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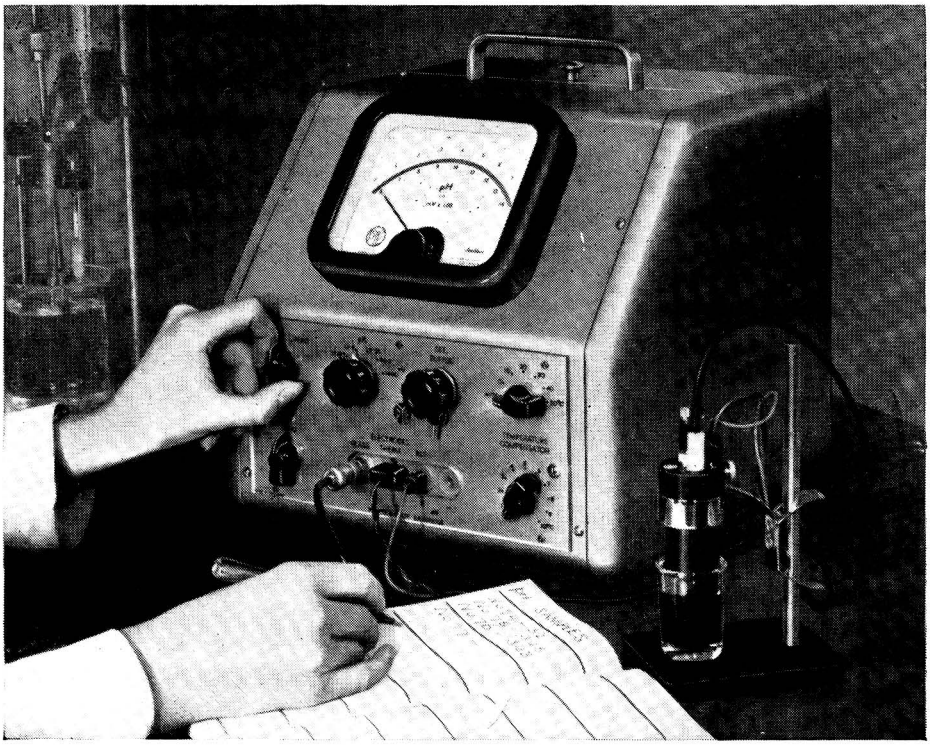

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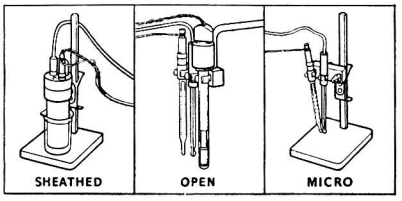

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

SPECIAL MEETING

A SPECIAL Meeting of the Society was held at 6 p.m. on Wednesday, July 21st, 1954, in the Lecture Theatre of the Royal Institution, 21 Albemarle Street, London, W.1. The Chair was taken by the President, Dr. D. W. Kent-Jones, F.R.I.C. The subject of the meeting was "The Use of Perchloric Acid in Analytical Chemistry," and the following papers were presented: "Perchloric Acid and Some Organic Perchlorates," by Professor Harold Burton, Ph.D., D.Sc., F.R.I.C., and P. F. G. Praill, B.Sc., Ph.D. "A Bomb in a Test Tube. Perchloric Acid Idiosyncrasies," by Professor G. Frederick Smith, Ph.D. Professor Smith made a special journey from America to London to give this lecture to the society. The discussion that followed was opened by Mr. B. Bagshawe, A.Met.

There was an attendance of 500 members and visitors, the large audience completely filling the lecture theatre. Professor Burton discussed the action of perchloric acid and some organic perchlorates on certain types of organic compounds, which could lead to the formation of explosive compounds. Professor Smith demonstrated by actual experiments in the lecture room the general properties of perchloric acid and how it could be used with complete safety for wet-oxidising such substances as chromacised cat-gut and a cigar, and for the analysis of metals, notably stainless steels. He stressed that safety depended on a knowledge of the reactions involved.

NEW MEMBERS

ORDINARY MEMBERS

Samuel Edward Qualtrough Ashley, B.S. (N.Y.), M.A. (Princeton); Arnold Eric Bender, B.Sc. (Liv.), Ph.D. (Sheff.), F.R.I.C.; Cyril Leake Grayson; Edmund Green, M.Sc. (Lond.); Noel Francis Maclagan, D.Sc., M.D., F.R.C.P. (Lond.), F.R.I.C.; William Joseph Sommerville Pringle, B.Sc. (Lond.); Muhammad Shafi, B.Sc.; John Frederick Harrop, B.Sc.

DEATHS

WE regret to record the deaths of

John Theodore Hewitt
Richard William Woosnam.

NORTH OF ENGLAND SECTION

A MEETING of the Section was held at 2.30 p.m. on Saturday, April 10th, 1954, at the City Laboratories, Mount Pleasant, Liverpool 3. Mr. T. W. Lovett, F.R.I.C., was in the Chair.

The subject of the meeting was the Food and Drugs (Amendment) Bill, a bill to amend the Food and Drugs Act, 1938, and the Food and Drugs (Milk and Dairies and Artificial Cream) Act, 1950. The Chairman reviewed the salient parts of the Bill, and his review was followed by a discussion.

In addition to the planned programme, Mr. N. Heron, F.R.I.C., kindly gave a talk on the determination of alcohol in body fluids and demonstrated the apparatus used for this determination. A discussion followed.

THE Seventeenth Summer Meeting of the Section was held at the Grand Hotel, Morecambe, from Friday, June 18th, to Monday, June 21st, 1954.

The Chairman, Mr. T. W. Lovett, F.R.I.C., presided. On the morning of Saturday, June 19th, a paper was presented by Mr. E. Green, M.Sc., on "Cosmetics." A motor-coach trip to Windermere was made on Sunday afternoon.

Obituary

HAROLD GOVETT COLMAN

HAROLD GOVETT COLMAN died on February 20th, 1954. His wide circle of friends will feel the loss of a great personality, wise counsellor and outstanding chemist.

Colman was born in 1866, studied chemistry under Roscoe at Owens College, Manchester, and took his B.Sc. (Vict.) in 1885. During the same year he successfully sat for the Associateship of the Institute of Chemistry. He also studied at Strasbourg and Wurzburg, taking his Ph.D. at the latter University in 1888. In that year he also took the M.Sc. (Vict.), and set out to follow an academic career, becoming private assistant to Sir Henry Roscoe and joint editor of Roscoe and Schorlemmers' famous "Treatise on Chemistry." He joined Professor Tilden's staff at Mason College, Birmingham, as demonstrator and assistant lecturer in 1891. About 1893, Professor Tilden advised the Birmingham Gas Committee to appoint a qualified and knowledgeable chemist to develop up-to-date chemical control of gas-manufacturing processes, and Dr. Colman was the chemist chosen and appointed. He spent ten years in the Gas Industry putting chemical control on a firm basis and became a Member of Council of the Institution of Gas Engineers. Attendance at Council meetings was not encouraged by the Gas Department, and this attitude, along with other drawbacks, caused Colman to embrace the freedom of the consulting world. He found his real life's work in this sphere, and the gas industry is greatly in his debt for his valuable investigations into the working efficiencies of large-scale carbonising plant and allied processes.

In 1904 he took his F.I.C., and in 1910 he received the degree of D.Sc. Manchester for research.

In the first World War he analysed and assessed for the Ministry of Supply the benzole and toluole recovered by the gas and allied industries for high explosives. This necessitated devising methods of analysis, and the term "to colmanise" in connection with such work came into everyday use in many laboratories. He was doing similar work for Midland Tar Distillers right to the end.

Colman's lectures on Gas-works Practice in the Fuel Department of Leeds University were classical, and when he gave up lecturing in 1924 it required the services of two chief chemists to carry on the work.

Colman was also a tower of strength in the development of gas-works refractories for retorts and other plant, being a great supporter of Dr. J. W. Mellor, F.R.S., of Stoke, another Manchester University man. Colman was Chairman of the Joint Refractories Committee of the Gas Industry and the Refractory Manufacturers, and it was the writer's privilege to make him a presentation on behalf of the Committee when he resigned in 1941.

He carried out a considerable amount of unpaid work from sheer generosity and sometimes because of the intellectual satisfaction he got from solving some particular problem. Had he been a really keen business man he would certainly have made a considerable fortune.

Dr. Colman had a delightful and charming manner, a keen sense of ethics and a profound knowledge of chemistry; he was always kindly disposed to juniors, a fact that many of us have cause to remember with gratitude. Chemistry and the gas industry have lost an outstanding personality and one whose name will be remembered with great esteem and affection.

T. F. E. RHEAD

The Micro-determination of Bromide in Presence of Chloride

By G. HUNTER and A. A. GOLDSPINK*

(*Presented at the meeting of the Society on Wednesday, May 5th, 1954*)

Conditions have been found for (*a*) quantitative conversion of micro amounts of bromide to bromate by hypochlorite and (*b*) quantitative formation of tetrabromorosaniline, as a basis for colorimetry, from bromine so formed by the interaction of the bromate formed in the above reaction with excess of added bromide. The colorimetric procedure is applicable in the range 0 to 5 μg of bromide in 5 ml of solution. In the same volume accurate measurements can be made of 0·1 μg of bromide, and of even smaller quantities by reducing the volume. The method is independent of the presence of chloride, although chloride has some effect on potential interference by chlorate and iodate. With a gross excess of chloride, about 200 equivalents of iodate or 6000 to 8000 equivalents of chlorate are necessary to cause appreciable interference with 1 equivalent of bromide. The production of chlorate in relatively large amounts is, however, an integral part of the method, but experimental conditions are such as to avoid interference from it. The method is simple and readily adaptable to multiple micro-determinations of bromide.

THE method is based on several well known reactions. Bromide is quantitatively oxidised to bromate, in suitably buffered solution, by hypochlorite[1]—

$$Br^- + 3ClO^- \rightarrow BrO_3^- + 3Cl^- \quad (1)$$

Bromate quantitatively gives six equivalents of bromine by reaction in acid solution with an excess of bromide—

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O \quad (2)$$

Bromine in minute amounts readily substitutes in rosaniline decolourised by acid, with the formation of a red solution[2] (3)

In this method reaction (2) is carried out in the presence of rosaniline, when the liberated bromine is found to react quantitatively.

The method dispenses with the various oxidising agents, such as potassium dichromate and sulphuric acid,[3] chlorine,[4] monopersulphuric acid,[5] that have been used to generate bromine from bromide for the rosaniline reaction. As is well known, their action is difficult to regulate, and the presence of small amounts of chloride, leading to the production of chlorine, usually vitiates the rosaniline - bromine reaction.

Although another reaction—

$$BrO_3^- + 6Cl^- + 6H^+ \rightarrow 3Cl_2 + Br^- + 3H_2O \quad (4)$$

also occurs, under the conditions of the present test reaction (2) takes precedence, even in the presence of a gross excess of chloride. The oxidising agent (BrO_3^-) is self-regulating as it uses itself up in the presence of an excess of bromide.

Iodide, if present, is quantitatively oxidised to iodate in the course of reaction (1) and the reaction—

$$IO_3^- + 6Br^- + 6H^+ \rightarrow 3Br_2 + I^- + 3H_2O \quad (5)$$

also occurs, but this is much slower than (2) and can only interfere when iodate is greatly in excess of bromate. Reducing agents, such as metals in a low state of valency or sulphur dioxide, are also oxidised by hypochlorite, so that all of the bromate formed in reaction (1) is available for reactions (2) and (3) together.

Presence of the chlorate ion is the most serious threat to the precision of the method. For, as is well known, when hypochlorite in slightly acid solution is heated, chlorate is formed by one molecule at the expense of the oxygen from two others—

$$ClO^- + 2ClO^- \rightarrow ClO_3^- + 2Cl^- \quad (6)$$

* Present address: Richard Thomas & Baldwins Ltd., "The Firs," Whitchurch, nr. Aylesbury, Bucks.

Now the reaction—

$$ClO_3^- + 6Br^- + 6H^+ \rightarrow 3Br_2 + Cl^- + 3H_2O \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

takes place in the presence of a high concentration of mineral acid at room temperature, and its velocity is increased by the presence of chloride. Reaction (2) requires for completion at least a 5 *N* concentration of mineral acid, but it is possible to reduce this to about 2·8 *N* by the use of molybdate as catalyst. This device greatly reduces reaction (7), which is not affected by the catalyst.

The extent of chlorate production in the course of reaction (1) has been determined, as well as the effect of chlorate under the conditions of reaction (2).

The procedure as finally developed will be described first and the evidence for its accuracy will follow. It is assumed that the material used for the test does not contain organic matter.

Method

Reagents—

Sodium hypochlorite, 1 N *in* 0·1 N *sodium hydroxide*—This is made as follows. In a 1-litre, 2-necked Wolff bottle place 500 ml of 1·1 *N* sodium hydroxide and set it aside in a refrigerator to cool. Bromine-free chlorine is generated, for example, from 130 ml of analytically pure hydrochloric acid and 40 g of manganese dioxide by gently heating the mixture in a 500-ml flask fitted with a safety tube. It is washed by passing it through 100 ml of water in a smaller 3-necked Wolff bottle, and it is led into the bottle containing the cooled sodium hydroxide solution, the bottle being in a bath of ice. From time to time, 1 ml of this is removed to a small flask, 5 ml of 3 per cent. hydrogen peroxide are added to destroy the sodium hypochlorite, and the solution is titrated with 0·1 *N* hydrochloric acid, with alizarin red as indicator, until about 1·0 ml of the 0·1 *N* acid is required for neutralisation. The solution is stable indefinitely when stored in a refrigerator.

Sodium formate, 50 *per cent. w/v.*

Buffer solution at pH 6·35—This is prepared by mixing 10 volumes of 40 per cent. w/v sodium dihydrogen phosphate dihydrate, $NaH_2PO_4.2H_2O$, 7 volumes of 2 *N* potassium hydroxide and 5 volumes of water.

Rosaniline solution—Prepared by dissolving 6 mg of rosaniline in 100 ml of 2 *N* sulphuric acid. The use of either the base or an equivalent amount of acetate, sulphate or chloride has been found suitable. After prolonged exposure to light this solution ceases to react quantitatively with bromine, but it keeps for several weeks in a brown bottle. It should be tested periodically with standard bromate solution.

Bromide - molybdate mixture—By dissolving 0·15 g of potassium bromide and 3·0 g of ammonium molybdate in water and diluting to 100 ml.

Sulphuric acid, 14 N.

tert.-*Butanol containing* 5 *per cent. v/v of absolute ethanol*—The ethanol is added to prevent freezing at room temperature.

Procedure, stage I: conversion of bromide to bromate—

To a test tube (16 mm × 130 mm) add 1 ml of buffer solution and then a suitable volume of bromide solution and make up with water to a total volume of 4·5 ml. To this add 0·25 ml of hypochlorite solution, mix the contents and immerse the tube in a bath of boiling water for 10 minutes. Add 0·25 ml of formate solution to destroy any excess of hypochlorite, mix the solution and replace the tube in the boiling water for a further 5 minutes. Cool the tube, add water to replace that lost (0·25 ml under our conditions) by evaporation, and mix the contents. This solution is used for the colorimetric procedure described in Stage II.

Procedure, stage II: colorimetry—

(*a*) *For standard bromate solutions containing less than* 5 μg *of bromine as bromate*—In a test tube, or colorimeter tube, place 0·1 ml of bromide - molybdate mixture, 0·1 ml of rosaniline solution per μg of bromine in the test solution to be used, 0·4 ml or less of water, 0·4 ml of 14 *N* sulphuric acid and 1 ml of the solution prepared in Stage I, in that order and mix them. The total volume at this stage is 2·0 ml, the amounts of bromide - molybdate and sulphuric acid being constant in all tests. Any volume of test solution less than 1·0 ml can be used if the balance is made up with water, which is added before the test solution. Leave the reaction mixture at 20° to 30° C for 3 minutes, add 2 ml of *tert.*-butanol and 1 ml

of 14 *N* sulphuric acid. Mix the solution and measure the optical density in a suitable absorptiometer, after all air bubbles have disappeared. The colour is stable for many hours even in bright light.

Maximum absorption by the bromorosaniline solutions is near 570 mμ and all optical densities are here recorded at this wavelength for solutions contained in 1-cm cells.

(*b*) *For solutions with unknown bromine as bromate*—In two test tubes place the solutions as in (*a*) on the assumption that 0·1 ml of test solution is to be used, and also that one test tube contains 0·05 ml and the other 0·50 ml of rosaniline. Now add 0·1 ml of the unknown solution to each test tube and continue as in (*a*). From the two observations one can determine approximately the amount of bromine present, unless the amount taken for the determination exceeds about 250 μg. Further test portions are then taken with suitable amounts of rosaniline solution until the optical densities are found to be proportional to the test portions taken.

When the liberated bromine is greatly in excess of the rosaniline necessary to absorb it, the readings will be low owing to the bleaching action of the bromine on any bromorosaniline that may be formed.

Results

A linear relationship is found to exist between the amount of bromate present and the optical density of the bromorosaniline formed in solutions resulting from the procedure described. When the optical density is measured at 570 mμ in 1-cm cells, the slope (μg of bromine in the volume of 5 ml divided by optical density) of the line that goes through the origin is 5·5.

When a series of standard bromide solutions was treated with hypochlorite and formate, as described above, and suitable test portions were taken, the relationship between bromide as bromate present and the density of the resulting bromorosaniline solutions was likewise found to be linear and coincident with the curve found for the standard bromate solution.

This conversion of bromide to bromate was shown to be precise from a series of 12 tests, in each of which 5 μg of bromide were used, and readings were taken for 1 ml of test solution. The extreme variation in readings was about 6 per cent. and the standard deviation 1·8 per cent.

That the presence of bromide-free sodium chloride has no effect either on the conversion of bromide to bromate or on the production of a colour by rosaniline is shown by the results of the six tests recorded in Table I.

Discussion and conclusions

Sodium hypochlorite solution—

Those who have used the Van der Meulen reaction for determining bromide by titrating the iodine freed by the bromate from iodide have always found an appreciable blank.[7] This

Table I

Optical density independent of the amount of chloride present

Six tests were carried out as described under Procedure, Stages I and II, each tube containing 5 μg of bromide and the amounts of sodium chloride shown

Sodium chloride in 5 ml, Stage I, mg ..	0	62·5	125	250	500	750
Sodium chloride in 1 ml, Stage II, mg ..	0	12·5	25	50	100	150
Optical density × 100	17·9	18·2	18·5	17·6	18·0	18·2

has commonly been attributed to the presence, either before or after a period of heating, of chlorate in the reagent. We tested several of our reagents prepared as described above, including one that had been stored in a refrigerator for over 30 months, and found that chlorate was absent. Foerster and Dolch[6] have noted the very slow change of hypochlorite to chlorate in 0·1 *N* sodium hydroxide. Nevertheless several such reagents had appreciable blanks by the titration method. The use of the colorimetric method has disclosed that the source of those blanks in hypochlorite solutions is not chlorate, but bromate. We have prepared a bromide-free hypochlorite solution from chlorine made by the action of sulphuric acid on sodium chloride freed from bromide by recrystallisation from methanol. This solution showed no titration blank.

We have determined the bromine in several commercial bleaches diluted to 1·0 *N* with respect to hypochlorite, by a titration method and by the present colorimetric method. If

bromine were introduced into a hypochlorite solution it would soon be changed to bromate, so that, by destroying the hypochlorite present with a formate, without any previous heating, the same value should be found as from a sample heated for 10 minutes on a bath of boiling water before addition of the formate. This is seen to be so from the observations shown in Table II.

TABLE II

PRESENCE OF BROMATE IN COMMERCIAL HYPOCHLORITE SOLUTIONS

Sample	Time of heating before formate added, minutes	Bromine found as mg per 100 ml at a N sodium hypochlorite concentration	
		Titration method	Colorimetric method
1	10	48·0	48·0
2	0	17·8	17·7
2	10	17·8	17·6
3	0	15·8	15·4
3	10	16·0	15·5

Clearly such hypochlorite solutions are too grossly contaminated with bromate to be of use as analytical reagents. Much better commercial samples have been found,[7] but such material has proved to be unreliable. The bromate in commercial bleaches is apparently dependent on the bromine content of the chlorine from which such hypochlorite solutions are made. With some A.R. hydrochloric acids it is, however, possible to make reagents, as described above, with quite low blanks. Our current reagent contains about 0·1 mg of bromine per 100 ml of solution. In the present method a 1-ml test portion would contain 0·05 μg of bromine, which gives a just perceptible blank, but is negligible for most analytical purposes.

CHLORATE—

It has been noted that chlorate is formed from slightly acid hypochlorite solutions according to reaction (6). In the Van der Meulen conversion of bromide to bromate under the conditions described by Hunter,[7] we have now found that some 60 equivalents of chlorate are produced for each equivalent of bromide converted to bromate. Indeed, the Van der Meulen principle can be applied despite the formation of clorate, because the reaction—

$$ClO_3^- + 6I^- + 6H^+ \rightarrow 3I_2 + Cl^- + 3H_2O$$

is negligible in extent compared with the reaction—

$$BrO_3^- + 6I^- + 6H^+ \rightarrow 3I_2 + Br^- + 3H_2O$$

under the usual conditions of titration with thiosulphate.[8]

As will be shown, sulphuric acid more concentrated than 6 N is necessary to suppress the colour of free rosaniline before colorimetry. In earlier attempts to develop the method we used such an acidity for reactions (2) and (3), and, as might be expected from a consideration of Figs. 1 and 2, we found that an appreciable colour was produced by amounts of chlorate necessarily formed in the process of conversion of bromide to bromate. It is clear from Fig. 1 that the amount of bromine liberated by the action of chlorate on bromide is greatly decreased at the lower normalities, and that the molybdate has no effect. From Fig. 2 it is seen that at a 3 N concentration of acid, an increasing concentration of chloride enhances the oxidising action of chlorate on bromide. As will be clear later, our test solutions contain less than 0·30 mg of chlorate as potassium chlorate, and this amount has no perceptible effect in the presence of less than 50 mg of sodium chloride and only a doubtful effect in the presence of 100 mg of sodium chloride. Hence it is apparent that the amount of chlorate formed in Stage I of the procedure will have no effect on the colorimetric procedure described.

The effect of iodate is also indicated in Figs. 1 and 2. It is of less interest, as its formation is not, unlike chlorate, inherent in the method. Iodate is more active than chlorate in freeing bromine from bromide, but, even in the presence of 50 mg of sodium chloride, 27 μg of potassium iodate produces only about as much colour as 0·05 μg of bromine as bromate. That is, on an equivalent basis, bromate is 200 times more sensitive than iodate.

THE CONVERSION OF BROMIDE TO BROMATE—

The conditions described under Procedure, Stage I, have been arrived at on the basis of the following observations.

To avoid unnecessary chlorate formation the amount of hypochlorite used should be kept at a minimum. The amount used, 0·25 ml, is shown to be adequate from the results in Table III. These results were obtained by carrying out the Procedure, Stage I, but cooling the tubes after 10 minutes in the bath of boiling water. Then 0·25 ml was removed from each tube, acidified, iodide was added and it was titrated with 0·005 *N* sodium thiosulphate. In blanks, without heating, 0·25 ml of hypochlorite ≡ 2·46 ml of the thiosulphate. The bromate present does not significantly affect the titration for hypochlorite, but it catalyses to some extent chlorate formation. It is of interest that upwards of 40 per cent. of the hypochlorite is oxidised even in the absence of bromide and less than 60 per cent. in the presence of 200 μg of bromide. That such amounts of bromide are converted to bromate may be seen from Fig. 3, although the method is not designed for the larger amounts, in whose presence great dilution of the test solution becomes necessary before absorptiometry. It can further be seen from Fig. 3 that a minimum of 6 minutes' heating is necessary for the conversion of 10 μg of bromide, while amounts of bromide up to 200 μg are converted after 12 minutes' heating. From many other observations, we have decided that 10 minutes in the bath of boiling water is enough for the amounts of bromide for which the method is suitable.

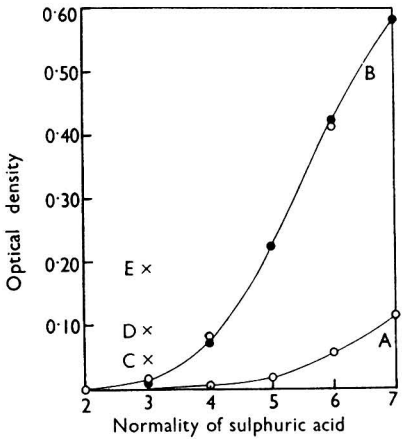

Fig. 1. The effect of concentration of acid on the action of chlorate on bromide and of iodate on bromide
—o——o—, chlorate
—●——●—, chlorate in presence of molybdate
X, iodate.
Curve A, 1·0 mg and curve B, 10·0 mg of potassium chlorate. The amounts of iodate at the 3 *N* concentration of sulphuric acid are point C, 0·7 mg; point D, 1·4 mg; and point E, 2·7 mg of potassium iodate

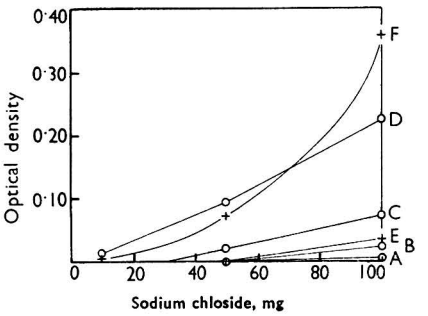

Fig. 2. The effect of concentration of chloride on the action of chlorate on bromide and of iodate on bromide in 3 *N* sulphuric acid. Curve A, 0·25 mg; curve B, 0·50 mg; curve C, 1·0 mg; and curve D, 2·0 mg of potassium chlorate. Curve E, 0·027 mg; and curve F, 0·13 mg of potassium iodate.

By the use of a series of buffers, a value of pH of 6·35 has been chosen on the evidence, shown in Fig. 4 (*a*), that the rate of conversion of bromide to bromate is at a maximum near this point. It may be noted that complete conversion occurs after heating for 6 minutes at this pH, but that conversion is not complete after 3 minutes heating. With a similar series of buffers, 0·25 ml of hypochlorite was treated as under Procedure, Stage I, and then the excess of hypochlorite was destroyed with formate. From each tube, 2-ml test portions were taken and titrated for chlorate by reduction with a ferrous salt and back titration with 0·1 *N* potassium dichromate, with diphenylamine as indicator (see Cumming and Kay[9]). As might be expected, the rate of chlorate formation is roughly parallel to that of bromate formation, but the amount of chlorate formed is much greater, about 1·35 mg as potassium chlorate. From such a test solution a 1-ml test portion thus contains 0·27 mg of potassium chlorate.

The proportion of chlorate to bromate formed under the above conditions is higher than it need be, with complete conversion of the bromide. For example, we have found that 10 μg of bromide is completely converted to bromate in the above buffer - hypochlorite

mixture in about 36 hours at 4° C, with the formation of only about 0·30 mg of potassium chlorate. At room temperature in the same time, about twice as much chlorate is formed. There might be circumstances calling for such treatment, but, as 0·30 mg of chlorate as potassium chlorate has no effect on the absorptiometric method, the saving of time would seem to justify the method adopted of heating for 10 minutes in the bath of boiling water.

The capacity of the buffer is sufficient to render unnecessary the neutralisation of the bromide solutions to be analysed unless they contain a gross excess of acid or base.

The volume of the solution at Stage I allows for a volume of up to 3·5 ml of solution for bromide determination.

The total volume of the resulting bromate (test) solution is 5 ml, which allows for many trial tests. Indeed, all volumes can often be reduced by half and still yield adequate material for the determination.

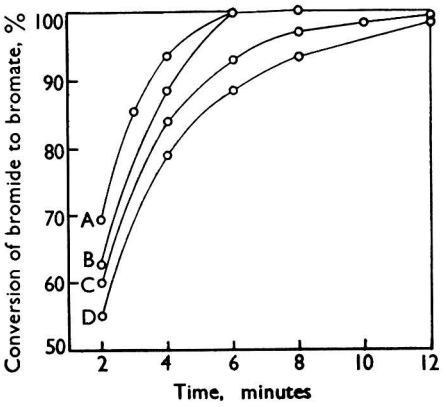

Fig. 3. Time of heating required for the conversion of bromide to bromate by hypochlorite. The amounts of bromide taken for Stage I are for curve A, 10 μg; curve B, 25 μg; curve C, 100 μg; and curve D, 200 μg

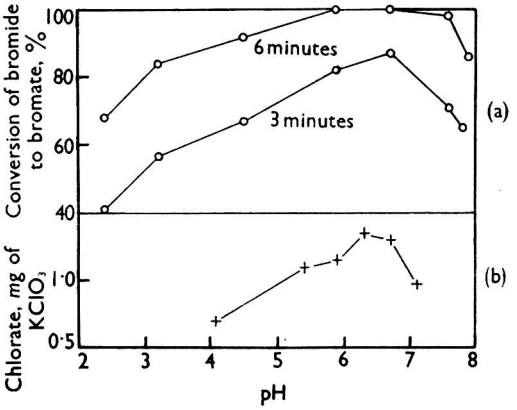

Fig. 4 (*a*). The effect of pH and time of heating on the rate of conversion of 10 μg of bromide to bromate by hypochlorite.

