammonium chloride buffer solution were supplied ready for use by Messrs. B.D.H., who market them under the "Alfloc" label.

TITRATION OF CALCIUM AND MAGNESIUM—

To the 100-ml sample, 2 ml of ammonia - ammonium chloride buffer solution and 10 drops of Eriochrome Black T indicator were added. The solution was titrated with 0·02 *N* di-sodium ethylenediamine tetra-acetate to a pure blue end-point.

TITRATION OF CALCIUM ONLY—

To the second 100-ml sample, 1 ml of 4 *N* sodium hydroxide solution and 0·2 g of Murexide were added. Titration with di-sodium ethylenediamine tetra-acetate was carried out to the violet end-point. The results of the tests on standard solutions are shown in Table I.

TABLE I

TITRATION OF MIXTURES OF STANDARD SOLUTIONS OF CALCIUM AND MAGNESIUM

Mixture No.	Calcium as CaO		Magnesium as MgO	
	Present, mg	Found, mg	Present, mg	Found, mg
1	9·81	9·82, 9·82	nil	nil, nil
2	8·83	8·77, 8·84	1·00	1·02, 1·00
3	7·85	7·82, 7·82	2·00	2·03, 2·05
4	6·87	6·84, 6·82	3·00	3·02, 3·07
5	5·89	5·97, 5·95	4·00	3·90, 3·97
6	4·91	4·91, 4·92	5·00	5·00, 4·98
7	3·92	3·92, 3·92	6·00	5·96, 5·96
8	2·94	2·97, 2·96	7·00	6·95, 6·93
9	1·96	1·99, 1·93	8·00	7·99, 8·02
10	0·98	1·01, 0·98	9·00	8·98, 8·99
11	nil	nil, nil	10·00	10·04, 10·04

To ascertain the reproducibility of the method, two operators determined the calcium and magnesium present in three samples containing various amounts of the test solutions. The results are shown in Table II.

TABLE II

REPRODUCIBILITY OF THE METHOD

Mixture No.	Calcium as CaO			Magnesium as MgO		
	Present, mg	Found, by operator A, mg	by operator B, mg	Present, mg	Found, by operator A, mg	by operator B, mg
1	8·34	8·42	8·46	1·50	1·58	1·58
2	4·51	4·50	4·51	5·40	5·52	5·45
3	1·18	1·17	1·12	8·80	8·80	8·81

PRACTICAL APPLICATION—

The determination of calcium and magnesium by di-sodium ethylenediamine tetra-acetate is subject to interference from most of the constituents of the materials under consideration, principally copper, iron, aluminium, manganese and phosphate.[8] All of these, however, are removed and determined in any scheme of analysis of such compounds before determining calcium and magnesium, which are left in a solution containing in addition only sodium, potassium and ammonium salts.

It was expected that the practical use of the method would be straightforward. The first tentative attempt to apply it was in the analysis of a sample of slimes from a surface condenser. After the removal of the interfering metals (copper, iron, aluminium and nickel were present, but no phosphate), the solution was diluted to 1 litre and 100-ml aliquots were treated as in the experimental titrations.

On addition of Eriochrome Black T to the solution after buffering it with 2 ml of ammonia - ammonium chloride buffer solution, the indicator assumed a purple tinge quite

different from the pink colour obtained in pure solutions. On titration, an end-point could not be reached.

In the calcium titration, the addition of Murexide after buffering with 4 *N* sodium hydroxide again gave an incorrect indicator colour and the violet end-point was unattainable.

The behaviour of Eriochrome Black T varies with the pH value. At pH values below 6·3, the indicator is red in colour; at all values of pH between 6·3 and 11·5, the colour is blue. At pH 10, however, magnesium in the solution causes the indicator to assume the red colour normally exhibited at pH 6·3 or less (it was found that calcium gave the same effect). At the end-point of the titration with di-sodium ethylenediamine tetra-acetate, the blue colour of the indicator is restored.

In the titration of calcium with Murexide indicator, the necessary pH value is also about 10. It was therefore apparent that, even after addition of the appropriate buffer, the solutions were not of the correct pH value. This was probably caused by the hydrolysis of the considerable quantity of ammonium chloride added in the removal of the iron group (a double precipitation was used, each preceded by the addition of 5 g of ammonium chloride). Further ammonium chloride is formed in the several neutralisation processes carried out during the analysis.

