THE ANALYST

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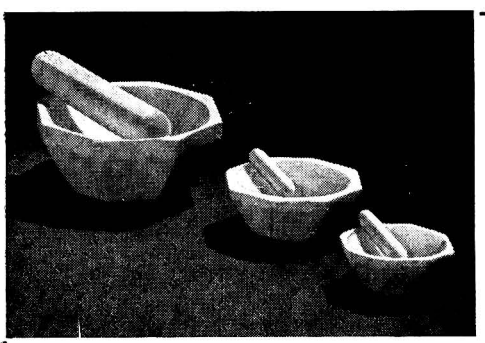

A group of Alumina 609 Mortars and Pestles

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

HONORARY ASSISTANT SECRETARYSHIP

THE Council has appointed Mr. N. L. Allport, Member of Council, to be Honorary Assistant Secretary of the Society. In this capacity, Mr. Allport will act as a link between the Council of the Society and the Committees of the Sections and Groups.

SECRETARYSHIP OF THE SOCIETY

WE have pleasure in announcing the marriage of Miss D. V. Wilson, Secretary of the Society, to Mr. J. P. Hicks.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held at 2 p.m. on Saturday, May 2nd, 1953, at the City Laboratories, Mount Pleasant, Liverpool, 3. Mr. A. A. D. Comrie, B.Sc., F.R.I.C., presided over an attendance of fifty-eight.

Mr. W. Gordon Carey, F.R.I.C., and Mr. J. G. Sherratt, B.Sc., F.R.I.C., introduced a comprehensive discussion on "The Analysis of Waters, Sewages and Effluents" to which a large number of members contributed.

THE Sixteenth Summer Meeting of the Section was held at the Imperial Hotel, Llandudno, from Friday, June 12th, to Monday, June 15th, 1953.

The Vice-Chairman, Mr. J. R. Walmsley, A.M.C.T., F.R.I.C., Ph.C., presided and thirty-eight members attended, including the President, Dr. D. W. Kent-Jones, F.R.I.C., and Mrs. Kent-Jones. On the morning of Saturday, June 13th, a paper was presented by Mr. C. A. Adams, C.B.E., B.Sc., F.R.I.C., on "Random Reflections on Food Legislation" (see below). A tour of the district was made by motor coach on the Sunday afternoon.

SOME RANDOM REFLECTIONS ON FOOD LEGISLATION

Mr. Adams said that the new Food and Drugs Bill now being drafted for presentation to Parliament was a matter of outstanding importance to Public Analysts and chemists engaged in food manufacture, but it was also of importance to the general public, as buyers and consumers of food. On the interest shown by the general public much of the success of the Bill would depend, and it was to be hoped that members of the Society and the general public would do their utmost to stimulate this interest—for legislation seldom ran ahead of public opinion.

The outstanding interest in the new Bill would be in the powers sought to transfer temporary war-time legislation into permanent form and in any additional powers that might be necessary to deal with the addition of chemicals to food, whether intentional in the course of manufacture, or adventitious in the form of pesticide residues or in any other way. These matters had recently been the subject of official enquiry in the United States of America. They had also been dealt with at length by Dr. J. R. Nicholls in his recent Presidential address, and were summed up from the official angle in the Annual Report of the Advisory Council on Scientific Policy for 1950–51 to the Lord President of the Council.

Mr. Adams said that in his view the main value of a change in the law would be in relation to the use of chemicals *newly* introduced into the food industry.

If the Orders under the Defence (Sale of Food) Regulations, 1943, were included in the new Bill, it would make them unalterable without appeal to Parliament. Instead, fuller powers to make such Orders would be sought in the Bill, and the Orders could then be re-enacted. Amongst these Orders the speaker allotted pride of place to the Labelling of Food Orders and the Food Standards Orders. All these Orders had been enforced by the Food and Drugs Authorities as though they were part of the Food and Drugs Act.

As the result of the experience in the working of these Authorities and food manufacturers, he was of the opinion that we had seen some welcome progress in what might be called the "co-operative administration of food legislation."

If the range of the Food and Drugs Act was to be extended to cope with problems arising from the use of chemicals, pesticide residues and the sale of sub-standard products, now was the time to make recommendations. Once the Bill became an Act, further changes might be long delayed.

Obituary

JOHN ROBERT STUBBS

JOHN ROBERT STUBBS, M.Sc., F.R.I.C., who passed away on April 17th, 1953, in his 73rd year, was educated at Warton Schools, Winsford and Witton Grammar School, Northwich. From Witton he proceeded by scholarship to Liverpool University, where he obtained his B.Sc. degree in 1900 and his M.Sc. degree in 1903.

He became an assistant in the laboratory of the late Dr. Campbell Brown, then Public Analyst for the County of Lancaster, in 1901. Under a succession of County Analysts, he continued to serve faithfully the Lancashire County Council and was eventually himself appointed County Analyst and Official Agricultural Analyst in 1938—a promotion he richly merited.

He was a most conscientious and painstaking analyst and these qualities are well exemplified in the many papers, some in collaboration with the late Dr. Elsdon, that he contributed to *The Analyst*.

He will always be remembered for his fundamental work in connection with the determination of extraneous water in milk by means of the freezing-point, work that the writer has good reason to know involved considerable physical effort and concentration in the early stages. The pages of *The Analyst* also contain examples of his researches in other fields of analytical work.

During the first world war he served in the R.A.O.C. for three years in charge of one of the two Schools of Ammunition in France, returning with the rank of Captain.

In 1929 he became Honorary Secretary of the North of England Section of the Society, the Section being then in its infancy. He continued in that office until 1940, when he was appointed Chairman, a position he occupied for three years. Both the Section and the Society must be ever deeply indebted to him for the wholehearted and splendid service he rendered during this period of 14 years. This service included the initiation and organisation of the highly successful pre-war Summer Meetings of the North of England Section of the Society, which began at Scarborough in 1930 and have contributed, by their sociable nature, so much to the personal friendships of professional colleagues.

Although what were known, in the County Laboratory, as "ersatz" Summer Meetings were held in Manchester during the late war, it is a matter for regret that Stubbs' retirement from the Chairmanship prevented him from presiding at a normal Summer Meeting, to the success of which he had so largely contributed.

He was laid to rest at Weaver Cemetery on April 22nd, 1953. The Society was represented by the writer.

ARNOLD LEES

A Reversion Method for the Absorptiometric Determination of Traces of Lead with Dithizone

By H. M. IRVING and E. J. BUTLER

(*Presented at the meeting of the Society on Wednesday, April 1st, 1953*)

A method is described for the determination of lead in small samples of biological materials. The procedure involves wet ashing of the sample, extraction of all the lead (and any bismuth present) with dithizone, a preliminary separation from the bulk of the bismuth, and a final absorptiometric determination of lead by the "reversion" technique.

Quantities of lead in the range 0 to 2 μg can be determined satisfactorily in the presence of up to 20 μg of bismuth and at least 100 μg of copper and zinc.

Although it seems to have no particular function, lead is invariably present in traces in most fluids and tissues as an "inevitable consequence of life on a lead-bearing planet."[1] In slightly larger amounts it is toxic, perhaps by competing with essential trace metals for the metal-binding components of enzyme systems. It has been suggested that lead may be a factor in the etiology of certain degenerative diseases of the nervous system, such as disseminated sclerosis,[2,3] and in the course of investigating this hypothesis the need was felt for a more sensitive, specific and convenient analytical method than any that was already available, particularly for samples that are necessarily limited in quantity, such as cerebrospinal fluid, specialised neurological tissue and biopsy specimens.

The use of dithizone for the absorptiometric determination of lead has been extensively studied, and even in the presence of other metals, *e.g.*, zinc, copper, iron and so on, the procedure can be made almost specific if the dithizone complex is extracted from an alkaline citrate buffer containing cyanide. Bismuth, tin[11] and thallium are to some extent co-extracted and estimated together with the lead.[4,5]

If a one-colour procedure is used for the subsequent absorptiometric determination of the red coloured lead - dithizone complex, $PbDz_2$,[6] for which $\epsilon = 68{,}600$ in carbon tetrachloride at $\lambda_{max.}$ of 520 mμ,[7] errors may arise if the "stripping" of excess dithizone (which absorbs appreciably at this wavelength) is incomplete, or is carried out so efficiently as to cause partial decomposition of the lead dithizonate.[8] The same criticisms apply to procedures in which the absorptiometric measurement is actually made on the equivalent amount of dithizone liberated from this stripped lead dithizonate by treatment with dilute acid.[9,10] If, however, the determination of lead is made to depend upon measurements of the absorbancy of lead dithizonate at 520 mμ, or on that of unreacted dithizone, a known excess amount having been added originally, the initial concentration of this dithizone ($\epsilon = 34{,}600$ in carbon tetrachloride at $\lambda_{max.}$ 620 mμ[7]), the pH of extraction and the phase ratio, which determine the extent of its partition into the aqueous phase, must be rigorously controlled. The difficulty of preserving solutions of dithizone of constant strength is well known, and although such variations in concentration are of no importance in the procedure described by Kozelka and Kluchesky,[11] in which absorbancies are measured at two selected wavelengths, an enormous amount of work is involved in preparing the necessary families of calibration curves. Moreover, these curves (like those for the other procedures mentioned above) would be invalidated by the co-extraction of other metal dithizonates or other coloured materials from the biological digest; the latter often appear on basification when nitric acid has been used to oxidise samples containing much fat.

Most of these objectionable features are removed by the process of "reversion."[6] If A_m is the absorbancy measured in a 1-cm cell at any wavelength of a mixed-colour extract containing molecular concentrations C_c of lead dithizonate, $PbDz_2$, and C_r of excess dithizone, HDz, and C_i of coloured impurities, of which the molecular extinction coefficients are ϵ_c, ϵ_r and ϵ_i, respectively, we have—

$$A_m = \epsilon_r C_r + \epsilon_c C_c + \Sigma \epsilon_i C_i \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

If now a part or all of this organic phase is shaken with an aqueous reagent that decomposes only the lead dithizonate, permitting a return of lead ions to the aqueous phase while an equivalent amount, $2C_c$, of dithizone appears in the organic layer (reversion), the absorbancy measured at the same wavelength will increase to a new value, A_r, given by—

$$A_r = \epsilon_r C_r + 2\epsilon_r C_c + \Sigma \epsilon_i C_i \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The increase in absorbancy, termed the reversion value, R, is then given by—

$$R = A_r - A_m = (2\epsilon_r - \epsilon_c)C_c \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The concentration of lead is thus proportional to the reversion value, and the sensitivity of the method will be greatest at that wavelength where $(2\epsilon_r - \epsilon_c)$ is greatest; for the lead - dithizone system 620 mμ is appropriate.

Although significant amounts of thallium are not normally present in biological fluids and tissues, bismuth is a common constituent of medicinal preparations, some of which may be prescribed for the treatment of disseminated sclerosis, and interference from this element may be serious in the analysis of specimens from diseased subjects. When determined under equilibrium conditions, the curve relating the percentage extraction of bismuth to the pH value of the aqueous phase lies to the left of that determined for lead under comparable conditions of reagent concentration and buffer composition, *i.e.*, its extraction is quantitative at a lower pH value. As the concentration of the dithizone solution is increased, the separation of these curves increases, since the extractability of lead depends on the square, whilst that of bismuth depends on the cube of the excess dithizone concentration.[12,13] Various workers have attempted to separate bismuth from lead by carrying out extractions from acid media (in the pH range of 2 to 4), when bismuth is preferentially extracted,[4,12] but inevitably some lead is co-extracted and lost. If, however, extraction is made from an alkaline citrate - cyanide buffer under optimum conditions for the removal of lead, all of the bismuth is co-extracted. Although treatment of the organic phase with a strongly acid solution restores both lead and bismuth to the aqueous phase, Bambach and Burkey[14] have shown that lead is preferentially reverted by a phthalate buffer at a pH of 3·4, and Kozelka and Kluchesky,[11] working with much stronger solutions of dithizone, advocate nitric acid at a pH of 2·3 to 2·5. Table I shows the extent of reversion of bismuth on shaking a solution of metal dithizonate in carbon tetrachloride (initial concentration of dithizone 12·5 mg per litre) with various reagent solutions (phase ratio 3 to 4) for different lengths of time.

TABLE I

REVERSION OF BISMUTH

Bismuth taken, μg	Lead taken, μg	Reversion mixture	Time, minutes	"Lead" found, μg
2·0	0·0	5 *N* sulphuric acid	10	2·71
2·0	0·0	3·5 per cent. sodium acetate - sulphuric acid buffer of pH 3·4	10	1·71
2·0	0·0	0·03 per cent. v/v nitric acid of pH 2·40	1	0·33
1·0	0·0	"	1	0·00
1·0	1·0	"	1	1·00

It can be seen that the reversion of 1 μg of lead is quantitative on shaking with dilute nitric acid of pH 2·4 for 1 minute, and that, although 1 μg of bismuth does not interfere, 2 μg is reverted to the extent of about 12 per cent. In the procedure described below lead is first removed completely from the biological digest by successive extractions with portions of dithizone (25 mg per litre in carbon tetrachloride), and a preliminary separation from bismuth is achieved by reversion with dilute nitric acid (pH 2·4). The small amount of bismuth remaining in this acid extract does not affect the precision of the subsequent determination of lead, which is carried out by extracting from an alkaline citrate - cyanide buffer under standard conditions with a more dilute solution of dithizone and subsequently determining the reversion value under standard conditions (see Tables III and IV). Tin does not interfere as it is oxidised to the stannic state during digestion.