(*b*) The effect of pH on the formation of chlorate from hypochlorite

THE FORMATION OF BROMINE FROM BROMATE AND BROMIDE—

According to Ephraim[10] this reaction is quadrimolecular and both hypobromous and bromous acid are probably among the intermediate entities. For the complete stoicheiometrical change shown in reaction (2), a high normality of a mineral acid is necessary.

Schirlow[12] observed that the related reaction—

$$BrO_3^- + 6I^- + 6H^+ \rightarrow 3I_2 + Br^- + 3H_2O$$

is catalysed especially by solutions of iron, chromium or molybdenum. We have found the colourless molybdate ion to be very effective as a catalyst and, as shown in Fig. 5, its presence enables the reaction to go to completion in 2·8 *N* acid at a temperature of about 25° C within three minutes. In the absence of the catalyst the optical density is not at a maximum until the acid concentration is greater than 5 *N*. The importance of thus lowering the normality has already been noted in relation to the potential interference by chlorate.

ROSANILINE AND BROMINE—

Guareschi[2] observed that bromine vapour gives a red colour with Schiff's reagent (rosaniline decolourised with sulphur dioxide), and Denigès[13] observed that rosaniline solutions are also decolourised by an excess of mineral acid, thus obviating the reducing action of sulphur dioxide on the halogens. A concentration of about 6 *N* sulphuric acid is necessary for maximum suppression of this colour, as shown in Table IV. Here the amount of 10 μg of rosaniline is somewhat greater than the small excess of rosaniline necessarily present for the procedure, but it serves to show that a considerable error might arise from the use of low normalities of acid. At the normality of 6·5, the bromo derivative retains its colour, and its optical density may, therefore, be accurately measured without interference by a small excess of rosaniline.

Fig. 6 shows the densities of the colours obtained with increasing amounts of rosaniline at levels of 1, 2, 3 and 4 μg of bromine as bromate in the test solutions. It is seen that about 6 μg of rosaniline per μg of bromine are necessary for maximum colour development. When there is gross deficiency of rosaniline, colour production is greatly hindered and, when there is gross excess of rosaniline, there is slightly depressed colour density.

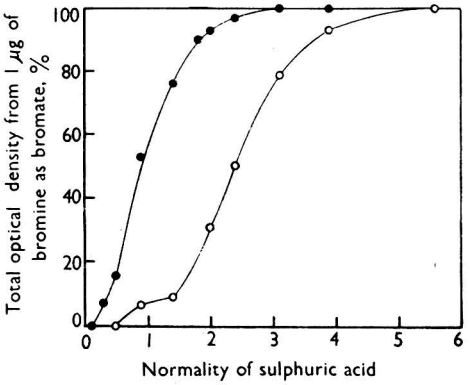

Fig. 5. The effect of concentration of acid, with and without molybdate, on the action of bromate on bromide.
—o——o— without molybdate.
—●——●— with molybdate

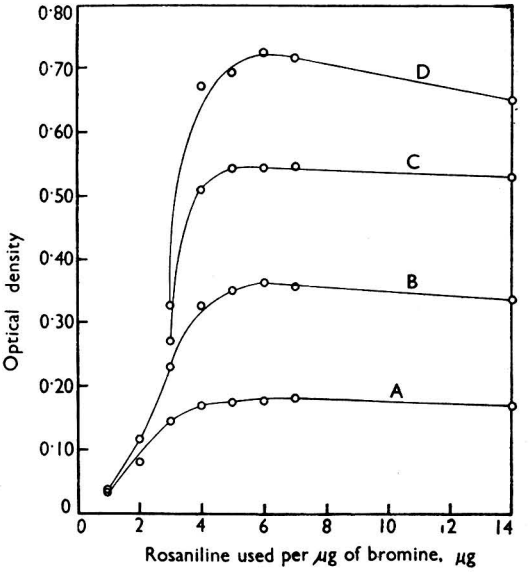

Fig. 6. The amount of rosaniline required for maximum optical density with a given amount of bromate. Curve A, 1 μg; curve B, 2 μg; curve C, 3μg; and curve D, 4 μg of bromine as bromate

The amount of rosaniline necessary, as indicated by Fig. 6, is precisely twice that required on Guareschi's evidence of the formation of tetrabromorosaniline. Turner[5] suggests that a pentabromorosaniline is formed. We have prepared crystalline material by adding bromine to rosaniline in 4 *N* acetic acid at room temperature, drying the product and recrystallising it from hot methanol. Analysis of two preparations by Weiler and Strauss of Oxford proved the substance to be tetrabromorosaniline acetate. (Calculated for $C_{22}H_{19}O_2N_3Br_4$: C, 38·91 per cent.; H, 2·83 per cent.; Br, 47·23 per cent. Found for preparation (*a*): C, 38·83 per cent.; H, 2·95 per cent.; Br, 47·4 per cent.; and found for preparation (*b*): C, 39·21 per cent.; H, 3·12 per cent.; Br, 47·8 per cent.)

Neither chlorine nor iodine substitutes in rosaniline as bromine does, but chlorine alters it to a brown water-soluble material similar to that produced by the action of light. By careful regulation, chlorine may be used to free bromine from bromide, but if there is more

TABLE III

UTILISATION OF HYPOCHLORITE IN CONVERSION OF BROMIDE TO BROMATE

Bromide added, μg ..	0	50	100	150	200
Thiosulphate used, ml ..	1·58	1·55	1·44	1·30	1·15
Hypochlorite used, % ..	35·8	37·0	41·5	47·0	53·3

TABLE IV

EFFECT OF INCREASING NORMALITY OF SULPHURIC ACID IN SUPPRESSING THE COLOUR OF ROSANILINE

Volume of solution 5 ml, as in Stage II. Present: 10 μg of rosaniline

Normality	0·47	0·94	1·4	1·86	2·3	3·0	4·9	5·8	7·0
Optical density × 100	9·4	7·6	5·8	4·1	3·5	2·3	1·5	1·0	0·5

present in solution than required for this action, the chlorine attacks the rosaniline and bromorosaniline and nullifies the method. The presence of chloride greatly increases this action of chlorine, presumably through increasing its oxidation potential.[11]

A similar bleaching action of bromine will be found if the amount of bromine liberated in reaction (2) exceeds the amount of rosaniline needed to absorb it. This point has been noted in the Procedure, Stage II. To get valid absorptiometric readings a slight excess of rosaniline must be present in the solution before the bromine is produced by reaction (2), for rather less colour is produced if the rosaniline is added after the test solution containing bromate. This finding would appear to be explained on the assumption that free bromine dissociates—

$$Br_2 \rightleftharpoons Br^+ + Br^-$$

The presence of chloride appears to catalyse reaction (2) or (3) or both, although the colour density is not affected.

It is clear that some chloride is necessarily present in the test portion. It may be readily calculated that a 1-ml test portion contains about 3·0 mg of chloride as sodium chloride, arising from the corresponding hypochlorite solution.

ACTION OF *tert.*-BUTANOL—

Immiscible solvents, such as chloroform, pentanol and benzyl alcohol, have been used by other workers to extract the bromorosaniline formed. A solvent is necessary, because the bromorosaniline is insoluble in the acid aqueous medium in which it is formed; indeed, there is little colour perceptible in the solution until after addition of a solvent. The addition of such solvents also suspends further oxidative changes in the solution. But we have found it more convenient to use *tert.*-butanol, as this is miscible with the acid aqueous medium and no separation of layers is necessary for absorptiometry. In the proportion stated the colour intensity is approximately the same as after extraction in 5 ml of benzyl alcohol.

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LECTRO-MEDICAL RESEARCH UNIT
MEDICAL RESEARCH COUNCIL
STOKE MANDEVILLE HOSPITAL
AYLESBURY, BUCKS.

December 16th, 1953

DISCUSSION

DR. J. HASLAM said that he was interested in the authors' observations on the oxidation of bromide by persulphate. He thought that a similar mechanism existed with other oxidising agents, such as chromic acid; they would oxidise bromide to bromine quantitatively, but would not oxidise chlorides directly. If the bromide and chloride were mixed, however, oxidation both of the bromide and of some chloride took place.

He confirmed Dr. Hunter's remarks about the presence of bromine compounds in commercial hypochlorite. It was, however, a fact that not all the hypochlorite produced contained the same amount of bromine. Years ago, when working in this field, he used to order bromine-free hypochlorite. With that material the blank in the hypochlorite oxidation was reduced to a very small figure.

He was of the opinion that the significant feature of the authors' method was the fact that it tolerated appreciable proportions of iodine; that would certainly be of value in industrial work. He asked whether the authors had had any experience of the application of their method to the determination of small amounts of bromide in iodides.

DR. R. F. MILTON asked whether the presence of reducing substances likely to occur in biological fluids would cause an uptake of bromine at the liberation stage and thereby affect the result.

MISS M. CORNER said that this paper seemed to her to be the answer to a maiden's prayer. She was working on the problem of determining small quantities of fluorine as lead fluorobromide (provided it was sufficiently insoluble for use on the microgram scale) and this would be the method she would chose for determining the bromide. She asked whether lead would act as a catalyst instead of molybdenum.

DR. HUNTER, in reply to Dr. Haslam, said they had had no experience in the application of the method to bromide in iodides.

In reply to Dr. Milton, Dr. Hunter stated that the method had been used with success on ashed biological fluids, and reducing materials in the ash were fully oxidised by the excess of hypochlorite in Stage I.

In reply to Miss Corner, Dr. Hunter said that lead had not been tried as a catalyst, but in the presence of large amounts of chloride it appeared that molybdenum could almost be dispensed with.

A Volumetric Procedure for the Determination of Zirconium in its Binary Alloys with Uranium

BY G. W. C. MILNER AND P. J. PHENNAH

Zirconium in acid solution can be readily determined by a volumetric procedure that involves the addition of an excess of a standard solution of ethylenediaminetetra-acetic acid to form a complex with the zirconium followed by the titration of unused EDTA with a standard iron solution and salicylic acid as the indicator. Under these conditions it is possible to determine up to at least 100 mg of zirconium with an accuracy of about $\pm$ 1 per cent. This procedure is also applicable after preliminary separation of zirconium from other elements with organic precipitants.

This titration has proved of advantage in the rapid analysis of uranium - zirconium binary alloys after the preliminary separation of the zirconium as the insoluble mandelate from a perchloric acid solution of the alloy.

FOR the analysis of binary alloys of zirconium with uranium, Milner and Skewies[1] recently reported a gravimetric procedure that consisted in separating the zirconium from most of the uranium by extracting it, as its cupferron complex, into chloroform. After the evaporation and ignition of the organic matter, the residue was re-dissolved and the zirconium was finally separated from small amounts of co-extracted uranium by precipitation as the insoluble mandelate. The zirconium mandelate precipitate was ignited to the oxide, and the zirconium content of the alloys was determined from the final weight of zirconium oxide. Although this procedure gave very reliable results for zirconium, it proved rather time-consuming because of the evaporations and ignitions that were needed to remove organic matter. Investigations were started to develop a more rapid procedure for the determination of zirconium in its binary alloys with uranium. The main requirements included a quick separation procedure that was specific for the separation of zirconium from uranium, and also a rapid technique for the determination of the final zirconium concentration that avoided the ignition to zirconium oxide.

Consideration was first given to the possibility of applying a physico-chemical technique to the rapid determination of macro amounts of zirconium. Mayer and Bradshaw[2] recently made use of the coloured lake that is formed by zirconium with alizarin in a direct absorptiometric procedure for the determination of small amounts of this element in magnesium-base alloys. This method was possible because the alloys contained only small amounts of zirconium and they were readily dissolved by hydrochloric acid. In contrast, some of the uranium - zirconium binary alloys contained substantial amounts of zirconium and this type of material could only be completely dissolved by the use of hydrofluoric acid. However, a procedure that is based on the formation of a coloured lake was not considered suitable for the determination of a main constituent of alloys and consideration was therefore given to other physico-chemical techniques.

The polarographic technique seemed to be completely unsuitable for the determination of zirconium, as the only step so far reported[3] for this element occurs after the discharge of hydrogen from a 0·1 *N* potassium chloride solution of pH 3. The half-wave potential of the step is $-1{\cdot}65$ volts against the saturated calomel electrode. Kolthoff and Johnson[4] have,

however, recently reported an amperometric titration procedure for the determination of milligram amounts of zirconium. The titrating reagent is *m*-nitrophenylarsonic acid, which precipitates the zirconium from its solution as the insoluble *m*-nitrophenylarsonate. A voltage of −0·5 volt against the S.C.E. is applied across the electrode system and after the titration end-point, a diffusion current results from the reduction of the nitro group of the excess of titrant at the dropping-mercury electrode. The graph for the determination of the position of the end-point is therefore of the reversed L-type. According to these workers the titration is best carried out in a 1·5 *N* hydrochloric acid medium containing about 20 per cent. of ethanol. This technique did not seem applicable to the direct titration of zirconium in the presence of greater amounts of uranium, as this latter element is reported by Kolthoff and Harris[5] to give a reduction step with an $E_{\frac{1}{4}}$ value of about −0·18 volt against the S.C.E. from 0·01 to 6 *N* hydrochloric acid solutions. The uranium diffusion current could be expected, therefore, to seriously interfere with the measurement of the current from the excess of *m*-nitrophenylarsonic acid.

Ethylenediaminetetra-acetic acid has recently been used for the volumetric determination of certain elements, including magnesium,[6] calcium,[6] zinc,[6] aluminium[7,8] and thorium.[9] Milner and Woodhead's procedure[7] for the determination of aluminium consists in first adding a small excess of a standard EDTA solution to the aluminium solution, which is adjusted to pH 6·5. The solution is then heated to form the aluminium - EDTA complex and, after cooling, the unused EDTA is titrated with a standard ferric iron solution with salicylic acid as the indicator. Cabell[10] recently reported the existence of a complex that is formed by zirconium with EDTA. He observed that whereas zirconium hydroxide is normally precipitated from solutions of pH 2, the presence of EDTA prevented this precipitation until pH values of about 7 were attained. It was evident from these observations that the zirconium - EDTA complex existed at pH values of less than 7 and that this behaviour can be a basis for the development of a volumetric method for zirconium.

Experimental

Development of a rapid method for the determination of zirconium—

In preliminary experiments the aluminium - EDTA volumetric procedure was applied directly to suitable aliquots of a zirconium solution that was prepared by dissolving 6·362 g of Specpure zirconium chloride, $ZrOCl_2.8H_2O$, in a total volume of 500 ml of distilled water that contained a small amount of hydrochloric acid to prevent hydrolysis. The standardisation of 20-ml aliquots of this solution, which was done by precipitation of the zirconium with ammonium hydroxide followed by filtration and ignition of the zirconium hydroxide to zirconium oxide, gave an average result of 73·6 mg of zirconium. Assuming that 1 molecule of zirconium reacted with 1 molecule of EDTA, the zirconium content of a 20-ml aliquot of the zirconium solution was found to be 73·9 mg, by the EDTA volumetric technique. The good agreement between these results justified a fuller investigation of the titration procedure.

Table I

The effect of pH on the recovery of zirconium by the EDTA titration procedure

pH before titration	Zr recovered, mg	Recovery, %
2·2	73·5	99·9
4·0	73·7	100·1
5·0	73·7	100·1
6·0	73·7	100·1

As the pH of the solution was quite important for the aluminium titration, the effect of pH was first studied for the zirconium titration so that the best conditions for the formation of the zirconium - EDTA complex could be selected. Further 20-ml portions of the standard zirconium solution were taken and they were adjusted to suitable pH values in the required range by means of ammonium acetate - acetic acid buffers; the volume of solution containing an excess of 0·1 *M* EDTA just before boiling was about 150 to 200 ml. After cooling, the amount of unused EDTA remaining in the solution was determined by titration with a standard 0·1 *M* ferric iron solution. The results for zirconium by this technique are shown in Table I and they indicate that the recoveries can be good over a wide range of pH values.

At values below pH 3, the titration end-point appeared to be sluggish, whereas at pH values of from 4 to 6·5 an excess of one drop of the iron solution was sufficient to develop the ferric salicylate colour rapidly at the titration end-point. The pH range of 5 to 6 was finally selected as the optimum range for this titration.

Method

Reagents—

Standard iron solution, 0·1 M—Dissolve 5·585 g of Specpure iron in 20 ml of hot hydrochloric acid, sp.gr. 1·16, and then oxidise the iron by boiling the solution after the addition of a few ml of nitric acid, sp.gr. 1·42. Dilute the solution to 1 litre with water. Suitably dilute an aliquot of this solution to give a 0·02 *M* solution.

Ethylenediaminetetra-acetic acid solution, 0·1 M—Dissolve 37·23 g of the disodium salt of ethylenediaminetetra-acetic acid in water and dilute it to 1 litre. Determine the exact molarity of this solution by the following standardisation procedure. Accurately transfer 20-ml aliquots of the EDTA solution to 400-ml conical flasks. Then to each flask add 3 g of ammonium acetate followed by about 150 ml of water. Dissolve 0·2 g of salicylic acid in each solution and then titrate with the standard iron solution until the colour of the solution just changes to brown owing to the formation of ferric salicylate. Suitably dilute an aliquot of this solution to give a 0·02 *M* solution.

Procedure—

To a solution of zirconium in hydrochloric acid, add sufficient EDTA solution to form a complex with the zirconium and to leave a slight excess of reagent. Then to the solution, add a few drops of cresol red indicator followed by ammonium hydroxide, sp.gr. 0·88, added dropwise, to develop the yellow colour of the indicator. Dissolve 3 g of ammonium acetate in the solution, cool it, and add either ammonium hydroxide or acetic acid to adjust the pH of the solution to between 5 and 6, as shown by a direct-reading pH meter. Dilute the solution with water to give a final volume of about 200 ml. Heat the solution to the boiling-point, continue boiling it for about 2 minutes and then cool it to room temperature. Dissolve 0·2 g of salicylic acid in the solution and, with the standard iron solution, titrate the EDTA that is in excess of the amount required to form a complex with the zirconium. For solutions containing less than 30 mg of zirconium, use a 0·02 *M* EDTA solution and a 0·02 *M* iron solution for the titration; for solutions containing more than 30 mg of zirconium use the 0·1 *M* reagents for the titration.

Calculate the weight of zirconium present as follows. Let the volume of EDTA solution added to the zirconium be V_1 ml of M_1 molar solution. Suppose that V_2 ml of M_2 molar ferric iron solution are needed for the back-titration. This is equivalent to $V_2 \times M_2/M_1$ ml of a M_1 molar solution of EDTA. Therefore the volume of EDTA solution needed to form a complex with the zirconium

$$= (V_1 - V_2 \times M_2/M_1) \text{ ml of a } M_1 \text{ molar solution.}$$

As the atomic weight of zirconium is 91·22, the weight of zirconium present

$$= 91{\cdot}22 \times M_1 \times (V_1 - V_2 \times M_2/M_1) \text{ mg.}$$

Results

By the procedure outlined above, with a standard zirconyl chloride solution, the results are those shown in Table II, and they indicate that the method is at least capable of determining amounts of zirconium up to about 100 mg with an accuracy of better than 1 per cent.

Table II

Determination of zirconium by the EDTA titration procedure

Weight of zirconium taken, mg	Weight of zirconium recovered, mg	Error, %
2·42	2·41	−0·4
4·85	4·81	−0·8
9·69	9·64	−0·5
19·28	19·25	−0·17
29·07	28·90	−0·6
50·5	50·8	+0·6
73·6	73·9	+0·4
99·6	100·0	+0·4

Further titrations were carried out in the presence of sulphuric acid to study the recovery of zirconium under these solution conditions. The procedure consisted of taking suitable aliquots of the standardised zirconyl chloride solution and, after the addition of 25 ml of a 25 per cent. v/v solution of sulphuric acid, evaporating each solution to fumes of the acid. On completing the EDTA titration procedure as above, the zirconium recoveries are those shown in Table III, and it can be seen that small amounts of sulphuric acid do not interfere with this determination. In addition, the results of titrations in the presence of larger amounts of sulphuric acid, up to a maximum of 25 ml of concentrated acid in the zirconium solution, showed that it did not interfere.

TABLE III

DETERMINATION OF ZIRCONIUM BY THE EDTA TITRATION PROCEDURE IN THE PRESENCE OF SULPHURIC ACID

Weight of zirconium taken, mg	Weight of zirconium recovered, mg	Error, %
2·42	2·51	+1·8
4·84	4·88	+0·8
9·68	9·69	+0·1
24·22	24·30	+0·4
48·0	48·0	Nil
96·6	96·7	+0·1

INVESTIGATION OF METHODS FOR THE SEPARATION OF ZIRCONIUM FROM OTHER ELEMENTS BEFORE ITS TITRATION WITH EDTA

Several organic acids, or their ammonium salts, have been used during the past few years for the precipitation of zirconium from solution to effect the separation of this element from other elements. These reagents include *m*-nitrobenzoic acid,[11] ammonium benzoate,[12] fumaric acid,[13] phthalic acid,[14] mandelic acid[15,16] and *m*-cresoxyacetic acid.[17] With all these reagents, the zirconium precipitate is ignited to zirconium oxide after filtration and the zirconium content is calculated from the weight of the oxide. On attempting to weigh zirconium mandelate precipitates directly after suitable drying, Oesper and Klingenberg[18] encountered difficulties that were caused by the use of large amounts of mandelic acid in the precipitation stage. After filtration, it proved very difficult to remove mandelic acid from the precipitate without redissolving some of the zirconium mandelate. After a study of various substituted mandelic acids, the above workers recommended the use of *p*-bromomandelic acid for the direct determination of zirconium. The precipitate that is produced by this reagent can be washed with water to free it from excess of precipitant and, after drying at 100° C, it can be weighed. Belcher, Sykes and Tatlow,[19] however, recently failed to substantiate the above findings and the results were reliable with this reagent only after igniting the zirconium *p*-bromomandelate precipitate to zirconium oxide. In addition, recent methods that use *p*-bromomandelic acid for the separation of zirconium in steel[20] and for the analysis of aluminium alloys[21] involve the ignition of the zirconium precipitate to the oxide.

Qualitative tests showed that many of the above complexes of zirconium with organic acids were soluble in strong mineral acids and that this behaviour presented the possibility of completing the zirconium determination volumetrically with EDTA, providing that no difficulties arose from the presence of organic molecules in solution. Ammonium benzoate was the first reagent to be investigated, as previous work[7] had shown that small amounts of benzoic acid did not interfere in EDTA titrations. Jewsbury and Osborn[12] reported that this reagent gave complete recovery of zirconium from hydrochloric and nitric acid solutions containing ammonium acetate starting from pH 1·0 to 1·5, whereas from sulphuric acid solutions the starting pH is given as from 2·0 to 2·5. In all the work the zirconium benzoate precipitates were ignited to zirconium oxide, which was then weighed. These results are in agreement with the complexing action of sulphuric acid with zirconium as compared with the weak or non-complexing powers of the other acids. These workers make no reference to the recovery of zirconium from perchloric acid solutions.

Jewsbury and Osborn's precipitation procedure was applied directly to pure solutions of zirconium in hydrochloric, nitric, sulphuric and perchloric acids. Each acid was investigated in turn by taking aliquots of a standard zirconium solution in the requisite acid, and the effects of pH on the recovery of zirconium were studied. After digestion, the benzoate precipitates were filtered through Whatman No. 40 filter-papers, and then they were washed

back into the precipitation beakers with water. Each precipitate was then dissolved in a 20-ml portion of hot hydrochloric acid, sp.gr. 1·18, first poured over the filter-paper, which was then washed with water. The zirconium content of these solutions was determined by the EDTA titration procedure and graphs were plotted showing the variations of the recovery of zirconium against pH. The results for the four acids are shown in Fig. 1 and these graphs agree reasonably well with Jewsbury and Osborn's statements for the recovery of zirconium from the first three acids. The precipitation of zirconium from perchloric acid solutions proved very interesting from the analytical point of view as its behaviour was similar to that in the weakly complexing acids (hydrochloric acid and nitric acid). Moreover, as the pH values for the complete recovery of zirconium from perchloric acid solutions are

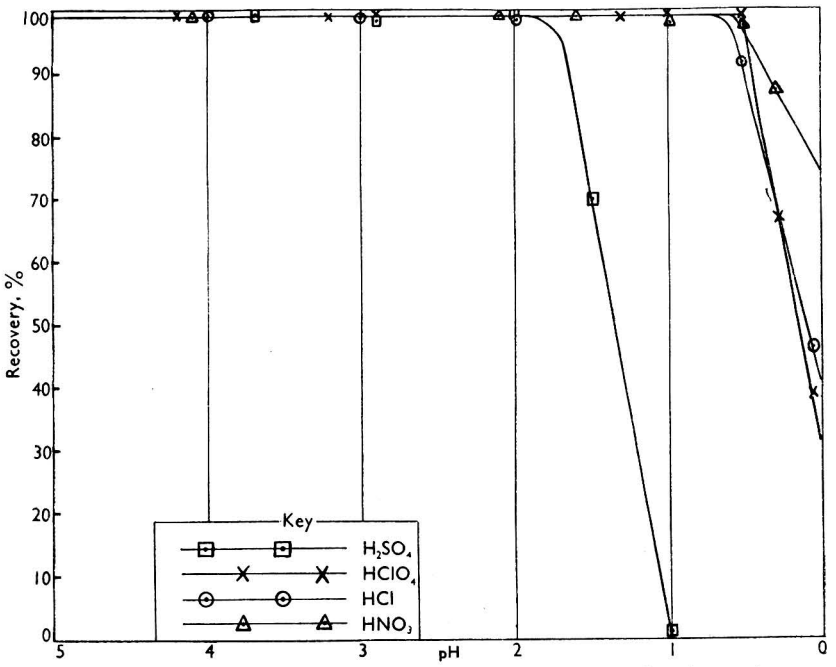

Fig. 1. Recovery of zirconium by precipitation with ammonium benzoate

lower than the values for sulphuric acid solutions, the former acid should be of greater value for separating zirconium from other elements; the chances of contamination of the zirconium benzoate precipitate with other elements should be less from perchloric acid solutions than from sulphuric acid solutions. The precipitation behaviour from perchloric and sulphuric acids was more important, because hydrofluoric acid is often needed to complete the solution of many alloys and this reagent must then be removed by evaporating the alloy solution to fumes of either perchloric or sulphuric acid, before carrying out the analysis. Unfortunately, certain other elements[12] are also precipitated by ammonium benzoate either completely or partially under the conditions necessary for the complete recovery of zirconium; the investigation was therefore extended to include a study of other possible precipitants.