Preliminary experiments—

Attempts were made to remove the ammonium chloride by (*i*) volatilisation and (*ii*) boiling with an excess of a concentrated sodium hydroxide solution. Both methods, besides being time-consuming, were unsuccessful in yielding the correct indicator colour. The possibility of overcoming the effect of ammonium chloride was investigated by (*i*) replacing the ammonia - ammonium chloride buffer solution by ammonium hydroxide only (since the chloride was already present) and (*ii*) increasing the volume of 4 *N* sodium hydroxide.

To 100-ml aliquots of the standard calcium solution, 1·5 g of ammonium chloride were added (this was the concentration calculated to be present in practice). To one aliquot, ammonium hydroxide (sp.gr. 0·880) was added in such quantity that the ratio of ammonium hydroxide to ammonium chloride was identical with that in the buffer recommended by Diehl, Goetz and Hach.[5] On addition of Eriochrome Black T, the correct red colour was obtained and a satisfactory end-point reached on titration with di-sodium ethylenediamine tetra-acetate.

The correct volume of 4 *N* sodium hydroxide was found by trial on a second 100-ml aliquot, and conditions were established which gave a satisfactory end-point with Murexide.

These results were encouraging, and the method was applied to a series of synthetic solutions designed to represent solutions resulting from 0·5-g samples of siliceous material after the removal of silica. The solutions contained known quantities of calcium and magnesium, and in addition iron (added as ferric ammonium sulphate), aluminium (as potassium aluminium sulphate) and in one experiment manganese (as manganous chloride). Each solution was acidified with concentrated hydrochloric acid.

After removal of the interfering elements, the calcium and magnesium were successfully determined by titration. The complete method is as follows.

Proposed method

Take 0·5 g of sample, determine the loss on ignition, remove silica, the iron group (by a double precipitation with ammonium hydroxide, each preceded by the addition of 5 g of ammonium chloride) and manganese (by the bromine - ammonium hydroxide separation).

Dilute the filtrate from the manganese precipitation to 1 litre. This is referred to subsequently as the bulk solution.

Joint titration of calcium and magnesium—

Transfer exactly 10 ml from the bulk solution to a 250-ml conical flask and dilute to 100 ml with distilled water. Add 2 ml of ammonium hydroxide (sp.gr. 0·880) and 10 drops of Eriochrome Black T indicator. Titrate with 0·02 *N* di-sodium ethylenediamine tetra-acetate until a blue colour free from any trace of pink appears. Shake vigorously near the end-point. This titre will probably be too small for practical use, but it can be used in calculating the volume of the aliquot necessary to provide a satisfactory titre (15 to 20 ml).

Transfer this volume by means of a pipette from the bulk solution, dilute to 100 ml with distilled water and add ammonium hydroxide (sp.gr. 0·880) as follows—

Volume of aliquot, ml	20	50	100
Volume of ammonium hydroxide (sp.gr. 0·880), ml	4	6	10

Add 10 drops of Eriochrome Black T and titrate as before.

TITRATION OF CALCIUM ONLY—

Transfer exactly 10 ml from the bulk solution and dilute to 100 ml with distilled water. Add 2 ml of 4 *N* sodium hydroxide solution and 0·2 g of Murexide. Titrate with 0·02 *N* disodium ethylenediamine tetra-acetate to the violet end-point. (The end-point is reached when the addition of 2 drops of titrant produces no further change in colour.)

Calculate the volume of aliquot required for a satisfactory titre, take this volume from the bulk solution and dilute it to 100 ml with distilled water. Add 4 *N* sodium hydroxide solution as under—

Volume of aliquot, ml	20	50	100
Volume of 4 *N* sodium hydroxide to be added, ml	5	8	15

Add 0·2 g of Murexide and titrate as before. Find the titre for the magnesium by difference.

If the concentrations of calcium and magnesium are expected to be small, the filtrate from the manganese may be diluted to a smaller bulk than 1 litre. If this is done, the volumes of ammonium hydroxide and of 4 *N* sodium hydroxide solution added for a given volume of aliquot must be increased in proportion, *e.g.*, if a bulk solution of 500 ml is used, the volume of buffer solution must be doubled.