METHOD

REAGENTS—

All reagents should be of recognised analytical purity.

Metal-free water—Redistil laboratory distilled water from an all-glass Pyrex still.

Carbon tetrachloride—Redistil reagent grade carbon tetrachloride in all-glass Pyrex apparatus.

Concentrated sulphuric and nitric acids—Redistil these acids in all-glass Pyrex apparatus under reduced pressure.

Perchloric acid—A 70 per cent. w/v solution redistilled in all-glass Pyrex apparatus under reduced pressure.

Hydrochloric acid—A redistilled constant-boiling mixture.

Nitric acid, 0·03, 1 and 2 per cent. v/v solutions—Dilute 0·3, 10 or 20 ml of the redistilled concentrated acid to 1 litre with metal-free water.

Ammonium hydroxide, approximately 4 N—Absorb the ammonia given off by heating reagent grade ammonium hydroxide, sp.gr. 0·880, in metal-free water cooled in an ice - salt mixture. All-glass Pyrex apparatus must be used.

Ammoniacal citrate solution A—A 50 per cent. w/v monohydric citric acid solution. Dissolve 500 g of citric acid monohydrate in approximately 500 ml of metal-free water and add about 1 ml of phenol red indicator (0·4 per cent. w/v). Add portions of ammonium hydroxide, sp.gr. 0·880, with continuous stirring until the colour of the indicator changes to red (pH about 8·5). Allow the solution to cool after each addition and finally make up the volume to 1 litre with metal-free water.

Purify the solution by shaking it with portions of a solution of dithizone in redistilled carbon tetrachloride (approximately 50 mg per litre) until two successive extracts show the unchanged green colour of the reagent. Shake the citrate solution with two small portions of any redistilled carbon tetrachloride to extract any dissolved dithizone.

This procedure was adopted since similar solutions prepared from analytical grades of ammonium citrate were found to be highly contaminated by traces of heavy metals.

Ammoniacal citrate solution B—A 20 per cent. w/v solution of monohydric citric acid. Dilute 400 ml of solution A to 1 litre with metal-free water.

Potassium cyanide solution, 5 per cent. w/v—Dissolve 50 g of potassium cyanide in about 100 ml of metal-free water and purify the solution as described for ammoniacal citrate solution A. Finally make the solution up to 1 litre with metal-free water.

Hydroxylamine hydrochloride solution, 20 per cent. w/v—Dissolve 200 g of hydroxylamine hydrochloride in about 600 ml of metal-free water and neutralise the solution with ammonium hydroxide, sp.gr. 0·880. Purify as described for ammoniacal citrate solution A and make up to 1 litre with metal-free water.

Dithizone solutions—Prepare a stock solution by dissolving 100 mg of good quality commercial dithizone reagent in 1 litre of redistilled carbon tetrachloride and dilute as required. Use Pyrex bottles covered with black paper and keep in a cold store.

Standard lead solutions—Dissolve lead nitrate in 1 per cent. v/v nitric acid to give a solution containing 1 mg of lead per ml. Dilute this stock solution with 1 per cent. v/v nitric acid to give a standard solution containing 1 or 2 μg of lead per ml. This shows no appreciable change in concentration over six months.

APPARATUS—

Corks and rubber bungs must not be used. All-glass apparatus must be made of Pyrex glass and it should be reserved for the estimation of lead or other trace metals. It must be specially cleaned with strong sodium hydroxide solution (about 50 per cent.), concentrated nitric acid (boiling where possible) and then rinsed thoroughly with distilled and metal-free water. Separating funnels should be washed with 50 per cent. nitric acid and then rinsed with water before use. The efficiency of the cleaning process should always be tested with a drop of dilute dithizone solution. Stainless steel instruments should be boiled with dilute acetic acid and then rinsed with water as above.

The tops of reagent bottles and standard flasks should be covered with small beakers or boiling tubes to exclude dust.

Pipettes may be stored in a covered 2-litre measuring cylinder and should be rinsed with metal-free water before and after use. Pyrex syringe-pipettes made from 10-ml pipettes

and discarded syringes with broken nozzles are convenient for transferring concentrated acids, ammonium hydroxide and potassium cyanide solution. The pipettes must not touch the bench.

Terry spring-clips are convenient for holding separating funnels on racks and on the mechanical shaker. The funnels should have short stems. White Vaseline petroleum jelly is a satisfactory lubricant for the taps.

The digestion of samples is best carried out in micro-Kjeldahl-type tubes of about 30-ml capacity on an electrically-heated rack with a rheostat control.

COLLECTION OF SPECIMENS—

Collect urine directly in a 2- or 3-litre bottle with the aid of a funnel if necessary. Dissolve any solids by adding hydrochloric acid. Take blood by veni-puncture with an ungreased Pyrex syringe and a stainless steel needle, and expel the blood into a tube provided with a stopper and containing a small amount of purified solid ammonium citrate as an anti-coagulant. Collect cerebrospinal fluid through a stainless steel needle in a tube provided with a stopper. Cover the tops of bottles and tubes with filter-paper secured with elastic bands when not in actual use, to avoid contamination from dust.

Take a large specimen of soft tissue in the normal way and then remove samples from the interior with specially cleaned stainless steel or glass instruments. Dry to constant weight in covered dishes by vacuum desiccation and heating at 110° C.

PROCEDURE—

Digestion—Heat the sample (20 ml of urine, 5 ml of blood, 5 to 10 ml of cerebrospinal fluid, or 100 to 500 mg of dry soft tissue) with 1·0 ml of concentrated sulphuric acid, 2·0 ml of concentrated nitric acid and 0·5 ml of 70 per cent. w/v perchloric acid until a colourless solution remains. Add further portions of nitric acid if necessary. Include a small piece of Pyrex glass to promote even boiling. Heat gently at first to avoid excessive frothing and finally raise the temperature until fumes of sulphuric acid appear.

Dissolve dry bone (about 1 g) in 10 ml of 50 per cent. v/v nitric acid with gentle heating. Take an aliquot containing 50 to 100 mg of bone and digest with 1·0 ml of concentrated nitric acid and 1·0 ml of constant-boiling hydrochloric acid solution until the nitric acid has distilled away.

Preliminary extraction—When the solution has cooled, add the appropriate volume of ammoniacal citrate solution A (containing phenol red): 5 ml for blood or soft tissue and 10 ml for urine or bone. Then add ammonium hydroxide until the colour of the indicator changes to red (pH about 8·5) and dissolve any solids by gentle heating.

Allow the solution to cool, re-adjust the pH with ammonium hydroxide if necessary, and transfer to a 60-ml separating funnel. Wash the digestion tube three times with metal-free water to make the total volume about 40 ml. Test the completeness of the transference by swirling a drop of dilute dithizone solution in the digestion tube; repeat the washing if this solution does not remain green. Add the washings to the contents of the separating funnel.

Add 3·0 ml of 5 per cent. w/v potassium cyanide solution and, for blood and liver, 1·0 ml of 20 per cent. w/v hydroxylamine hydrochloride solution. Dry the stem of the funnel with a roll of "ashless" filter-paper and plug it with a small piece of cotton wool freed from metals by dithizone extraction.

Extract the lead (plus bismuth) by shaking manually with 2-ml portions of a solution of dithizone in carbon tetrachloride (25 mg per litre) until two successive extracts show the unchanged green colour of the reagent. Note the volume of the dithizone solution required; this serves as an indication of the total amount of lead (plus bismuth) present. Four extractions are usually sufficient for a normal specimen. Run the organic extracts into a 20-ml separating funnel, having previously dried the stem of this funnel and the bore of the tap with a roll of filter-paper. Do not allow the aqueous phase to enter the bore of the tap of the 60-ml funnel and take care that no drops of the alkaline aqueous phase accompany the organic extracts, as they will raise the pH of the unbuffered reversion reagent and may lead to incomplete reversion of lead.

Shake the organic extracts mechanically for 1 minute (200 shakes) with 5 ml of 0·03 per cent. v/v nitric acid. Separate the organic phase carefully, without allowing the aqueous

phase to enter the bore of the tap, and discard it unless the bismuth content is to be determined. Take an appropriate aliquot of the aqueous phase for the determination of lead if it is judged that the total amount of the metal present will not lie on the linear portion of the calibration graph.

Determination of lead by reversion—Add 3 ml of a solution of dithizone in carbon tetrachloride at the appropriate concentration. This should be about 12·5 mg per litre ($A_o \simeq 0{\cdot}5$) if a Spekker absorptiometer model H546 is used to record absorbancy readings in the most sensitive region of the logarithmic scale. With the new version of this instrument (H760) the concentration is limited principally by the deviation from Beer's law, and it may be increased to about 20 mg per litre ($A_o \simeq 0{\cdot}7$). Then add 1·0 ml of ammoniacal citrate solution B, 1·5 ml of 5 per cent. w/v potassium cyanide solution and 2·5 ml of 2 per cent. v/v nitric acid solution and shake mechanically for 1 minute (200 shakes). The pH value of the aqueous phase should be between 8 and 9.

Dry the stem of the funnel if necessary and plug it with a small piece of purified cotton wool to prevent small droplets of the aqueous phase from being carried through into the optical cell (where they would make variable contributions to the absorbancy) or into the reversion funnel (where they may cause incomplete reversion of lead). Discarding the first few drops, which serve to wash out the stem, run about 1·5 ml of the mixed colour extract into a 10-ml separating funnel (with a dry, plugged stem) calibrated at 1·5 ml. Add 2 ml of 0·03 per cent. v/v nitric acid solution and shake mechanically for 1 minute (200 shakes).

Determine the absorbancy of the organic phases at 620 mμ before and after reversion (A_m and A_r, respectively), using a Spekker absorptiometer with 1-cm micro-cells of 0·5 ml capacity and the tungsten-filament lamp and Ilford No. 607 orange filter combination. Wash the cell with a small amount of the organic phase and cover with a glass slide to prevent evaporation. Calculate the reversion value, R, by subtracting A_m from A_r and correct this for the reversion value for the blank, which must be included with each batch of samples and treated in exactly the same way. Calculate the amount of lead present in the original sample by reference to a previously determined standard curve.

Prepare a standard curve by mixing x ml of a standard solution of lead in 1 per cent. v/v nitric acid (see below) with $(5 - x)$ ml of 1 per cent. v/v nitric acid solution. Add 1·5 ml of 5 per cent. w/v potassium cyanide solution, 1·0 ml of ammoniacal citrate solution B, 2·5 ml of metal-free water and 3 ml of dithizone solution (see below). Shake for 1 minute (200 shakes) and measure the absorbancy before and after reversion as described above (p. 574). If a model H546 Spekker absorptiometer is used, the concentration of the standard lead solution should be 1 μg per ml and that of the dithizone solution about 12·5 mg per litre ($A_o \simeq 0{\cdot}5$). The standard graph obtained was linear for up to 3·5 μg of lead and in this range the reversion value was 0·075 per μg of lead. With a model H760 Spekker absorptiometer, a standard lead solution containing 2 μg per ml and a dithizone solution containing about 20 mg per litre ($A_o \simeq 0{\cdot}7$) was used, when the standard graph was linear over the range 0 to 7 μg of lead and the reversion value was 0·070 per μg of lead.

Consistent readings could not be obtained with the model H546 absorptiometer when working in a dark room and using the technique recommended by the makers. Hence the lamp-house shutter was fixed permanently in a fully open position and the light switched on and off for each measurement. The position of the cell carriage was also found to be critical, and to ensure that it is reproduced exactly each operator must follow strictly his personal technique. These difficulties appear to have been eliminated by mechanical improvements in the new model (H760).

Discussion of results

Satisfactory recovery of lead from small biological samples is indicated by the typical results given in Table II for blood, dry bone and two different samples of urine.