Derivatives of benzoic acid with higher dissociation constants were briefly investigated in the hope that they would give complete recovery of the zirconium from solutions of higher acidity than those necessary for benzoic acid. Toluic acid, with a dissociation constant roughly twice that of benzoic acid, appeared to offer very little advantage, nor did *m*-nitrobenzoic acid according to the results of Osborn's investigations[11]. *o*-Chloro- and *o*-nitrobenzoic acids, with dissociation constants roughly 10^3 times that of benzoic acid, quantitatively precipitated zirconium from 0·2 to 0·5 *N* hydrochloric acid solutions. These acids are, however, very insoluble and filtration of the zirconium precipitates proved very difficult owing to the excess of reagent crystallising out of solution. *p*-Hydroxybenzoic acid, gallic acid, anthranilic acid and vanillic acid failed to precipitate zirconium from 0·1 *N* hydrochloric acid solutions and they are therefore inferior to unsubstituted benzoic acid.

m-Cresoxyacetic acid[17] has been recommended as a reagent for the precipitation of zirconium; the precipitation is complete from 0·20 to 0·25 *N* hydrochloric acid solutions. However, on dissolving this type of precipitate in hydrochloric acid before the application of the EDTA titration of the zirconium, red cresol products, presumably from the breakdown of the reagent, were formed. This effect masked the end-point of the iron back-titration and the use of this reagent was therefore not investigated further. Phthalic acid,[14] fumaric acid[13] and mandelic acid[15,16] have also been recommended as precipitants for the separation of zirconium. Phthalic acid was not studied, however, because a 2-hour digestion period

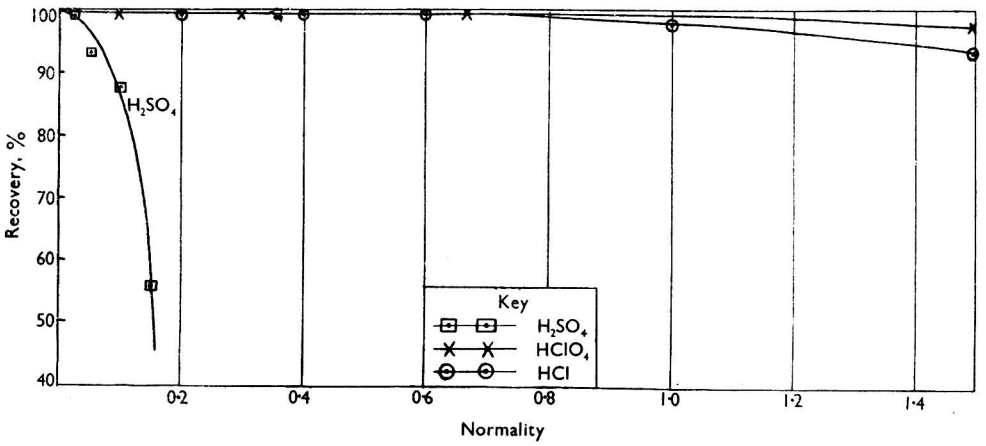

Fig. 2. Recovery of zirconium by precipitation with fumaric acid

is needed to give the complete precipitation of the zirconium. In the investigation of the other two acids, some difficulty was initially experienced in the re-solution of the zirconium fumarate and zirconium mandelate precipitates in hydrochloric acid, before the EDTA titration. However, both of these precipitates were found to dissolve in boiling dilute sulphuric acid and this allowed the EDTA titration to be applied, as the results of previous

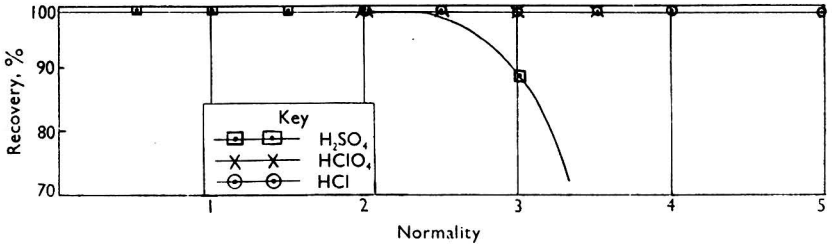

Fig. 3. Recovery of zirconium by precipitation with mandelic acid

work (see p. 478) showed that some sulphuric acid can be present in the titration procedure without affecting the zirconium results. On re-dissolving the fumarate precipitates from 50-mg amounts of zirconium in 25-ml portions of 25 per cent. sulphuric acid before titration with EDTA, the results are those shown in Fig. 2, for the recovery of zirconium from hydrochloric, sulphuric and perchloric acid solutions. Venkataramaniah and Rao[13] reported the complete recovery of zirconium from solutions up to 0·35 *N* in hydrochloric acid. This is confirmed in Fig. 2 and, in addition, the recovery is seen to be essentially complete from slightly more acid solutions. As with benzoic acid, the recoveries from perchloric acid solutions proved to be very similar to those from hydrochloric acid solutions and recoveries from sulphuric acid solutions were definitely inferior.

According to Kumins' work,[15] zirconium is quantitatively precipitated by mandelic acid from stronger hydrochloric acid solutions (2*M*) than is possible with the other organic acids. Furthermore, Hahn[16] claims that the hydrochloric acid concentration is not critical, as his results were quantitative from solutions 0·1 *M* to 8 *M* with respect to hydrochloric acid.

This behaviour undoubtedly accounts for the more specific nature of mandelic acid for separating zirconium from other elements; zirconium is quantitatively separated by this acid from Ti, Fe, V, Al, Cr, Th, Ce, Sn, Ba, Ca, Cu, Bi, Sb, Cd, Mg, Hg, Ni, U, Zn, Co and Mn. With Kumins conditions for precipitating 50-mg amounts of zirconium and allowing the solutions to stand for 60 minutes before filtration, the mandelate precipitates were dissolved in 25-ml portions of 25 per cent. sulphuric acid and the results of the EDTA procedure for precipitations from hydrochloric, sulphuric and perchloric acids are shown in Fig. 3. Again the behaviour of zirconium in perchloric acid solutions agreed closely with that in hydrochloric acid solutions, and it indicated the advantages of the use of this acid over those of sulphuric acid in the analysis of alloys, especially for those samples where hydrofluoric acid is used to dissolve the material completely.

The analysis of uranium - zirconium alloys

From the results of the previous section, mandelic acid appeared to be the precipitant likely to give the best separation of zirconium from uranium before the EDTA titration procedure. In preliminary experiments to test this precipitant in the presence of uranium, synthetic solutions were prepared each containing a 10-ml aliquot of a standardised zirconyl chloride solution (48·4 mg of zirconium) and 2·5 g of uranyl nitrate dissolved in water. After the addition of 25 ml of 72 per cent. perchloric acid to each solution, they were evaporated to heavy fumes of this acid. On cooling, 25 ml of hot water was added to each beaker followed by 50 ml of hot 16 per cent. mandelic acid solution to precipitate the zirconium. The solutions were next heated at about 80° C for 60 to 90 minutes and then the zirconium mandelate precipitates were filtered on Whatman No. 40 filter-papers; a hot 5 per cent. mandelic acid solution in 2 per cent. hydrochloric acid was used for washing the precipitates. After the resolution of each precipitate in 25 ml of 25 per cent. v/v sulphuric acid, the EDTA titration technique gave results of 99·6, 99·2 and 100·0 per cent. recovery of zirconium.

The above satisfactory results led to the development of a suitable procedure for the determination of the zirconium content of uranium - zirconium binary alloys. The most suitable sample weights required for this analysis are given in Table IV and the best solution technique consisted of attacking the sample with hot nitric acid to dissolve as much material as possible, followed by the addition of a small amount of hydrofluoric acid to complete the dissolution.

Table IV

Sample weights for the analysis of uranium - zirconium alloys

Nominal zirconium content, %	Sample weight, g	Remarks
0 to 5	2·0	Use all the sample for analysis
5 to 10	2·5	Dilute solution to 250 ml and take 50 ml for analysis
10 to 25	2·0	Dilute solution to 250 ml and take 50 ml for analysis
25 to 50	2·0	Dilute solution to 250 ml and take 25 ml for analysis
>50	0·5	Dilute solution to 250 ml and take 50 ml for analysis

Procedure

Transfer the requisite weight of sample to a 100-ml platinum dish, add to it 25 ml of nitric acid, sp.gr. 1·42, and warm. When the action of the nitric acid ceases, add dropwise the smallest amount of 40 per cent. hydrofluoric acid necessary to complete the solution of the sample. Then add 25 ml of 72 per cent. perchloric acid and evaporate the solution to strong fumes of this acid, making use of infra-red heaters. Continue the fuming for about 10 minutes. Cool the solution and dilute it to 250 ml, if necessary, and take an aliquot in accordance with Table IV. Place the sample solution in a 400-ml squat-type beaker, add 7 ml of 72 per cent. perchloric acid for alloys containing 5 per cent. of zirconium or less and 25 ml of this acid for all other samples (to ensure a final acidity of about 3 *N* with respect to perchloric acid). Evaporate the solution on the hot-plate to dense fumes of perchloric acid, and heat strongly for about 20 minutes to remove the hydrofluoric acid. Cool the beaker

and contents, add 25 ml of water, and heat the solution almost to boiling. Then slowly add 50 ml of a hot 16 per cent. mandelic acid solution and stir the solution whilst heating until precipitation of the zirconium mandelate is appreciable. Maintain the solution at above 80° C for about 90 minutes.

Filter the solution through a Whatman No. 40 filter-paper, washing the precipitate several times with a hot 5 per cent. mandelic acid solution in 2 per cent. hydrochloric acid. Wash the precipitate from the filter-paper back into the precipitation beaker, pour 25 ml of hot 25 per cent. v/v sulphuric acid over the paper, and wash it with hot water. Return the beaker to the hot-plate and heat it to gentle boiling until complete solution of the precipitate is produced. Cool this solution and then complete the zirconium titration with EDTA, as described previously.

The results for zirconium found by applying the above procedure to typical uranium - zirconium alloys and results for the same samples when analysed by the cupferron procedure reported earlier,[1] showing the good agreement between the results by these procedures, are as follows—

Zirconium by gravimetric procedure, % ..	2·2	2·85	4·80	6·1	59·0
Zirconium by volumetric procedure, % ..	2·19	2·70 2·70	4·98 4·90	5·88 5·88	59·2

NOTE—After the completion of this investigation, information was received about an independent development of a titration procedure for zirconium with ethylenediaminetetra-acetic acid by J. S. Fritz and M. O. Fulda, Institute of Atomic Research, Iowa State College, Ames, Iowa. Details are published in Report I.S.C. 382.

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A Study of the Separation of Zinc from Certain Other Elements by Means of Anion Exchange

By CHRISTINA C. MILLER and JOHN A. HUNTER

Five to 50 mg of zinc, in 2 *N* hydrochloric acid solutions containing a maximum of 100 mg of metal ions, can be quantitatively adsorbed on a 15-cm column prepared from 3 g of the strong-base anion-exchange resin, Amberlite IRA-400 (Cl). After the percolation of 2 *N* hydrochloric acid to a total of 50 ml, aluminium, magnesium, copper, cobalt, nickel, manganese^{II}, chromium^{III}, iron^{III}, thorium, zirconium, titanium^{IV}, uranium^{VI}, beryllium and calcium are found almost entirely in the eluate. Cadmium, tin^{IV}, antimony^{III} and bismuth behave similarly to zinc, and some lead and indium are held. Zinc, cadmium and indium are eluted by means of water and 0·25 *N* nitric acid, which also remove 20 per cent. of tin, small amounts of antimony, bismuth and lead, and small residual amounts of other metals. If water alone is used, various small quantities of zinc are tenaciously retained by the resin. Methods are proposed for the separation of zinc from solutions free from cadmium and indium and for its determination with 8-hydroxyquinoline. In the analysis of a few alloys containing 2·5 to 34 per cent. of zinc, the results have been promising and of moderate precision. The quantitative separation of 0·5 mg of zinc from 100 mg of aluminium or magnesium has been achieved.

There is marked activity in the field relating to the analytical chemistry of zinc, which presents difficulty in analysis because of the lack of specificity in the reactions available for its determination. Polarographic methods have been applied in the analysis of light alloys.[1] Bishop and Liebmann[2] separated zinc from numerous other elements by cellulose chromatography before determining it polarographically. Recently, Kinnunen and Wennerstrand[3] used the disodium salt of ethylenediaminetetra-acetic acid for determining zinc that had first been separated from other metals by extracting it as the thiocyanate from an acid solution by means of an organic solvent. Flaschka,[4] who used potassium cyanide as a complexing agent, successfully determined zinc and cadmium in the presence of large amounts of cobalt, nickel, copper and mercury. Cation exchange was utilised by Brown and Hayes[5] for separating most of a large amount of magnesium from zinc before determining the latter with disodium ethylenediaminetetra-acetate. Earlier,[6] the selective extraction of zinc by means of an organic solvent mixture from a cation-exchange resin on which zinc, cobalt and nickel had previously been adsorbed was reported.

We were primarily interested in the determination of small to large amounts of zinc in association with other metals that might normally be expected to interfere. Moore and Kraus,[7] after studying the anion-exchange behaviour of iron, cobalt, nickel, copper, manganese and zinc in hydrochloric acid solutions on the strong-base anion-exchange resin, Dowex 1, indicated that the separation of these elements from one another should be possible on a resin column of moderate length. All the metals except nickel were adsorbed from concentrated hydrochloric acid solution. By examining the behaviour of the various elements in different concentrations of hydrochloric acid, they found that only zinc was very strongly adsorbed from 2 *N* acid. Elution of zinc was effected readily with 0·005 *N* hydrochloric acid. If a 2 *N* hydrochloric acid solution containing zinc and other elements was placed in a resin column and certain of the elements were washed out with an excess of the same acid, it seemed possible that the zinc could then be eluted with water.

With the anion-exchange resin Amberlite IRA-400, in the chloride form, we have succeeded in effecting the separation of 5 to 50 mg of zinc from a considerable number of elements in solutions containing a maximum of about 100 mg of metals. We have also studied the behaviour of elements whose separation is incomplete. Certain irregularities that occurred in the use of the resin are detailed. Methods adopted for the separation and determination of zinc have been applied tentatively to several alloys, and preliminary experiments have been made on the separation of half-milligram amounts of zinc.

Experimental

The determination of zinc with 8-hydroxyquinoline—

As ion-exchange separations might be incomplete, a method of fairly general applicability, namely precipitation with 8-hydroxyquinoline, was chosen for the determination of zinc. In buffered acetic acid medium, zinc is separable from small amounts of lead, manganese and magnesium, because the 8-hydroxyquinoline complexes of these metals begin to form at a pH above that required for quantitative deposition of the zinc complex. But in acid solutions, zinc cannot be separated from iron, cobalt, nickel, aluminium, copper, tin, bismuth, and so on. In alkaline tartrate solutions, cadmium, copper, magnesium, iron^{II} and a few other elements, if present, are precipitated with the zinc - 8-hydroxyquinoline complex, whereas moderate amounts of bismuth, antimony, tin, arsenic, iron^{III}, aluminium, chromium^{III}, manganese^{II}, cobalt and nickel are probably tolerable. Berg's[8] methods were slightly modified to give the following reasonably reliable gravimetric procedures for all subsequent determinations of zinc in this work. The first method is simpler and, when it is applicable, is preferred to the second.

Method

Apparatus and reagents—

Use Pyrex or similar glassware throughout and reagents of recognised analytical grade. Prepare 6 *N* ammonium hydroxide from cylinder ammonia and store it in a polythene bottle.

Procedure 1—

Precipitation in acid solution—To 60 ml of an approximately 0·25 *N* hydrochloric acid solution containing 20 to 50 mg of zinc, add 6 *N* ammonium hydroxide until the solution is neutral to methyl red indicator, and then add 0·1 ml of glacial acetic acid and 6 ml of 30 per cent. w/v ammonium acetate solution. Heat the solution to 60° C and add a 2 per cent. w/v solution of 8-hydroxyquinoline in 0·8 *N* acetic acid at the rate of 1 drop per second, until an excess of 1 ml has been added. Heat the mixture gradually just to the boiling point and maintain it there for 2 to 3 minutes. Cool the mixture in running water for 30 minutes, then remove the precipitate on a weighed sintered-glass crucible of No. 4 porosity, using 50 ml of hot water for transferring the precipitate and washing it. Stir up the precipitate on the filter during washing, and dry it at 160° C to constant weight.

For 5-mg amounts of zinc proceed as above, but replace the crucible with a filter-tube provided with another as a tare, and give both tubes the same heating and cooling treatment. If only 2 to 3 mg of zinc are present, reduce all volumes to one-half and use a semimicro-balance. When 0·5-mg amounts of zinc are dealt with, have initially 3 ml of solution in a 6-ml beaker, carry out the precipitation on a micro-scale, with one-twentieth of the usual amounts of reagents, suitably diluted if necessary, and filter the precipitate through a sintered-glass filter-stick of No. 4 porosity. Proceed with the wiping, heating and cooling of the beaker and filter and the associated counterpoise in the manner described by Miller and Chalmers,[9] and weigh them on a semimicro-balance. Dissolve the precipitate in a concentrated hydrochloric acid - ethanol mixture (1 + 2, v/v), remove the solution and wash, dry and weigh the beaker and filter-stick.

The zinc - 8-hydroxyquinoline complex contains 18·49 per cent. of zinc. Apply a correction of +0·3 mg to all results for about 50-mg quantities of zinc.

Notes on procedure 1—Ammonium acetate had to be added before 8-hydroxyquinoline. If it were added after, with perhaps a little ammonium hydroxide to adjust the pH of the solution, or if ammonium hydroxide was added to increase the pH of the solution after the zinc - 8-hydroxyquinoline complex had been precipitated as described, difficulty was experienced in attaining constancy of weight. Although, according to Borrel,[10] precipitation is complete within the pH range 4·5 to 12, increasing the pH from 4·7 to 5·5 increased the negative error in the determination of 50 mg of zinc, probably because the zinc was not entirely in the correct form. Increasing the excess of 8-hydroxyquinoline used in precipitating 50 mg of zinc led to an increase in the weight of the precipitate, despite a fall in the pH of the solution. A constant volume excess of reagent was eventually used, although, as is shown in Table I, there was a consistent negative error in determining 50 mg of zinc.

Experiments were made with weighed aliquots of standard solutions of zinc chloride prepared from Hilger's "H.H.P." (99·99 per cent.) zinc and AnalaR concentrated hydrochloric acid.

TABLE I

DETERMINATION OF ZINC IN BUFFERED ACETIC ACID SOLUTION

Zinc taken, mg	Error, mg	pH of filtrate	Zinc taken, mg	Error, mg	pH of filtrate	Zinc taken, mg	Error, mg	pH of filtrate
52·75	−0·29	4·7	20·90	−0·10	5·1	5·68	+0·09	5·4
52·75	−0·29	4·7	21·05	+0·05	5·1	6·07	+0·01	5·4
47·18	−0·24	—	17·60	+0·02	—	5·67	+0·02	—
48·63	−0·37	—	18·29	+0·03	—	5·62	+0·04	—

The influence of other substances—Only small amounts of tin and antimony^{III}, but more lead, may be present, as is shown by the following results on the determination of 5 mg of zinc in the presence of these elements—

	Tin				Lead		Antimony	
Weight of other metal, mg ..	0·3	0·5	3	5	2	2	0·5	0·75
Error on zinc, mg	+0·11	+0·12	+0·17	+0·19	+0·03	+0·08	+0·14	+0·05

Antimony^{V} was so slowly hydrolysed that 10 mg of it did not affect the determination of 5 to 50 mg of zinc.

The addition of 0·8 g of ammonium nitrate to solutions containing 5 to 50 mg of zinc was without significant influence on the results.

PROCEDURE 2—

Precipitation in alkaline tartrate solution—To 60 ml of an approximately 0·25 *N* hydrochloric acid solution containing 5 to 50 mg of zinc, add 1 g of tartaric acid and 0·05 ml of 0·002 per cent. w/v *o*-cresolphthalein solution, and then 5 ml more of carbonate-free 2 *N* sodium hydroxide than is required to make the indicator pink. If the amount of zinc is near the maximum, increase the excess of 2 *N* sodium hydroxide, if necessary, to 6·6 ml in order to ensure complete solution. To the cold solution add 3 to 5 mg of sodium tauroglycocholate, which prevents the precipitate adhering to the beaker and assists filtration, and then add, at the rate of 1 drop per second, with stirring, a fresh 2 per cent. w/v solution of 8-hydroxyquinoline in 0·75 per cent. w/v sodium hydroxide solution, until an excess of 0·5 ml has been

TABLE II

DETERMINATION OF ZINC IN ALKALINE TARTRATE SOLUTION

Standard amount of 2 *N* sodium hydroxide			Effect of additional 2 *N* sodium hydroxide		
Zinc taken, mg	Excess of 2 *N* sodium hydroxide, ml	Error, mg	Zinc taken, mg	Excess of 2 *N* sodium hydroxide, ml	Error, mg
47·67	6·6	+0·09	48·25	8	+0·19
48·40	6·6	+0·03	48·57	8	+0·26
			52·75	15	−0·50
18·15	5	+0·12	52·75	25	−1·86
17·91	5	−0·09	17·54	8	−0·40
			18·92	8	−0·33
5·35	5	−0·04	5·18	8	−0·63
5·37	5	−0·04	4·58	8	−0·38

added. For amounts of zinc under 10 mg (precipitation may not take place in the cold) add 2·5 ml of the precipitant. Digest the mixture at 70° to 80° C for 30 minutes in order to induce the precipitation of small amounts of zinc and to flocculate the precipitate already present. Filter the hot mixture through an unweighed sintered-glass crucible (the alkaline solution causes loss of weight), and continue the determination as described in procedure 1. Finally dissolve the precipitate in a concentrated hydrochloric acid - ethanol mixture (1 + 2, v/v). Wash, dry and weigh the crucible.

Notes on procedure 2—The method differs from Berg's in that 8-hydroxyquinoline is dissolved in a minimum excess of sodium hydroxide solution instead of in ethanol or acetone, both of which markedly inhibited the precipitation of small amounts of zinc. As was found

by Berg, variations in the excess of sodium hydroxide used had an influence on the results, and with small amounts of zinc it was necessary to restrict the quantity of alkali used. More latitude was permissible with large amounts of zinc. In the determination of 20 mg of zinc, the 8-hydroxyquinoline could be present in a 10 to 50 per cent. excess without significant effect. With small amounts of zinc, precipitation did not occur readily in the cold and therefore a fixed quantity of precipitant was added for all amounts below 10 mg. Berg did not heat the solutions until a precipitate appeared, but heating them immediately after the addition of the precipitant did not have an adverse influence. The results in Table II, which are for the same standard solutions as were used in procedure 1, show that the method is adequate for the present purpose.

The influence of other metals—As the following results show, large amounts of tin could be tolerated, except perhaps with the smallest amounts of zinc, when contamination by hydrated stannic oxide occurred and made filtration very slow—

Zinc taken, mg	46·97	48·99	18·15	17·70	4·71	5·86
Tin taken, mg	50	50	80	80	100	100
Error, mg	+0·33	+0·05	−0·03	+0·07	+0·15	+0·11

Even 100 mg of iron^{III} gave no precipitate with 8-hydroxyquinoline in cold or hot solutions. Five milligrams of cobalt gave some precipitation in hot solutions, so that only a small amount could be tolerated.

PRELIMINARY EXPERIMENTS ON ION EXCHANGE—

In the preparation of materials containing zinc for analysis, it will often be possible to arrange to have a solution consisting mainly of chlorides of metals in hydrochloric acid. It was proposed to apply to a column of resin in the chloride form, in equilibrium with 2 *N* hydrochloric acid, about 5 ml of a 2 *N* hydrochloric acid solution of the metals, then to wash the column with sufficient of the same acid to remove unadsorbed and weakly adsorbed ions, to elute zinc by means of water, and then to determine it with 8-hydroxyquinoline.

Moore and Kraus used Dowex-1, a strong-base anion-exchange resin, but, as this was not readily available, we used Amberlite IRA-400 resin (Analytical Grade), and preferred it to the comparable product De-acidite FF, because of its somewhat greater uptake of zinc. Qualitative experiments, which were made with 1 to 5 mg of metals on columns containing 0·25 g of resin, provided information for testing more exactly the separation on larger columns of 5 to 50 mg of zinc from other metallic elements in solutions containing a maximum of 100 mg of metals. With these small columns, 5 mg of nitrate, sulphate, arsenate, phosphate or molybdate, present initially with 2·5 mg of zinc, were found only in the hydrochloric acid eluate, which was free from zinc. All other experiments to be described were made at about 17° C on resin beds, 15 cm deep and about 7 mm in diameter, containing about 3 g of resin.

When several millilitres of a 2 *N* hydrochloric acid solution containing 50 mg of zinc were allowed to pass on to the chloride form of the resin, which was in contact with the same acid, and the column was then further washed with the acid, the first 50 ml of eluate were found to contain less than 0·01 mg of zinc. Probably 75 ml of acid could be used without significant loss of zinc. It was thought that 50 ml would effectively remove maximum amounts of copper, cobalt, nickel, manganese^{II}, chromium^{III}, aluminium, thorium, zirconium, titanium, uranium^{VI}, beryllium, magnesium and calcium. Much iron^{III} was readily eluted, but a little was retained by the resin. Cadmium behaved like zinc, and the removal of indium was incomplete. All of 100 mg of tin^{IV} and of 50 mg of antimony^{III} or bismuth were thought to remain on the column, but when antimony was in the quinquevalent form 25 per cent. of it was found in the eluate. Lead chloride, in the amount required to saturate the small volume of acid solution added to the column, was held by the resin.

Qualitative tests were similarly made in order to find how much water would be required to elute 50 mg of zinc from the resin and the effect of that volume on other adsorbed elements. Twenty millilitres of water removed over 80 per cent. of the zinc, and, after 60 ml had been used, two further 20-ml portions showed the presence of 0·02 mg and less than 0·01 mg, respectively. Sixty millilitres of water were considered adequate. Of the other metals that were adsorbed from 2 *N* hydrochloric acid solutions, 100 mg of tin^{IV} yielded 20 mg to the water eluate, 50 mg of antimony^{III} yielded 2 mg, 50 mg of antimony^{V} yielded 10 mg, a few mg of lead yielded 1 mg and 50 mg of bismuth yielded nothing. Most, if not all,

of 50 mg of cadmium was also removed, as was residual indium. Traces of iron and copper were in the eluate.

When elution was effected first with 20 ml of water and then with 40 ml of 0·25 *N* nitric acid, instead of entirely with water (see p. 486), the amount of antimony^{III} accompanying zinc was doubled, and there were 2 to 3 mg of bismuth when 5 to 50 mg were present initially. Contamination by iron and copper was greater. By direct quantitative test, 100 to 5 mg of copper, in the presence of some zinc, were found to yield 0·2 per cent. of the amount applied to the solution containing zinc. Similarly, 100, 10, 5 and 0·5 mg of iron^{III}, the last three in association with 10 mg of zinc, gave 0·6, 0·07, 0·04 and 0·015 mg, respectively. A blank test with 10 mg of zinc and no added iron gave 0·002 mg of iron. On occasion, however, larger amounts of iron were noted than would be predicted from these results, indicative of some erratic effect.

None of the foregoing elements when present in the amounts quoted, except cadmium and indium, would be expected to interfere seriously with the determination of zinc with 8-hydroxyquinoline in an alkaline tartrate solution. The copper would be quantitatively precipitated, but a correction seemed readily applicable. It was therefore concluded that it should be possible to evolve a method for the determination of zinc originally in association with a large number of other metals, excluding cadmium and indium.

Quantitative experiments with zinc alone and also in association with other elements—

The first experiments on the determination of zinc that had been eluted with water from a resin column on which it had previously been adsorbed were sufficiently satisfactory to justify a test of the behaviour of mixtures. In experiments involving the separation of the smaller amounts of zinc from metals that were not held on the resin, there were a few rather large negative errors. In general, resin columns that could be freed of adsorbed ions when eluted with water had been regenerated with 2 *N* hydrochloric acid and used again, but fresh columns were also needed at intervals. As the more significant errors were associated with the latter, a thorough investigation was made of the behaviour of zinc on fresh resin and on regenerated resin, in an attempt to locate the source of the irregularities. The results, shown in Table III, indicate that larger negative errors were associated with resin that was in use for the first time. With fresh resin the amount of zinc lost from 50 mg originally taken ranged from 0·1 to 0·7 mg, and that lost from 5 mg of zinc was from 0·04 to 0·4 mg. A different batch of resin showed comparable irregularities. Decreasing the flow-rate of solutions through the columns did not effect any improvement. Lost zinc was not found in the acid eluate, and in an experiment in which 50 mg of zinc had been used and 0·34 mg had been lost, only 10 per cent. of this amount was recovered from a further 40 ml of eluting water. As slight hydrolysis might have caused loss of zinc, eluting with 0·005 *N* hydrochloric acid instead of with water was tried, but there was no improvement.

Table III

Determination of zinc alone, after adsorption on and elution from the anion-exchange resin Amberlite IRA-400 (Cl)

Approximate weight of zinc taken, 10^{-3} g	Elution with 60 ml of water: Resin not previously used. Error, 10^{-5} g	Elution with 60 ml of water: Regenerated resin. Error, 10^{-5} g	Elution with 20 ml of water and 40 ml of 0·25 *N* nitric acid. Error, 10^{-5} g
50	−12, −31, −72, −34, −15, −34	+2, −24, −30, −11	+18, −17, +30, −11, +6
20	−12, −17	−5, −8	+4, +9
5	−41, −15, −18, −23 −33*, −20*, −4	−6, +7	+6, +3, +6, +3

* Slower rate of flow.