CALCULATIONS—

$$\text{Calcium present as CaO, mg} = \text{Calcium titre} \times 0{\cdot}56 \times \frac{\text{Volume of bulk solution}}{\text{Volume of aliquot}}$$

$$\text{Magnesium present as MgO, mg} = \text{Magnesium titre} \times \frac{0{\cdot}40 \times \text{Volume of bulk solutions}}{\text{Volume of aliquot}}$$

The results given by the synthetic solutions are shown in Table III.

TABLE III

ANALYSIS OF SYNTHETIC SOLUTIONS

Solution No.	Calcium as CaO		Magnesium as MgO		Other constituents present		
	Present, mg	Found, mg	Present, mg	Found, mg	Iron as Fe_2O_3, mg	Aluminium as Al_2O_3, mg	Manganese as MnO, mg
1	28·0	28·0	13·1	13·6	317·0	103·0	—
2	280·0	274·4	196·5	196·0	31·7	10·3	—
3	28·0	28·0	325·0	324·0	95·1	30·9	—
4	56·0	55·4	6·5	6·6	317·0	103·0	—
5	5·6	6·2	6·5	6·1	317·0	103·0	—
6	280·0	274·4	32·5	30·0	317·0	20·6	136·0

DISCUSSION—

The results generally show a satisfactory degree of accuracy over a wide range of calcium and magnesium concentrations and in the presence of various quantities of interfering elements. It may be that a stronger solution of the titrant, say 0·1 *N*, would give even better results at the higher concentrations, by making it possible to use larger aliquots or less dilution.

The time saved by using the volumetric method is very great. After the interfering elements have been removed, the calcium and magnesium can be determined well within an hour.

No trouble was found in judging the end-points, and titration of calcium in the absence of magnesium was straightforward and accurate. This is contrary to the findings of other workers,[2,3,5] who state that the presence of magnesium sharpens the end-point.

Copper was not added to the synthetic solutions, as it does not appear frequently in samples of refractories, etc. It can be removed after the silica by hydrogen sulphide precipitation. Traces of copper can be suppressed by adding sodium diethyl-dithiocarbamate after addition of the buffer.[5] Phosphate ion is completely removed with the iron group.

CONCLUSION—

The volumetric determination of calcium and magnesium is rapid and reasonably accurate. It is applicable to all normal samples of siliceous material after the removal of interfering ions.

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Notes

THE BROMATE TITRATION OF TERVALENT ARSENIC

FOR the titration of arsenic^{III} in hydrochloric acid solution, potassium bromate has been recommended as titrant.[1] Of the many indicators suitable for this purpose[2] either methyl orange or methyl red have been most commonly used.

During an investigation of the distillation and separation of arsenic^{III} from hydrochloric acid solution, titrations of the distillate with standard potassium bromate solution and methyl orange as indicator gave low and erratic results.

A search of the literature failed to disclose that much attention had been paid to the effect of acidity during the titration.[3,4] Schreyer, Thompson and Ockerman[5] suggested that the hydrochloric acid concentration should be limited to the range 1·5 to 2·0 *N*, but gave no figures to substantiate this suggestion.

This note reports further work performed to determine precisely the safe limits of acidity within which the bromate titration of tervalent arsenic gives quantitative recoveries.

EXPERIMENTAL

REAGENTS—

Arsenious oxide—"Baker's Analysed" tested for impurities.[6] Purity, 99·94 per cent.

Potassium bromate—Analytical reagent grade prepared by Standard Laboratories. After drying to constant weight at 120° C the purity was determined[6] and found to be 99·98 per cent.

Methyl orange—A 0·1 per cent. solution of the sodium salt in distilled water.

Hydrochloric acid—A chemically pure product containing 34·5 per cent. of hydrogen chloride by weight.

PROCEDURE—

A standard solution of potassium bromate was prepared by accurately weighing approximately 2·8 g of the dried solid and dissolving it in sufficient distilled water to make 1 litre of solution. The normality was calculated on the tested purity of the sample and the calibrated volume of the glassware used.

Accurately weighed 0·2-g portions of arsenious oxide were dissolved in 10 ml of 2 *N* sodium hydroxide solution, and then sufficient water and hydrochloric acid added to give the various acidities at the end-point (volume 170 ml). After heating to 80° C, the potassium bromate solution was added slowly with vigorous stirring to within 1 to 2 ml of the expected end-point, reheated to 80° C, 0·5 ml of indicator solution added and the titration continued dropwise until

the orange-red colour of the indicator was just discharged. Wash water was added at intervals from a graduated cylinder to ensure constant end-point volume.