Wet oxidation was preferred to dry methods,[5] which have given much lower recoveries in the hands of some workers owing to volatilisation or loss of lead by fusion into the surface of the vessel used for ignition[15]; fictitious high lead figures will result if ignition is carried out in porcelain crucibles fired with a lead glaze. Any precipitate of calcium phosphate obtained on basification, or calcium sulphate formed when sulphuric acid has been used in the oxidative digestion mixture, carries lead down with it and must therefore be redissolved before extraction. Although this can be effected by warming with alkaline citrate solution,

the over-all concentration of citrate required (approximately 6 per cent. for blood and soft tissues, 12 per cent. for urine and considerably higher for bone after digestion with a sulphuric - nitric acid mixture) reduces the efficiency with which lead is later extracted. For example, a graph relating the percentage of lead extracted by 3 ml of dithizone solution (12·5 mg per litre in carbon tetrachloride) on shaking for 1 minute with 10 ml of alkaline citrate buffer

TABLE II

RECOVERY OF LEAD FROM BIOLOGICAL SPECIMENS

Specimen	Lead added, μg	$R_{corr.}$*	Lead found, μg	Average lead found, μg	Recovery, %
18 ml of urine	0·0	0·020	0·27	0·30	
	0·0	0·025	0·33		
	1·0	0·092	1·23	1·24	94
	1·0	0·094	1·25		
18 ml of urine	0·0	0·033	0·44	0·45	
	0·0	0·035	0·47		
	2·0	0·186	2·51	2·43	99
	2·0	0·177	2·36		
2 ml of blood	0·0	0·059	0·79	0·82	
	0·0	0·064	0·85		
	1·0	0·138	1·84	1·78	96
	1·0	0·129	1·72		
27·4 mg of dry tibia	0·0	0·020	0·27	0·23	
	0·0	0·014	0·19		
	1·0	0·096	1·28	1·26	103
	1·0	0·093	1·24		

* $R_{corr.}$ is the reversion value corrected for the blank.

containing 2 μg of lead to the pH was constructed in the usual way. Extraction was found to be quantitative over the pH range 8 to 9 when 2 per cent. citrate was used; but on increasing the concentration to 5 per cent., the extraction curve had a flat maximum covering the same pH range, and only 75 per cent. of the lead was extracted. For this reason the extraction of all biological digests was carried out with successive portions of dithizone until the colour of the last extract was a clear green. To minimise the precipitation of calcium salts at any

TABLE III

SEPARATION OF LEAD FROM BISMUTH BY REVERSION WITH 0·03 PER CENT. W/V NITRIC ACID

Lead taken, μg	Bismuth taken, μg	$R_{corr.}$ (A)	$R_{corr.}$ (B)	"Lead" found, μg (A)	"Lead" found, μg (B)
0·0	2·0	0·000	0·000	0·00	0·00
0·0	5·0	0·051	0·000	0·73	0·00
0·0	10·0	0·079	0·000	1·13	0·00
0·0	15·0	0·091	0·000	1·30	0·00
0·0	20·0	0·096	0·000	1·37	0·00
2·0	0·0	0·138	0·142	1·97	2·03
2·0	1·0	0·139	0·138	1·99	1·97
2·0	2·0	0·144	0·136	2·06	1·94
2·0	5·0	0·140	0·143	2·00	2·04
2·0	10·0	0·123*	0·136	1·76*	1·94
2·0	15·0	0·155*	0·139	1·64*	1·99
2·0	20·0	0·113*	0·140	1·61*	2·00

NOTE—The initial concentration of dithizone was 20 mg per litre. Measurements under the heading B refer to mixtures of lead and bismuth that have been subjected to the full procedure (p. 574) of two cycles of extraction and reversion. Results under heading A were obtained on mixtures subjected to the second stage only; the fixed amount of dithizone was here insufficient for the complete extraction of lead in the presence of large amounts of bismuth, which accounted for the low recoveries indicated by asterisks.

* Low recoveries.

stage, the citric acid was invariably added before basification, and a nitric - hydrochloric acid mixture was used when bone samples were digested; this had the added advantage of preventing the separation of solids and any consequential bumping. In the analysis of blood and liver, excessive oxidation of dithizone by ferricyanide derived from the high iron content was prevented by the hydroxylamine.

Table III illustrates the efficiency with which lead can be separated from bismuth. The final absorptiometric determination by the reversion technique deals effectively with lead in the presence of 5 μg of bismuth (column A); the two-stage process described in the full procedure (p. 574) is effective in the presence of at least 20 μg of bismuth. Since the extractability of bismuth depends on the cube and that of lead upon the square of the concentration of excess dithizone,[13] the retention of bismuth in the organic phase when reverting lead with dilute nitric acid is strongly favoured by using a dithizone concentration of 20 mg per litre (Table III) rather than 12·5 mg per litre (Table I).

The efficient separation and recovery of lead from biological fluids containing bismuth and other metals is illustrated by the data in Table IV.

TABLE IV

RECOVERY OF LEAD FROM 18-ml SPECIMENS OF URINE IN THE PRESENCE OF ADDED BISMUTH, COPPER AND ZINC

Metal added						
Pb, μg	Bi, μg	Cu, μg	Zn, μg	$R_{corr.}$	Lead found, μg	Recovery, %
0·0	0·0	0·0	0·0	0·032	0·43	—
1·0	0·0	0·0	0·0	0·104	1·39	96
1·0	2·0	0·0	0·0	0·108	1·44	101
1·0	5·0	0·0	0·0	0·104	1·39	96
1·0	10·0	0·0	0·0	0·111	1·48	105
0·0	0·0	0·0	0·0	0·021	0·28	—
1·0	0·0	0·0	0·0	0·101	1·35	107
0·0	0·0	100·0	100·0	0·024	0·32	—
1·0	0·0	100·0	100·0	0·096	1·28	96

An abnormal amount of bismuth in a biological digest is readily detected, as its dithizone extract (clear orange) appears before that of the lead (full red). The bismuth is retained in the organic phases from the initial and the final reversion stages whence it can be removed by shaking with 1 per cent. nitric acid solution. It may then be determined by extraction with dithizone from an ammonium hydroxide - cyanide buffer[4] solution followed by reversion with N sulphuric acid.[6]

Apart from the effective determination of lead in the presence of bismuth and other metals we would emphasise that the principle advantages of the procedure described above are flexibility of conditions and economy of time and materials. Any good commercial sample of dithizone can be used without purification, as any coloured impurities initially present or subsequently introduced do not give rise to errors, provided that they are unaffected by the reversion process. Day to day changes in the concentration of dithizone solutions are without significance. For conventional mixed-colour methods it is necessary to remove all dithizone remaining in cyanide and citrate solutions after purification; this tedious operation need not be carried to completion in the proposed procedure. The adjustment of pH is not critical provided it remains between 8 and 9. Finally, the sensitivity of the reversion method (R per μg of Pb $\simeq$ 0·075) is about twice that of the mixed-colour method.

The above procedure has been in frequent use over the past three years for the determination of lead in fluid and tissue specimens from patients with chronic neurological diseases[16] and lead poisoning, and has proved satisfactory in the hands of laboratory technicians. It should be possible to apply it to other materials with little modification.

Our thanks are due to Mr. J. R. P. O'Brien for his interest in this work and for providing laboratory facilities. We are also indebted to Mr. G. E. Newman for skilled technical assistance and to Dr. L. A. Stocken of the Biochemistry Department, Oxford, for the gift of a digestion apparatus.

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THE DEPARTMENTS OF BIOCHEMISTRY AND NEUROLOGY
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OXFORD

July 30th, 1952

Discussion

MR. N. L. ALLPORT, in congratulating the authors on their work, recalled that it was just 21 years ago that, in association with G. H. Skrimshire, he had presented a paper in that same room showing how lead might be extracted from an alkaline aqueous phase containing citrate and cyanide by means of a chloroformic solution of dithizone. It was then pointed out that bismuth interfered, but there did not seem to be any way of overcoming the difficulty. Since that day a great deal of work had been published on the use of dithizone for the isolation and determination of trace metals and in the intervening years great advances had been made in the design and construction of spectrophotometers. It was therefore of particular interest to learn that the problem of bismuth interference had at last been overcome.

DR. D. C. GARRATT said that, as the cause of the irrelevant absorption was unknown and hence it was impossible to say that it was unaffected by the extraction, he questioned whether the authors' correction could be considered valid.

MR. R. C. CHIRNSIDE said that a number of people appeared to be worried as to the possible effect of foreign ions the identity and amounts of which were not known.

DR. IRVING said that Dr. Garratt's and Mr. Chirnside's questions referred to the rather ill-defined term $\Sigma\epsilon_i C_i$ of equations (1) and (2). It must be remembered that the reversion technique was applied to the quantitative determination of lead only after preliminary stages of extraction (p. 574) and reversion (p. 575) under carefully controlled conditions designed to concentrate all the lead, and essentially only the lead, in an aqueous acid. Here it would be accompanied possibly by some bismuth, but by very little else. Other water-soluble impurities would have remained almost exclusively in the aqueous phase rejected after the initial extraction, whilst organic-soluble impurities would have passed into the chloroform extract and remained there after treatment with the 0·03 per cent. nitric acid. The irrelevant absorption in the final dithizone extract used for measuring A_m was therefore not really unknown, for it would be due primarily to the bismuth - dithizone complex.

MR. A. A. SMALES said he thought that the doubt that had arisen about the reversion stage in the authors' procedure was best expressed by saying that the specificity of hydrogen ion for reverting lead but not bismuth at this stage was open to question. He suggested that this specificity might well be improved by the use of ethylenediaminetetra-acetic acid or one of the other "complexones" described by Schwarzenbach, which formed strong complexes with lead but very weak ones with bismuth.

DR. IRVING agreed that the hydrogen ion did not have the specific character theoretically postulated for a reversion agent (*cf.* reference 6). At best it was a "selective" reagent (*cf.* Irving, H. M., and Williams, R. J. P., *Analyst*, 1952, **77**, 813), and in the proposed procedure its use inevitably led to a degree of compromise. The principle of the method described in this paper was in fact devised by the senior author in 1946, *i.e.*, in pre-complexone days, and there was no doubt that considerable improvements in the choice of reversion agents for lead should now be possible.

DR. E. C. WOOD said that the authors had stressed at the beginning of the paper that there was no pH at which a quantitative extraction of lead from dithizone could be made without at the same time extracting at least some of any bismuth present; yet the theory of the reversion technique seemed to require that this should be done in between the two optical measurements. He asked Dr. Irving to clarify this point.

DR. IRVING said that in order to save space they had not included in their paper details of the very extensive preliminary work (*cf.* reference 12) involving studies of (*a*) the variation of percentage extraction with pH for various aqueous salt media, and (*b*) the effect of variations in pH on (*i*) the rate of extraction of lead and of bismuth by a solution of dithizone in chloroform and (*ii*) the rate of reversion of chloroform solutions of various compositions. In the procedure described only a small fraction of any bismuth extracted as dithizonate was decomposed by the acid reversion mixture, partly because of the choice of pH, partly because the time of shaking specified did not permit this reaction to reach the equilibrium appropriate to the pH chosen, although the relatively more rapid decomposition of lead dithizonate did so, and partly because the dithizone set free during the reversion of the lead dithizonate favoured the retention of the bismuth as the dithizone complex in the organic phase (p. 577; *cf.* reference 15 and also Irving, H., Bell, C. F., and Williams, R. J. P., *J. Chem. Soc.*, 1952, 356). In short, conditions of acidity were so chosen that theoretically (*i.e.*, under equilibrium conditions) the minimum amount of bismuth should be reverted along with all the lead, and then the time of shaking was further chosen so that non-equilibrium conditions prevailed and the kinetics of reaction were made to weight the balance still more heavily in favour of extracting lead in preference to bismuth. That the procedure was successful in practice followed from the figures shown in Tables III and IV.

DR. J. HASLAM asked if there was much difference in the extinction coefficients of different samples of so-called "purified" dithizone. He was worried about the effect of this variation on the extinction term for dithizone in the equation $R = A_r - A_m = \text{etc.}$

DR. IRVING said that there were considerable variations in the molecular extinction coefficients of different samples of so-called "purified" dithizone. In the customary "two-colour" methods such differences between samples could easily make nonsense of even the most carefully constructed calibration curves (*cf.* reference 11 and the reply to Mr. Wyatt, below). But it was generally agreed that one of the best ways of determining the molecular extinction coefficient of a pure sample of dithizone (Cooper, S. S., and Sullivan, Sister M. L., *Anal. Chem.*, 1951, **23**, 613), or of determining the content of pure reagent in an impure sample (Irving, H., and Bell, C. F., *J. Chem. Soc.*, in the press), was to make use of the fact that dithizone, HDz, formed metal complexes with bivalent metals, M^{II}, of exactly stoicheiometric composition MDz_2, and that these dithizonates contained two equivalents per molecule of absolutely pure dithizone. The change in absorbancy when a concentration, C_c, of pure metal dithizonate was "reverted" to give a concentration, $2C_c$, of perfectly pure dithizone of molecular extinction coefficient ϵ_r was thus $C_c(2\epsilon_r - \epsilon_c)$, as given in equation (3), where ϵ_r and ϵ_c referred to perfectly pure substances. Of course, they had stated that one of the advantages of the reversion method was that the use of highly purified dithizone (or of dithizone of invariable purity) was unnecessary. If one distinguished between reagent dithizone of extinction coefficient ϵ'_r and the dithizone *puriss.* of extinction coefficient ϵ_r liberated by reversion, equations (1) and (2) of the paper could be rewritten as follows—

$$A_m = \epsilon'_r C_r + \epsilon_c C_c + \Sigma \epsilon_i C_i \quad \ldots \quad (1a)$$

and

$$A_r = \epsilon'_r C_r + 2\epsilon_r C_c + \Sigma \epsilon_i C_i \quad \ldots \quad (2a)$$

from which equation (3) followed. Hence the reversion value was independent of the absolute purity of the reagent.