The use of resin pre-saturated with zinc—

As zinc was evidently sometimes retained on the resin, experiments were made on a resin that had been previously treated with an excess of zinc chloride. Resin, in the chloride form, was soaked overnight in an excess of a solution of zinc chloride in 2 *N* hydrochloric acid,

and a column was prepared from the suspension. The resin was washed with 2 *N* hydrochloric acid and then with 60 ml of water. With further 20-ml water washings, a significant amount (0·05 mg) of zinc was found only in the first eluate. The resin was left in contact with water overnight and then 50 ml of 2 *N* hydrochloric acid were passed through it and tested for zinc, when 0·05 mg was found. Further treatments with 60 ml of water and 50 ml of acid yielded 0·1 and less than 0·01 mg of zinc. A final wash with water, after the resin had been in contact with acid for 3 days, gave 0·05 mg of zinc. These results showed how tenaciously a small amount of zinc was held by the resin.

Two columns of zinc-saturated resin were repeatedly treated alternately with water and 2 *N* hydrochloric acid before they were used for testing the recovery of 5-mg amounts of zinc, which were applied to them in the usual way. But the negative errors still persisted and were similar to the maximum error previously recorded for this amount of zinc. This showed that the resin, originally saturated with zinc, had for all practical purposes been freed from zinc by the successive acid and water treatments and was reclaiming some from the test solution.

Water was left in both columns overnight and then 50 ml of 0·005 *N* hydrochloric acid were passed through, with removal of about 0·05 mg of zinc. Further washing of one column with 20 ml of 0·25 *N* sulphuric acid yielded 0·05 mg of zinc, and of the other with two 20-ml portions of 0·25 *N* nitric acid yielded 0·1 and 0·05 mg of zinc. It was therefore confirmed that the manner of attachment of some of the zinc to the resin may be different from that of the rest. As the amount retained will presumably be related to the period of contact of the resin with water, it seemed that zinc might be determined correctly in the test solutions if the zinc-treated resin were so handled beforehand that none of the firmly held zinc was detached. Accordingly, columns prepared from zinc-saturated resin were, after being washed with 20 ml of 2 *N* hydrochloric acid and 100 ml of water, immediately treated with 50 ml of 2 *N* hydrochloric acid and used for experiments with 50 and 5-mg amounts of zinc. The recovery of zinc was quantitative; the errors for 50-mg amounts were +0·01 and +0·08 mg, and for 5-mg amounts were nil and +0·01 mg. As resin columns prepared in this way had been found to yield an amount of firmly held zinc similar to that detached from columns through which 5 mg of zinc had been passed and also to the average amount that had been lost in the passage of 50 mg, it was evident that columns to which 5 to 50-mg quantities of zinc had been applied and from which the bulk was afterwards removed with water, should, if regenerated immediately with 2 *N* hydrochloric acid, give similar results. Two experiments with mixtures containing 50 mg of zinc and 50 mg of aluminium, one made on a column suitably prepared from zinc-saturated resin, the other on resin regenerated after passage of zinc in the ordinary way, gave similar small positive errors.

In Table IV (columns *a* and *b*) are recorded all the results hitherto determined for mixtures, with fresh resin and with regenerated resin, but without special attention being paid to the period during which the resin had stood in contact with water before regeneration. In addition, results (marked §) are included for determinations since made on resin immediately regenerated after passage of at least 5 mg of zinc, in experiments or in trials. With reference to the results for mixtures that left negligible amounts of metals on the column, it is evident that there is a lack of precision throughout. Investigation of the manner of regenerating the resin has effected no important improvement. There was no evidence that repeatedly used columns settled down and gave better results. There seemed to be an irregularity associated with the resin, and we can offer no explanation for this.

THE ELUTION OF ZINC FROM RESIN COLUMNS BY MEANS OF DILUTE NITRIC ACID—

Since the attempt to counteract the error due to the retention on the resin of a variable residue of zinc had only partially succeeded, a means of ensuring complete removal of zinc was next sought. As 0·25 *N* nitric acid eliminated strongly attached zinc much more readily than water and more effectively than 0·25 *N* sulphuric acid (above), its use for breaking down and removing the zinc chloride complex from the resin was examined. Elution with water had led to retention of bismuth and much of the tin and antimony owing to the hydrolysis of their salts. Therefore 20 ml of water, which eluted 80 per cent. of 50 mg of zinc, were applied first, and then sufficient nitric acid to elute the rest of the zinc; 40 ml of acid provided a safe excess. Under these conditions the results were reasonably accurate (last column of Table III) when various amounts of zinc were adsorbed on the resin, eluted and determined with 8-hydroxyquinoline in acid solution. A tendency for positive errors to occur suggested that

traces of metallic impurities, most likely iron and copper, were intruding. Precipitation with 8-hydroxyquinoline in alkaline tartrate solution will avoid the effect of iron, but not that of copper, which requires the presence of some potassium cyanide.[11]

When the method was applied to mixtures of zinc with other elements, the same trends as were noted for zinc alone were indicated by the results, which are shown in the last column of Table IV. Where sufficient experiments have been done, the results show greater precision than those in which water alone has been used for elution. Excellent separations of zinc from

TABLE IV

DETERMINATION OF ZINC IN MIXTURES AFTER ADSORPTION ON, AND ELUTION FROM, THE ANION-EXCHANGE RESIN AMBERLITE IRA-400 (Cl)

Approximate weight of zinc taken, 10^{-3} g	Other metals	Approximate weight of other metals taken, 10^{-3} g	Elution with water: (a) Resin not previously used. Error, 10^{-5} g	Elution with water: (b) Regenerated resin. Error, 10^{-5} g	Elution with water and 0·25 *N* nitric acid. Error, 10^{-5} g
50	Al	50	—	−16, +4	—
20		80	—	−14, +1; +10,§ −19§	+3, +1
5		100	—	−12, −2; +23,§ −11§	−1, +3
50	Mg	50	−16, −4	−13, +14	−12, −11*
20		80	−1	+1	−3*
5		100	−5, −4	—	+4, (−43*); +4,* +3*
50	Cu	50	—	+19, −25	−18†
20		80	—	−3, −7	—
5		100	−38, −38	−1, −2	+13†
50	Co, Ni and Mn (1:2:2)	50	−9, −7	+28,§ −35§	−21, +3
20		80	−19, −12	+7,§ −5*	+2, +6
5		100	−15, −8	−8,§ +9§	+11, +8
50	Fe^{III}, Be, Ti^{IV} and Ca (1:1:1:1)	20	—	−5, +12	0*
20		20	—	−11, +20	+8,‡ +3*
5		20	—	+6, +10	+5,† +7*
50	Sb^{III}, Bi, Th, Zr and Cr^{III} (1:1:1:1:1)	25	—	−27	—
20		25	—	−24	—
5		25	—	−17	—
50	Th, Zr, U^{VI} and Cr^{III} (1:1:1:1)	20	—	—	+4
20		20	—	—	+5
5		20	—	—	+13
50	Sn^{IV}	50	—	−8,* +9*	−54*
20		80	—	−23,* −13*	−30*
5		100	—	−34,* −5*	−26*
50	Bi	50	—	−63	−4*
20		50	—	−26	—
5		50	—	−12	−12*
50	Sb	50	−11	+39*	−12 / −8*
20		50	−22 Sb^{V}	+15* Sb^{III}	— Sb^{V} / — Sb^{III}
5		50	−31	−9*	+4 / −2*

* Precipitation with 8-hydroxyquinoline in alkaline tartrate solution.
† Corrected for copper, see p. 487.
‡ Corrected for iron, 0·05 and 0·18 mg, respectively.
§ Immediate regeneration of resin after passage of a zinc-containing solution, see p. 488.

aluminium and magnesium have been achieved. If these elements had been present, the aluminium - 8-hydroxyquinoline complex would have been precipitated in acid solution and the magnesium complex in alkaline tartrate medium, so causing positive errors. With mixtures containing iron, which tended to behave a little erratically, precipitation in alkaline tartrate solution is desirable. As traces of iron were frequently noted, it may be that they are at least partly responsible for the small positive errors found in some other experiments. A correction has been made for copper in accordance with the results given on p. 487. The

possibility of avoiding its interference in the final determination of zinc has not yet been examined. As indicated on p. 487, iron and copper seemed to behave like zinc; small amounts of these elements are held on the resin in the elution with water, but are liberated by treatment with nitric acid. Nitric acid separates zinc more satisfactorily from bismuth than does water, whereas the reverse holds for tin, which evidently traps some zinc in the resin. One could easily reduce this error by eliminating part of the tin as stannic halide before effecting the separation on the resin.

ANALYSES OF ALLOYS—

In the course of this investigation some alloys were analysed and, when necessary, additional zinc was added to raise the content to the minimum under consideration. Latterly, however, it was desired to find whether the separation of less than 5 mg of zinc would be practicable, and a bronze and an aluminium alloy yielding half this amount were included, the final determination of zinc being effected in the customary manner, but on an appropriately reduced scale (p. 484). Because of the high tin content, the determination of zinc in the bronze had to be done in alkaline tartrate solution. As the risk of faulty adjustment of the alkalinity was greater on the small scale, precipitation of the zinc - 8-hydroxyquinoline complex in acid solution was preferred for the aluminium alloy, which contained an insignificant amount of tin. The precipitates were afterwards examined quantitatively for iron and copper and corrections applied, namely, for 6 μg of iron and 10 μg of copper in the first precipitate, and for 8 μg of each element in the second.

All results are shown in Table V. Those referring to elution with water are to be compared with the results given in column (*b*), Table IV. The results in the last columns of Tables IV and V are similarly comparable. Although some of the results in the second from last column are apparently good, the method relating to elution with water and nitric acid is believed to be more reliable, and it is therefore recommended. Smaller amounts of zinc than 5 mg can obviously be dealt with, but it would be preferable to have a simpler means of determining them than precipitation of the zinc - 8-hydroxyquinoline complex in alkaline tartrate solution.

TABLE V

DETERMINATION OF ZINC IN ALLOYS

Alloy	Approximate composition of alloys									Zinc found	
	Cu, %	Sn, %	Fe, %	Pb, %	Al, %	Mn, %	Mg, %	Ni, %	Sb, %	Zn, %	After elution with water, %	After elution with water and nitric acid, %
Manganese brass No. 179 ..	59	2	1	1	2	1	—	1	—	33·9§	33·5, 33·6	33·8,*† 33·7*†
Brass No. 37*b* ..	70	1	0·2	1	—	—	—	<1	—	27·09‖	26·9, 27·2	—
Bronze No. 183 with added zinc	83	10	0·1	2	—	—	—	—	1	(1) 5·01§ (2) 5·18§	4·95* 5·26*	—
Bronze No. 207..	87	10	0·1	<1	—	—	—	<1	≪1	2·53§	2·28,* 2·24*	2·46,*† 2·40*†
Aluminium alloy	1	—	0·3	—	89	1	3	—	—	5·80¶	5·70, 5·78	5·97, 5·81
Aluminium alloy No. 181 with added zinc	Essentially as below									(1) 5·26§ (2) 5·53§	5·24 5·60	—
Aluminium alloy No. 181 ..	5	≪1	0·5	2	87	—	1	2	—	2·37§	—	2·40,‡ 2·41‡

* Precipitation with 8-hydroxyquinoline in alkaline tartrate solution.
† Corrected for copper (0·2 per cent. of the original copper content of the alloy), see p. 487.
‡ Corrected for copper and iron by direct quantitative test.
§ British Chemical Standard certificate value plus added zinc where required (1·86 per cent. of zinc in bronze No. 183).
‖ U.S.A. Bureau of Standards certificate value.
¶ Value supplied by the British Aluminium Company.

RECOMMENDED PROCEDURE FOR THE SEPARATION OF ZINC BY ION EXCHANGE IN ALLOY ANALYSIS—

Preparation of the resin column—For the glass column take a piece of Pyrex tubing, 16 cm long and of 6 to 7 mm internal diameter, and to the top seal a cup 7 cm long and of 18 mm diameter, and to the bottom a piece of glass tubing, 3 cm long and of 3 mm internal

diameter. Above the lower joint, place a small pad of glass wool as a support for the resin. Attach a short length of polythene tubing with a screw clip to the bottom of the column.

Slightly grind the dry Amberlite IRA-400 (OH) resin, as supplied, and collect the solid that passes a 50 and is held on a 100-mesh sieve. Soak the resin overnight in slightly warm 2 *N* hydrochloric acid, remove fine particles, and then, after filling the glass column with 2 *N* hydrochloric acid and leaving the screw-clip open, transfer sufficient of the slurry of resin to give a 15-cm bed when the resin has packed down. Allow 20 ml of 2·0 *N* hydrochloric acid to percolate through the resin, and adjust the rate of flow to 3 to 4 ml per square cm per minute. On top of the resin pack tightly a thin pad of cotton wool to prevent the column from running dry. Finally pass 50 ml of 0·25 *N* nitric acid and 50 ml of 2·0 *N* hydrochloric acid through the column, which is then ready for use.

The adsorption and elution of zinc—Transfer to the column about 5 ml of 2 *N* hydrochloric acid containing up to 50 mg of zinc and a total of not more than about 100 mg of metallic elements, and allow the solution to percolate at the rate specified. Rinse the container five times with 1-ml portions of 2·0 *N* hydrochloric acid, allowing the column to drain between additions, and then add more 2·0 *N* acid to bring the total volume of solution added to 50 ml. Now elute the zinc by allowing 20 ml of water, followed by 40 ml of 0·25 *N* nitric acid, to percolate through the column. Collect the solution and proceed with the determination of zinc by the appropriate method (p. 484). Precipitation of the zinc - 8-hydroxyquinoline complex in alkaline tartrate solution is generally applicable. Precipitation in acid solution is simpler and, with reference to the information given on p. 485 and p. 487, may be used for materials that contain no bismuth, a limited amount of iron and less than 2 per cent. of tin or antimony. Whichever method is used, a correction is required for copper, and when precipitation in acid solution is adopted, it is desirable to examine precipitates for the presence of iron. For 50-mg amounts of zinc, precipitated in acid solution, a correction of +0·3 mg is required.

Reject the resin if the original solution contained metals such as lead, antimony, bismuth and tin, which are retained by it. Otherwise prepare the column for further use by immediately passing through it 50 ml of 2·0 *N* hydrochloric acid.

Preparation of alloy solutions—Disintegrate 100-mg amounts of alloys that contain not more than 50 per cent. of zinc with hydrochloric and nitric acids. Remove the excess of nitric acid by evaporation in the presence of an excess of hydrochloric acid, and dissolve the residue in a minimum volume of 2 *N* hydrochloric acid. If necessary, remove insoluble matter and lead chloride (after cooling to 0° C) by filtration, and then transfer the filtrate and washings to the resin column. Proceed with the adsorption and elution of zinc as indicated.

EXPERIMENTS ON THE SEPARATION OF 0·5-mg AMOUNTS OF ZINC FROM OTHER METALS BY ION EXCHANGE—

As the separation of 5-mg amounts of zinc from an excess of other elements was reasonably satisfactory and a few analyses of alloys gave good recovery of 2·5 mg, it was of interest to investigate the recovery of much smaller quantities of zinc and to see how the irregularities associated with the columns would affect them. The 8-hydroxyquinoline procedure for the determination of zinc in acid solution was adapted to the micro-scale, and, after trial determinations of 0·5 mg of zinc, the water - nitric acid eluates from normal blanks on two resin columns were evaporated and added to the same amounts of zinc, which were then determined. Half-milligram quantities of zinc, first alone and then in association with 100 mg of aluminium or magnesium, were next adsorbed on the resin and eluted with water and nitric acid in the usual way. The eluates were evaporated to small bulk, transferred by a capillary tube to 6-ml beakers and evaporated to dryness in the presence of hydrochloric acid before determination of the zinc with 8-hydroxyquinoline in acid solution as described on p. 484. Precipitation was effected in acid solution in order to ensure the recovery of likely adventitious impurities. In all experiments, except those in the direct determination of zinc, the precipitates were disintegrated with concentrated nitric and sulphuric acids and prepared for the colorimetric determination of iron and copper with potassium thiocyanate and a chloroform solution of diethylammonium diethyldithiocarbamate, respectively. Both metals were found in amounts that tallied well with the positive errors recorded throughout. When corrections for them were made, it was evident, as is shown in Table VI, that the recovery of 0·5 mg of zinc was virtually complete, even in the presence of a 200-fold excess of aluminium or magnesium. Complications may arise, however, when more complex mixtures are examined.

TABLE VI

DETERMINATION OF APPROXIMATELY 0·5-mg AMOUNTS OF ZINC UNDER VARIOUS CONDITIONS

Remarks	Weight of zinc taken, μg	Weight of zinc found, μg	Weight found in precipitate: Fe, μg	Weight found in precipitate: Cu, μg	Corrected weight of zinc found, μg	Error, μg
Direct determination of zinc	491	477	—	—	—	−14
	615	618	—	—	—	+3
	541	547	—	—	—	+6
	476	477	—	—	—	+1
Direct determination of zinc after addition of the blank from the resin	458	488	12	6	463	+5
	463	503	15	8	471	+8
Zinc put through the ion-exchange procedure	478	516	lost		—	—
	472	518	21	10	474	+2
100 mg of magnesium present	465	488	15	8	456	−9
	486	492	7	3	478	−8
100 mg of aluminium present	551	570	7	7	552	+1
	495	520	12	8	493	−2

The separation of zinc and cadmium is now under consideration and a simpler means of determining small amounts of zinc is being sought.

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The Determination of Traces of Boron with Quinalizarin

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The well known method for the determination of boron with quinalizarin has been investigated, with particular reference to the concentration of the sulphuric acid that is used in the production of the quinalizarin - boron colour. The acid concentration has been found to be of importance in establishing the sensitivity and the range of the method, and this has led to the discovery that a fairly accurate determination of boron can be made without the use of a spectrophotometer or colour standards, by a simple titration with water and subsequent reference to a calibration graph.

Quinalizarin dissolves in concentrated sulphuric acid, sp.gr. 1·84, to give an intense violet-blue solution. With increasing additions of water this solution changes colour through violet and mauve to red,[1] so that a solution of quinalizarin in 93 per cent. w/w sulphuric acid exhibits a colour that is intermediate between the deep blue and red. When 1 ml of water is added to 10 ml of this solution, the colour changes towards red, but if the 1 ml of water contains borate, the change towards red is less and if sufficient boron is present there is a reverse so that the resulting colour is more blue. This colour change is the basis of the usual method for the determination of boron. If a reference solution containing known amounts of quinalizarin, sulphuric acid and water is compared with other solutions of exactly the same reagent, acid and water contents, but containing in addition known amounts of borate, it will be seen that the resulting increases of the blue colour are related to the boron concentration. By carrying out spectrophotometric measurements with an Ilford orange filter, a standard graph can be prepared and the boron content of any solution is then deduced by similar treatment with quinalizarin in sulphuric acid and subsequent reference to this graph.[2,3,4]

Much of the past work on this subject has been concerned with the effect of various concentrations of the reagents,[1,2,3,4] and it may be of interest at this stage to describe what we consider to be a standard spectrophotometric procedure before discussing the newer titration method.

There are three variables to be considered: concentration of quinalizarin, concentration of sulphuric acid and the working range of boron over which measurements are to be made. Fig. 1 shows a composite picture of the colour changes that occur for different amounts of boron with various concentrations of sulphuric acid, a 0·001 per cent. quinalizarin solution being used. Curve A shows decrease in blue colour as water is added to 10 ml of a 0·001 per cent. solution of quinalizarin in sulphuric acid, sp.gr. 1·84. The values used to plot this curve are determined by measuring the colour intensity of the solution after small additions of water, making use of a Spekker absorptiometer with water as reference solution and the Ilford No. 607 orange filter. The readings are corrected for the difference in volume brought about by the additions of water. Curve B is produced similarly, but 10 μg of boron are now present in the solution. The vertical distance YZ then represents the greatest difference in blue colour that there can be between a blank and a test solution containing 10 μg of boron, and its position indicates that the optimum water content for these conditions is 1·5 ml. It follows that for the best results over the range 0 to 10 μg of boron, the 0·001 per cent. reagent solution should be prepared from an acid mixture containing 100 ml of acid and 15 ml of water. Similarly for curve C, the 0·001 per cent. reagent solution should be prepared from an acid mixture containing 100 ml of acid and 28 ml of water. For any particular concentration of quinalizarin and range of boron the strength of sulphuric acid may thus be adjusted to take full advantage of the red - blue colour change.

Variations in reagent concentration appear to have less effect on the sensitivity of the method once the optimum concentration of sulphuric acid has been determined (see Fig. 2), although there is some advantage in the use of a stronger reagent solution.[3,4] By constructing a series of curves, similar to those in Fig. 1, for other concentrations of the reagent, the most appropriate conditions for any particular range of boron concentrations can be predicted.

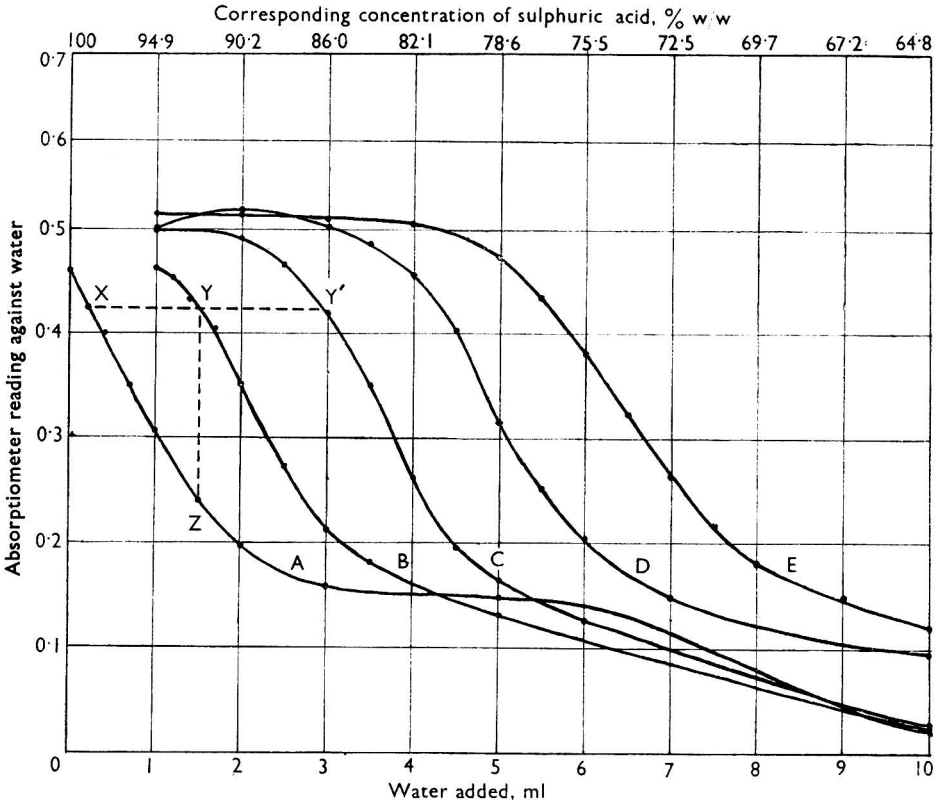

Fig. 1. The effect of the addition of water to quinalizarin solutions containing boron. Curve A, blank; curve B, 10μg; curve C, 100 μg; curve D, 1000 μg; and curve E, 10,000 μg of boron

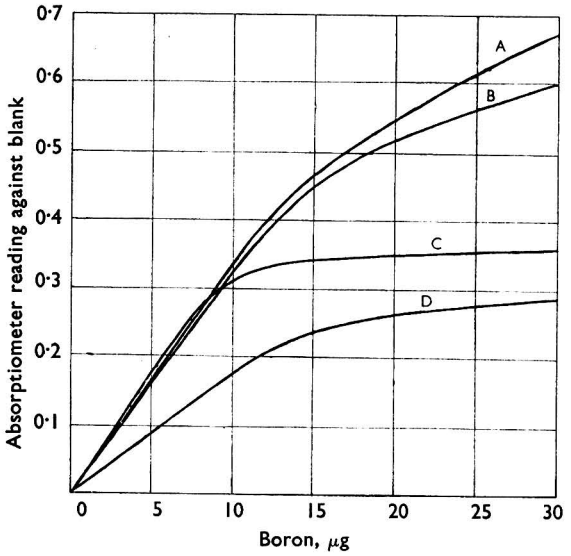

Fig. 2. Calibration curves for: A, 10 ml of 0·01 per cent. quinalizarin + 2 ml of water; B, 10 ml of 0·003 per cent quinalizarin + 2 ml water; C, 10 ml of 0·003 per cent. quinalizarin + 1 ml of water; and D, 10 ml of 0·01 per cent. quinalizarin + 1 ml of water

With increase of reagent concentration these curves move to the right and become more elongated in a vertical direction.

Fig. 2 shows several curves of the intensity of the blue colour plotted against the boron content, each curve being for a different concentration of either quinalizarin or acid. The concentration of acid is the important factor in this method; decrease in acid concentration (*i.e.*, increase in water above the optimum amount) renders the method less sensitive, but it can be used over a wider range of boron concentration.[1]

The curve A shows a good standard line for the determination of 0 to 30 μg of boron. It is determined by treating suitable aliquots of a standard sodium borate solution as described below.

Absorptiometric procedure for the determination of boron over the range 0 to 30 μg of boron

With a pipette, place 2 ml of the aqueous solution containing borate into a dry boiling tube and to it add 10 ml of a 0·01 per cent. solution of quinalizarin in sulphuric acid, sp.gr. 1·84, by means of a pipette fitted with a safety bulb. Cool the tube to room temperature and

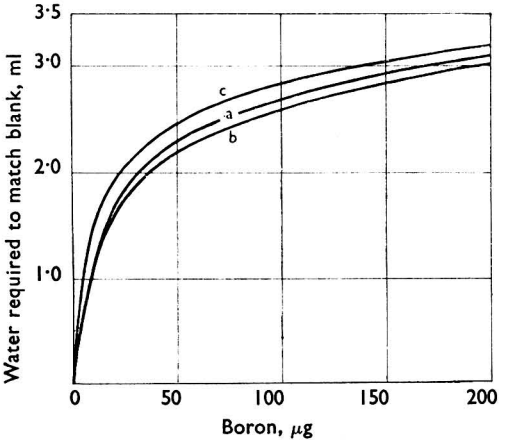

Fig. 3. Calibration curves for (*a*) 0·003 per cent. quinalizarin, 1 ml of water in blank; (*b*) 0·003 per cent. quinalizarin, 2 ml of water in blank; and (*c*) 0·001 per cent. quinalizarin, 1 ml of water in blank

leave it to stand for 30 minutes.[3] Measure the optical density by means of the Spekker absorptiometer making use of the No. 607 orange filter and the 1-cm cell, with, as a reference solution, a blank containing 10 ml of the reagent solution and 2 ml of water. A new calibration curve must be worked out for each batch of reagent solution, as a variation of 0·2 per cent. w/w in the strength of the sulphuric acid will affect the readings. For this reason the reagent solution should be kept in a closed vessel that is protected from moisture; under these conditions it keeps indefinitely. Over the range 0 to 10 μg the method gives results correct to within $\pm$ 0·2 μg of boron.

Upon further examination of Fig. 1 it can be seen that XY represents the amount of water that must be added to a solution of 10 ml of a 0·001 per cent. solution of quinalizarin in sulphuric acid, sp.gr. 1·84, containing 10 μg of boron, to bring it back to the colour of a similar solution without boron. Similarly, XY' corresponds to the larger amount of water that is needed to restore the colour of the solution containing 100 μg of boron, to that of the blank. Hence the boron content can be related to water additions and a standard curve can be made by plotting volume of water in ml against amount of boron in μg. The calibration curve is prepared as described below.