RESULTS—

Five determinations were made at each of the ten levels of acidity shown in Table I; blank determinations were run at each level of acidity, due allowance for their magnitude being made in calculating recoveries. Burette corrections were noted for the same purpose.

TABLE I

RECOVERY OF ARSENIC AT DIFFERENT ACIDITIES

Acidity at end-point, hydrochloric acid, N	As_2O_3		
	taken, mg	found, mg	difference, mg
0·3	200·1	199·6	−0·5
0·6	201·5	201·1	−0·4
1·2	199·9	199·9	0
1·7	200·3	200·2	−0·1
2·2	200·8	200·7	−0·1
2·8	199·6	199·4	−0·2
3·5	201·0	200·8	−0·2
4·0	199·7	199·0	−0·7
4·6	200·1	198·0	−2·1
5·2	200·6	197·2	−3·4

Blank titrations at all levels of acidity were no greater than 0·02 ml, but end-point colour changes at acidities less than 1·2 N and greater than 3·5 N with respect to hydrochloric acid took a measurable time for completion. Within these limits, colour changes occurred instantaneously.

Statistical analysis[7] of all results indicates that titrations performed at end-point acidities outside the range 1·2 to 3·5 N are significantly in error at the 95 per cent. level of probability.

CONCLUSIONS—

Titrations of tervalent arsenic at 80° C with potassium bromate and methyl orange as indicator have been found to be accurate provided the acidity at the end-point is maintained between 1·2 and 3·5 N with respect to hydrochloric acid. Both above and below these acidities significant errors occur, the indicator becoming pronouncedly sluggish in action.

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VICTORIA, AUSTRALIA

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Ministry of Food

STATUTORY INSTRUMENT*

1952—No. 1283. The Food Standards (Ice-Cream) (Amendment) Order, 1952. Price 2d.

This Order, which came into operation on July 7th, 1952, amends the Food Standard (Ice-Cream) Order, 1951 (S.I. 1951, No. 13; Analyst, 1951, 76, 120), by substituting for the Schedule thereto, which specifies the standard for ice-cream, the following, in which heavy type indicates changes made by this order—

STANDARD FOR ICE-CREAM

1. The standard for ice-cream shall be as follows:—

Ice-cream shall contain not less than **4** per cent. fat, 10 per cent. sugar and **5** per cent. milk solids other than fat:

Provided that—

(i) ice-cream containing any fruit, fruit pulp or fruit puree shall either conform to the standard set forth above or, alternatively, the total content of fat, sugar and milk solids other than fat shall be not less than **21** per cent. of the ice-cream, including the fruit, fruit pulp or fruit puree, as the case may be, and such total content of fat, sugar and milk solids other than fat shall include not less than **6** per cent. fat, 10 per cent. sugar and 2 per cent. milk solids other than fat;

(ii) "Parev" (kosher) ice sold, offered or exposed for sale under that description shall contain not less than **8** per cent. fat and not less than **14** per cent. sugar, and the standard for ice-cream set forth above shall not apply to this product.

2. For the purpose of the standards prescribed above "sugar" means sucrose, invert sugar or the solids of any sweetening material derived from starch so however that no ice-cream shall contain less than 7½ per cent. sucrose.

3. Each reference in this Schedule to any proportion or percentage means that proportion or percentage by weight.

CIRCULAR MF 5/52*

Approved Oxidising and Preservative Agents

This circular (price 2d.), dated July 4th, 1952, refers to Circular MF 11/50 (*Analyst*, 1950, **75**, 504) *and to Circulars MF* 17/50, *MF* 5/51 *and MF* 19/51 (*Analyst*, 1951, **76**, 321 *and* 735), *and gives the name of a further product whose use for the cleansing of milk tankers, vessels or appliances has been approved by the Minister of Agriculture and Fisheries and the Minister of Food, as follows:*

ALFAKLOR

FOOD STANDARDS COMMITTEE

SACCHARIN AND OTHER SWEETENING TABLETS CONTAINING SACCHARIN

THE Minister of Food has authorised the publication of a Report of the Food Standards Committee, who have reviewed the existing temporary standards for saccharin tablets contained in the Saccharin Order, S.I., 1949, No. 945.