MR. C. H. PRICE asked if there was not a risk that some of the excess of dithizone might be oxidised between the determinations of A_m and A_r, *i.e.*, between the final two readings of absorbancy, and if it would not be an advantage to use some sulphurous acid or a reducing buffer.

DR. IRVING replied that they had found no signs of oxidation during the few minutes that separated the determinations of A_m and A_r and there need be no fear of error from this cause.

MR. E. E. ARCHER asked whether a buffer solution could be used to extract lead in one stage directly from the first dithizone extract, so eliminating one stage from the procedure.

DR. IRVING replied that this simplification could be justified in practice only if the method was to be applied to routine samples of the same type and approximately the same lead content in which the presence of interfering elements, especially bismuth, was known to be constant and very small. The two-stage procedure described in the paper allowed for a considerable degree of variation from sample to sample and this flexibility was gained at the expense of only one additional stage.

MR. P. F. WYATT said that he had tried the reversion method soon after its publication in the *Journal of the Chemical Society* in 1949, but had found a disadvantage in that the standard curve for dithizone when the Ilford orange 607 filter was used was only rectilinear below an optical density of about 0·5; above this value there was an increasing deviation from Beer's law, and he had had difficulty in getting reproducible results, although he agreed that for a limited low range of lead concentrations results were good.

For this reason he had developed a "mixed colour" method, which he preferred to use, whereby the optical density of the chloroform extract containing the lead dithizonate plus an excess of free dithizone was measured at two wavebands corresponding to the Ilford orange 607 and green 604 filters. The absorption due to lead dithizonate with the orange filter was very small, so that the optical density measured with an Ilford 607 filter could be assumed to give a measure of the excess of dithizone alone, E (Dz). From appropriate standard curves for free dithizone, the absorption in the waveband passed by the Ilford 604 filter that was due to the excess of dithizone could be calculated and deducted from the total optical

density measured with the 604 filter. The difference gave the optical density corresponding to the lead concentration, E (Pb). Typical values for the calibration graphs were as follows, a dithizone solution containing 35 to 40 mg per litre being used.

1. *Standard curves for dithizone in chloroform*—Approximately 37 mg of dithizone per litre; 25-ml dilution; 1-cm cell.

Dithizone solution, ml ..	1	2	3	4	5	6	7	8
E (Dz) with 607 filter ..	0·170	0·330	0·485	0·625	0·75	0·875	0·975	1·065
E (Dz) with 604 filter ..	—	0·084	—	0·165	—	0·251	—	0·329

2. *Standard curve for lead*—10-ml dilution; 1-cm cell.

Lead, μg	Volume of dithizone solution used, ml	Observed optical density: Ilford 607 filter	Observed optical density: Ilford 604 filter	E (Dz) with 604 filter calculated from E with 607 filter	Net reading E (Pb) with 604 filter
8	2·5	0·155	0·265	0·038	0·227
16	4·0	0·165	0·50	0·041	0·459
24	6·0	0·23	0·735	0·058	0·677
32	7·0	0·17	0·955	0·042	0·913
40	9·0	0·24	1·20	0·060	1·140

A graph of the net reading, E (Pb), against weight of lead taken was a straight line over the whole range.

With regard to bismuth, Mr. Wyatt agreed that quantitative separation of bismuth from lead by extraction from a solution buffered at about pH 3 was difficult. A slight increase in pH caused some lead to be lost by extraction with the bismuth, while a slight drop in pH led to incomplete removal of bismuth. He preferred to remove bismuth by extraction with diethylammonium diethyldithiocarbamate from a solution of high acidity, 4 to 6 *N*, depending on the relative amounts of sulphuric and hydrochloric acids present (Strafford, N., Wyatt, P. F., and Kershaw, F. G., *Analyst*, 1945, **70**, 232), and then to isolate the lead by extraction with the same reagent at a much lower acidity. The extract containing the lead was then decomposed and the determination completed by the dithizone "mixed colour" method already outlined.

Dr. Irving said that he was not surprised that Mr. Wyatt had had trouble when working with the more concentrated solutions of dithizone and lead dithizonates to which Beer's law no longer applied. But this was scarcely a reflection on the reversion method and he was glad to learn that Mr. Wyatt had had good results from it in the low lead range where it could most legitimately and profitably be used. He asked whether, in Mr. Wyatt's problems, there was any objection to using a smaller sample, or to using a smaller aliquot of the digest from a large sample if there was any danger of segregation. In the "mixed colour" method he described, variations in the quality of the dithizone used would, of course, affect the calibration. Unlike the reversion procedure it did not discriminate between lead and bismuth and could, therefore, only form the final determination step after some previous stages of quantitative separation.

Mr. Smales said he wondered whether the brilliant colours of the dithizone complexes were not, in fact, dazzling us, and whether it might not have been better if the reagent and the complexes had been colourless. More use might then have been made of dithizone for its remarkable separating powers, the final determination being by some alternative to absorptiometry, such as polarography. Polarographic instruments were available with a sensitivity of 0·02 μg of lead per ml of solution, more than adequate for the class of determination being considered.

A Colorimetric Determination of Dihydrostreptomycin

By G. C. ASHTON, M. C. FOSTER and M. FATHERLEY

A colorimetric method for the determination of dihydrostreptomycin is presented. It is based on a reaction between guanido materials and diacetyl, alkali and α-naphthol. The colour formed is not subject to interference at high salt concentrations and can be used in the routine analysis of factory samples. Penicillin and its compounds do not interfere with the reaction, so the method can be applied to the determination of dihydrostreptomycin in mixtures with penicillin.

The standard error of analyses made by the method is ±1·3 per cent., which compares favourably with that found in microbiological assay.

Several chemical methods are available for determining dihydrostreptomycin. In general they are either tedious or subject to interference from substances other than dihydrostreptomycin in the samples submitted for assay. We required a simple method for determining dihydrostreptomycin in a variety of samples ranging from process control samples to samples of the pure antibiotic alone and in admixture with other antibiotics.

Oxidimetric, spectrophotometric and colorimetric methods have been published for the determination of dihydrostreptomycin. The oxidimetric procedures depend on the oxidation of dihydrostreptomycin by sodium metaperiodate[1] or periodic acid.[2,3] Formaldehyde is produced, and, after distillation under carefully controlled conditions, the formaldehyde is determined with chromotropic acid. The necessity for distillation was overcome by Vail and Bricker,[3] who removed the interfering periodic and iodic acids by lead acetate precipitation and developed the colour of chromotropic acid with formaldehyde in the clear centrifugate. Methods based on periodate oxidation suffer from the disadvantage that streptomycin produces different amounts of formaldehyde under different conditions. In our experience such careful control of this procedure is required as to make it inconvenient for routine use.

A method based on the spectrophotometric absorption of dihydrostreptomycin at 265 mμ after acid hydrolysis was suggested by Hiscox[4] and is suitable for pure dihydrostreptomycin, but it cannot be applied to mixtures of antibiotics without the use of appropriate correction factors, which reduce its precision.

As the dihydrostreptomycin and streptomycin molecules are identical except that the aldehyde grouping of the streptose moiety is hydrogenated in dihydrostreptomycin and is therefore not reactive, colorimetric methods for streptomycin based on the streptidine or N-methylglucosamine moieties are potentially applicable to the determination of dihydrostreptomycin. Hence, the streptidine assay developed by Sullivan and Hilmer[5] for streptomycin has been applied to dihydrostreptomycin by Monastero.[6] The streptomycin assay described by Scudi, Boxer and Jelinek[7] is based on the Elson - Morgan reaction for glucosamine and, similarly, can be used for dihydrostreptomycin estimation on the nearly pure substance, but dihydrostreptomycin gives only one twenty-fourth of the colour given by streptomycin with the same technique and reagents.

Our objective being to develop for dihydrostreptomycin an assay that could be applied routinely to a variety of samples, as mentioned above, we investigated a number of reactions dependent on the streptidine moiety of the molecule. Such a reaction is not specific for dihydrostreptomycin; it is also given by streptomycin and mannosidostreptomycin. Streptomycin and dihydrostreptomycin occur together at certain stages during dihydrostreptomycin manufacture, and in assaying these samples for dihydrostreptomycin by a streptidine assay it is necessary to allow for the streptomycin present. This can be done conveniently with the ferric maltol assay,[8] which determines streptomycin but not dihydrostreptomycin. Mannosidostreptomycin is not usually present in the streptomycin used for the manufacture of dihydrostreptomycin, but if any were present it would be estimated by the ferric maltol assay. It is unusual to find streptomycin and dihydrostreptomycin associated in samples of mixed antibiotics. Hence, for practical purposes a streptidine assay can be used as a measure of dihydrostreptomycin content in mixed antibiotic samples.

Experimental

Several possible methods based on reactions of the guanidine groups of the streptidine moiety were examined. The method of Monastero[6] with the oxidised nitroprusside reagent

of Weber[9] is subject to interference by salts. A method for aromatic amidines[10] is applicable to dihydrostreptomycin, but was found also to be subject to salt interference.

We also investigated extensively the Sakaguchi reaction,[11] using combinations of α-naphthol or 8-hydroxyquinoline with hypochlorite[12] or hypobromite.[13] We found the resulting colours to be unstable and could not obtain reproducible results with any combination of the reagents.

A recent paper by Halliday[14] described a spray reagent for streptomycin chromatograms. The reaction involved is attributed to Voges-Proskauer, and the spray reagent used is based on Barritt's[15] procedure for guanidine materials, involving the use of diacetyl, α-naphthol and potassium hydroxide. This reaction was finally selected as the basis of our method.

Halliday[14] said that the diacetyl colour with streptomycin developed slowly and faded after reaching a maximum optical density. We have confirmed this, although the fading rate and time at which the maximum optical density occurs depend on the relative concentrations of the three reagents.

ESTABLISHMENT OF OPTIMUM CONCENTRATIONS—

The original instructions[15] for applying the diacetyl reaction to guanidine materials were as follows. To 1 ml of guanidine solution add 1 ml of a 0·1 per cent. solution of diacetyl in water, 2·25 ml of water, 0·5 ml of 5 per cent. ethanolic α-naphthol and 0·25 ml of 40 per cent. alcoholic potassium hydroxide. These amounts of reagent proved suitable for aqueous solutions of dihydrostreptomycin, but from solutions containing much salt the salts were precipitated owing to the high alcohol concentration of the final solution. This was prevented by preparing the caustic potash solution in water rather than in methanol, but on addition of the α-naphthol solution this reagent was itself precipitated. If, however, the potash was added before the α-naphthol, no precipitation occurred. Changes in reagent volumes were then made, so that 2 ml of sample were diluted with 15 ml of water and 1 ml of each of the three reagents was added.

As already mentioned, the time of maximum colour development and the amount of colour formed depend on the relative concentrations of the three reagents. Experiments with different combinations of 0·1, 0·2 or 0·4 per cent. diacetyl, 10, 20 or 40 per cent. aqueous potassium hydroxide and 2·5, 5 or 10 per cent. alcoholic α-naphthol were carried out. The optical density of each solution after standing for 30, 60 and 90 minutes was measured. From these results it appeared that 0·4 per cent. diacetyl, 20 per cent. potassium hydroxide and 10 per cent. α-naphthol solutions gave maximum colour formation. This combination of reagents gave a colour that remained at its maximum value for 10 to 15 minutes.

WAVELENGTHS OF MAXIMUM ABSORPTION—

The wavelengths at which the solutions from the first experiment absorbed most strongly were determined; $\lambda_{max.}$ ranged from 505 mμ to 530 mμ. With the combination of reagents finally adopted, the maximum absorption was at 525 mμ.

EFFECTS OF TIME AND TEMPERATURE—

A solution of dihydrostreptomycin in water was treated with 0·4 per cent. diacetyl, 20 per cent. potassium hydroxide and 10 per cent. α-naphthol solutions at 21°, 23°, 25° or 27° C. Aliquots were removed at 5-minute intervals and the optical densities measured at 525 mμ against water. The results are shown in Fig. 1. Both time and temperature have a marked effect on the rate at which the colour develops, but the final colour is always maximal after about 40 minutes and is fully developed between 23° and 27° C. It is, therefore, recommended that the temperature at which colour development is carried out should be not less than 23° C, although satisfactory results can be obtained at lower temperatures. Further, it is necessary to measure the developed colours at a fixed time after adding the reagents; we have found 40 minutes to be suitable.

BLANK VALUE OF THE REAGENTS—

The reagents themselves give rise to a coloured solution, the intensity of the colour increasing with time (see Fig. 1). Therefore, if a series of samples is being assayed at timed intervals it is not practicable to use the same reagent blank value. Rather than develop a separate reagent blank for each sample, we find it convenient to measure the developed

sample colour against water, and subtract from this measurement the optical density value of a separately developed and similarly timed reagent blank measured against water.

CALIBRATION GRAPH—

An apparently linear calibration graph is obtained with dihydrostreptomycin solutions containing up to 400 units per ml when the final coloured solution is measured in a 1-cm cell at 525 mμ against water. This line passes through a point on the ordinate corresponding to the blank value of the reagent. Calibration is also linear with a Spekker absorptiometer and No. 604 filters.