Preparation of calibration curve for the water titration method over the range 0 to 10,000 μg of boron

Prepare a series of standard solutions containing 0 to 10,000 μg of boron from a solution prepared from AnalaR quality sodium borate so that each amount of boron is contained in exactly 1 ml of water, and place the solutions in boiling tubes. To each tube add 10 ml of a 0·001 per cent. solution of quinalizarin in sulphuric acid, sp.gr. 1·84, from a pipette

fitted with a safety bulb, and shake and cool the tube. Prepare a blank solution similarly, with 1 ml of water in place of the borate solution. This blank solution will then be red when compared with the increasing blue colours of the standards. Titrate dropwise each one of the standard solutions in turn with water from a 1-ml micro-burette and cool the mixture, so that when the colour is viewed vertically against a white background it matches that of the blank when both are at room temperature. It will be found, and it can also be deduced from Fig. 1, that the relation between the amounts of boron and water is nearly independent of the intital amount of water, but it does depend to some extent on the reagent concentration. We have found experimentally that the two calibration curves (*a*) for 10 ml of a 0·003 per cent. quinalizarin in sulphuric acid, sp.gr. 1·84, and 1 ml of water, and (*b*) for 10 ml of a 0·003 per cent. quinalizarin solution and 2 ml of water, are almost identical, whereas the curve (*c*) for 10 ml of a 0·001 per cent. quinalizarin solution and 1 ml of water is slightly different (see Fig. 3). The weaker reagent is more suitable for this method, as the colours produced with it are more easily matched visually. By plotting $\log_{10}$ (amount of boron in μg) against volume of water added in ml, a straight line results over the range 1 to 1000 μg of boron. For an initial 1 ml of water and the 0·001 per cent. reagent solution the equation of this line is—

$$\log_{10} (\mu\text{g of boron}) = 0{\cdot}78 \times \text{water titration in ml} - 0{\cdot}2.$$

Determination of boron over the range 0 to 10,000 μg by the water titration method

Treat exactly 1 ml of boron solution with 10 ml of a 0·001 per cent. solution of quinalizarin in sulphuric acid, sp.gr. 1·84, from a pipette, cool the mixture and titrate it in the manner described for the preparation of the calibration curve. The temperatures of the two solutions at the end of the titration must not differ by more than 5° C. Substitute values in the equation given above to find the amount of boron in μg. The precision over the range 1 to 1000 μg of boron is within $\pm$10 per cent., but below 1 μg it decreases rapidly. When the calibration curve is used instead of the equation, a precision of within $\pm$10 per cent. can be attained over the entire range 1 to 10,000 μg of boron. Variations of up to 10 per cent. in the reagent concentration and up to 2 per cent. w/w in the concentration of sulphuric acid are permissible provided that the same reagent and acid are used both for the test and blank.

This method is remarkably free from interference. The following list gives some idea of the amounts of other ions that can be tolerated over the range 10 to 1000 μg of boron—

Magnesium, calcium, aluminium and phosphate	At least 10 mg of any of these has no effect.
Chloride	At least 30 mg has no effect.
Sodium and ammonium	At least 5 mg of either of these has no effect.
Ferrous iron	This does not alter the colour, but it gives a white precipitate that obstructs the colour matching.
Ferric iron	Ten milligrams of this has no effect in the absence of chloride, but only 1 mg can be tolerated if chloride ions are present in the solution; a strong yellow colour, which masks the characteristic colours, is produced when large amounts of ferric iron are present together with chloride ions.
Nitrate	One milligram of this completely removes the colour, 100 μg inhibits the colour change, 30 μg increases the water to boron ratio required to match blank and with 10 μg there is no interference.
Silica	Three milligrams of soluble silica is the maximum permissible amount without interference. With more than this amount, the silica is precipitated by the acid as a gel. This gel remains blue and does not revert to red on dilution with water. Insoluble silica does not interfere.

The authors are indebted to the Directors of Hopkin and Williams, Ltd., for permission to publish this paper.

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Phosphate Interference in the Flame-photometric Determination of Calcium

By L. LEYTON

In the air - acetylene flame, calcium emission from solutions of calcium chloride decreases linearly with increasing phosphate content up to a point where the phosphorus to calcium ratio is 1 to 2; beyond this point, calcium emission is independent of the phosphate concentration. The influence of up to 100 p.p.m. of potassium and up to 50 p.p.m. of sodium on the degree of phosphate interference is negligible. Phosphate interference is attributed to the formation of an equivalent amount of calcium phosphate, which is only slightly excited in the air - acetylene flame and hardly at all in the coal-gas flame. With an air - acetylene flame, calcium determinations can be corrected for phosphate interference if the phosphate content is known or, in the presence of an excess of phosphate, they can be made directly if a calibration curve for calcium phosphate is available. With a coal-gas flame, elimination of the phosphate is essential and a method is suggested in which the calcium is adsorbed on a cation-exchange resin column; it is subsequently eluted and is free of phosphate.

Whereas it has long been known that the determination of calcium by the flame-photometric procedure can be subject to appreciable error in the presence of phosphate (*cf.* Riehm[1]), only a few systematic investigations appear to have been made into the nature and extent of the interference. Fieldes *et al.*,[2] with the Beckman spectrophotometer and a propane - butane gas mixture, found that the calcium emission, relative to that in the absence of phosphate, decreased with increasing phosphate content, rapidly at first (over the range 0 to about 0·1 per cent. of phosphorus pentoxide) and then more gradually, finally reaching a constant value. Up to a concentration corresponding to 0·1 per cent. of phosphorus pentoxide, the relative emission curves for 0·01 *N* and 0·03 *N* calcium chloride solutions were identical; beyond this concentration the relative emission was lower for the more concentrated calcium solution. As a result of these investigations, the authors recommended the complete elimination of phosphate, if reliable calcium determinations were to be made with the flame photometer. Similar depressions in calcium emission by phosphate were demonstrated by Brealey *et al.*[3], who used a propane - air flame; they found that the emission from solutions containing 5 and 50 p.p.m. of calcium was independent of phosphate concentration when the latter exceeded 0·5 mg of phosphate per ml. Chen and Toribara[4] with the Weichselbaum - Varney apparatus and a coal-gas flame also reported decreasing calcium emissions with increasing phosphorus concentrations; for solutions containing 1 p.p.m. of calcium, the maximum inhibition was 25 per cent. at a concentration of 1 p.p.m. of phosphorus. The suppression due to inorganic phosphate was found to be much greater than that due to organic phosphate (as glycerophosphate), but it was partially obviated in the presence of protein. In a more comprehensive investigation covering a wide range of calcium concentrations, Kohnlein and Lücke[5] found that there was a linear fall in calcium emission with increasing phosphate content up to a certain level beyond which the emission was independent of the phosphate concentration. In a coal-gas flame, the initial fall in emission was more pronounced than in an acetylene - air flame and the final constant emission was practically nil. Over the whole range of calcium concentrations, the inflection in the emission curves occurred at a point where the calcium oxide to phosphorus pentoxide ratio was 1·23 to 1 and, although this aspect was not investigated, it suggested the formation of a compound, which corresponded to this composition and was only slightly excited in the acetylene - air flame and hardly at all in the coal-gas flame. Further work suggested that both potassium and sodium affected the depressing influence of phosphate on calcium emission and, in view of the practical difficulties involved in the elimination of phosphate from solution, these authors concluded that the flame-photometric technique was not suitable for the determination of calcium in solutions containing various amounts of sodium, potassium and phosphate.

These findings are of considerable importance in view of the widespread adoption of the flame-photometric technique for the analysis of biological fluids and extracts, many of which

contain appreciable quantities of phosphate. It is necessary to indicate, however, that the results of Kohnlein and Lücke covered a range of concentrations (calcium up to 700 p.p.m., phosphorus up to 430 p.p.m., and potassium and sodium up to 750 p.p.m.) that generally exceeds those encountered in normal practice and that their phosphate interference curves do not quite agree with those determined by previous investigators, especially with regard to the degree of suppression of calcium emission at different calcium concentrations. It was therefore considered worth while to repeat the investigation over a lower range of calcium and phosphate concentrations and to study in rather more detail the particular nature of the interference effect with the object of devising some means of overcoming it.

Experimental

A series of solutions was prepared from pure calcium carbonate, which was dissolved in hydrochloric acid, and from potassium hydrogen phosphate, to cover the range 20 to 100 mg of calcium per litre containing 0 to 150 mg of phosphorus per litre of solution. A parallel series was prepared containing, in addition, various concentrations of potassium (as potassium chloride) to cover the range 0 to 200 mg of potassium per litre of solution. These solutions

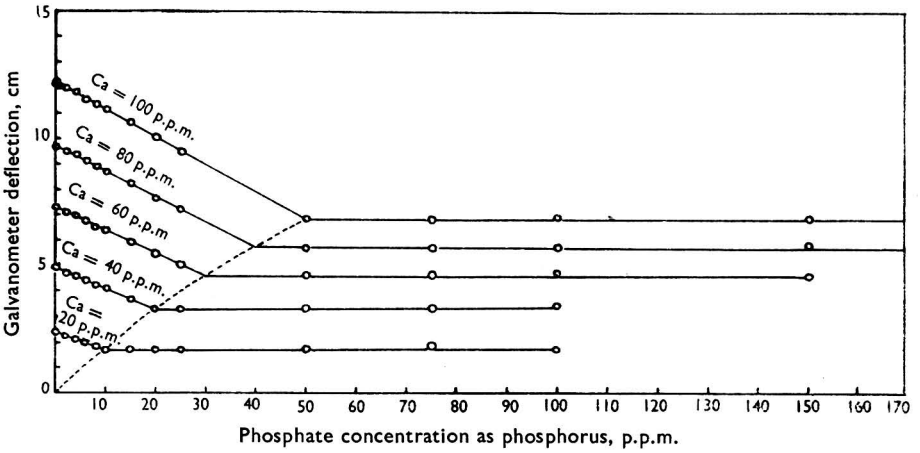

Fig. 1. The effect of increasing the phosphate concentration on the calcium emission from solutions of calcium chloride in an acetylene - air flame

were analysed by the flame photometer previously described[6] with an acetylene - air flame and an interference filter for calcium with a maximum transmission at 616 mμ. The instrument was used at one-fifth of its full sensitivity.

Discussion of results

In Fig. 1, the galvanometer deflections corresponding to the emission at each level of calcium have been plotted against the phosphorus concentrations. The results are identical with those of Kohnlein and Lücke[6] for more concentrated solutions: at each calcium concentration there is a linear fall in calcium emission with increasing phosphorus concentration up to a point at which the phosphorus concentration equals one-half that of the calcium concentration (*i.e.*, ratio of calcium oxide to phosphorus pentoxide is 1·23:1); beyond this point, the calcium emission is independent of the phosphate concentration.

There is no evidence to support the claim that potassium influences the degree of phosphate interference on calcium emission. For two series of solutions containing 50 and 100 p.p.m. of calcium, respectively, and phosphate concentrations ranging from 0 to 40 p.p.m. of phosphorus, various potassium concentrations between 0 and 100 p.p.m. of potassium had no apparent influence on the intensity of the calcium radiation. At higher potassium concentrations there was a slight increase in emission, but, even at a concentration of 200 p.p.m. of potassium, the rise was equivalent to no more than 1 to 2 p.p.m. of calcium. It is possible that much of the variation reported by Kohnlein and Lücke is attributable to the relatively inefficient filter systems used, rather than to a more fundamental relationship between the various elements in solution. Further investigations with sodium, which, compared with

potassium, is more difficult to isolate from calcium, reveal that it has a somewhat greater influence on calcium emission and they confirm the importance of a sufficiently selective filter-system. It is interesting to note that even with solutions containing no calcium, Chen and Toribara found that there was an increase in the emission in the calcium region of the spectrum, 620 mμ, on increasing the concentration of either sodium or potassium. In the present investigation, however, the influence of sodium on calcium emission over the range 0 to 50 p.p.m. of sodium was found to be negligible.

The constancy of the calcium to phosphorus ratio at the points of inflection on the interference curves suggests, as Kohnlein and Lücke have already noted, the formation of a compound that contains these elements in this ratio and that is excited in the flame to a much smaller extent than calcium chloride. The possibility that this compound is tricalcium phosphate, $Ca_3(PO_4)_2$, is supported by the fact that the calcium emission curve for various concentrations of this compound (dissolved in hydrochloric acid) corresponds exactly with the curve drawn through the points of inflection in Fig. 1. Furthermore, it seems that when the phosphorus concentration is less than one-half that of the calcium, the emission represents the sum of the emissions due to the equivalent amount of calcium phosphate that is formed and to the remaining "free" calcium chloride. Thus in Fig 1, for a solution containing, say, 60 p.p.m. of calcium and 20 p.p.m. of phosphorus, the galvanometer deflection of 5·5 cm represents the sum of the deflections due to 40 p.p.m. of calcium as calcium phosphate (3·2 cm) and to 20 p.p.m. of calcium as calcium chloride (2·3 cm).

Photographs of the emission spectra of calcium chloride and tricalcium phosphate, excited in the acetylene - air flame, reveal only the calcium oxide bands; even for the chloride salt there is no evidence of a chloride band. It is possible therefore that, compared with other salts, the reduced emission of calcium as phosphate is due to a smaller degree of dissociation into the oxide rather than to a different type of emission. It is also necessary to emphasise that these results refer to the acetylene - air flame; in the cooler coal-gas and propane flames it is possible that other phenomena are involved, and this would account for the differences observed in the interference curves (*cf.* for example, the curves determined by Chen and Toribara).

The correction and elimination of phosphate interference

From the above findings, a method is suggested whereby flame-photometric determinations of calcium can be corrected for phosphate interference, if the concentration of phosphate is known and if calibration curves for both calcium chloride and calcium phosphate are available. The amount of calcium that is volatilised as phosphate is equal to twice the amount of phosphorus in solution and the resulting deflection can be determined from the

Table I

Results for the determination of calcium after addition of phosphate

Extract	Phosphorus concentration, p.p.m.	Apparent calcium concentration (direct photometry), p.p.m.	Corrected calcium concentration, p.p.m.	Actual calcium concentration (oxalate method), p.p.m.
Larch seedlings	6·8	84	91	94·0
	36·6	74	107	108·4
	38·5	52	88	92·0
Spruce seedlings	19·0	92	110	114·2
	28·7	71	98	101·6
	53·8	87	140	146·6

calcium phosphate calibration curve. Subtracting this deflection from the total deflection gives the deflection due to any calcium still present as chloride; the amount of this calcium is determined from the calcium chloride calibration curve. An estimate of the total calcium present can now be made by summing the amount of calcium that is volatilised in the flame as the phosphate and as the chloride. As shown above, this correction applies reasonably well to artificial solutions, but to check its applicability in actual practice tests have been made on a number of solutions that were prepared by dissolving plant ash of various origins in dilute hydrochloric acid. The true concentration of calcium in these extracts was determined by an oxalate precipitation followed by titration against standard potassium permanganate

and that of the phosphorus by colorimetric analysis of the molybdenum-blue complex. In these particular tests, complications were introduced by the presence in the extracts of small amounts of aluminium, which also suppresses calcium emission (*cf.* Mitchell and Robertson[7]) and which therefore had to be removed by precipitation as ferric aluminium phosphate before determining the calcium. Once this had been done, the corrected values for calcium were found to be in very good agreement (to within 5 per cent.) with the calcium contents. Typical results are shown in Table I.

Although the above correction would appear to be generally applicable, a more practicable way of overcoming phosphate interference is to add to the test solution enough phosphate (as potassium dihydrogen phosphate) to ensure a phosphorus concentration of more than one-half that of the calcium; in this way calcium may be determined directly by comparison with calibrated calcium phosphate solutions. A somewhat similar scheme has already been suggested by Brealey *et al.*, although they added the excess of phosphate (as ammonium phosphate) to both samples and standards. This method has proved satisfactory for artificial solutions of various composition and for plant extracts. When the test solutions contained an excess of phosphate, they could be analysed directly against standard calcium phosphate solutions without the addition of extra phosphate. Typical results for extracts of this kind are shown in Table II.

TABLE II

RESULTS FOR THE DETERMINATION OF CALCIUM IN THE PRESENCE OF AN EXCESS OF PHOSPHATE

Extract	Phosphorus concentration, p.p.m.	Calcium concentration (oxalate method), p.p.m.	Calcium concentration (photometry), p.p.m.
Spruce seedlings ..	63·4	92·0	88
	62·8	79·0	83
	62·7	106·0	107
	50·4	81·2	81
	58·2	80·8	78

When a coal-gas flame is used (as with certain commercial instruments), the low excitation of calcium phosphate renders the above methods too insensitive for general work and elimination of the phosphate from solution is then essential. The method of elimination, however, must not invalidate the particular advantages of the flame-photometric technique and as yet no suitable chemical method has been found. Attempts to separate the phosphate anion from solution by direct adsorption on anion-exchange resin columns (*e.g.*, De-acidite FF) have met with little success, but an indirect method whereby the calcium is selectively adsorbed and subsequently eluted from a cation-exchange resin column (Zeocarb 215) has proved a much more promising technique. The method is a modification of that proposed by Mason[8] for the preparation of phosphate-free solutions in the ethylenediaminetetra-acetate method for calcium. Although the method may appear to be time-consuming, the actual working time involved is relatively short and the method would appear very suitable for routine determinations of calcium. From a practical point of view it appears to possess distinct advantages over other procedures, such as the preliminary precipitation of the calcium as oxalate as recommended by Chen and Toribara.

The author is indebted to Mr. J. W. Dexter (of the Forestry Department) for help in the analyses, to Mr. I. H. Rorison (of the Botany Department), for details of the cation-exchange method and to members of the Physical Chemistry Department for help in the preparation and interpretation of the emission spectra photographs.

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Investigation of the Kokatnur - Jelling Method for the Determination of Benzoyl Peroxide

By A. J. RADFORD*

The iodimetric method of Kokatnur and Jelling has been re-investigated in view of the difficulties encountered in attaining reproducible results, and the results are compared with those from the arsenious oxide reduction method of Siggia. The factors that affect the accuracy of the method are detailed.

THE simplest method for the determination of organic peroxides is that depending upon the liberation of iodine from an acidified solution of an iodide. Of the many variations of this method that of Kokatnur and Jelling[1] has been widely used for organic peroxides and for benzoyl peroxide in particular.

As sometimes the results were different for the same material when analysed by different operators and the agreement was frequently poor between duplicate determinations by the same operator, it was decided to re-investigate the accuracy of the method.

The original procedure of Kokatnur and Jelling is as follows. Add 0·1 g of benzoyl peroxide to 25 ml of *iso*propanol, followed by 1 ml of a saturated aqueous solution of potassium iodide and 1 ml of glacial acetic acid. Heat the mixture almost to boiling and maintain it at incipient boiling for 2 to 3 minutes with occasional swirling. Without cooling the mixture, titrate the liberated iodine with 0·1 *N* sodium thiosulphate to the disappearance of the yellow colour of the iodine.

When the method was first tried in these laboratories, the titration value of approximately 8 ml of sodium thiosulphate, which was required after taking 0·1 g of the peroxide, was considered to be too low for assaying the purity of commercial benzoyl peroxide. Accordingly, the size of the sample was increased fivefold, the volume of the alcohol to 50 ml, and the volumes of iodide and acid to 2 ml each, with satisfactory results. However, it was considered that the first factors that required checking were the quantities of iodide and acid necessary for the determination on 0·5 g of benzoyl peroxide.

Secondly, it was suggested that the time of "incipient" boiling, two minutes, was insufficient and that a minimum of three minutes was required. Although this was not confirmed during the early experiments, it was decided to investigate the effect of a longer period of boiling, *viz.*, 15 minutes.

A third factor was the effect of water, which could be present in either a damp sample of peroxide or in the *iso*propanol. Kokatnur and Jelling state that the presence of water causes inaccurate results, although they do not quote any figures, and they recommend the use of 99 per cent. v/v *iso*propanol and not the constant boiling mixture of 91 per cent. v/v.

In place of potassium iodide, the use of sodium iodide has been recommended by other workers, in particular, by Wagner, Smith and Peters,[2] for various peroxides, including benzoyl peroxide. In fact, the present author has found that, in the determination of *tert.*-butyl perbenzoate, the use of sodium iodide gave values approximately 3 per cent. higher than those recorded when potassium iodide was used. This was presumed to be due to (*a*) the greater solubility of sodium iodide in water (124·4 g per 100 ml of solution at 25° C) compared with that of potassium iodide (102·8 g per 100 ml of solution at 25° C), which gives a greater concentration of iodide in the saturated solution, and (*b*) the greater solubility of sodium iodide in *iso*propanol (19·65 g per 100 ml of solution at 25° C) compared with that of potassium iodide (0·138 g per 100 ml of solution at 25° C), which gives a greater iodide ion concentration.

Experimental

Recommended Method—

Based on this work, the following is the method proposed for the determination of commercial benzoyl peroxide.

Add approximately 0·5 g of benzoyl peroxide, accurately weighed, to a 600-ml tall-form beaker containing 50 ml of *iso*propanol, and then 5 ml of a saturated aqueous solution of

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potassium iodide and 5 ml of glacial acetic acid. Cover the beaker with a clock-glass, and heat it for 2 to 3 minutes at "incipient" boiling on a temperature-controlled hot-plate at low heat so that the uncondensed alcoholic vapours do not reach higher than half-way up the sides of the beaker. Without cooling the solution, titrate the liberated iodine with 0·1 *N* sodium thiosulphate to the disappearance of the yellow colour of the iodine.

Carry out a blank determination on the reagents by exactly the same procedure. The titration value should not exceed 0·1 ml of 0·1 *N* sodium thiosulphate.

However, when this method was subsequently tried by other members of the staff, difficulties were still encountered in the poor reproducibility of results between certain operators. Careful observations of the technique of these operators showed that there was a difference of opinion regarding the interpretation of the term "incipient" boiling and moreover that the lower results were always due to a more vigorous heating procedure. By careful control of the heating procedure, as given above, these discrepancies disappeared.

This latter requirement cannot be too strongly emphasised.

THE EFFECT OF THE CONCENTRATION OF POTASSIUM IODIDE AND ACETIC ACID—

To determine the optimum concentration of the saturated solution of potassium iodide and that of the glacial acetic acid required for accurate results, series of analyses were carried out with various volumes of potassium iodide solution from 2 to 10 ml and of acetic acid from 2 to 5 ml.

TABLE I

RESULTS SHOWING THE EFFECT OF THE CONCENTRATION OF POTASSIUM IODIDE AND ACETIC ACID

Series	A	B	C	D
Volume of potassium iodide solution, ml ..	2	5	5	10
Volume of acetic acid, ml	2	2	5	5
Benzoyl peroxide, per cent. w/w ..	94·78	95·88	95·82	95·83
	93·53	95·20	95·77	95·23
	92·12	94·97	95·73	95·17
	93·34	96·37	95·52	95·74
	93·69	95·77	95·33	95·98
Mean	93·49	95·64	95·63	95·59
Maximum deviation from mean	1·4	0·7	0·3	0·4

From the results, shown in Table I, there is a definite indication that 2 ml of a saturated potassium iodide solution is insufficient to ensure complete reduction of 0·5 g of benzoyl peroxide and that an increased quantity was necessary. The increased concentration of acetic acid left the mean result unaltered, but it led to closer agreement between duplicates and so, although this beneficial effect may have been coincidental, 5 ml of acetic acid is recommended.

THE EFFECT OF TIME OF "INCIPIENT" BOILING—

Although, during the course of the above analyses, the actual time of "incipient" boiling over the period of 2 to 3 minutes could not in any way be related to the benzoyl peroxide value determined, it was decided to extend the period of boiling to 15 minutes. As it was quite conceivable that losses of iodine would occur on heating the solution in a beaker for a prolonged period, it was decided to heat the solution under a reflux condenser. The results determined under these conditions were: 94·45, 96·31, 89·13, 89·57, 94·56, 89·15, 88·89 and 91·03 per cent. w/w of benzoyl peroxide. These results were disappointing and they showed that there was considerable loss of iodine in most determinations.

THE EFFECT OF WATER—

As Kokatnur and Jelling state that the presence of water has an adverse effect on the accuracy of the method, two series of analyses were carried out with the addition of 5 ml and 10 ml of water, equivalent to 9 and 17 per cent. v/v of water, respectively, to the 50 ml of *iso*propanol used. The volumes of potassium iodide solution and acetic acid used were 5 ml for each experiment.

From the results, shown in Table II, it appears that the presence of water has very little effect upon the benzoyl peroxide value and they certainly do not confirm Kokatnur and

TABLE II

RESULTS WITH THE ADDITION OF WATER TO THE *iso*PROPANOL

Series	E	F	G
Volume of water, ml	nil	5	10
Benzoyl peroxide, per cent. w/w	95·83	96·13	96·38
	95·91	95·93	95·69
	96·78	96·70	96·03
	96·12	96·21	96·03
	96·03	95·62	—
Mean	96·13	96·12	96·03
Maximum deviation from mean	0·6	0·6	0·4

Jelling's statement. Finally, in view of the difficulty in purchasing analytical-reagent grades of *iso*propanol, a laboratory-reagent grade was used with equally satisfactory results.

THE USE OF SODIUM IODIDE IN PLACE OF POTASSIUM IODIDE—

The possibility that the replacement of potassium iodide by sodium iodide might improve the accuracy of the results was investigated, the sodium iodide being added as a saturated aqueous solution. The results in Table III when compared with those for Series E

TABLE III

RESULTS WITH SODIUM IODIDE AS REDUCING REAGENT

Series	H	J
Volume of sodium iodide solution, ml	2	5
Volume of acetic acid, ml	2	5
Benzoyl peroxide, per cent. w/w	95·44	96·39
	95·82	96·23
	96·79	96·44
	96·55	96·43
	96·64	96·35
	96·20	96·02
Mean	96·24	96·31
Maximum deviation from mean	0·8	0·3

in Table II, which were found for the same batch of material, show that for the determination of benzoyl peroxide the use of sodium iodide has no great advantage over that of the potassium salt, although 2 ml of the sodium iodide solution are sufficient for complete reduction of the peroxide. But, whereas the sodium iodide dissolves readily in the *iso*propanol, potassium iodide is precipitated on addition of its solution to the alcohol, being subsequently redissolved on liberation of the iodine, and it acts as an anti-bumping agent in the preliminary stages of heating.

COMPARISON OF THE RECOMMENDED METHOD WITH AN INDEPENDENT METHOD—

It appeared essential at this stage to have a comparison of the results with those found by an independent method. The most suitable method appeared to be that of Siggia,[3] which involves dissolving the sample in ethanol and reducing it with an excess of arsenious oxide followed by removal of the ethanol by evaporation and titration of the excess of arsenious oxide with standard iodine solution. Unfortunately, this method is somewhat time-consuming and therefore it is not suitable for rapid process-control work.

Three analyses of a benzoyl peroxide sample gave the following results: 96·75, 96·66 and 97·09 per cent. w/w, with a mean value of 96·83 ($\pm$0·3) per cent. w/w of benzoyl peroxide.

When compared with series E, H and J, these results are in fairly good agreement, although they are higher by approximately 0·5 per cent., and it was considered that the iodimetric method was sufficiently accurate for process control.

THE ANALYSIS OF RECRYSTALLISED BENZOYL PEROXIDE—

As a final check, a quantity of commercial benzoyl peroxide was recrystallised twice by precipitation from chloroform solution with methanol at room temperature. Certain hazards are associated with this recrystallisation and they are referred to in Organic Syntheses,[4] which should be consulted before carrying out any experimental work. This material was analysed by the iodimetric and the arsenious oxide methods.

TABLE IV

THE ASSAY OF RECRYSTALLISED BENZOYL PEROXIDE

Series Method		K Iodimetric	L Arsenious oxide
Benzoyl peroxide, per cent. w/w		98·06	98·63
		99·17	98·64
		99·06	99·06
		98·27	98·91
		98·38	99·09
		98·26	98·74
Mean		98·53	98·84
Maximum deviation from mean		0·6	0·2

Hence, from Table IV it can be seen that, although the iodimetric method gives a slightly lower average value, owing most likely to slight losses of iodine vapour, the accuracy and reproducibility of the method are sufficient for process control.

CONCLUSIONS—

It is recommended that for quantities of 0·5 g of benzoyl peroxide, the Kokatnur - Jelling method should be carried out with 5 ml of saturated aqueous solution of potassium iodide and 5 ml of glacial acetic acid in 50 ml of *iso*propanol.

This work was carried out in the Research Laboratories of Laporte Chemicals Limited, and I therefore thank the Directors for permission to publish this paper. I also acknowledge the help and advice given me by Mr. W. S. Wood and Mr. P. F. Kane.

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The Absorptiometric Determination of a Non-ionic Detergent

BY D. G. STEVENSON

Two procedures for the determination of a non-ionic detergent are described. Both procedures involve precipitation of the detergent by phosphomolybdic acid and a subsequent absorptiometric determination of the precipitate. Both methods will detect 10 μg of detergent in 10 ml of solution; one method is quicker but somewhat less accurate and sensitive than the other.

FEW methods are available for the determination of non-ionic detergents, as they are remarkably resistant to any chemical action. However, Oliver and Preston[1] have described a gravimetric method that can be modified for use absorptiometrically.