The Committee recommend that the standard for saccharin tablets shall be maintained and extended to cover "other sweetening tablets containing saccharin." They also recommend that the provisions with regard to the packaging and labelling of saccharin tablets shall be continued and extended to cover sweetening tablets containing saccharin, but they do not think it necessary in future to apply the description "standard" to saccharin tablets.

The Committee recommend that the standard for saccharin tablets and for other sweetening tablets containing saccharin shall be as follows—

A saccharin tablet or other sweetening tablet containing saccharin—

(*a*) shall contain not less than 0·18 grain and not more than 0·22 grain of saccharin, or the equivalent weight of soluble saccharin;

(*b*) may contain as excipient sodium bicarbonate with or without other suitable substances. The total amount of excipient shall not exceed four times the maximum quantity of saccharin;

(*c*) shall not contain more than five per cent. of water-insoluble matter, nor less bicarbonate than that required to render the saccharin completely soluble.

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

British Standards Institution

NEW SPECIFICATIONS*

B.S. 501, 554: 1952. Reports on Metric Units of Volume and Standard Temperature of Volumetric Glassware. Price 2s. 6d.
B.S. 612 : 1952. Nessler Cylinders. Price 2s.
B.S. 769 : 1952. Methods for the Chemical Analysis of Butter. Price 3s. 6d.
B.S. 1847 : 1952. Graduated Beakers for Injectable Fluids (for Hospital Use). Price 2s.
B.S. 1848 : 1952. Glass Condensers. Price 3s.
B.S. 1864 : 1952. Milk Piping and Milk Pipe Fittings. Price 5s.

AMENDMENT SLIPS*

Printed slips bearing Amendments to British Standards have been issued by the Institution as follows—

PD 1420—Amendment No. 2 (June, 1952) to B.S. 508 : 1950. Normal butyl alcohol (Butanol).
PD 1421—Amendment No. 1 (June, 1952) to B.S. 579 : 1951. Technical ether.

DRAFT SPECIFICATIONS

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

Draft Specification prepared by Technical Committee LBC/4—Thermometers.
CO(LBC)3323—Draft B.S. for General Purpose Laboratory Thermometers (Revision of B.S. 593).
Draft Specification prepared by Panel CHE/37/4/1—Analysis of Boiler Scale (Drafting).
CO(CHE)2986—Draft B.S. for Sampling and Testing of Boiler Water Deposits.

Book Reviews

VITAMIN METHODS. Edited by PAUL GYÖRGY. Volume I. Pp. x + 571. Volume II. Pp. xi + 740. New York: Academic Press Inc. 1950 and 1951. Prices $11.00 and $14.50.

Willy-nilly, we may have to acknowledge that the holographic general textbook is on its way out. Few already, and for obvious reasons, are the scientists prepared singlehandedly to write up more than one facet of a subdivision of a main subject—that is, in anything other than an elementary fashion. Increasingly, the coalition of specialists is being recognised as the most rewarding authorship of our time. Of course, this type of coalition, no less than another that comes to mind, entails a special operational problem, and this we shall return to presently. Meantime, a current example of the product of a coalition of scientists demands our attention—a lot of attention indeed, for "Vitamin Methods" is a *handbuch* of no mean size and scope.

There are perhaps half a dozen books devoted to vitamin estimation, none of which, however, can usefully be compared with the present work. "Vitamin Methods" presents not only full details, but also the history, of practically all known methods for every important vitamin. Additionally, there is a notable chapter by Norman Jolliffe giving just the right amount of information on the avitaminoses that the non-specialist needs, while, for good measure, C. I. Bliss has contributed, with his usual competence, what amounts to an internal textbook on the statistics of bio-assay. The work is therefore much more comprehensive than others of like design, or than its own modest but accurate title immediately conveys. It is devised to "serve not only workers in food technological, agricultural, chemical, commercial, pharmaceutical and clinical laboratories, but also physicians interested in problems of nutrition" and the intention is "to spare the reader as far as possible, the necessity of consulting other reference sources before a given assay method could be considered complete and workable"—a high ambition that comes a good deal closer to realisation than even the keenest might expect.