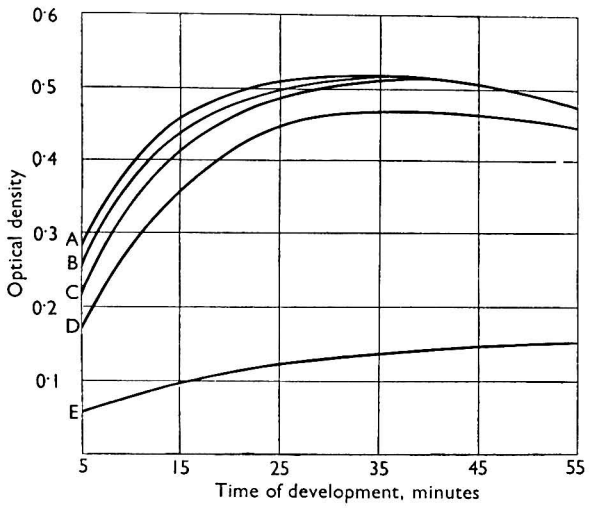

Fig. 1. Effect of time and temperature on development of colour between dihydrostreptomycin and the diacetyl - α-naphthol reagent. Curve A, 27° C; curve B, 25° C; curve C, 23° C; curve D, 21° C; curve E, reagent blank

STABILITY OF REAGENTS—

From experiments with combinations of reagents of different ages, it appears that the diacetyl and potassium hydroxide solutions are stable for at least 2 weeks. The slope of the graph relating optical density to concentration increases slightly as the α-naphthol solution ages, an effect not solely due to an increased blank value, but good results have been obtained with α-naphthol solutions 2 weeks old.

EFFECT OF CONTAMINANTS ON COLOUR—

The effect on the diacetyl determination of substances that might be encountered in pharmaceutical preparations of mixed antibiotics, or during the production of dihydrostreptomycin, was determined. The substances chosen were added to a solution containing 250 units of dihydrostreptomycin per ml.

No colour suppression or intensification was obvious when the dihydrostreptomycin solutions contained 10 per cent. w/v of anhydrous sodium sulphate, 5 per cent. w/v of anhydrous sodium citrate, 1 per cent. w/v of calcium chloride ($CaCl_2.6H_2O$), 0·2 per cent. w/v of sodium benzylpenicillin, 0·03 per cent. w/v of procaine penicillin or 0·03 per cent. w/v of penethamate hydriodide.

The effect on the diacetyl reaction of substances other than those mentioned has not been investigated. Caution should be exercised in applying the method to pharmaceutical preparations containing vegetable oils, ointment bases or unknown excipients.

The diacetyl reaction is also given by streptomycin and mannosidostreptomycin, streptidine, arginine and other guanido derivatives.

Method

Reagents—

Diacetyl solution—A 0·4 per cent. w/v solution of diacetyl in distilled water.

Potassium hydroxide solution—A 20 per cent. w/v solution of potassium hydroxide in distilled water.

α-Naphthol solution—A 10 per cent. w/v solution of α-naphthol in absolute ethanol.

Procedure—

Either dissolve the sample in distilled water or dilute liquid samples to give a solution containing between 100 and 400 units of dihydrostreptomycin per ml (0·1 to 0·4 mg per ml of dihydrostreptomycin base).

Transfer 2 ml of the sample solution to a 6-inch × 1-inch test tube, add 15 ml of water and then 1 ml of diacetyl solution, 1 ml of potassium hydroxide solution and 1 ml of α-naphthol solution, in that order. Mix the contents of the tube by inversion after each addition. Start timing when the diacetyl solution is added. Precisely 40 minutes later determine the optical density at 525 mμ in a 1-cm cell against water. Alternatively, measure the optical density on an absorptiometer with a suitable filter, *e.g.*, a Spekker absorptiometer with No. 604 filters.

Prepare a standard graph from suitable dilutions of a standard dihydrostreptomycin solution, using the same procedure for colour development as described for the sample. Determine the potency of the diluted sample solution from the standard graph. A standard graph should be prepared for each determination to allow for differences in room temperature, reagents, and so on.

Results and discussion

Precision—

An examination of the results from replicate determinations on different samples at one concentration level showed the apparent standard error of a determination to be ±0·6 per cent. However, observations on replicate determinations at several concentrations of one sample showed the standard error to be greater than this. It seems likely, therefore, that the graph of optical density and concentration has a slight curvature over the range 0 to 400 units per ml.

To determine the precision attainable over the recommended range, three different dihydrostreptomycin samples were dissolved in water to give solutions containing approximately 400 to 450 units per ml. Each solution was then diluted accurately with water to give solutions of concentrations 75, 50 and 25 per cent. of the original solutions. The three original solutions and their nine dilutions were assayed in duplicate by the method described. The potencies of the twelve solutions were determined from a standard graph prepared from a standard dihydrostreptomycin material.

The results of this experiment are shown in Table I.

Table I

Potencies of dihydrostreptomycin solutions at four concentrations

Dilution level, %	Sample A	Sample B	Sample C	Level average
25	423·1	426·1	420·1	427·02
	431·9	429·0	431·9	
50	424·5	417·2	409·9	415·25
	418·7	414·3	406·9	
75	420·1	417·2	410·3	416·55
	424·1	419·2	408·4	
100	421·6	426·7	414·3	420·02
	419·4	423·8	414·3	
Sample average	422·93	421·69	414·50	419·71

An examination of the results showed that the major part of the error is due to the non-agreement between results from different levels on any one sample, caused by slight curvature of the graph of optical density and concentration. The error between duplicate determinations at one level on one sample is considerably less than the major error due to curvature.

For routine purposes, however, this curvature error is insufficient to warrant special precautions (such as restricting values to the linear portion of the graph or making allowances for the curvature). The standard error of a pair of duplicate determinations, including the error due to curvature, is about $\pm$ 1·3 per cent.

COMPARISON WITH BIO-ASSAY—

A number of dihydrostreptomycin sulphate samples and various dihydrostreptomycin solutions were assayed both microbiologically by the *Klebsiella pneumoniae* plate assay and chemically by the method described. The results are shown in Table II.

TABLE II

COMPARISON BETWEEN PROPOSED METHOD AND MICROBIOLOGICAL ASSAY

Solid samples		Solutions	
By diacetyl method, units per mg	By microbiological method, units per mg	By diacetyl method, units per ml	By microbiological method, units per ml
765	750	343	365
778	780	341	340
770	780	354	350
765	767	318	340
		326	335
		326	330

There are no significant differences between the chemical and microbiological results.

APPLICATION TO STREPTOMYCIN—

It is clear that the method described is also applicable to streptomycin and gives results similar to those by the ferric maltol method,[8] both with simple streptomycin solutions and with mixed antibiotic samples. The method cannot, however, be used for the determination of the streptomycin in fermentation broths, as it gives a measure of the total guanidine-reacting material present in the broth. We believe the chief value of the method lies in its application to dihydrostreptomycin, for which the more specific ferric maltol method cannot be used.

We are indebted to Mr. J. P. R. Tootill not only for carrying out the statistical analysis of the results, but also for useful criticism and helpful suggestions in the planning of experiments during the development of the method. We wish also to thank Mr. K. A. Lees for carrying out the microbiological assays.

Note added in proof.—After this paper had been accepted for publication, a paper appeared describing the application of the Voges - Proskauer reaction to the quantitative assay of streptomycin (Szafiv, J. J., and Bennett, E. O., *Science*, 1953, **117**, 717).

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A Systematic Approach to the Choice of Organic Reagents for Metal Ions

By R. J. P. WILLIAMS

The choice of an organic reagent for any particular metal ion is limited by the nature of the free energy change occurring on the formation of the complex of the reagent and the metal ion in aqueous solution. A broad general division of such reactions is made that is based upon whether the entropy change or the heat change of the reaction is the more important term in the free energy change. Small cations of large charge (usually ions of low electronegativity) are divided from the larger and more electronegative cations in this way. The choice of the type of reagent suitable for selective reaction with a particular metal ion can be based on these principles. Minor factors, such as the nature of the available orbitals, also affect the free energy of formation of complexes with particular metal ions. Many examples of the successful use of organic reagents are examined and some suggestions are made for the design of further reagents.

In their search for new organic reagents for metal ions, analytical chemists generally prepare an organic compound without regard to the requirements of any special metal ion, unless the compound happens to be a simple modification of an organic reagent already in use for the determination of a particular cation. The testing of the reagent then proceeds by a series of experiments of a trial and error nature. Often the new compound is rapidly shown to be to some extent selective but in no way specific. It is proposed to give, in this paper, some general approach to the problem of the selectivity of organic reagents.

The formation of a complex in aqueous solution and its subsequent transfer to a second solvent or its subsequent precipitation can generally be represented by the series of equilibria—

$$M + L \overset{K}{\rightleftharpoons} ML \begin{cases} \overset{K_2}{\rightleftharpoons} ML \text{ (precipitate)} \\ \overset{K_1}{\rightleftharpoons} ML \text{ (in a second immiscible solvent)} \end{cases} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

M and L represent a metal ion and a reagent, respectively, charges are omitted for convenience, and the constants K, K_1, K_2, are the equilibrium constants for the three possible stages: formation of the complex, its partition and its precipitation. K_1 is simply related to the partition coefficient and K_2 to the solubility of the anhydrous complex, as defined by Irving and Williams.[1] Previous discussions of these equilibria have been based on the equilibrium constant K, its variation from metal to metal and from one ligand to another[1] and on the pH control in the formation of precipitates or in the partition of the complexes.[2] However, a better understanding of the equilibria can be obtained by resolving the free energy changes, ΔG, into their component entropy and heat changes, ΔS and ΔH, respectively.

$$\Delta G = -2{\cdot}303\ RT \log_{10} K = \Delta H - T\Delta S.$$

First, it is important to realise the kind of change that can occur in ΔS and ΔH. This is not possible in terms of the above equilibria (1), as they misrepresent the reactions that take place, in so far as they omit the water molecules involved in the reactions. The reactions are—

$$M(H_2O)_a + L(H_2O)_b \overset{K}{\rightleftharpoons} ML(H_2O)_c + (a + b - c)H_2O \begin{cases} \overset{K_2}{\rightleftharpoons} ML \text{ (precipitate)} + dH_2O \\ \overset{K_1}{\rightleftharpoons} ML \text{ (second phase)} + fH_2O \end{cases} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The three reactions are accompanied by considerable changes in the number of water molecules bound to the reactants and therefore in the total number of molecules "free" in the solution. This means that an entropy change takes place. If L is an anion and M is a cation, the number of "free" molecules in the solution will increase upon complex formation because of the release of some of the molecules of water co-ordinated to the ions concomitant with

the neutralisation of charge. Table I shows some examples of entropy changes on complex formation, which illustrate the large changes that favour the complex formation when a neutralisation of charge occurs.[3,4] Many further examples will be published later.[5] The

TABLE I

MOLAR HEATS AND ENTROPIES OF FORMATION OF SOME COMPLEXES AT 25° C

Reaction	ΔG, K cals.	ΔH, K cals.	ΔS, cals. per ° C	Reference
$U^{\cdots\cdot}$—OH'	−17·2	−2·1	+50·5	Nat. Bur. Stand. Circular[6]
$Fe^{\cdots}$—OH'	−16·0	−1·2	+50·0	Evans and Uri[3]
$Fe^{\cdots}$—F'	−6·9	+7·5	+49·0	»
$Fe^{\cdots}$—Cl'	−2·0	+8·5	+35·0	»
$Fe^{\cdots}$—Br'	−0·8	+6·1	+23·0	»
$Fe^{\cdots}$—C_2O_4''	−13·2	−0·3	+43·0	»
$Cr^{\cdots}$—OH'	−14·0	−1·0	+43·3	Nat. Bur. Stand. Circular[6]
$Cr^{\cdots}$—Cl'	−2·0	+5·0	+23·3	»
$Sn^{\cdot\cdot}$—OH'	−17·0	−10·0	+23·0	Vanderzee[7]
$Sn^{\cdot\cdot}$—Cl'	−1·6	+2·6	+14·0	»
$Sn^{\cdot\cdot}$—Br'	−1·0	+1·4	+8·0	»
$Mg^{\cdot\cdot}$—SO_4''	−3·6	+5·7	+31·0	Evans and Monk[8]
$Mg^{\cdot\cdot}$—$CH_2(CO_2)_2''$	−4·0	+3·2	+24·0	»
$Ba^{\cdot\cdot}$—S_2O_3''	−3·1	+2·6	+19·0	Davies and Wyatt[9]
$Zn^{\cdot\cdot}$—$CH_2(CO_2)_2''$	−5·0	+3·1	+27·0	Evans and Monk[8]

decrease in hydration must depend to some extent upon the initial degree of hydration of the reacting ions. Table II gives some examples of the entropy of hydration of anions