The non-ionic detergent Lissapol N (I.C.I.), which is used in the anhydrous form, Lissapol NXA, has been found to give a clear red colour on the addition of a trace of it to concentrated sulphuric acid. This colour was found to persist on diluting the acid with up to three or four parts of water. It has also been found that a small quantity of phosphomolybdic acid gives a more sensitive colour, violet to rose-pink, with the detergent in sulphuric acid. This violet colour appears to be specific for the non-ionic detergent, as it is not given by the anionic alkyl sulphates or alkyl aryl sulphonates. Hence the Oliver and Preston precipitation with barium chloride and phosphomolybdic acid is

convenient for the combined concentration of the detergent and for its absorptiometric determination; for this the precipitate is dissolved in concentrated sulphuric acid and the colour is measured photometrically at 520 mμ.

Method I

Reagents—

Hydrochloric acid, diluted (1+4).
Barium chloride solution—A 10 per cent. w/v solution of $BaCl_2.2H_2O$.
Phosphomolybdic acid, 10 per cent. solution.
Sulphuric acid, concentrated.

Procedure—

An aliquot containing between 10 and 500 μg of detergent is transferred to a 10-ml graduated centrifuge tube, and it is diluted to 5 or 10 ml as desired. Three drops of hydrochloric acid, 2 drops of barium chloride solution and 2 drops of phosphomolybdic acid are added and the contents of the tube are well stirred with a platinum wire whisk. The resultant precipitate is well centrifuged and the supernatant liquid is decanted. Washing of the precipitate is unnecessary as no coloration is caused by the reagents alone. The tube is left inverted for a minute or two to drain.

The precipitate is dissolved in concentrated sulphuric acid, with stirring and warming, if necessary, and more acid is added to a total volume of 4 ml. The violet to rose-pink acid solution is transferred to a 1-cm diameter colorimeter tube and, after 40 minutes, the absorption is determined at 520 mμ. The detergent concentration may be determined from a previously constructed calibration curve prepared from standard aliquots.

The colour observed in the above method develops to a slight extent at first, and then fades slowly. The results are consistent when readings are taken after about 40 minutes. The above procedure will detect 10 μg of detergent which, if it is contained in a 10-ml aliquot, corresponds to 1 p.p.m. This method is quick and simple, and the accuracy seems reasonable.

Method II

A second procedure is available when the presence of sulphuric acid in the colorimeter tube is undesirable, and when greater dilution of the colour is required, as in work at higher concentrations.

This method is based on the direct determination of molybdenum in the phosphomolybdate complex precipitate. Snell and Snell[2] describe a method for determining molybdenum by the thiocyanate coloration in the presence of stannous chloride; this can be adapted to the present determination. The amber molybdenum thiocyanate may be determined in the aqueous phase, in which slow fading again occurs, or it may be extracted into butyl acetate. However, some difficulty can be experienced in the extraction and a determination in the aqueous phase is usually preferable. In both solvents the amber colour shows an absorption peak at 470 mμ.

Reagents—

The reagents as before.
Ammonium thiocyanate, 5 per cent. w/v solution.
Stannous chloride solution—Dissolve 35 g of stannous chloride, $SnCl_2.2H_2O$, in 10 ml of concentrated hydrochloric acid and dilute the solution to 100 ml with water. Keep it reduced by the addition of tin.

Procedure—

An aliquot containing 10 to 500 μg of detergent is transferred to a 10-ml graduated centrifuge tube and is diluted to 5 or 10 ml. Three drops of hydrochloric acid, 2 drops of barium chloride and 2 drops of phosphomolybdic acid are added, and the contents of the tubes are well stirred with a platinum wire whisk. After the precipitate has been centrifuged, the supernatant liquid is decanted and the precipitate is washed with 5 ml of distilled water. After further centrifuging and decantation, the tube is inverted and allowed to drain.

The precipitate is mixed in 1·2 ml of concentrated sulphuric acid and, when it has fully dissolved, the acid is diluted to about 6 ml with water, 1 ml of ammonium thiocyanate solution and 0·5 ml of stannous chloride solution are added, the mixture is then diluted to 10 ml and stirred well. The resultant amber solution may be centrifuged if desired. In the

aqueous phase some initial development of the colour occurs before a slow fading. The colour is best determined after about 20 minutes and at a wavelength of 470 mμ.

Should extraction be required, the above solution is transferred to a small separating funnel and the molybdenum thiocyanate is extracted with butyl acetate previously saturated with ammonium thiocyanate and stannous chloride.

A very slight turbidity can be seen in the amber solutions obtained by this method; this turbidity is not removed by centrifuging, but it does not appear to interfere with the determination. The second procedure is rather more sensitive than the sulphuric acid method, but it is more time-consuming, especially in that washing of the precipitate is essential. Snell and Snell include a little ferric iron in the molybdenum determination, but higher blanks were recorded with this addition and so it has been omitted.

Discussion of results

Figs. 1 and 2 show calibration curves for 1-cm tubes in a Unicam SP 350 spectrophotometer. With the sulphuric acid method, the whole aliquot is contained in 4 ml of sulphuric

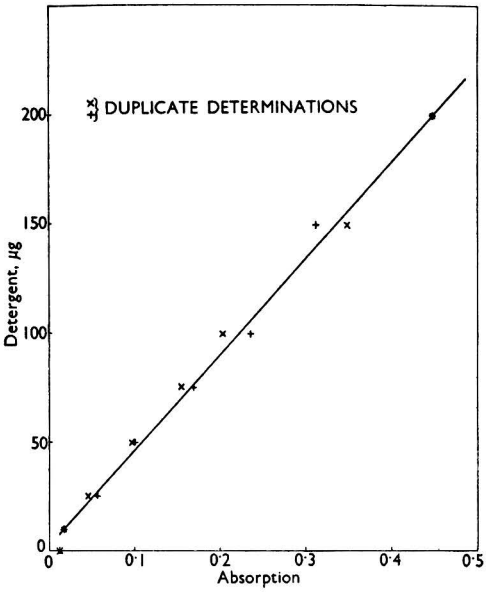

Fig. 1. Calibration curve by sulphuric acid method

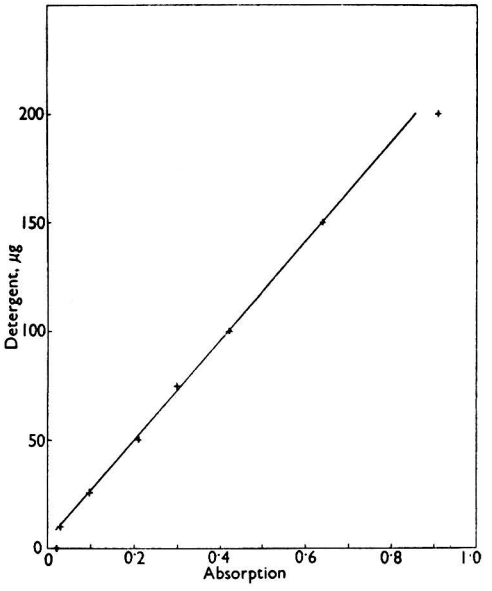

Fig. 2. Calibration curve by molybdenum thiocyanate method

acid; with the thiocyanate method the coloured solution occupies 10 ml: the latter method is thus some five times more sensitive to a given aliquot. The lower limit of detection by both methods is about 10 μg or 1 p.p.m. of detergent and it is limited by the amount of blank and also probably by the general technique of centrifuging.

Interference of other compounds

Inorganic salts, such as sodium metasilicate and phosphates, do not interfere with the gravimetric determination by Oliver and Preston's method. Alkyl sulphate, alkyl aryl sulphonate and sodium carboxymethylcellulose cause high results; they appear to be associated with the non-ionic detergent when the latter is precipitated.

In the present methods the presence of alkyl aryl sulphonates caused a heavier precipitate, but a barely significant increase in absorption was observed when 1 mg of the sulphonate was added to 100 μg of non-ionic detergent. When salts of ethylenediaminetetra-acetic acid were added in a similar quantity they had no effect. A similar addition of an alkyl sulphate gave a reading corresponding to about three-quarters of the expected value. Alternatively, when 100 μg of carboxymethylcellulose were added to 100 μg of non-ionic detergent the readings were very low. A similar behaviour was observed in both methods of determination.

The interference caused by the alkyl sulphate and carboxymethylcellulose appeared to be due to a protective action on the phosphomolybdate precipitate, which results in an incomplete separation on centrifuging. By varying the conditions this difficulty could possibly be overcome.

Acknowledgment is made to the Director of the Atomic Weapons Research Establishment for permission to publish this paper.

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Atomic Weapons Research Establishment
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February 8th, 1954

Carotene: Effect of Antioxidant on its Determination

By V. H. BOOTH

The interference of NN′-diphenyl-*p*-phenylenediamine in carotene determinations, which was reported by Beauchene *et al.*, was not observed when light petroleum was used for the extractions and aluminium oxide for the chromatographic separation.

In America NN′-diphenyl-*p*-phenylenediamine (DPPPD) is sometimes used for treating alfalfa (lucerne) meals to reduce the loss of carotene during storage,[1] and there is a possibility that it may be regularly used in Britain. Beauchene, Mitchell, Parrish and Silker[2] found that more carotene appeared to be present in lucerne meal immediately after treatment with DPPPD or other antioxidants than in untreated meal. They found that this anomaly was due to the production of a yellow pigment by reaction between DPPPD and the magnesium oxide that is used in the chromatographic assay of carotene.

Comparison of treated and untreated meals

During the past four years, many experiments have been made in this laboratory in which dried grass and lucerne meals were treated with DPPPD, in order to determine the best conditions for its commercial application. Control tests were made to see whether the diamine affected the carotene assays. Specimens of DPPPD were obtained from two sources and they were applied to the meals in diverse ways, either mixed in as dry powders, or dissolved in acetone or oils, or suspended in aqueous solutions, and sprayed by high-pressure or low-pressure apparatus into vigorously agitated meals. Both treated and untreated meals were assayed at once or after the solvents had evaporated. In this laboratory carotene was extracted from leaf meals by heating them for an hour with light petroleum, boiling range 80° to 100° C, according to Duodecim Viri,[3] and it was purified chromatographically on a mixture of aluminium oxide and anhydrous sodium sulphate (Booth,[4] Analytical Methods Committee[5]). Carotene in solution was determined in some experiments with a Pulfrich gradation photometer and in others with a Unicam photo-electric spectrophotometer having a glass prism. The results of paired determinations are shown in Table I. Each value is the mean of triplicate or higher replicate determinations. The standard deviation of a single determination varied a little with carotene level; for carotene values of about 250 p.p.m. it was $\pm 3{\cdot}1$, estimated from many results. Hence the standard error of the mean of triplicate determinations was $\pm 1{\cdot}8$. The mean ratio shows that DPPPD had no effect on carotene determinations when the extraction was made with light petroleum before purification on aluminium oxide.

Results with other adsorbents

The apparent higher carotene value for treated than for untreated meal, which was observed by Beauchene *et al.* when magnesium oxide was used as adsorbent, has been confirmed. An adsorbent recommended by Duodecim Viri,[3] by the Analytical Methods Committee,[6] and commonly used in England (Mann[7]) is defatted bone meal. Two specimens of

bone meal were obtained and each was used for triplicate determinations on lucerne meal and on grass meal, untreated and treated with 200 and 2000 p.p.m. of DPPPD. The average ratio

TABLE I

EFFECT OF ANTIOXIDANT ON THE DETERMINATION OF CAROTENE IN DRIED LEAF MEALS, ALUMINA BEING USED AS ADSORBENT

DPPPD applied, p.p.m.	Carotene found, p.p.m.		Ratio of treated to untreated	
	Untreated	Treated		
Dried grass meals—				
100	125	120	0·960	
100	375	370	0·987	
100	395	395	1·000	
105	300	285	0·950	
105	390	395	1·013	
150	330	335	1·015	
150	395	380	0·962	
200	245	260	1·061	
1200	265	275	1·038	
2000	245	250	1·020	
Dried lucerne meals—				
100	165	170	1·030	
100	185	190	1·027	
100	215	210	0·977	
140	190	195	1·026	
150	175	170	0·971	
1000	300	305	1·017	
2000	30	30	1·000	
2000	100	105	1·050	
2000	215	215	1·000	1·006*
2000	355	350	0·986	
2000	375	370	0·987	
2000	380	380	1·000	
		Mean ratio	1·003	

* Mean for heavy treatment.

of treated to untreated meals was 1·03. Hence the error likely to occur in a carotene determination in which bone meal is used, namely about 3 per cent., although ten times greater than that when aluminium oxide is used, is not serious.

CAROTENE SIMULATION BY THE DIAMINE

From carotene-rich meals samples of about 0·25 g were usually taken for extraction, and from a carotene-poor meal this weight was increased to about 0·5 g. If the latter had been very heavily treated with DPPPD (2000 p.p.m.), the amount of DPPPD in each extraction vessel might be 1 mg. To determine the error that might be produced through carotene simulation by DPPPD, various amounts of it from 1·1 to 2·6 mg were "extracted" and separated by chromatography exactly as in the method for leaf meal. No pigment was visible in any of the eluted solutions, but when the solutions from 7 mg were pooled and concentrated a brown pigment was discernible. The extinction at 450 mμ was such that the extract from 1 mg of DPPPD was equivalent to 0·78 μg of β-carotene. Therefore a poor meal (carotene content of 100 p.p.m.), if treated with 2000 p.p.m. of DPPPD, might be over determined by 1·5 p.p.m. As the coefficient of variation of one determination by this method is nearly 2 for medium meals and a little greater for poor meals, this error would be barely discernible. With moderate applications of DPPPD to good quality meals the error would be below the experimental error.

DISCUSSION OF RESULTS

The divergence of these results from those of Beauchene *et al.* is doubtless due mainly to the chromogenic reaction observed by these authors between magnesium oxide and DPPPD. They did not try another adsorbent, but the present findings suggest that aluminium oxide does not show the effect. The divergence may also be due partly to differences in eluting technique. We adsorb carotene from light petroleum, elute it with 2 per cent. of acetone in light petroleum and use the moving sharp band of carotene as an

indicator of progress. By this means the protracted tailings of Beauchene *et al.* are avoided. A minor contributory reason for lack of interference may have been the low solubility of DPPPD in the light petroleum that was used for the extraction. Beauchene *et al.* used for their extractions a mixture of light petroleum and acetone in which DPPPD is much more soluble and more easily conveyed to the adsorbent.

Since this work was done, Mitchell and Silker[8] have reported a modification of the A.O.A.C. method for carotene determination. They evaporate the extract to remove acetone, add light petroleum to it, carry out chromatography on tricalcium phosphate instead of magnesium oxide and then measure the extinction of the eluate at 436 mμ. This method gave carotene determinations for meals treated with 0·2 per cent. of DPPPD that were only 5 per cent. high. The method used in the present investigation avoids most of this small error and is quicker.

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January 25th, 1954

Determination of Fat in Canned Cream Soup

REPORT OF A COMMITTEE OF THE FOOD MANUFACTURERS' FEDERATION

A Committee of the Food Manufacturers' Federation has made the following report on the determination of fat in canned cream soup.

The members of the Committee were Messrs. S. Back, T. E. Barnes and J. H. Pennington (Food Manufacturers' Federation), D. Dickinson (Fruit and Vegetable Canning and Quick Freezing Research Association), S. G. Burgess (Society for Analytical Chemistry) and W. M. Shortt (Ministry of Food), with Dr. F. H. Banfield (British Food Manufacturing Industries Research Association) as Chairman.

Report

The voluntary Code of Practice agreed between the industry and the Ministry of Food lays down a specific minimum fat content to justify the style Cream Soup. (This minimum is at present 3·5 per cent.) Using the method of extraction with ether after drying with sand, some analysts have obtained results as low as 2 per cent., whereas others using the Röse - Gottleib or Werner - Schmidt methods obtained results averaging 3·7 per cent. on similar samples.

It would appear that the presence of carbohydrate is responsible for the retention of the fat within the hard impervious mass formed during the drying process, as tests have shown that the amount of fat not extracted depends on the degree of drying. Dehydration of the mass by the addition of anhydrous sodium sulphate has not proved satisfactory. Collaborative work has shown that satisfactory and uniform results are attained by the use of a method drawn up by the Analytical Sub-Committee of the Comité International Permanent de la Conserve. This is based on Methods given in *The Analyst*, 1943, **68**, 50, and in "Official Methods of Analysis of the Association of Official Agricultural Analysts," Seventh Edition, 1950, 13: 96, and is given below.

Method

Hydrolysis—

Hydrolysis with hydrochloric acid can be carried out in a beaker or similar vessel, after which the mixture is transferred to a small cylinder. Weigh the sample (5 to 10 g) and place

it in the hydrolysis vessel, add 2 ml of ethanol and mix it with the sample. This prevents the formation of lumps when the acid is added. Then add 10 ml of hydrochloric acid (25 ml of hydrochloric acid, sp.gr. 1·184 + 11 ml of water) and mix well. Set the vessel in a bath of water kept at 70° to 80° C for 30 to 40 minutes or until the liquid is virtually clear. Cool the contents, add 10 ml of ethanol and mix. If the sample is to be transferred to an extraction tube, wash the vessel used for hydrolysis with ethanol and ether.

EXTRACTION—

Add 25 ml of ether (or rinse the vessel into the extraction tube with 25 ml of ether in three portions), stopper the tube and shake it vigorously for 1 minute. Add 25 ml of light petroleum, boiling range 40° to 60° C, and shake the mixture for 1 minute. Set the tube aside for 10 to 20 minutes until the upper liquid is practically clear.

With the aid of a fine siphon, draw as much as possible of the ethereal solution of fat through a filter consisting of a plug of cotton-wool packed just tightly enough to allow the ether to pass freely into a flask of about 200 ml capacity, which for convenience should fit a suitable distilling apparatus.

Blow out any remaining drops of ethereal fat solution in the siphon tube and rinse it out with a mixture of equal parts of ether and light petroleum, boiling range 40° to 60° C, or with the re-distillate from previous determinations.

Re-extract the remaining liquid in the tube three times, each time with about 15 ml of each solvent, *i.e.*, ether and light petroleum, boiling range 40° to 60° C. Shake the sample cautiously after each addition. Draw the clear ether solutions through the filter into the same flask as before and wash the tip of the siphon, the funnel and the end of the funnel stem with a few millilitres of a mixture of ether and light petroleum in equal quantities.

Distil the ether as soon as possible. Do not allow the vessel containing the ether solution to stand overnight, as the fat solution may then creep over the side of the vessel.

Transfer the flask to an oven at 100° C, dry it for one hour and then remove and cool it. Re-dissolve the dried fat in 15 to 20 ml of light petroleum, boiling range 40° to 60° C, and filter the solution through a small fat-free filter-paper into a flask similar to that used before, but previously dried at 100° C, cooled in air and weighed. Wash the first flask, filter-paper and funnel into the tared flask with a jet of light petroleum, boiling range 40° to 60° C, and evaporate the solvent. Dry the flask at 100° C for 1 hour, cool it in air for 20 minutes and then weigh it. Repeat this procedure until the difference between two successive weighings is less than 1 mg.

RESULTS

By this method, the results shown in Table I were found for various cream soups.

TABLE I

FAT IN CREAM SOUPS

Variety	Number of determinations,	Minimum, %	Maximum, %	Average, %
Cream of tomato ..	17	3·70	3·92	3·8
Cream of chicken ..	21	3·30	3·72	3·4
Cream of celery ..	24	5·11	5·44	5·3
Cream of green pea ..	24	3·74	4·12	3·9
Cream of mushroom ..	23	7·78	8·18	8·0
Number of laboratories	7			
Total number of analysts ..	15			

For each variety, one case of 24 tins was taken from an experimental batch made on works production scale and distributed among the different laboratories for the purpose of examining the method.

With the exception of the Cream of Tomato variety, the soups contained garnish such as peas or pieces of chicken meat, which were suitably dispersed through a sieve; high-speed disintegrators, which might have broken the emulsion, were avoided.

When distribution and sampling difficulties are taken into consideration the agreement between analysts in several different laboratories is considered to be good, and the method is accordingly recommended.

FOOD MANUFACTURERS' FEDERATION
57, CATHERINE PLACE
LONDON, S.W.1

December 29th, 1953

Symposium on the Comparison of Chemical and Biological Estimation of Drugs in Quantitative Pharmacology

AT an Ordinary Meeting of the Society, organised by the Biological Methods Group, held on Wednesday, April 7th, 1954, at the Wellcome Research Institution, Euston Road, London, N.W.1, six papers were presented and discussed under the Chairmanship of Professor J. H. Burn, M.A., F.R.S. The papers and discussions are summarised below.

CHEMICAL METHODS FOR THE DETERMINATION OF DIGITALIS

DR. J. M. ROWSON said that the following methods were in use for the estimation of *Digitalis purpurea*—

1. Colorimetric methods (*a*) based upon the aglycone portion of the molecule: reaction with 3: 5-dinitrobenzoic acid; 2: 5-dinitrobenzoic acid; trinitrophenol (Baljet); sodium nitroprusside (Legal); *m*-dinitrobenzene (Raymond); 1:3:5-trinitrobenzene; (*b*) based upon digitoxose: reaction with Keller - Kiliani reagent; phosphoric acid (Pesez); orcinol (Bial).

2. Fluorimetry in the presence of either phosphoric acid or hydrochloric acid to determine glycosides of the B series.

3. Ultra-violet light absorption at 217 mμ; polarographic determination with half-wave potential at $-1{\cdot}9$ to $-2{\cdot}0$ volts.

4. Paper or column chromatography for qualitative separation or quantitative determination of the individual components.

Of the methods in group 1 (*a*), the use of 3: 5-dinitrobenzoic acid was the most accurate and convenient in the author's experience; reagents should be prepared with analytical accuracy and observations should be made at 20° C because the reaction was sensitive to changes in temperature. The Baljet process, although frequently used, was sensitive to the presence of impurities in leaf extracts and tended to give high values. The Raymond process called for very critical use of the spectrophotometer in measuring rates of colour fading. All reactants in this group failed to distinguish between individual glycosides or between glycosides and aglycones. Colour reactions in group 1 (*b*) necessitated separation of glycosidal material from any free digitoxose present in the leaf extracts; by their use, true glycoside was determined and not aglycones, which were of lower therapeutic efficiency. By the use of each reagent, only two of the three digitoxose molecules present in primary glycosides could be determined, but all three digitoxose molecules were determined in the secondary glycosides, digitoxin and gitoxin. Enzymic degradation of primary to secondary glycosides had been used by several workers to overcome this difficulty and also to determine their relative amounts present: little or no aglycone had been found in Digitalis leaves by these methods.

The author had shown that complete extraction of total glycosides from Digitalis leaf was achieved by maceration with gentle agitation for 1 hour in either 10 volumes of 70 per cent. ethanol or 100 volumes of water. Chloroform failed to extract primary glycosides or gitoxin (also digitalinum verum and gitorin) from aqueous or dilute alcoholic extracts; these were, however, removed by a mixture of chloroform and ethanol.

The comparative values of chemical and biological assays for the assessment of digitalis had been the objective of a number of research workers. Eastland, Lawday and Sellwood (*J. Pharm. Pharmacol.*, 1952, **4**, 811) had claimed that mixtures of chloroform-soluble glycosides could not be adequately assessed by the picrate method, which generally gave results two to three times as high as the pharmacological method: with increasing purity of these mixtures, the ratio of chemical to biological value approximated more and more closely to unity. Soos, Fuchs and Kabert (*Sci. Pharm.*, 1951, **19**, 73) had compared the determinations by means of the Keller - Kiliani, dinitrobenzoic acid, picrate and Legal reactions with those obtained by frog and guinea-pig biological assays for seven separate species of *Digitalis*.

They found no general agreement between the four chemical methods, but some agreement between the Keller - Kiliani and the biological methods. The extraction methods used by Soos and his collaborators were erroneous, and would explain these deviations. Neuwald (*Arch. Pharm.*, 1950, **283**, 93; *Ibid.*, 1952, **285**, 22) had found reasonable agreement between the picrate or genin assays and biological assays. Langejan and van Pinxteren (*Pharm. Weekbl.*, 1953, **88**, 529) had found good agreement between biological estimation and dinitrobenzoic acid determinations; the Bial results had shown wider deviation.

Rowson and Dyer (*J. Pharm. Pharmacol.*, 1952, **4**, 840) had examined a large number of leaf samples; for all of those grown and processed under their own control, there had been good agreement between dinitrobenzoic acid and biological estimations; for many commercial samples they had also found good agreement between the two methods, but for two commercial samples (values for one as yet unpublished), they had found a wider divergence between chemical and biological methods, the chemical value being the higher.

At present the author did not consider that the determination of each individual glycoside was necessary for the therapeutic assessment of leaf samples; until the pharmacologist presented clearer evidence of differential potencies in these glycosides, evaluation of total materials by dinitrobenzoic acid or other similar reagent and an estimate of true glycosides by the Keller - Kiliani or similar procedure were all that was necessary. A large difference between the two would indicate the presence of large amounts of genins, which must be excluded: otherwise there appeared to be good parallelism between the two chemical procedures, and between either of them and the biological estimation.

For the manufacture of digitoxin or the other glycosides, however, it was more essential to know the true composition of the glycosidal mixture present in the leaf than to assess its total potency in terms of international units per gram, and here chromatographic and fractional solvent extractions offered good promise for the detailed qualitative and quantitative examination of the glycosidal complex present in the leaf of *D. purpurea* or *D. lanata*.

The Biological Standardisation of Digitalis Preparations

Mr. G. A. Stewart stated that, of the four cardiotoxic methods described in the British Pharmacopoeia, 1953, for the standardisation of digitalis preparations, those depending on the intravenous infusion of the drug into the guinea-pig or pigeon were commonly used to-day.

He described the guinea-pig assay method used in his laboratory since 1947. The animals were anaesthetised with urethane given intraperitoneally, artificial respiration was applied, and the thorax was opened before beginning the slow constant infusion of the drug into the accessory cephalic vein. The digitalis standard was extracted with absolute alcohol by the 6-hour continuous procedure detailed in the British Pharmacopoeia, 1953.

From accumulated data the following partial regression coefficients were found for the First, Second and Third Digitalis International Standards—

$$(0{\cdot}1311 \pm 0{\cdot}0533)X_1 \text{ and } (0{\cdot}6631 \pm 0{\cdot}0668)X_2,$$

where X_1 was $\log_{10}$ (concentration as units per 10 ml) and X_2 was $\log_{10}$ (body weight in g)/100. (Each coefficient has been given with its standard error.) For the British Standard of Strophanthin-K, the corresponding partial regression coefficients were $(0{\cdot}3148 \pm 0{\cdot}0846)X_1$ and $(0{\cdot}7403 \pm 0{\cdot}1459)X_2$.

The weight factors were in agreement with those found by other workers (Braun, H. A., and Siegfried, A., *J. Amer. Pharm. Ass., Sci. Ed.*, 1947, **36**, 363; Jacobsen, E., and Larsen, V., *Acta Pharmacol. et Toxicol.*, 1951, **7**, 35) and indicated that it was not strictly correct to express the lethal dose in units or mg per kg.

Statistical analyses were conducted on a number of assays, selected at random, by the ratio method on lethal dose per kg, without reference to logarithms, and also after covariance analysis based on the logarithm of lethal dose and the logarithm of body weight. When the mean infusion times for both standard and test preparations and the body weights of the two groups of animals used were similar, the mean potencies and limits of error obtained by the two methods of analyses were in excellent agreement. However, if assays were not balanced in all respects, covariance analysis would have to be applied.

From the accumulated data on the assays of six types of digitalis preparation, and of strophanthin-K, against their common standards, it was possible to test for the significance of the difference between the standard and test error mean squares, and so to obtain a measure of the significance of the difference of the distribution of lethal doses of the materials compared.

Digitalin, a preparation from the seeds of *Digitalis purpurea*, produced a different distribution of lethal doses from that of the International Standard Preparation of *Digitalis purpurea* ($P < 0{\cdot}002$), and the differences for strophanthin-K, tincture of *Digitalis purpurea* and *Digitalis lanata* leaf approached significance ($P = 0{\cdot}1$ to $0{\cdot}2$). It was therefore necessary that each type of preparation should have its own standard.