Dr. György tells us that the planning was begun more than five years before Volume I, covering chemical, physical and microbiological methods, was published in 1950. The appearance of Volume II, mainly devoted to biological and clinical matters, but including a 100-page modernising supplement to the first volume, now allows us to contemplate the stature and shape of the treatise as a whole. We see that the editor and his team of twelve have distilled the essence

* Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London, S.W.1.

of the research of some 1900 scientists, themselves included, into over 1300 pages. This is large-scale work. The main partitioning is into methods, a scheme whose *pros* and *cons* need not be argued here, although it is proper to mention that the consequent absence of what might be called, in the statistician's language, the interaction of vitamins and methods, is not, as it could be, made good in a separate section. In other words, the relative worth of different kinds of analytical methods for any given vitamin is seldom apparent, although the final remark in the section on the chemical estimation of pteroylglutamic acid (Vol. I, p. 259) is an instance of its being unmistakably apparent.

At this point a breakdown of the contents may be worth while. Grouped together, the chemical and microchemical methods, in the hands of György, Rubin, De Ritter and Bessey, account for 18 per cent. of the space. Stiller's section on physical methods occupies 13 per cent. The microbiological section by Snell, Wright, Skeggs, Rubin and De Ritter takes almost as much, 17 per cent., as the chemical section. There are three other big sections: macrobiological, by Bliss and György (18 per cent.); bio-assay statistics, by Bliss (14 per cent.); and clinical estimations, by J. H. Jones (10 per cent.). The remaining 10 per cent. is shared between Guerrant's general introduction to the practice of small animal experimentation, Jolliffe on malnutritional symptomatology, and Hirschberg's concise survey of optical instruments. Two features of this allocation are striking: firstly, the massive demands of microbiological assay, emphasising a realistic modern outlook; secondly, the fact that the macrobiological methods section, plus that large part of the statistical chapter concerned with animal assay, plus the chapter on general animal experimentation —in other words, all the exclusively small-animal material—account for a third of the entire work. The dilettante reader would never suspect from this, and would be surprised to learn, that of the thirteen vitamins dealt with in these 400 or so pages only one, and that one far from the most important, is ever actually *estimated* in the animal laboratory. For the other twelve, macrobio-assay is now important only in the research assessment of the relative potency of the vitamers, and while this is a role whose importance may match the ample space given to it, the situation might have been made clearer to the reader. It is true that three of the chapters in Volume II begin with paragraphs on the principles of bio-assay, but none of them is wholly satisfactory. Nor is the rationale of the broad approach noticeably clarified by a prefatory statement that, compared with other methods, "animal assays reflect more truly the nutritional value of vitamins."

The co-existence of three opening sub-sectional paragraphs on the same theme itself raises a question. Replication of material in a book of this nature is to some extent unavoidable and in some departments judicious, but just where is the line to be drawn between the acceptable and the *outré*? Mild recapitulation of the principles of absorptiometry may be permissible, but how to defend the printing of a 3-page extract of an official method twice within the space of 28 pages (Vol. II, pp. 614 and 639)? To argue the individual requirements of the separate authors is to remit the larger problem of how much latitude should be given to the members of a coalition of authors. My own answer, which is patently not everyone's, is "Very little." The unity and stylistic form of the bound book is an important thing whose preservation should be ardently striven for even in the most mixed technical company. Yet the tendency to-day is in the other direction, and "Vitamin Methods" is not exceptional. To my mind the editor of a miscellany of authors who between them are to produce a book should make the late Harold Ross his exemplar. Ross made *The New Yorker* incomparably the best of its kind in the world by sheer ruthlessness, by insisting on a standard of clarity and conformity from which no contributor, be he never so eminent and individualistic, was allowed to depart. And technical writing, with years of preparation, is simpler to handle than non-technical writing with a weekly deadline. On this view, a textbook editor should not only prevent, for instance, some of his authors' using $m\mu$ and cc and γ where others use Å and ml and μg, but should relentlessly cut out overlaps and factual clashes and inconsistencies.