TABLE II

ENTROPIES OF HYDRATION OF IONS EXPRESSED AS CALORIES PER ° C

	$U^{\cdots\cdot}$	$Fe^{\cdots}$	$Cr^{\cdots}$	$Cd^{\cdot\cdot}$	$Sn^{\cdot\cdot}$	$Mg^{\cdot\cdot}$	$Ca^{\cdot\cdot}$	$Sr^{\cdot\cdot}$	$Ba^{\cdot\cdot}$
Hydration entropy	−78	−70	−73	−14·6	−9	−28	−13	−9	+3
Entropy change on formation of hydroxide	+50	+50	+43	—	+23	—	—	—	—
Entropy change on formation of chloride..	—	+35	+23	+8	+14	—	—	—	—

	F'	OH'	Cl'	Br'	I'
Entropy of hydration ..	−2·3	−2·5	+13·2	+19·2	+26·1
Entropy change on ferric complex formation ..	+49	+50	+35	+23	—
Entropy change on stannous complex formation ..	—	+23	+14	+8	—

and cations. Latimer[10] has shown that these values can be expressed in a general formula—

$$S^\circ = \frac{3}{2}\text{R.log M} + 37 - \frac{270z}{r^2}.$$

where M, z and r are the molecular weight, charge and effective radius of the ion. Thus it is to be expected that the entropy change upon complex formation should be related to z/r^2. Definite proof of such an exact relationship is not available as yet, but the data in Table I indicate that in the reaction between one cation and a series of anions, the entropy change is related to the inverse of the anionic radius, $F' \geqslant OH' > Cl' > Br'$. Again, in the reactions of a series of cations with one anion, *e.g.*, chloride or hydroxide in Table I, the order of the entropy changes is $U^{\cdots\cdot} = Fe^{\cdots} \geqslant Cr^{\cdots} > Cd^{\cdot\cdot} = Sn^{\cdot\cdot}$, an order that coincides with that of the order of the ionic potentials z/r. If further water molecules are released when the complex is precipitated or extracted, the entropy changes will again follow the same sequence. The general parallel between precipitation and complex formation will be discussed in more detail on p. 589.

No specificity of reaction can be expected from the entropy changes on complex formation, but it is clear that these changes can result in a high degree of selectivity. Organic reagents that are anions will react preferentially with highly charged small cations on this basis. Furthermore, as it is known that the reaction of cations with neutral molecules involves only small, or even opposed, entropy changes on complex formation (see Table III),

highly charged small cations are to be expected to react rather with anions than with neutral molecules, provided that the heat of reaction is not overwhelmingly large for the latter.

TABLE III

HEATS AND ENTROPIES OF FORMATION OF COMPLEXES BETWEEN CATIONS AND NEUTRAL MOLECULES

Reaction	ΔG, K cals.	ΔH, K cals.	ΔS, cals. per ° C	Reference
Mg^{++}—$2NH_3$	−0·3	−1·2	−3·0	Nat. Bur. Stand. Circular[6]
Cu^{++}—$4NH_3$	−16·6	−19·7	−10·0	"
Hg^{++}—$4NH_3$	−26·2	−28·5	−7·7	Fyfe[11]
Ag^{+}—$2NH_3$	−10·0	−13·3	−11·0	"
Co^{+++}—$5NH_3$	−42·4	−49·4	−23·3	Nat. Bur. Stand. Circular[6]
Cu^{++}—En	−14·2	−18·9	−15·0	Williams[12]
Zn^{++}—En	−7·7	−9·8	−7·0	"
Fe^{++}—3Dipy	−24·5	−24·5	0·0	Williams[5, 12]
Ag^{+}—Py	−2·8	−4·7	−6·3	"

NOTES—1. In this table "En" represents ethylenediamine, "Dipy" dipyridyl and "Py" pyridine.
2. Many other examples will be given in a further publication.[5]

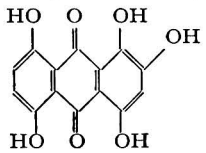
HEAT CHANGES ON FORMATION OF COMPLEXES

A consideration of reaction (2) shows that the loss of free energy in the formation of a complex, ML, must be considered in conjunction with the gain in free energy consequent upon the decrease in hydration. In the same way as the entropy changes in the reaction are dependent upon the entropy of hydration of the reactants, so is it to be expected that the heat of reaction will be dependent upon the heat of hydration of the ions and molecules involved. However, whereas the entropy change in the reaction is more favourable for reactants of greater hydration, the heat change will be more unfavourable.[5] Hence ions that form mainly ionic complexes, such as aluminium,[III] thorium[IV] and magnesium[II]—all ions of low electronegativity and weak acceptors—form complexes with anions of high electronegativity, *i.e.*, weak donors, only through the agency of large entropy changes that are opposed by the heat of reaction. Table I contains many examples of complexes formed in this way; several other examples are discussed elsewhere.[5] It is only to be expected that the heats of formation of complexes of organic reagents that are anions and weak donors, and which, in addition, are large molecules, will be even further opposed to complex formation (by these ions), as the "interference volume" of the ligands around the cations will be large; large repulsion terms between the co-ordinated residual water molecules and the combined reagent will be inevitable. This repulsion term will be much smaller if the reagent is a chelating agent; much of the stability of chelate complexes arises from the small interference volume of these co-ordinated molecules.[5] Metal ions that are weak acceptors will have little tendency to bind electropositive neutral molecules that are good donors. Furthermore, the entropy changes in these reactions are not favourable to complex formation. The over-all result is that these cations of high ionic potential and which are also poor acceptors will always form hydroxide complexes (with the aid of large favourable entropy changes) at a much lower pH value than they will form complexes with neutral molecules. It is now possible to discuss the type of organic molecule that will be suitable as a reagent for these cations.

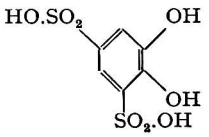
CATIONS OF LOW ELECTRONEGATIVITY AND HIGH IONIC POTENTIALS—

The extreme members of this first group of cations are the very small quadrivalent ions of the metals of group IVA of the periodic table, namely, titanium, zirconium, hafnium and thorium. A suitable organic reagent for these very small ions must be anionic and, preferably, a dibasic acid, so that two molecules of the reagent can completely neutralise the cation charge (four molecules of a monobasic acid would have a much larger interference volume). The reagent must co-ordinate through oxygen atoms, weak donor groups, for otherwise it will react with the larger, more electronegative cations, such as thallium[III]. Finally, the insolubility of the complex produced should not depend upon the formation of a continuous lattice, for small cations are not easily built into such structures because of the radius ratio effect.[4] An ideal reagent for these cations is an organic arsenite, such as phenylarsonic acid, $C_6H_5.AsO(OH)_2$. The sulphinic acids are also suitable, but a disulphinic acid

might be even better. As the co-ordinating groups are made more electropositive, *i.e.*, better donors, the selectivity of the reagent is reduced and the conditions for reaction with certain tervalent cations coincide with those for the reactions of the quadrivalent ions. This is already apparent with the reactions of the lake-forming phenols, such as purpurin, **I**, and titan yellow, **II**. Ferric, gallium and chromic ions react with these reagents under the

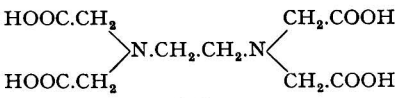


I



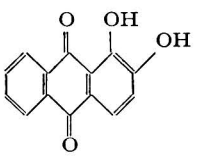
II

same conditions as zirconium. If the co-ordinating groups are greatly increased in volume, the region of overlapping reactions is still further extended. The stability of the ethylenediaminetetra-acetic acid complex of titanium^{IV}, $\log_{10} K = 17{\cdot}3$, is much lower than that of the ferric complex, $\log_{10} K = 25{\cdot}3$, and is not much greater than that of lanthanum^{III}, $\log_{10} K = 15{\cdot}4$. The two nitrogen atoms in ethylenediaminetetra-acetic acid, **III**, are good donors.

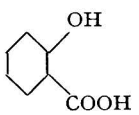


III

When devising a reagent for a tervalent ion that is a poor acceptor, such as aluminium, scandium, or a rare earth ion, maximum selectivity from the reactions of bivalent ions can again be achieved by making use of the larger entropy changes of the reactions of the tervalent ions. Thus carboxylic acids, phenols and carbonyl groups, which can form chelates with the cations, make excellent reagents. Their reactions selectively depend upon the size of the cation. Typical reagents of this kind are alizarin, **IV**, aurin tricarboxylic acid and salicylic acid, **V**. The size of the ligand is not so critical as it is with quadrivalent cations, but the

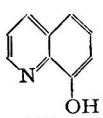


IV



V

reagent still must not be a good donor. An increase in donating power immediately introduces an overlap of reactions with the more electronegative metal ions, such as cupric. For this reason oxine, **VI**, discriminates poorly between bi- and tervalent ions. Its nitrogen group is a good donor.



VI

For a bivalent metal ion that is also a poor acceptor, such as magnesium, calcium, strontium or barium, it is clear that, as the importance of the entropy of complex formation is now smaller, it will not be possible to separate it from other, larger, bivalent ions that are good acceptors. The procedure is now to find reagents for the good acceptors instead (see p. 591). It is still interesting to know if it is possible to separate the four ions of group IIA from one another by means of organic reagents. Table IV illustrates the principles on which the analytical chemist should act in approaching this problem.

The greatest difference between the stabilities of the magnesium and the calcium complexes occurs with glycine. A much smaller difference is found for the oxalate complexes and, in the lattice of these salts, calcium is more stable, as shown by the much greater solubility of the magnesium complex. A reagent for magnesium that will not react with calcium

must, clearly, have a small interference volume and, also, must not form a continuous lattice, such as that found in oxalates.[4] Oxine is one such a reagent; it is selective for magnesium in the presence of calcium. "Magneson," **VII**, is a similar reagent. Other reagents that also suggest themselves are compounds such as **VIII**. Such a molecule would, in some respects, resemble chlorophyll, which is a very selective magnesium reagent.[13]

Selectivity for calcium must be based on the size of the ion. Many examples are given elsewhere, most of them from the work of Schwarzenbach.[4]

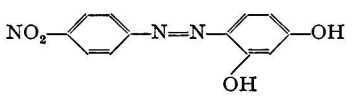
TABLE IV

THE LOGARITHM OF STABILITY CONSTANTS OF SOME ALKALI - EARTH METAL COMPLEXES

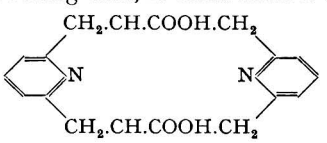
Reagent	Metal ion			
	Mg	Ca	Sr	Ba
Glycine	3·44	1·43		0·77
$N(CH_2.COOH)_3$	5·41	6·41	4·94	4·82
Oxalate	3·43	3·00	2·54	2·33
	(4·07)	(8·64)	(7·25)	(6·96)
Tartrate	1·36	1·80	1·65	1·62
Thiosulphate	1·84	1·92	2·04	2·33
	(sol.)		(3·00)	(4·00)
Nitrate	0·00	0·28		0·92
	(sol.)			(2·35)
Sulphate	2·15	2·28		
	(sol.)	(4·64)	(6·55)	(10·00)

NOTE—The numbers in parenthesis are logarithms of solubility products.

The data in Table IV show that the stabilities of the complexes of the strong acid anions follow the sequence of the radii: $Ba^{\cdot\cdot} > Sr^{\cdot\cdot} > Ca^{\cdot\cdot} > Mg^{\cdot\cdot}$. This sequence can only arise in one way. The heat of formation of these complexes so strongly opposes the reaction that it controls the order of the stabilities despite the contrary influence of the entropy changes.[4] This only occurs with the strong acid anions. It must result from the variation in the hydration of the cations. The difference between the reactions of the various ions is much more noticeable in the solubility products and these differences too can be traced to the large differences in the heats of solution for the various cation salts.[4,5] If an organic reagent is to be prepared for barium, it must be similar in character to the sulphate anion. It must be a derivative of a dibasic and, preferably, strong acid, it must form a continuous

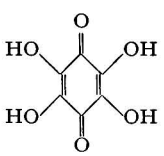


VII



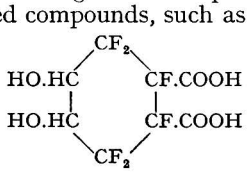
VIII

lattice and it must be a large molecule. One or two reagents of this type are known, *e.g.*, rhodizonic acid, **IX**. Molecules such as those of the sugar acids, which also contain large

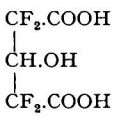


IX

numbers of hydroxyl groups, would also seem suitable. Note the way in which hydroxyl groups stabilise the larger ions on passing from the oxalates to the tartrates in Table IV. Highly fluorinated compounds, such as **X** and **XI**, or nitrated compounds, might also serve.

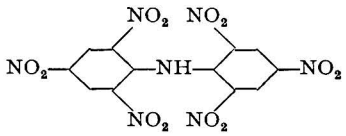


X



XI

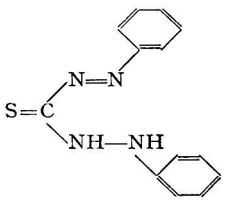
The principles on which a search should be made for organic reagents for the larger group IA metals should be related to the above, but there should be only one ionising group in any molecule chosen and as many other co-ordinating groups of a highly polar character as possible. An excellent example of such a reagent is compound **XII** for potassium. Reagents for magnesium should always be tried as reagents for lithium.