Experiments were also described in which the potencies obtained for four digitalis leaf preparations were compared after estimation by the infusion methods in the pigeon under ether anaesthesia, and in the guinea-pig under urethane and sodium pentobarbital anaesthesia. The four preparations used were the Third International Standard of *D. purpurea*; a sample obtained by mixing 9 batches of *D. purpurea* obtained from various sources; and two other samples, one of *D. purpurea*, and one of *D. lanata* obtained from single sources. The powders were each extracted by the room temperature and hot methods of extraction described in the British Pharmacopoeia, 1953, making 8 tinctures in all. The potency found for each sample was shown to depend on the anaesthetic and species of animal used, and on the method of preparation of the tinctures.

To check whether such differences could occur in different laboratories, *D. purpurea* preparations of recent manufacture obtained from the British market were assayed by the guinea-pig infusion method, urethane being used as the anaesthetic. Of five preparations examined, the potencies found varied from 63·4 to 116·9 per cent. of the labelled potencies.

Comparisons were made between the assays in man, and those conducted in cats, described by H. Gold (*J. Amer. Pharm. Ass., Pract. Ed.*, 1947, **8**, 594), in which large discrepancies were found between the therapeutically determined oral potencies in man and the cardiotoxically determined intravenous potencies in the cat. The differences might have been due to the form in which the preparations was given, the route of administration and the effect observed.

The author finally stated that the aim in the biological standardisation of cardiac glycoside preparations, potential and established, should be to obtain an animal in which there was cardiac failure and in which there was absorption of glycoside of the same kind as that occurring clinically, and to determine a cardiotonic response, finally to be compared with bio-assay in man.

Discussion on the foregoing two papers

Dr. G. F. Somers said that the biological assay of digitalis violated the basic principles of biological assay, as the constituents of the standard and test samples might differ. He asked if it should not merely be called a biological evaluation. Results by different laboratories conflicted, even when the same method was used. It was questionable whether the British Pharmacopoeia was justified in admitting four different methods, as then results must be expected to conflict with each other. The only reasonable procedure appeared to be an estimation by each procedure and a determination of the mean, as was done in the establishment of the Third International Standard; but this was hardly practicable as a routine.

Dr. G. E. Foster said that he was surprised that Dr. Rowson had not made greater reference to the application of paper chromatography for the examination of digitalis preparations. This technique was more specific for the individual glycosides. Dr. Foster suggested that use might be made of chromatography to standardise digitalis leaf on the basis of the most important glycoside present.

Mr. A. L. Bacharach said that the only measure of therapeutic activity for any digitalis preparation must be expressed by $\Sigma(G\alpha)$, where G is the amount of individual glycoside and α its individual activity. To this must be added an unknown quantity, X, expressing the summated effect of all potentiating and inhibiting substances present. Most, if not all, of the information needed for such a calculation seemed at present to be lacking. On the other hand, biological methods seemed at least of doubtful validity in the absence of evidence that any one of them gave results parallel with therapeutic activity in man. It did not appear that anything beyond a lower limit test for cardiac activity could at present give quantitative information of value to the clinician.

Dr. J. I. M. Jones suggested that, as digitalis preparations contained several different substances having different and possibly overlapping pharmacological actions, the scientific and only really satisfactory way of dealing with the problem, whether considered as an analytical problem or a manufacturing problem, was to isolate the active ingredients. The intrinsic activities of the pure isolated compounds would have to be determined once and for all by a co-operative experiment. For the physician's purpose, if he so desired it, the pure compounds could be mixed in any way to produce the effects wanted. From the analyst's point of view, it would be sufficient to determine the amounts of the different substances in such mixtures by chemical methods, which would doubtless be readily available after a technique of separation by chromatographic or other methods had been achieved. No doubt or confusion would then arise either for the

physician or for the analyst from the potentiating or inhibiting effect of the unknown materials present in naturally occurring materials or in concentrates made therefrom.

DR. F. J. DYER asked whether Mr. Stewart thought that the use of 70 per cent. ethanol, or a concentration similar to that used for tincture, was preferable to the use of 80 per cent. ethanol as prescribed in the British Pharmacopoeia, 1953, p. 828. It was the tincture that was applied clinically, not the 80 per cent. ethanolic extract.

DR. H. O. J. COLLIER said that Mr. Stewart had shown that discrepancies existed between the results of the different methods of chemical extraction and biological assay of digitalis. Although they were undoubtedly real, it did not seem to him that the discrepancies were great enough to destroy confidence in the biological evaluation of digitalis. It had been suggested that pure glycosides should alone be used clinically, but this seemed to be wasteful, because the side-effects of digitalis were not particularly associated with any unwanted substance in the preparation and because the physician had a chance to assess the effect of a preparation in his patient before any harm was done.

DR. ROWSON replied that, in general, comments on the variation in biological assay values might be taken as criticisms of chemical methods. In reply to Dr. Foster, he said that chromatography was being investigated in his laboratory and was a useful technique. The suggestion that the potency of digitalis was due to one component was incorrect; several components contributed to the potency. It had been found by experience that most leaf samples gave good comparative values by biological and chemical methods.

In reply to Mr. Bacharach, Dr. Rowson said that a limiting value for the assay was not accurate enough, but a wider range of tolerance for the assay was desirable. It was contended that a chemical determination would be at least as accurate as a biological assay. To Dr. Dyer, he replied that in his own experiments he used 70 per cent. ethanol as solvent.

MR. G. A. STEWART, in reply to Dr. Dyer, said that the use of 70 per cent. ethanol for the preparation of tinctures from both standard and test materials was preferable to the use of the higher concentrations given in the B.P. under the assay of digitalis only when the final commercial preparation was to be Tincture of Digitalis, B.P. The clinical use of the tincture in this country was now, however, not very great.

In reply to a question by Dr. Trevan, Mr. Stewart agreed that the description of one method only in the B.P. would be of great help in obtaining agreement between laboratories assaying samples from the same batch if a standard similar in type to the preparation under test was adopted. It would not necessarily, however, ensure that the samples were adjusted to a uniform clinical potency.

Finally, in reply to Dr. Collier, Mr. Stewart stated that the real difficulty in assessing the confidence to be placed in any biological evaluation of digitalis was that, in the last resort, the patients themselves acted as their own assay subject, and the amount taken was adjusted until the desired effect was obtained.

There was a case for having clinical and pharmacological tests performed in this country on a number of different batches of digitalis preparations to clarify this problem.

THE CHEMICAL DETERMINATION OF VITAMIN D

DR. J. GREEN said that the absence of methods of chemical analysis capable of measuring vitamins D in foodstuffs or biological tissues had made progress in the understanding of the physiological role of these vitamins very slow. However, certain advances had been made in recent years. There were four main reasons that accounted for the extreme difficulty of the problem of determining vitamin D. These were—

(*a*) The high biological potency of vitamin D, which made it desirable to measure small amounts.

(*b*) The paucity of even reasonably specific chemical reagents.

(*c*) The facts that vitamin D was always a relatively minor constituent of a complex sterol mixture and that many of these sterols must be removed before assay.

(*d*) The frequent occurrence of vitamin D with vastly greater amounts of vitamin A, which masked vitamin D in all known reactions and gave rise to the most serious elimination problem.

In the present state of knowledge, it was important that chemical analyses should be carried out with frequent recovery experiments, and new methods should be rigorously checked by bio-assays.

A number of reagents had been proposed for the colorimetric determination of vitamin D, most of them depending on carbonium-ion formation. Probably the most useful was the antimony trichloride - acetyl chloride reagent, which gave a deep orange to pink colour with a peak at 500 mμ and $E^{1\%}_{1cm}$ of about 2000. The usual first step in the analytical procedure was saponification of the fat fraction, which was easily done without loss of vitamin D. Elimination of interfering sterols was often the next stage, and this was usually done by a

combination of digitonin precipitation and chromatography. Absorbents such as alumina, Superfiltrol, magnesium oxide - kieselguhr, bentonite and floridin had been used.

Few techniques were available for the quantitative separation of vitamins A and D. A number of workers had proposed methods involving selective condensation of vitamin A with maleic or citraconic anhydride. This method did indeed remove very large amounts of vitamin A, but not all of it. Also, careful experiments had shown that large losses of vitamin D, especially vitamin D_3, might occur during the procedure, and the method could not be recommended. Selective irradiation at 300 to 400 mμ was suitable for removing small amounts of vitamin A, but was of little value from a practical point of view. Chromatography was the method most often used. Superfiltrol, magnesium oxide - kieselguhr and the acid clays were all claimed to be suitable, but there was no doubt that the ideal chromatographic separation was a long way off.

Recent advances had been made with both adsorption and reversed-phase paper chromatography. On paraffin-coated paper, good separation of vitamin D from many substances, including the important tachysterol and small amounts of vitamin A, were claimed. This technique held promise, and might well be essential to the final solution of the vitamin-D assay problem.

Biological Methods of Assay of Vitamin D

Dr. M. E. Coates said that vitamin D had long been recognised as essential for the prevention of rickets, but its precise biochemical role was not fully understood. There was evidence that it facilitated absorption of calcium from the gut and was involved in the deposition of minerals in bone. The condition of rickets was accompanied by a raised plasma alkaline phosphatase. Vitamin D was essential for optimal growth in young animals, probably because of its effect on skeletal growth.

These physiological effects had all been used as criteria of response in methods of biological assay. A further property of vitamin D, its capacity to reverse the bacteriostatic effects on *Lactobacillus casei* of long-chain unsaturated fatty acids, had been made the basis of a microbiological assay by Kodicek (*Biochem. J.*, 1950, **46**, xiv).

Specificity of vitamin D assays—The characteristic action of vitamin D in bone formation had made possible methods of assay with very specific criteria of response. It was also possible to distinguish by means of rat and chick tests between the forms of vitamin D, as although mammals utilised both vitamins D_2 and D_3 indiscriminately, vitamin D_2 was utilised by birds most inefficiently. The present International Standard, being a solution of vitamin D_3 (cholecalciferol) in oil, was suitable for both rat and chick assays.

Criteria of response—The methods of assay in most frequent use depended on some measurement of bone formation. In the classic "line test," bones were removed, split longitudinally and stained with silver nitrate. The extent of the stained area, which could be assigned an arbitrary value, was proportional to the degree of healing of rickets. Inevitably such a method had a large personal error, and probably a better assessment of calcification could be obtained radiographically, when some distance such as that between tarsals and metatarsals in the hock joint could be measured. Even then the judgment was somewhat subjective, particularly in poorly calcified bones. Determination of the bone ash was the most satisfactory criterion of calcification, but this was tedious and expensive. Use of toe ash instead of tibia ash reduced both time and expense and gave, according to Bliss (*Poult. Sci.*, 1945, **24**, 534), equally reliable results. In a technique proposed by Snyder, Eisner and Steenbock (*J. Nutrit.*, 1951, **45**, 305), bone formation was assessed by the determination of radioactivity in the forepaws of rats after injection of phosphorus-32.

Other criteria of response had been suggested. For instance, Jones and Elliot (*Biochem. J.*, 1943, **37**, 209) had shown that gain in weight of chicks was, under certain dietary conditions, quantitatively related to their intake of vitamin D. Motzok (*Biochem. J.*, 1950, **47**, 196) had found a linear relationship between the plasma alkaline phosphatase in chicks and dose of vitamin D. Fournier (*Compt. Rend.*, 1951, **232**, 1019) had related excretion of calcium in the faeces of rats to the dietary level of vitamin D.

Precision of the methods—The precision of the methods mentioned above had been considered by the respective authors. Bills ("Biological Symposia," 1947, XII, p. 409) had discussed the probable error of the "line test," and Waddell and Kennedy ("Biological Symposia," 1947, XII, p. 435) that of the chick assay. Campbell and Emslie (*Poult. Sci.*,

1945, **24**, 296) had suggested that, in chick tests, calculation of fiducial limits from error variance underestimated the variance of an assay. Instead they recommended replicate groups of chicks at each dose level and the use of variance between groups in the estimate of error.

Use of biological methods—Although biological assays were not sufficiently sensitive for fundamental research on the biochemical role of vitamin D, they were the only true measure of its physiological availability. A biological method was therefore to be preferred when the vitamin-D content of a foodstuff was determined.

Discussion on the foregoing two papers

Dr. Ethel M. Cruickshank said that, in making experiments on vitamin-D balance and distribution in rats given massive doses of ergocalciferol, workers in The Dunn Nutritional Laboratory had used the radiographic technique and preferred it to the bone-ash method or the "line test" because (*a*) the degree of rickets in individual animals could be determined before assay and (*b*) records of the experiments were available for reference. The reproducibility of the assays was also satisfactory. They found that little or no loss of vitamin D occurred when the tissues were freeze-dried and stored at −20° C for up to 4 months.

Dr. E. Kodicek said that, with regard to the microbiological procedure referred to by Dr. Coates, the growth of gram-positive bacteria, especially that of lactobacilli, could be inhibited by the presence of small amounts of long-chain unsaturated fatty acids in the medium. The inhibition of growth, most likely owing to permeability changes, could be prevented by vitamin D. He was tempted to call this state "bacterial rickets," as many of the criteria of mammalian rickets could be observed: the response to vitamin D, replacement of the vitamin effect by calcium ions, dependence of these effects on phosphate concentration, and the production of toxic effects by large amounts of vitamin D. The growth response to vitamin D was, within limits, proportional to the vitamin concentration. Cholesterol was partly active (about 10 per cent.), but not the other sterols studied; phospholipids and vitamin E had a slight reversing activity. When the procedure was applied to natural material, difficulties were experienced; for instance, the non-saponifiable matter from fish oils contained a potent inhibitor for bacteria. For this and other reasons, the paper-chromatographic procedure for separating sterols was developed to permit the identification of vitamin D in presence of other sterols and the quantitative determination of vitamin D in enriched natural material, such as liver, faeces and oils, provided that the vitamin concentration was greater than 5 μg per gram and that vitamin A was not present in excess. This procedure, combined with the microbiological technique, should prove useful in the study of certain problems, but was not yet sensitive enough for the assay of amounts present naturally.

Dr. G. E. Foster asked Dr. Kodicek what methods had been used to detect the spots of vitamin D and related compounds on paper chromatograms.

Dr. Kodicek replied that the spots could be detected by spraying the chromatogram, dried and warmed to 50° to 70° C, with a 24 per cent. solution of antimony trichloride in chloroform. The paper was then warmed until the colours formed with the sterols appeared. As little as 1 μg of vitamin D could then be detected by its fluorescence in ultra-violet light.*

Dr. J. I. M. Jones enquired what was the minimum quantity of vitamin D required for determination by the chemical techniques described. He pointed out that the low value for $E^{1\%}_{1cm}$ at the point of maximum light absorption necessitated, in a 1-cm cell of 5 ml volume, 8000 international units to produce a reading on the most reliable part of the scale. Although this quantity could be reduced by reducing the volume of the cell, a relatively large amount would still be necessary. He asked to what extent this quantity could be reduced by the colorimetric methods.

Dr. Green replied that one was limited by the gross extinction of vitamin D at 265 mμ, which was small, and it was not going to be easy to measure the small amounts of vitamin D by this method. Also, it is a low-wavelength region, where the necessity for rigorous elimination of impurities was bound to cause difficulty.

Dr. A. E. Bender asked whether oxidation products of vitamin D reacted with the chemical reagents mentioned by Dr. Green, and whether they reacted microbiologically in the method described by Dr. Kodicek; in other words, whether the true vitamin D content could be measured by these methods in a preparation that had deteriorated.

Dr. Green replied that oxidation products would not react. Trials over several years had shown that the assay measured deterioration quite well. The products of decomposition of vitamin D did not appear to interfere.

Dr. Kodicek replied that some oxidation products reacted microbiologically. They could, however, be differentiated by the paper-chromatographic method mentioned before.

* Summaries describing these methods have since appeared (*Biochem. J.*, 1954, **57**, xii and xiii).

Chemical and Biological Methods for the Determination of Adrenaline and Noradrenaline

Dr. G. B. West said that many colorimetric tests for these amines, varying widely in sensitivity and specificity, had been devised. Most depended upon the presence of the phenolic groupings, and frequently these estimates of decomposed or contaminated adrenaline or noradrenaline solutions were higher than those derived from biological assay. The most widely used were those of Folin, Cannon and Dennis (1913), Folin and Ciocalteu (1927) and Shaw (1938). In the first two methods, the catechol amines produced equivalent colours when present in similar concentrations, but in the last test, in which arsenomolybdic acid was used, the colour produced by noradrenaline was about 15 times less than that given by adrenaline. The production of coloured "chrome" compounds with iodine had long been known to give reasonable estimates of the two amines. Euler and Hamberg (1949) showed that, at pH 4 for $1\frac{1}{2}$ minutes, only about 10 per cent. of the noradrenaline was oxidised, whereas, at pH 6 for 3 minutes, oxidation was complete. As adrenaline was completely oxidised in both conditions, a simple calculation gave the individual amounts of the two substances in the mixture. The method lacked sensitivity and results might be erroneous when some tissue extracts were used.

The fluorimetric methods seemed most likely to reach a degree of sensitivity sufficient for the measurement of concentrations as low as those that occurred in peripheral venous blood. Lund (1949) had used the property that adrenaline formed a fluorescent oxidation product in alkaline solution to measure concentrations as low as 10^{-8}. More recently, Weil-Malherbe and Bone (1952) had found that more satisfactory results could be achieved by trapping the adrenochrome or noradrenochrome by condensation with ethylenediamine hydrochloride in the presence of free ethylenediamine. Recovery experiments had a standard deviation of 5 per cent., which was very good for estimations of concentrations as low as 10^{-9}. The fluorescent intensity produced by noradrenaline was one-fifth of that produced by an equi-molar amount of adrenaline. Initial separation of the amines by column chromatography was usually carried out.

Colorimetric methods, however, because of their lack of specificity, could not entirely replace the pharmacological methods when the composition of the solution was unknown. The latter methods were usually based on the quantitative difference between the activities of the two amines on certain preparations. Parallel quantitative assays were made on at least two biological preparations, one that was much more sensitive to adrenaline than to noradrenaline (*e.g.*, the isolated uterus of a non-pregnant rat or the isolated rectal caecum of the chick or hen), and the other for which noradrenaline was the more active amine (*e.g.*, the blood pressure of the cat, the isolated colon of the rat or the isolated ileum of the rabbit). From the results so found, the relative and absolute amounts of both amines could be calculated by mathematical formulae. Other biological methods involved the use of the nictitating membrane of the cat (both normal and denervated), the uterus of the cat *in situ*, the blood pressure of the rat or rabbit, or the isolated frog heart.

If the amines were first separated so that each could be assayed individually against a standard solution on a suitably sensitive pharmacological preparation or by fluorescence, then sensitivity and accuracy were increased. There were at present several solvents available for the paper-chromatographic separation, and at least seven spray solutions that were suitable for locating and roughly estimating the desired areas. This analytical procedure represented an important advance over the methods in which the original extracts were used without chromatography. Considerable purification was effected, thereby increasing the accuracy of the subsequent assay method on the blood pressure, say, of the rat or cat.

Routine Methods used in the Quantitative Determination of Adrenaline

Dr. G. F. Somers said that although numerous methods were available for the determination of adrenaline, few of them were suitable for routine purposes. The method used should be capable of statistical design, so that the precision of the determinations could be evaluated and evidence obtained that the actions of the standard and test were identical. The (2 + 2) dose design, in which large and small doses of standard and test samples were given in random order, was most commonly used. An alternative design was the constant-standard method of Vos, which appeared to be more accurate but did not provide a measure

of the slope due to the standard. Of the biological methods, the blood pressure of the spinal cat had been widely used in this country, while the atropinised dog was the official preparation in the United States.

The hexamethonium-treated cat provided a useful alternative. The cat was anaesthetised with chloralose and a dose of 50 mg per kg of hexamethonium bromide was given subcutaneously. The addition of atropine, 1 mg per kg, would improve an irregular baseline. From nineteen assays, with a single Latin square design, the mean slope, b, was 53, compared with 73 for the atropinised dog quoted by Noel from twenty-seven assays. The value for s—the standard deviation of an individual result—was 2·09 in the cat, compared with 2·44 in the dog. The corresponding s/b values were 0·042 for the cat and 0·034 for the dog. The dog method was therefore slightly more accurate. Four comparative assays on the spinal cat gave a mean s/b value of 0·028, which suggested that this was the most accurate method.

Methods involving isolated organ preparations were generally more time-consuming and troublesome. They included the isolated duodenum or ileum of the rabbit, the rat colon and the rat uterus. The rabbit-gut method was simple and satisfactory, provided that the tissue contracted regularly. From seven assays with the (2 + 2) design, the author had obtained a mean value for b of 79 and an s/b value of 0·063. The rat uterus and rat colon methods were based on the antagonistic action of adrenaline to acetylcholine or carbachol. Constant amounts of acetylcholine were added to the bath at 2 or 3-minute intervals and the reduction in the contractions was measured after the dose of the standard or unknown was given; this dose was administered 1 minute before the next acetylcholine dose was due. Each response therefore involved at least five dosing procedures, and properly designed assays were laborious in the absence of some automatic dosing apparatus. For the rat colon, acetylcholine being used, the slope b from four assays was 128 and s was 7·55, giving for s/b a value of 0·059. The precision was greater than that reported by Gaddum and Lembeck who, using carbachol, had obtained a mean s/b value of 0·13. The same authors had obtained a mean s/b value of 0·062 for the rat uterus.

Discussion on the foregoing two papers

Dr. J. Haslam asked Dr. West whether adrenaline and noradrenaline responded to the indophenol test. He also asked if any investigators had been able to take advantage, analytically, of the fact that one of the substances was a substituted primary aliphatic amine whilst the other was a substituted secondary aliphatic amine.

Mr. H. E. Brookes said that the chief concern in the control of pharmaceutical preparations of adrenaline was the degradation to a coloured compound, presumably adrenochrome. He asked if adrenochrome was biologically active and, if not, whether chemical methods depending on a reaction with adrenochrome could be considered satisfactory.

Mr. F. W. Diggins asked why rats were not used more in biological assay, as they were cheaper and simpler to use than were cats. He instanced the method of Dr. W. S. Peart at St. Mary's Hospital for phaeochromocytoma, in which a urine extract was used, and asked Dr. West if the chromatographic method necessitated an initial preparation of the urine.

Dr. F. Wokes said that the problem of replacing biological assays of drugs by physico-chemical methods should be studied in the light of the remarkable successes achieved when the same problem was tackled for vitamins. The value of suitable physico-chemical methods lay not only in the economy of time and expense in routine assays, but also in the opportunities they afforded for the closer study of the pharmacological and physiological actions of drugs and vitamins. The past lack of suitable physico-chemical or microbiological methods for vitamin D explained why so little had yet been learned about its pharmacological properties and emphasised the importance of the recent work described at this meeting. For other vitamins there had been many large-scale collaborative investigations in which experts in biological methods had co-operated with experts in physico-chemical methods. The earliest of these, on vitamin A, had been carried out under the direction of the Chairman, Professor Burn, and had led to the development of rapid and accurate physico-chemical methods that had been of the greatest value in World War II. Similar collaborative investigations on provitamin A and on various members of the vitamin-B group had been inaugurated by the Society for Analytical Chemistry. The use of standard samples in these investigations had greatly helped the analysts.

Turning to smaller points, Dr. Wokes suggested that some of the difficulties encountered by the authors of the papers in attaining a sufficiently high sensitivity in physico-chemical methods might be overcome by a closer study of those methods. Fluorimetric methods for digitalis and for adrenaline and noradrenaline might be improved by the use of more sensitive fluorimeters, such as that described in *The Analyst* in 1949.

The ultra-violet absorption method for vitamin D could be made more sensitive by the use of micro-cuvettes, in which a light path as long as 10 cm through the liquid could be obtained with a volume of less than 0·5 ml.

DR. H. WEIL-MALHERBE said that they had compared the sensitivity of the two fluorimetric methods mentioned by Dr. West and had found that they gave closely similar fluorescent intensities. A lower efficiency in Lund's apparatus might have been the reason why he had not attained the same sensitivity as they did. The importance of using an efficient fluorimeter had been stressed by Dr. Wokes, but the degree to which the sensitivity of a fluorimeter could be increased was limited by the background fluorescence and by the stability of the light source and recording circuit.

DR. WEST admitted, in reply to Dr. Haslam, that more work could be done on methods involving the primary and secondary aliphatic amine groups. Both adrenaline and noradrenaline responded to the indophenol test when they were present in high concentrations, but determinations based on this property were not very accurate owing to interfering reactions.

To Mr. Brookes, he replied that adrenochrome had some biological activity—probably about 0·05 to 0·01 of that of adrenaline—but this did not interfere with the biological estimation. Because of this, chemical methods depending on a reaction with adrenochrome would not give a true estimate of biological potency.

In reply to Mr. Diggins, Dr. West said that the biological assay in which the rat's blood-pressure was used was satisfactory, but they preferred to keep to the cat. Urine had to be concentrated before being subjected to paper chromatography.

Notes

A SPECTROPHOTOMETRIC MICRO-TITRATION OF CALCIUM

ALTHOUGH accurate results have been reported when relatively large amounts of calcium are titrated with 0·02 *N* disodium ethylenediaminetetra-acetate with murexide as indicator,[1] the end-point is not easy to detect[2] and this can cause errors in the micro-determination of calcium. Bricker and Sweetser[3] have recently reported a spectrophotometric method of detecting the end-point in titrations with disodium ethylenediaminetetra-acetate, and their apparatus has been modified, as described below, for the micro-determination of calcium.

METHOD

APPARATUS—

The cell carrier of a Unicam SP600 spectrophotometer was replaced by a Perspex cover, which was painted black and fitted with a slot to carry the specially constructed Perspex cell shown in Fig. 1. The cell extended into the light-path of the instrument and was equipped with a polythene stirrer. The stirrer had a projection on its stem so that it could be hooked on the top of the cell while optical density measurements were being made. The projection was far enough up the stem to avoid contact with the liquid in the cell.

REAGENTS—

Disodium ethylenediaminetetra-acetate—An approximately 0·01 *N* solution standardised against 0·01 *N* calcium chloride by the method described below.

Calcium chloride solution—A 0·01 *N* solution accurately prepared by dissolving pure calcium carbonate in the minimum quantity of hydrochloric acid and diluting to volume.

Murexide indicator powder—A mixture of 0·2 g of murexide powder with 40 g of potassium sulphate.

All reagents should be of recognised analytical grade.

DETECTION OF THE END-POINT—

The absorption spectra of murexide solution and of the calcium - murexide complex, both at pH 10, are shown in Fig. 2. At wavelengths between 600 and 625 mμ the murexide alone has an appreciable absorption whereas that of the calcium complex is small. Measurement of the optical density of the solution at a wavelength of 610 mμ during the titration of the calcium - murexide complex with disodium ethylenediaminetetra-acetate produces results of the type shown graphically in Fig. 3. The optical density rises slowly at first and then more rapidly until at the end-point, when all the calcium has been removed from the calcium - murexide complex, it becomes

constant. When the optical density is plotted against the volume of titrant added, the point of intersection of the two branches of the curve represents the end-point. It can be seen from Fig. 3 that the proportion of purple murexide colour increases from 50 to 100 per cent. during the addition of about 0·02 ml of 0·01 *N* disodium ethylenediaminetetra-acetate, which explains the difficulty of accurate visual detection of the end-point.