This personal attitude to a feature that "Vitamin Methods" shares with many other publications does not detract from the solid achievement that the book represents. The range of information and the fullness of detail are impressive, and the avoidance of two faults all too frequent in conspectuses of this kind can here be warmly acclaimed: firstly, the origins and courses of development of most methods are accurately chronicled (and may surprise in some instances); secondly, the surveys are truly international in character. Oddly enough, the one exception to both these generalisations is the newest and most unusual vitamin method: the microbiological plate-assay. The publication date of Volume II was not too early to have enabled the responsible authors to draw attention to the interesting fact, extractable from the literature, that this method was borrowed from the antibiotic field and first applied to the water-soluble vitamins independently

in four different laboratories in England and the U.S.A., and was first applied to vitamin B_{12}, again independently, by Cuthbertson in England and by Foster, Lally and Woodruff in the U.S.A., both in 1949. It would perhaps be unfair to criticise the treatment, bits of it incomprehensible to me, of the theoretical background of the estimation of vitamin A, because of the highly controversial views still held in this field. Incontrovertibly, however, "vitamin A_3" (Vol. I, p. 6) is no longer an entity, being a tentative name for what was later designated "kitol." And even if the misuse of "precision" is waived, there are still five mistakes in the sentence "On the whole the British correction factor of 1600 was justified if irrelevant adsorption could not be allowed for, but the use of a correction factor of 1800 and corrected E values permits the assay of individual oils with considerably improved precision" (Vol. II, p. 617).

Misprints are few, but one persistent one should be noted: confusion of "absorb" and "adsorb" and their derivatives. This extends even to a bold-type section title (Vol. II, p. 611), and at one juncture I had almost concluded that the distribution of *ad*'s and *ab*'s was purely aleatory, when I was halted by the discovery of a cluster of 15 *ab*'s (Vol. I, p. 60), 13 of which are wrong. And what of that Aunt Sally of reviewers, the index? It is a pleasure to report that the indexing, especially that of authors, has been done with enough thought and care to make it a model of its kind. This also applies to the system of references and the bibliographies (although p. 444, a bibliographic page in Volume II, is unprinted in the two copies I have seen). In similar connection the elaborate symbol glossary and equation index appended to Bliss's statistical chapter deserve mention among the many attractive characteristics that combine to make "Vitamin Methods" a work that everyone it is addressed to will itch to possess. N. T. GRIDGEMAN

THE CARE AND BREEDING OF LABORATORY ANIMALS. Edited by EDMOND J. FARRIS. Pp. xvi + 515. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1950. Price $8.00; 64s.

The British bio-assayist will find in this book edited by the present Director of the Wistar Institute, Philadelphia, some expert information not available to him in the U.F.A.W. handbook, which he will undoubtedly possess, although it is currently out of print and shortly, I learn, to appear in a second edition. One of the major differences between the two books lies in the subject-matter, for the American book contains chapters on the opossum, on reptiles—which include snakes, lizards, turtles and crocodilians—and on Drosophila. The British handbook, on the other hand, has space to devote to the cotton rat, an American animal described in a chapter written by an American author, and mentioned nowhere in the American book. In the U.F.A.W. publication are also chapters, albeit some of them are not more than a page or two long, on the black rat (*Rattus rattus*), the wild house-mouse, the wood mouse, the deer mouse, the common and the Orkney voles, the hedgehog, the pigeon and the canary.

Whereas the Wistar Institute volume devotes a chapter each to the dog, the cat and the monkey (none of which are specially bred for scientific use in this country), these three species are given only cursory notice in a chapter on "Some Species not Dealt with in Detail" in the U.F.A.W. handbook. Common to both books are chapters, and pretty extensive ones, on the species of animals most abundantly raised as laboratory tools, that is, in rough order of numerical importance, mice, guinea-pigs, rats, rabbits, hamsters and ferrets.

It is a curious thing that neither book approaches the many problems of laboratory animal husbandry from the standpoint of those who are the most extensive users of laboratory animals. The U.F.A.W. handbook was conceived by humanitarians—"animalitarians" would be a more logical name for them—and written mainly by biologists, especially pathologists. The Wistar team are equally expert and many of them as universally known as the British writers: their outlook and motivation appear to have been primarily that of the biological research worker. Although both books contain a few general chapters, on such matters as the law (in Britain) and the control of pests in the animal laboratory (in both books), neither contains any general discussion on the general problem of reproductivity, the qualitative and quantitative factors that have to be weighed when deciding what strains of what species to breed, or buy, for biological assay work, often carried out on a very large scale. Of the million and a half mice used in this country yearly, it is certain that well over two-thirds, and probably nine-tenths, are required for the more or less routine determination, as accurately as may be, of toxicity, antigenicity and other biological properties or constituents that can only be estimated on living animals.