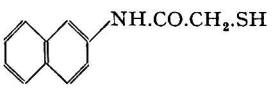
XII

ELECTRONEGATIVE METAL IONS—

Molecules such as ammonia and ethylenediamine, cyanide ions, iodide ions and bromide ions react with metal ions such as mercury^{II}, platinum^{II}, platinum^{IV}, thallium^{III}, gold^{I} and silver^{I} with a considerable evolution of heat,[5] and with but small entropy changes. Not all these groups can be introduced into organic reagents without modifying their activity to such an extent as to make them unreactive, *e.g.*, iodide. However, both nitrogen and sulphur co-ordinating groups are easily prepared in many organic compounds. As specific reagents for the electronegative metals such compounds are ideal. A great number are already known, amongst which are dithizone, **XIII**, thionalide, **XIV**, substituted thioureas,

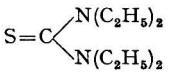


XIII

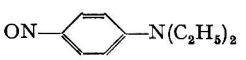


XIV

XV, and substituted nitroso-anilines, **XVI**. As the metal ions are large it is an advantage if the combining ligand molecule occupies a large volume around the cation so that steric factors act to increase the selectivity of the reagent for these large ions. It is noteworthy that the selectivity of these reagents is of a different order from that discussed in the previous section. For example, apart from osmium and ruthenium, few metal ions are able to react



XV



XVI

with tri- and tetra-ethyl thioureas, even at high pH values. As has been pointed out elsewhere, the selectivity of a reagent such as dithizone differs considerably from that of oxime.[1] The most electronegative cations react with many of the reagents in the above group even in strong mineral acid solutions.

Between this group of cations and the cations in the large first group come a large number of ions with somewhat similar properties; these vary from cupric ion, which is almost to be classed with the good acceptors, and manganous ion, which is not much different from a poor acceptor such as magnesium. For example, along the series of bivalent ions, manganese, iron, cobalt, nickel and copper, the acceptor properties increase steadily, and selectivity of reaction will depend largely upon a careful choice of conditions. The problem has been discussed in detail in an earlier paper.[1] In general, the larger the ion and the higher its electronegativity as measured by its ionisation potential, the more easily it will react with compounds that are good donors. Furthermore, the lower the charge on a subgroup-B metal, the more easily does it form complexes with strong donors. These and other trends amongst the stability constants of such metal ions have been discussed already.[1] There are two other methods of obtaining selective reactions, which are more important in the present discussion: a change of valency and a change of ground-state of the ion involved in the reaction. Often the two changes are interdependent.

CHANGE OF VALENCY—

In general, if there is a lower valency state readily available, a change to this state from the stable valency of the metal in aqueous solution will take place on the replacement of water by a more electropositive ligand provided that no radical electron rearrangement is involved. A change to a higher valency state will be brought about by a change of ligand to a more electronegative group than water, again provided there are no radical rearrangements of the non-valency electrons. High-valency states are usually found in oxide (O″) complexes; manganese^{VII}, chromium^{VI}, molybdenum^{VI} and iron^{III} are amongst many others. Low-valency states are usually found with cyanides, *e.g.*, with such cations as nickel^{I}, palladium^{I}, manganese^{I} and molybdenum^{II}. Such changes of valency have great importance for the analyst, because a cation can be made to react selectively first in one valency state and then in another. The reduction of cupric ions to cuprous with iodide and the reduction of both ferric and cupric ions with thiols are well-known examples. Auric and thallic ions are most stable when they are bound to oxygen in anionic complexes, whereas the lower valency states of gold and thallium are most stable when bound to cyanide. There are very few organic compounds that bring about these changes and little effort seems to have been made to find them. Reagents that increase the valency of the metal ion must be oxidising agents that form strong complexes through oxygen atoms. Hydrogen peroxide is a clear example. It is used in the preparation of peroxy-acids of chromium from a chromic salt solution. Hence it seems that other per-acids of organic carboxylic acids should produce the same effect. The efficacy of reducing agents is well known, the best examples being with thio-compounds such as thiourea and thioglycollic acid. Moreover, dithiocatechol is a common reagent for molybdenum^{II}. There might be phosphorous compounds that would also bring about such reactions.

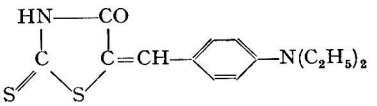
ORBITAL CHANGES—

Many ions of the transition metals are able to change their electronegativity by a change of electronic structure. The greater the *d*-orbital character in the bonds of these ions the more electronegative they become. These changes have often been suggested as a possible source of specific reactions. Some clear examples are known. The ferrous state is stabilised relative to the ferric state by complex formation with dipyridyl, *o*-phenanthroline or cyanide, but not by complex formation with hydroxyl, chloride, ethylenediaminetetra-acetic acid or oxine. The former are the more electropositive reagents, but they also form complexes with ferrous ions that are diamagnetic. In these complexes ferrous ion binds the ligands with d^2sp^3-orbitals. A selective valency change that is different from the one discussed above, and is opposed by the tendency of the lower valency state to combine with the good donor, can be brought about if on losing an electron the ion reaches an upper state in which the possible orbital combinations are much stronger acceptors. A well-known example is the reaction of cobaltous ion with cyanide. The reaction of this ion with α-nitroso-β-naphthol also depends upon the change in electronegativity in the tervalent state. Other selective reactions caused by orbital changes are those of nickel and palladium with dimethylglyoxime. The problems of whether nickel can be made ter- or quadrivalent in solution have yet to be tackled and it may be possible to stabilise manganese in a quadrivalent state. The stabilisation of such valency states may require very powerful donor ligands such as arsenic or phosphorus compounds.

Orbital changes often involve changes in stereochemistry, and specificity can arise through the choice of the reagent to fit the steric requirements of the cation. For this reason 2:2′-diquinolyl reacts with cuprous (tetrahedral) ion, but not with ferrous (octahedral) ion.[1] No systematic search has been made for other examples of this kind, but one or two suggest themselves.

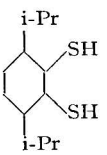
The hydrate of nickel is peculiarly stable. The heat of hydration is hardly different from that of the cupric ion. The reason for this stability is not known, although it is either due to the Stark splitting of the *d*-energy levels in the octahedral field or else to the interaction with the incompletely filled 3*d* band. The *d*-orbitals can only supply additional stabilisation if the nickel ion is surrounded by six octahedrally placed molecules or four planar molecules. Now, a ligand can be chosen that is too large to fit anything but a tetrahedral arrangement. This complex will be unusually unstable relative to the nickel hydrate. Some examples

are known. Nickel chloride is less stable than either cobaltous or cupric chloride; the substituted 2-methyl oxine complex of nickel is less stable than the corresponding cobaltous

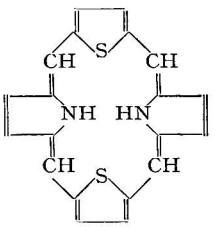


XVII

complex or, at least, it is no more stable than the latter despite the considerably greater stability of the nickel oxinate. Surely, if much larger substituents are used this effect must be greatly enhanced. If Orgel's calculations are correct, it might well be possible to reduce the stability of nickel complexes by a factor of 10 logarithm units relative to the corresponding zinc complex.[13] The dithizonates may be a case in point.



XVIII



XIX

The difference in hydration can be used in other metal complexes. The reaction of argentous ions with ammonia is somewhat sensitive to the substitution of alkyl groups for nitrogen. However, this ion will form amine complexes even with tertiary amines. Although cupric and nickel ions form amines of a stability greater than that of argentous ions, the stability of their complexes is considerably reduced by substitution of the ammonia molecule. Tertiary amine complexes have not been reported. At the same time, a specific reagent for argentous ion in the presence of large amounts of either nickel or cupric ions is a tertiary amine of formula **XVII**.

There must be other instances of complex formation being made deliberately to favour a large ion of slight hydration as opposed to a small ion of large hydration energy. The selectivity of dithiols must be greatly increased if large groups are substituted *ortho* with respect to the thiol groups. Such a reagent as that shown in **XVIII** might be of use for stannous or plumbous ions and might not react with the other group elements of the analysis tables, *viz.*, copper[11] and cadmium.

The bias in the design of the above reagents has been in the favour of the large ions. Surely there must be ways of designing molecules so that the co-ordinating groups are too close together to react with large ions. The porphyrins are natural compounds of this kind, but thiophene derivatives of the same kind (**XIX**) might be more selective.

The approach to the design of organic reagents can be greatly improved and it is hoped that this article has shown a few possibilities. In conclusion, it must be pointed out that it has only been possible in this article to quote a few examples from the reagents commonly used for different classes of cations. Reference to standard works on the subject of the selectivity of organic reagents[15] will immediately supply many more examples that will confirm some of the general remarks made.

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MERTON COLLEGE
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Micro-determination of Iodides by Arresting the Catalytic Reduction of Ceric Ions

BY B. ROGINA AND M. DUBRAVČIĆ

A method is described for the determination of small amounts, 0·01 to 1·00 μg, of iodine as iodide. The method is based on the catalytic effect of iodides on the reduction of a ceric salt by arsenious acid. The rate of reduction is readily determined by arresting the reaction at a given time by the addition of ferrous and thiocyanate solutions and by measuring the resulting red colour of ferric thiocyanate by means of a photometer. The method offers advantages over that described by Chaney in which the rate of reduction is measured while the reaction is in progress.

Results are quoted to show the accuracy of the proposed method. A reproducibility of ±0·002 μg has been attained in the range from 0·01 to 0·10 μg of iodide in 8 ml of aqueous solution.

IT has been known for some time that iodides have a catalytic effect on the reduction of ceric sulphate by arsenious acid in sulphuric acid solution. The reaction takes place in the following way—

$$2Ce^{\cdots\cdot} + As^{\cdots} \rightarrow 2Ce^{\cdots} + As^{\cdots\cdot\cdot}$$

The gradual progress of the reaction can be followed by the steady disappearance of the yellow colour of ceric ions. Sandell and Kolthoff[1,2] found the speed of the reaction to be nearly proportional to the iodide concentration, and they used this reaction for the determination of small amounts of iodides. The conditions of the reaction (concentration of reagents, acidity and temperature) were fixed, and iodides were determined by measuring the time required for the complete disappearance of the yellow colour.

A more convenient method based on the same reaction was developed by Chaney.[3] He preferred to measure the concentration of the remaining non-reduced ceric ions after a fixed time, instead of waiting for complete decoloration (reduction).

It has been observed that some inevitable technical difficulties occur in measuring the concentration of ceric ions while the reaction is in progress. The catalytic reaction takes place in a thermostatically controlled enclosure, whereas the concentration of the remaining ceric ions is measured elsewhere with a photo-electric absorptiometer. Because of instability of the electricity supply, frequent adjustments to the photometer are necessary and the measurements are therefore protracted. It is difficult to carry out the measurement quickly enough to avoid inaccuracies arising both from the reaction being still in progress and from changes of temperature. This results in errors in the determination of iodides.

We found that, after a suitable time had elapsed, the progress of the catalytic reaction could be arrested. By addition of an excess of ferrous ions at a given moment the remaining ceric ions are immediately reduced as follows—

$$Ce^{\cdots\cdot} + Fe^{\cdot\cdot} \rightarrow Ce^{\cdots} + Fe^{\cdots}$$

The resulting amount of ferric ions is equivalent to that of ceric ions present immediately before the catalytic reaction was arrested. It is also proportional to the intensity of the red colour that appears after addition of a thiocyanate solution. The depth of this colour is inversely related to the iodide concentration and can be determined photometrically. The amount of iodide is deduced from a previously prepared calibration graph.

By the method described, the colour can be measured with a colour comparator or a visual type of photometer without undue haste and therefore more accurately. In the method described, a Zeiss - Pulfrich photometer with a green filter has been used.

Experimental

Ratio of extinction to ceric ion concentration—

As the first step in the development of the method, the relation between the rate of reduction of ceric salt and the photometer drum readings was examined. To solutions of ceric salt of different concentrations, ferrous and thiocyanate solutions were added and the optical densities were measured. It was found that a 2×10^{-3} N concentration of ceric salt produced a considerable extinction (about 2) which, however, could be measured with sufficient accuracy. Therefore, a concentration of 2×10^{-3} N of ceric ions was chosen as the most suitable initial (maximum) concentration.

To 10 ml of different concentrations of ceric salt in solutions N in sulphuric acid, 1 ml of 1·5 per cent. ferrous solution and 1 ml of 4 per cent. thiocyanate solution were added. The extinctions of the red colour so produced were measured in 0·5-cm cells with the green filter S 50 (maximum transmission at 488 mμ). These extinctions, plotted against the concentration of ceric ions, gave a straight line (Fig. 1, graph A).