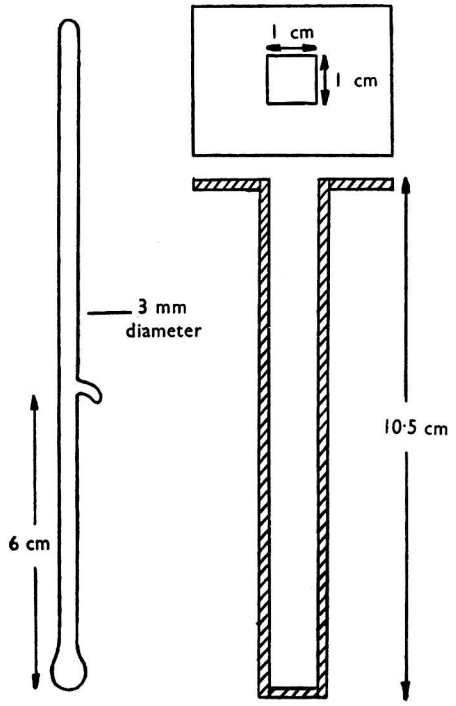

Fig. 1. Absorption cell and stirrer

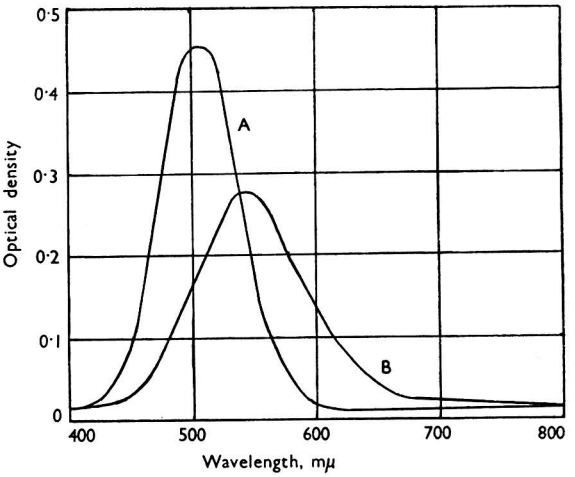

Fig. 2. Absorption spectra. Curve A, calcium - murexide complex; curve B, murexide

PROCEDURE—

Transfer the calcium solution, which should have a volume of not more than 1 ml and contain 10 to 20 μg of calcium, to the titration cell, add 4 to 8 mg of murexide indicator powder, stir, and then add 2 *N* sodium hydroxide solution dropwise until a strong pink colour appears, and add a further 2 to 4 drops. Dilute the solution with water to about 3·5 ml. Allow the spectrophotometer to warm up and set the wavelength at 610 mμ. Place the titration cell in position, adjust the dark current, and then, with the switch in the "Test" position and the transmission dial set at zero optical density, balance the meter needle with the slit control. Add 0·01 *N* disodium ethylenediaminetetra-acetate from a syringe micro-burette that has its jet just dipping into the solution. Stir the solution between additions of the titrant and determine the optical density with the stirrer out of the light-path. Towards the end-point add the titrant in 0·005-ml increments. When the optical density has increased sharply and then become constant for three consecutive readings, stop the titration, plot the optical density readings against volumes of titrant and determine the end-point as in Fig. 3.

RESULTS

When disodium ethylenediaminetetra-acetate was standardised by titrating it with various amounts of standard calcium solution by the method described above, a straight-line relationship of the type $y = a + bx$ was found between the two variables. A small excess of disodium ethylenediaminetetra-acetate over the stoicheiometric amount was always necessary to attain the end-point when it was measured spectrophotometrically. For the particular solutions used—

$$y = 0{\cdot}007 + 0{\cdot}777x,$$

where x is ml of 0·00999 N calcium chloride solution and y is ml of disodium ethylenediaminetetra-acetate solution. The equation was calculated by the method of least squares from ten results, and the standard deviations of a, b and y were calculated and found to be 0·0016, 0·0027 and 0·0022, respectively. Examination of the reagents and the water used failed to reveal sufficient calcium or other interfering metal to account for the whole of the excess of titrant used. The murexide was found to yield 0·2 per cent. of ash, but this would only account for a maximum of

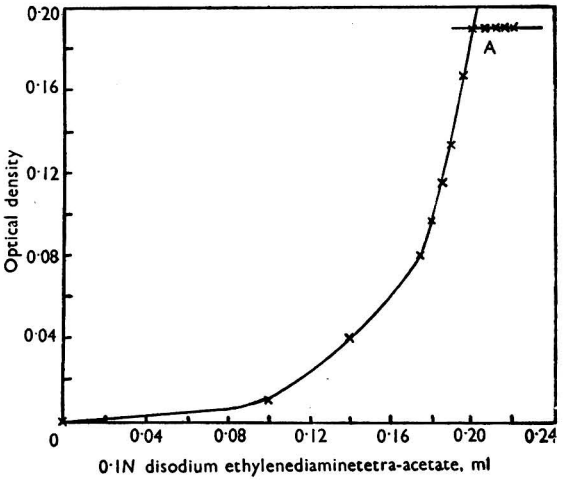

Fig. 3. Titration curve; the end-point is at A

0·003 ml of titrant. In view of the standard deviation of the intercept, however, it seems likely that the murexide was the source of this error.

Various amounts of calcium were titrated alone and in the presence of 200 μg of magnesium, and the results are in Table I.

TABLE I

RESULTS OF RECOVERY EXPERIMENTS

Calcium taken, μg..	90	74	67	39	Magnesium absent
Calcium found, μg	89	74	66	41	
Calcium taken, μg..	139	109	60	28	With 200 μg of magnesium present
Calcium found, μg..	140	109	61	27	

It is evident that the new method of detecting the end-point in this titration permits the determination of calcium in the range 20 to 150 μg with a maximum error of about 2 μg.

NOTE—

After submission of this Note it was found that methods substantially similar to this had been proposed by Kibrick, Ross and Rogers[4] and Fales.[5] A theoretical treatment of the problem has since been published by Fortuin, Karsten and Kries.[6] The present work was done independently, but acknowledgment is due to these authors.

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R. A. CHALMERS
March 17th, 1954

INDIRECT ABSORPTIOMETRIC DETERMINATION OF MAGNESIUM WITH 4-AMINOPHENAZONE (4-AMINOANTIPYRIN)

THE usual absorptiometric methods for the determination of magnesium after its precipitation as the complex with 8-hydroxyquinoline are based upon the bluish-green colour that results when 8-hydroxyquinoline reacts with ferric ions in dilute acetic or hydrochloric acid solutions.[1] In other methods,[2,3] the 8-hydroxyquinoline is coupled with diazobenzenesulphonic acid to give an azo dye.

In the method to be described in this note, magnesium is precipitated from ammoniacal solution with 8-hydroxyquinoline. The precipitate is washed and redissolved in dilute acid, and the 8-hydroxyquinoline is determined colorimetrically by means of the orange-red colour that it gives when it is coupled with 4-aminophenazone, and the complex is oxidised with potassium ferricyanide in alkaline solution. This is a sensitive reaction for 8-hydroxyquinoline. It was first proposed by Emmerson[4] for the detection of phenols, and it has been applied to the colorimetric determination of phenols[5,6] and phenolic fungicides.[6]

METHOD

REAGENTS—

8-Hydroxyquinoline—A 5 per cent. solution in 2 *N* acetic acid. The solution is stable for at least a month.

Ammonium hydroxide, approximately 10 N—Dilute 50 ml of ammonium hydroxide, sp.gr. 0·880, to 100 ml with water.

Ammonium hydroxide, approximately 0·8 N—Dilute 4 ml of ammonium hydroxide, sp.gr. 0·880, to 100 ml with water. It is preferable to saturate this solution with the magnesium - 8-hydroxyquinoline complex.

4-Aminophenazone hydrochloride—An 0·5 per cent. solution of the compound in distilled water. At this concentration some of the reagent remains undissolved, but this does not affect the determination.

Potassium ferricyanide—A 10 per cent. aqueous solution. Store the solution in a dark bottle, when it will keep for about a week.

Sodium carbonate, 5 per cent. solution—A 5 per cent. solution of anhydrous sodium carbonate in water.

Hydrochloric acid, 0·1 N.

Sodium hydroxide, 0·1 N.

PROCEDURE—

The following precipitation method is slightly adapted from Hoffman.[1]

The sample should consist of up to 50 μg of magnesium in about 4 ml of solution. To the neutral or slightly acid solution add 0·2 ml of the 8-hydroxyquinoline solution, and heat it in a water-bath at 70° to 80° C until it reaches this temperature. Stir the mixture with a fine glass rod, add 10 *N* ammonium hydroxide until the solution is basic (usually about 3 drops are required) and then add a further 5 drops. Allow the mixture to stand for 1 hour at room temperature. Remove the precipitate by filtration on a micro sintered-glass filter (porosity 4). Wash out the precipitation vessel with three successive 1·0-ml portions of the 0·8 *N* ammonium hydroxide, and transfer the washings to the funnel.

When the precipitate has drained, dissolve it in 1 ml of hot (about 70° C) 0·1 *N* hydrochloric acid. Collect the acid solution in a clean dry vessel. Wash the filter with three 1-ml portions of hot distilled water, and then transfer the solution and washings quantitatively to a 50-ml calibrated flask. Add 1 ml of 0·1 *N* sodium hydroxide to the contents of the flask and then 5 ml of 5 per cent. sodium carbonate. Cool the solution and to it add 4 ml of 0·5 per cent. aminophenazone hydrochloride. Mix the contents of the flask, then add 1 ml of 10 per cent. potassium ferricyanide, make the solution up to 50 ml with distilled water and allow it to stand for 15 minutes. Measure the optical density with a Spekker absorptiometer making use of the 1-cm cell and a Chance OB1 or Ilford No. 602 spectrum blue filter. A blank determination should be carried out at the same time. The calibration curve is prepared by taking solutions containing 10, 20, 30 and 40 μg of magnesium, each solution being about 4 ml, through the whole procedure. There is a linear relationship between optical densities and these concentrations.

Results

Amounts of magnesium up to 50 μg in 4 ml of solution give recovery values in the region of 95 per cent. There is close agreement between the results for a series of magnesium standards and those for equivalent amounts of 8-hydroxyquinoline, as shown in Table I.

Table I

Comparison of the optical densities found for 8-hydroxyquinoline in the presence and absence of magnesium

Amount of 8-hydroxyquinoline, μg	Magnesium present, μg	Optical density
100	8·44	0·10
200	16·88	0·21
300	25·32	0·32
400	33·76	0·43
100	nil	0·09
200	nil	0·19
300	nil	0·29
400	nil	0·41

References

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R. A. McAllister
March 1st, 1954

THE IDENTIFICATION OF THE NITROPHENOLS AS THEIR ARYLOXYACETIC ACIDS

The conversion of compounds containing the phenolic group into the corresponding aryloxyacetic acids is an established method for the identification of these compounds.[1]

Koelsch[2] has examined the reaction for a number of phenols, and he developed a general method of preparing the derivatives. This method is, however, inapplicable to the nitrophenols. The method of Koelsch is based upon the methods of Jacobs and Heidelberger[3] and of Minton and Stephen[4]; these methods do satisfactorily give 2-, 3- and 4-nitrophenoxyacetic acids, but they are successful only on the macro scale and are quite unsuitable for the preparation of derivatives.

Kym[5] successfully condensed 2- and 4-nitrophenols, as their dry sodium salts, with ethyl chloroacetate at 180° C in sealed tubes, and the method has been applied to higher esters.[6] Hewitt, Johnson and Pope[7] prepared a variety of aryloxyacetic esters by reaction of the sodium phenoxides with ethyl chloroacetate in ethanol, the mixture being heated on a water-bath. It was found that cresols, halogenated phenols and 3-nitrophenol reacted, but these workers failed to condense 2- and 4-nitrophenols under these conditions, as had Auwers and Haymann[8] previously. On the basis of theoretical consideration, it is expected that 2- and 4-nitrophenoxides will be more stable than the parent phenoxide, but again the phenoxide structures are favoured instead of the possible quinoid forms. It therefore seemed that the failure to promote reaction with 2- and 4-nitrophenoxides was due to the temperature factor. This has now been demonstrated, and a general technique has been evolved for the rapid preparation of the nitrophenoxyacetic acids in effectively quantitative yields.

The sodium salt of the nitrophenol is prepared and is dissolved in triethylene glycol, then ethyl chloroacetate is added, and the mixture is heated at 180° to 200° C. A smooth but rapid reaction occurs, and the ester that is produced can be hydrolysed directly to the acid. The product, if prepared from pure materials, does not need further purification. Higher esters, such as *n*-propyl, *n*-butyl, *n*-pentyl and *n*-hexyl, can replace the ethyl ester; their only advantage is that, as they are less volatile, the reaction time is slightly less.

Method

Sodium (0·005 g-atom) is added to 2 ml of anhydrous methanol contained in a 5-ml flask fitted with a reflux condenser. The mixture is now warmed in an oil-bath to effect solution.

When the sodium has dissolved, the nitrophenol (0·005 mole) in 1 ml of methanol is added, and the apparatus then set for distillation. The temperature of the oil-bath is raised to and maintained at 100° to 140° C until all the methanol has been removed. Three millilitres of triethylene glycol are added, and the heating is continued until all the solid sodium salt has passed into solution. A slight excess of ethyl chloroacetate (or a higher ester) is now added and the mixture is heated at 180° to 200° C for 30 minutes. Finally, the hot mixture is poured into 20 ml of 10 per cent. w/w sulphuric acid contained in a 50-ml flask, and this is heated under a reflux condenser. The disappearance of the oily ester, which is precipitated on pouring into acid, indicates the completion of the hydrolysis. The clear solution is cooled, and the pure crystalline acid is collected, washed with cold water and dried.

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THE TECHNICAL COLLEGE
BLACKBURN

J. GRUNDY
March 24th, 1954

Apparatus

AN ABSORBER FOR MISTS AND GASES IN AIR

The apparatus consists of a cylindrical glass vessel, as shown in Fig. 1, fitted with two sintered-glass discs, one disc close to the bottom and the other near the top. A stoppered filling tube is

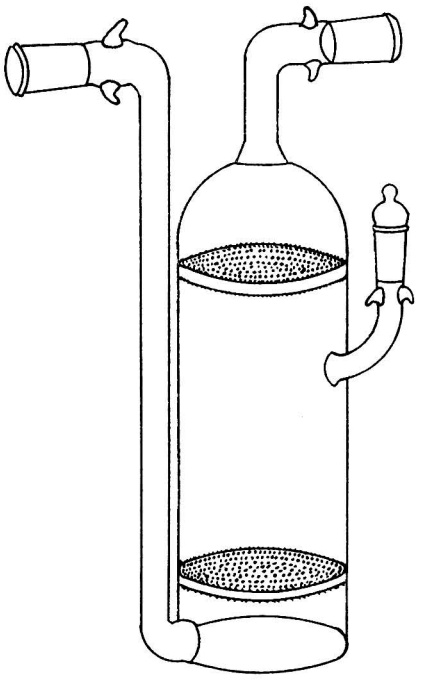

Fig. 1. Absorber

inserted just below the upper disc and offset from the gas exit tube for convenience in filling. In use, enough absorbent is poured in to give a layer about 1 inch deep above the lower disc. The sample is drawn in at the bottom and through the lower disc. The upper disc serves as an impinger and also catches any spray formed by the bubbles from the lower disc.

In this laboratory the absorber has been used successfully during the past five years for mists

that are difficult to absorb. For example, it has been used for sampling the air over chromium-plating baths and for absorbing traces of hydrofluoric acid in air.

The apparatus in use has discs 6 cm in diameter, the lower of porosity B.S. grade II and the upper of porosity B.S. grade I.

The results of tests when two absorbers were used in series and air was drawn in at a rate of flow of 2 litres per minute were as follows—

Air containing chromic acid spray: first absorber collected 17·0 mg and the second absorber collected 0·001 mg.

Air containing hydrogen fluoride: first absorber collected 14·4 mg and the second absorber collected 0·04 mg.

The maximum flow rate is about 5 litres per minute, above which channelling causes loss of efficiency.

I wish to thank the Government Chemist for permission to publish this note.

GOVERNMENT LABORATORY
CLEMENT'S INN PASSAGE
STRAND, LONDON, W.C.2

A. A. W. RUSSELL
January 13th, 1954

Ministry of Food

LIST OF CURRENT STATUTORY INSTRUMENTS AND STATUTORY RULES AND ORDERS RELATING TO FOOD

The List of Current Statutory Instruments and Statutory Rules and Orders, Sectional List No. 33, has been revised to March 31st, 1954, and can be obtained from H.M. Stationery Office at cost of postage. See Analyst, 1954, 79, 111.

British Standards Institution

NEW SPECIFICATIONS*

B.S. 458:1953. Xyloles. Price 7s. 6d.
B.S. 2069:1954. Gas Sampling Tubes. Price 2s. 6d.
B.S. 2461:1954. Gas Washing Bottles. Price 2s. 6d.

Book Reviews

APPROVED METHODS FOR THE PHYSICAL AND CHEMICAL EXAMINATION OF WATER. Second Edition. Pp. ii + 59. London: The Institution of Water Engineers. 1953. Price 7s.

The first edition of this collection of tested and approved methods for the examination of potable and raw waters was a paper-covered reprint from the October, 1949, issue of *The Journal of the Institution of Water Engineers*; the demand for it has supplied sufficient justification for the publication of this, the revised and enlarged second edition, in a more permanent form, as a strongly bound cloth-covered book. It is the work of a Joint Committee of five members, representing the Institution of Water Engineers (the convening body), the Institute of Chemistry and The Society for Analytical Chemistry, appointed for the purpose of recommending methods for the analysis and reporting of water samples that could be adopted in waterworks laboratories and by consultants as standard practice.

The Joint Committee have carried out the terms of reference with usefulness to their fellow analysts and with credit to themselves. It is to be hoped that water analysts everywhere will reward their labours by adopting their recommendations forthwith.

The need for standardising empirical methods, such as those for determining organic nitrogen, oxygen absorbed from permanganate and the residual chlorine in treated waters, is self-evident; that uniformity in the presentation of results is desirable, all who have ever had the task of correlating the reports from different laboratories to make them intelligible to those without special technical knowledge will readily agree.

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

Comparison of this book with the original text shows that revision has been thorough; almost every page shows evidence of the care that has been taken to ensure accuracy, clarity and freedom from ambiguity. Typographical imperfections are but few, and such as occur will not cause any trouble to a chemist.

Some of the methods approved and described are of the old and well-tried kind that, after prolonged and heated controversy, to which the early volumes of *The Analyst* bear witness, won general acceptance nearly eighty years ago; others, of more recent introduction, have still to undergo a further period of trial and probation before they can be said to have attained the status of standard methods.

In addition to the time-honoured methods for the determination of the free and albuminoid ammonia, chlorine, total solids, nitrates and nitrites, and so on, methods are included for metallic impurities, phenols, thiocyanates and cyanogen.

Of special interest is the inclusion of Palin's ferrous ammonium sulphate method for determining free chlorine, chloramines and nitrogen trichloride.

Amongst the newer methods described, an important advance in analytical technique has received approval by the inclusion of Schwarzenbach's ethylenediaminetetra-acetate reagent for the determination of total hardness and calcium; this now replaces the palmitate method for hardness, with its somewhat insensitive and uncertain end-point.

The words "Physical and Chemical" in the title and the warning in the text about the importance of a bacteriological in addition to a chemical examination seem to imply the prospect of a companion volume on the bacteriological examination of water. If this is so, its publication will be eagerly awaited by users of the present volume; if not, its production is recommended for consideration by the Joint Committee responsible for this, the chemical, side of the subject.

Although the five members of the Committee responsible for the selection of these recommended methods were appointed as representatives of three independent bodies, it is interesting to note that they are also all members of the Society for Analytical Chemistry. This is as it should be and is in keeping with tradition; for in the list of the founder members of the old Society of Public Analysts, dated 1874, are to be seen the names of at least three chemists to whose early work on water the present methods owe so much. Chief among these names is that of J. Alfred Wanklyn, the originator of the alkaline permanganate method for the determination of albuminoid ammonia. The first volume of *The Analyst* contains a notice of Wanklyn's book on water analysis, in which the reviewer uses words that cannot be bettered in recommending to all interested in water analysis this new and important collection of approved methods—"We can confidently recommend the little book to all requiring a guide to the analysis of water." F. L. OKELL

PHARMACOLOGY. By J. H. GADDUM, Sc.D., F.R.S., M.R.C.S., L.R.C.P. Fourth Edition. Pp. xviii + 562. London, New York and Toronto: Geoffrey Cumberlege, Oxford University Press. 1953. Price 35s.

It is a little over thirteen years since Gaddum's "Pharmacology" was originally published and a little less than thirteen years since its first edition was reviewed in *The Analyst* (1941, **66**, 511). This fourth edition bears many indications of change, as witness the increase in length from 383 to 516 pages of text and from 23 to 35 pages of index.

Much chemotherapeutic water has indeed passed under the bridges since those early days of World War II, when the discovery of penicillin just failed to get mentioned in Gaddum's first edition (and the word antibiotic had not even been invented), when the "anti-pernicious anaemia factor" was still the object of baffled pursuit on two continents, when a single page on "Cortin activity" dealt in this book simply with extracts of adrenal cortex.

In this edition all the antibiotics currently available in Great Britain are discussed (but why aureomycin instead of chlortetracycline, when oxytetracycline is given as the "official" name of terramycin?): the cobalamins are described—but in the chapter on "Blood," not in the one covering the vitamins: and there is an account of six different specific corticosteroids. Examples of changes could be multiplied *ad nauseam*, although the short section on emetics seems to have remained unaltered (in Chapter XII, "The Alimentary Canal")!

Another useful innovation is an alphabetical "Key to the Interpretation of Chemical Names," accompanied by a figure showing some of the main organic ring structures. This is evidently intended to help the medical student, to whom the book is primarily directed, which is doubtless why it has a strong practical emphasis, being more concerned with therapeutics, including chemotherapy, than with research or academic pharmacology. The very fact makes it peculiarly useful to the chemical analyst, who, without any formal training in pharmacology, may find that his work

takes him into the fascinating field of drug action, including that of biological assay. Nothing could be clearer than Professor Gaddum's writing (occasionally there is a distinctly dry humorous flavour to it): he has the ability to make the subject seem easy—perhaps almost too easy—to the novice, although he has clearly taken much trouble to avoid sacrificing accuracy to simplicity.

Although he has been at pains to correct any errors in the first edition pointed out by reviewers or by colleagues, one still wonders on what basis he has chosen to give some proprietary names of some drugs, and not those of others—as, for instance, when describing the organic iodine compounds used in radiography, he seems positively to prefer the foreign trade names to the British ones. It would have been better to omit all except the Pharmacopoeial or other official designations, possibly listing the proprietary names in an appendix, though this would admittedly have been prodigal of space. The information is, after all, to be found in "Martindale," to which a reference is, in fact, given, but its wealth of information on this particular matter is not mentioned.

In one or two directions Professor Gaddum does not seem to have thought it necessary to bring his text up to date. Thus, on the very first page, the list of "essential" amino-acids needs revision, neither histidine nor arginine being any longer so regarded. Again, his account of the blood coagulation mechanism is not consonant with modern knowledge: the facile four-factor scheme, no longer acceptable, has apparently been transferred through all editions to date with trifling changes of detail and none of substance.

Revision has, however, led to a number of drastic changes. Thus the chapter (VII) now called "The Brain" (next to Chapter VI, on "Narcotics") was formerly "Other Depressants of the Nervous System," although it covers much the same ground. Of particular interest to analysts will be the change in scope and title of the chapter on "Heavy Metals and Metalloids," now called "Toxic Elements" (XVIII): those formerly and those now discussed make an interesting comparison. Copper and zinc have been removed, the latter now appearing under "Diet: Inorganic Salts and Fats" (Chapter I) and the former in Chapter XIV on "Blood." On the other hand, the toxic elements considered now include gold, beryllium, molybdenum and selenium—as well as the ten elements covered in the first edition; the chapter also contains a section on dimercaprol. It looks, incidentally, as if molybdenum also would next have to be moved for some more "physiological" consideration. But Professor Gaddum is doubtless prepared for this and other changes in the many future editions that must surely lie in store for his book: he knows full well that the price of sound scientific tutelage is, like that of liberty, eternal vigilance.

A. L. BACHARACH

METHODS FOR EMISSION SPECTROCHEMICAL ANALYSIS. Sponsored by A.S.T.M. Committee E-2 on Emission Spectroscopy. Pp. viii + 309. Philadelphia, Pa.: American Society for Testing Materials. 1953. Price (cloth) $5.15; (paper) $4.50.

For many years the American Society for Testing Materials has given strong support to the improvement of spectrographic methods of analysis through the activities of Committee E-2 and its various Sub-Committees. As a step towards the preparation of new A.S.T.M. tentative methods, the Committee has reviewed many spectrographic procedures representing current laboratory practice. An extensive collection of more than fifty of these suggested methods has now been published, together with four spectrochemical methods that have achieved the status of tentative methods.

Although the suggested methods originate in a large number of different laboratories, the individual authors being named, severe editing has brought all the material into a standard form, which makes comparison between methods extremely simple. Lay-out and numbering of subsections is to a consistent plan throughout the book, so that sections 4.3, 4.4 and 4.5, for example, always relate to electrode system, excitation conditions and exposure conditions, respectively.

About three-quarters of the methods are devoted to emission spectrochemical analysis of metals; the remainder includes cement, slags, solutions and chemical reagents. The book also gives chapters on suggested practices in installation and safe operation, photographic processing, photographic photometry and nomenclature. The volume is well indexed and will certainly be welcomed by all spectroscopists whose practice includes a miscellany of inorganic samples.

Comments are solicited on all the suggested methods included in this collection, and it will be some return to the A.S.T.M. for its liberal publication policy if readers will provide suggestions and criticism that will help in establishing tentative standard methods from these suggested procedures.

B. S. COOPER

Publications Received

MINERALS FOR THE CHEMICAL AND ALLIED INDUSTRIES. By S. J. JOHNSTONE, O.B.E., B.Sc., F.R.I.C., M.I.M.M. Pp. 692. London: Chapman & Hall Ltd. 1954. Price 75s.

ORGANIC CHEMISTRY. By R. C. FUSON and H. R. SNYDER. Second Edition. Pp. viii + 544. London: Chapman & Hall Ltd.; New York: John Wiley & Sons Inc. 1954. Price 52s.

SPOT TESTS. Volume II. ORGANIC APPLICATIONS. By F. FEIGL, Eng., D.Sc. Translated by R. E. OESPER, Ph.D. Fourth Edition. Pp. xvi + 436. Amsterdam and New York: Elsevier Publishing Co. Ltd.; London: Cleaver-Hume Press Ltd. 1954. Price 37s. 6d.

VEGETABLE FATS AND OILS. By E. W. ECKEY. Pp. x + 836. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1954. Price 132s.

QUALITATIVE INORGANIC ANALYSIS. A NEW PHYSICO-CHEMICAL APPROACH. By G. CHARLOT. Translated by R. C. MURRAY, Ph.D. Pp. xii + 354. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1954. Price 42s.

MICROBIOLOGY. AN INTRODUCTION. By E. GRAY, M.Sc., M.R.C.V.S. Pp. xii + 175. London: Crosby Lockwood & Son Ltd. 1954. Price 10s. 6d.

MANURES AND FERTILISERS. POTENTIAL PROGRESS IN EUROPE. Pp. 120. Paris: The Organisation for European Economic Co-operation. 1954.

THE INFRA-RED SPECTRA OF COMPLEX MOLECULES. By L. J. BELLAMY, B.Sc., Ph.D. Pp. xviii + 323. London: Methuen & Co. Ltd.; New York: John Wiley & Sons Inc. 1954. Price 35s.

HANDBOOK OF COSMETIC MATERIALS. By L. A. GREENBERG, Ph.D., and D. LESTER, Ph.D. Pp. xii + 455. New York and London: Interscience Publishers Inc. 1954. Price $12.50; 95s.

TRAITÉ DE MICRO-ANALYSE MINÉRALE. QUALITATIVE ET QUANTITATIVE. Volume I. By C. DUVAL. Pp. 552. Paris: Presses Scientifiques Internationales. 1954.

THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Fourth Edition. Volume XI. SOIL, ORGANIC CONSTITUENTS OF—ZYMURGY. Edited by M. A. WHITELEY, O.B.E., D.Sc., F.R.I.C. Pp. x + 1145. London and New York: Longmans, Green & Co. Ltd. 1954. Price 140s.

PAPER CHROMATOGRAPHY. By Dr. F. CRAMER. Second Edition translated by L. RICHARDS, B.Sc. Pp. xii + 124. London: Macmillan & Co. Ltd.; New York: St. Martin's Press Inc. 1954. Price 25s.

Errata

FEBRUARY (1954) ISSUE, p. 110, key at top of Fig. 1. *For* "Extracted liquid" *read* "Extracting liquid" *and vice versa.*

APRIL (1954) ISSUE, p. 221, 2nd line of *Ammonium hydroxide – ammonium chloride buffer solution* (*pH* 10) in list of reagents. *After* "sp.gr. 0·880" *add* "and make up to 1 litre with water."

JUNE (1954) ISSUE, p. 346. The first 4 lines under Table I were inserted in error and should be deleted.

IBID., p. 347. The heading at the top of the page should read "METHOD FOR THE DETERMINATION OF TANTALUM."

IBID., p. 348. *For* "METHOD FOR THE DETERMINATION OF TANTALUM" *read* "METHOD FOR THE DETERMINATION OF NIOBIUM."

PORTRAITS OF PAST PRESIDENTS

A FURTHER photogravure portrait in the series of Portraits of Past Presidents, that of Mr. S. E. Melling (President: 1943–44), is in preparation. Members of the Society and subscribers to *The Analyst* who wish to receive a gratis copy of this portrait should apply to the Editor, *The Analyst*, 7–8, Idol Lane, London, E.C.3, before September 30th, 1954.

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