Bio-assayists will certainly need to possess the book edited by Dr. Farris, for all information on this tricky but fascinating subject is welcome, nay essential, to them, and the Wistar volume, in spite of the overlap in subjects briefly indicated above, contains much information not to be

found in the U.F.A.W. handbook. This they must also possess, and for the analogous reason. But there still remains to be written an authoritative monograph, sound in theory but informed by practice, on the production and care of laboratory animals bred specifically for use in quantitative biology and chemical analysis. A. L. BACHARACH

HORMONES: A SURVEY OF THEIR PROPERTIES AND USES. Pp. xii + 220. London: The Pharmaceutical Press. 1951. Price 35s.

The main object of this book is to provide pharmacists with a compact account of those hormones that have well-defined pharmacological effects and therapeutic applications. Sections have been written by H. E. Dale (Physiology and Action and Uses), C. W. Emmens (Standardisation), D. H. Hey (Chemistry) and T. D. Whittet (Pharmacy). The book is attractively printed with 34 illustrations and contains much information and a large number of references to the original literature. An enormous number of papers about hormones have appeared in recent years and it would, of course, be quite impossible for a comparatively small book to give anything like a complete account of the whole field. It has been necessary to select the material for inclusion in this book, but it may be doubted whether the selection has always been wisely made. More space is devoted to the fact that deoxycortone does not cure rheumatism than to all the work on cortisone. The account of the actions and uses of oxytocin and vasopressin is confused. It is difficult to imagine what can have led to the inclusion on p. 159 of the astonishing statement that 1 mg of adrenaline does not affect the blood pressure of animals. The chemical chapters contain a detailed account of what is known about the organic chemistry of the hormones and there is a useful list of commercial preparations.

The most interesting and important chapter is that dealing with biological standardisation. Professor Emmens is universally recognised as one of the leaders in this field, and he has given a simple account of a complex branch of knowledge in 27 pages. There is a list of international standards, a discussion of general principles and, after a small dose of mathematics, an up-to-date review of the best methods of assay for nearly all the hormones. Few details are given, but there are plenty of references to full accounts of the different techniques. The errors of 19 different tests are compared in an interesting table that is easy to understand. These tests are all supposed to have been (2 + 2) assays designed to give limits of error (P = 0·95) of 80 to 125 per cent. Errors due to uncertainty of the slope of the log-dose - effect curve and to incorrect forecasting of the result are neglected, and the number of animals that would have to be used in each test is given. The number of rabbits in a crossover test for insulin is 16. All the other tests depend on comparisons between different individual animals, and the numbers vary from 25 to 350. This is a useful way of comparing the different tests. The book is worth having for this chapter alone. J. H. GADDUM

Publications Received

IODINE CONTENTS OF FOODS. ANNOTATED BIBLIOGRAPHY, 1825-1951. Pp. vii + 183. London: Chilean Iodine Educational Bureau. 1952. Price 21s.

THEORY OF SUPERCONDUCTIVITY. By M. VON LAUE. Translated by Lothar Meyer and William Band. Pp. x + 140. New York: Academic Press Inc. 1952. Price $4.00.

ANNUAL REPORTS ON THE PROGRESS OF CHEMISTRY FOR 1951. Pp. 429. London: The Chemical Society. 1952. Price 25s.

WAX CHEMISTRY AND TECHNOLOGY. By L. IVANOVSZKY, D.Sc., Dipl. Ing., F.R.I.C., M.I.Chem.E., F.Inst.Pet. Pp. 44. Published by the author at "Glenbrook," 68, Park Street, Bridgend, Glamorgan. 1952.

Notice to Authors

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

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

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

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

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

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

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

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

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

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

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

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

NOTES ON THE WRITING OF PAPERS FOR "THE ANALYST"

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

The title should be brief but descriptive.

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

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

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

(*a*) Synopsis.

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

(*c*) Preliminary experimental work.

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

(*e*) Presentation of results.

(*f*) Scope and validity of results.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

IF there is a research-minded chemist of enterprising character who feels that he has no scope in his present calling, the Managing Director of a well-established manufacturing concern having many fields of interest would welcome hearing from him with details of his career to date, salary suggestion, etc. Location of plant Leatherhead, Surrey. Write Box No. 3821, THE ANALYST, 47, Gresham Street, London, E.C.2.

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CONTENTS

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