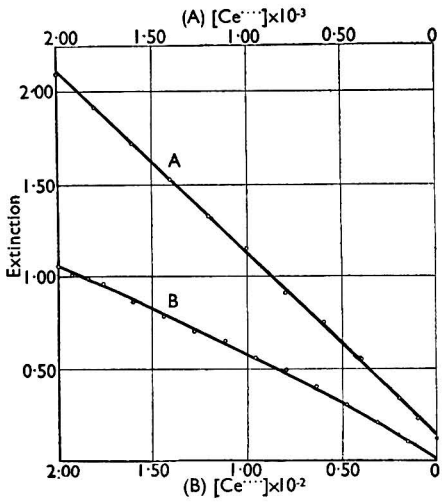

Fig. 1. Extinction values of ferric thiocyanate solutions plotted against concentration of ceric ions. Measurement in 0·5-cm cells with a green filter of maximum transmission at 488 mμ

Considerably higher initial concentrations of ceric salt were used by the authors quoted.[2,3] For comparison, solutions of ceric salt of various concentrations to a maximum of 2×10^{-2} N, made 1·2 N in respect of sulphuric acid, were measured directly. As shown by Fig. 1, graph B, the extinction values are considerably lower and there is not such a good straight line relation as was found with the lower concentrations.

The catalytic reaction and calibration graphs—

The amount of iodide could only be determined on the basis of the rate of the reduction of ceric ions if all the conditions under which the catalytic reaction takes place were fixed and adhered to. In laying down the conditions we availed ourselves partly of the experiences of other authors[2,3] after taking into consideration our particular circumstances. For the reasons mentioned above, a reaction mixture 2×10^{-3} N in ceric ions was used. A solution 5×10^{-3} N in arsenious acid, and N in sulphuric acid was found to be suitable.

For a satisfactory rate of reduction, the temperature and duration of the reaction should be adapted to the range in which lie the iodide concentrations that have to be determined.

The most suitable conditions for the "high range," *i.e.*, for amounts of iodide between 0·1 and 1·0 μg, were found to be a temperature of 20° C and 8 minutes as the limit for the duration of the reaction. For the "low range," *i.e.*, for 0·01 to 0·10 μg of iodides, a temperature of 30° C and a reaction time of 20 minutes were used.

With the above conditions and the technique described in the procedure, calibration graphs as shown in Figs. 2 and 3 were plotted.

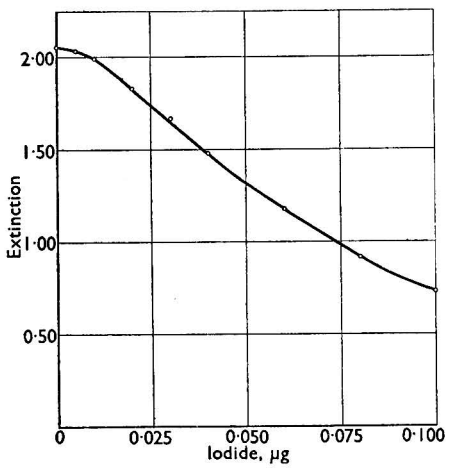

Fig. 2. Calibration graph for 0·01 to 0·10 μg of iodide ("low range"). Temperature, 30° C; reaction time, 20 minutes

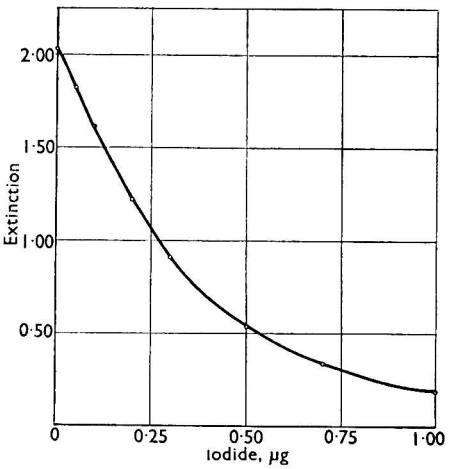

Fig. 3. Calibration graph for 0·1 to 1·0 μg of iodide ("high range"). Temperature, 20° C; reaction time, 8 minutes

Interferences with the catalytic effect of iodides by other ions were not especially studied in this work; this point is covered in the literature.[2,3,4,5] It need only be mentioned here that chlorides, bromides and, especially, osmium ions have a catalytic effect on the reduction of ceric salt, and cyanides, thiocyanates, mercuric and silver ions have an inhibitory effect.[2,3]

Indifferent ions, such as the sodium ion, decrease the speed of the catalytic reaction when present in high concentrations. If such ions are present, the reference standards must contain the same amount of indifferent ion, and a corresponding calibration graph must be drawn.

STABILITY OF THE COLOUR—

The stability of the colour of ferric thiocyanate was found to be satisfactory. Temperature has most influence on the colour. The rate of fading of ferric thiocyanate increases with increasing temperature. To minimise any differences in temperature, the solutions must be set aside at room temperature for 45 minutes after the red colour has been developed. The measurement can be made at any time after this waiting period has elapsed, in contrast to the usual practice in iron determinations. Owing to the lower concentration of potassium thiocyanate, the colour of the ferric thiocyanate solution is stable enough and does not fade, provided the room temperature remains substantially constant.

The temperature of measurement should not differ widely from that at which the calibration curve was recorded. Readings taken at different temperatures can nevertheless be compared if reference standards or numerical corrections are used. Ovenston and Parker[6] found that the extinction of ferric thiocyanate solutions decreased by about 1 per cent. for every degree centigrade rise of temperature. We found such a correction to be useful. It is more accurate, however, to make in every set of determinations a few measurements of known amounts of iodides in order to obtain reliable reference values.

METHOD

APPARATUS—

A rack with test tubes of about 2 cm in diameter and 18 cm in length.
Pipettes.

A water-bath controlled at 20° and 30° C (±0·2° C), fitted with a transparent front, large enough to contain the rack with the test tubes and the bottle of ceric salt solution.

A Pulfrich photometer with green filter No. S 50 (with maximum transmission at 488 mμ) and 0·5-cm cells.

A stop-watch.

NOTE—All glassware should be soaked in a strong sulphuric acid - bichromate mixture and then thoroughly rinsed, first with tap water and then with distilled water.

REAGENTS—

Sulphuric acid, 60 per cent. w/w.

Arsenious acid—A 0·1 *N* solution in about 0·01 *N* sulphuric acid. Dissolve by heating 4·946 g of arsenic trioxide in about 500 ml of water acidified with 10 drops of sulphuric acid. Dilute to 1 litre.

Ceric ammonium sulphate solution—A 0·02 *N* solution in 1·6 *N* sulphuric acid. Dissolve 13·38 g of $Ce(SO_4)_2.2(NH_4)_2SO_4.4H_2O$ in water, add 44 ml of sulphuric acid, sp.gr. 1·84, and dilute to 1 litre.

Ferrous ammonium sulphate solution—A 1·5 per cent. w/v solution of $FeSO_4.(NH_4)_2SO_4.6H_2O$ in 0·6 per cent. sulphuric acid.

Potassium thiocyanate solution, 4 per cent. w/v.

Standard potassium iodide solutions—Dissolve 261·6 mg of potassium iodide in 1 litre of water to give a solution containing 200 μg of iodine per ml. Dilute the solution to 1 in 200 and 1 in 2000 to make "high range" (1·0 μg per ml) and "low range" (0·1 μg per ml) standard solutions.

NOTE—Stock solutions must be stored in a dark place and kept tightly closed.

PROCEDURE—

If the approximate concentration of the iodide solutions is unknown, make a preliminary test to determine what dilution should be prepared to obtain concentrations corresponding either to the "high range" (0·1 to 1·0 μg in 8 ml of sample solution) or to the "low range" (0·01 to 0·10 μg in 8 ml of sample solution).

The "high range" concentrations give at 20° C an appropriate rate of reduction after 8 minutes. The conditions laid down should be scrupulously observed (±0·2° C and ±5 seconds), both in the preparation of the calibration graph and for the determination of iodides in samples.

For lower concentrations, higher temperatures and longer periods of time should be used. For the "low range," use a water-bath temperature of 30° C ± 0·2° C and a reaction time of 20 minutes.

Several samples can be analysed at intervals of 1 minute in one set of determinations. As the reaction lasts 8 or 20 minutes, depending on the range used, and as 1 minute is required for the necessary laboratory work with the test tube, a maximum of 8 or 20 test tubes, respectively, should be used.

To increase the accuracy, every set of determinations should include from two to four appropriate reference standards. These standards will indicate any difference, however small, between the conditions used in determination and those used in the preparation of the calibration graph, and a correction to the photometer readings can be made, if necessary.

Place in each test tube 8·0 ml of iodide solution, 0·5 ml of arsenious acid and 0·5 ml of sulphuric acid. If less then 8 ml of iodide solution is taken because the concentration is above the normal range, add water to make the total to 8 ml.

Shake each tube thoroughly to mix the contents, rinse the inside walls of the tubes by rotation, and place the rack with the tubes in the water-bath at the appropriate temperature (20° or 30° C). After about 20 minutes, the temperature of the tube contents will be that of the bath.

Add to the first tube 1 ml of ceric ammonium sulphate solution (at the same temperature), start the stop-watch, mix the contents of the tube by shaking and put it back on the rack. Repeat the procedure with the second tube, timing the addition of the ceric ammonium sulphate to coincide with a reading of 60 seconds on the stop-watch. At 1-minute intervals repeat the addition of the ceric ammonium sulphate solution to the other tubes.

After 8 or 20 minutes, according to the range, arrest the catalytic reaction in the first tube by adding 1 ml of ferrous ammonium sulphate solution and mixing. The remaining

yellow colour disappears suddenly. Add 1 ml of potassium thiocyanate solution, which will cause the red colour of ferric thiocyanate complex to appear. Place the tube on a second rack beside the water-bath. Arrest the reaction in the other test tubes at 1-minute intervals, and develop the red colour as described.

Place the rack with red coloured solutions near the Pulfrich photometer and allow the solutions to attain room temperature. After 45 minutes, or later, measure the extinction of the solutions with a green filter, S 50, and 0·5-cm cells, with water in the reference cell. From the measured extinctions evaluate the iodide concentrations from a calibration graph prepared at a temperature not differing greatly from the prevailing room temperature. If the values for the standard solutions included in the set do not exactly fit in the calibration graph, draw a parallel line through the new standard points, and read the iodide concentration from this.

Results

The reproducibility of determinations of iodide by the method described is shown in Table I. Four different amounts of iodides (two at "low range" and two at "high range") were each determined 12 times, the determinations being spread over 3 days. The calibration graph was prepared on another day. The results show that at the "low range" the amount of iodide found is within $\pm 0{\cdot}002$ μg of the amount taken. At the "high range" the error is greater, but it does not exceed 4·2 per cent. of the iodide taken.

Table I

Reproducibility of results

Day	Iodine taken, μg	Iodine found, μg				Range of errors, μg	Error, %
First	0·030	0·032	0·029	0·031	0·029	±0·002	±6·7
Second	"	0·028	0·030	0·029	0·028		
Third	"	0·030	0·031	0·031	0·029		
First	0·080	0·081	0·082	0·079	0·078	±0·002	±2·5
Second	"	0·078	0·078	0·079	0·079		
Third	"	0·080	0·080	0·082	0·080		
First	0·300	0·310	0·307	0·304	0·304	+0·010	+3·3
Second	"	0·295	0·295	0·296	0·307	−0·005	−1·2
Third	"	0·304	0·295	0·296	0·300		
First	0·800	0·817	0·817	0·817	0·808	±0·034	±4·2
Second	"	0·783	0·834	0·800	0·766		
Third	"	0·800	0·792	0·800	0·808		

Discussion of results

It is reasonable to suppose that Chaney, too, met with difficulties in measuring the concentration of the remaining ceric ions while the reaction was in progress. In his later publication[4] he proposed a technical improvement of the photometer. The catalytic reduction was carried out in a special photo-electric absorptiometer with a built-in thermostat. A vacuum-type photo-tube, an electronically regulated power supply and a continuous recording device were used to improve sensitivity. Chaney claimed that the ultimate sensitivity of this method of measurement was 0·001 μg of iodine in 5 ml of reaction mixture. His reproducibility figure of $\pm 0{\cdot}001$ μg was attained in the range of 0·05 to 0·10 μg. He did not state whether the same reproducibility could be attained for amounts below 0·05 μg.

Table I shows that, in the range of 0·01 to 0·10 μg of iodides, we attained in a much simpler way a reproducibility of $\pm 0{\cdot}002$ μg in 10 ml of reaction mixture. For less than 0·01 μg of iodide the accuracy was not so great because of the variable catalytic effect of the lowest iodide concentrations. The effect is often upset by the traces of other elements present as impurities. Sometimes there is a slight catalytic effect, as shown in Fig. 2. These concentrations of iodides could, therefore, be considered a "region of uncertain reaction".[7]

The red colour of the ferric thiocyanate complex has advantages for measurement as compared with the yellow colour of ceric salt solutions. Although the latter show the most significant absorption in the violet and ultra-violet region of the spectrum, solutions of ferric

thiocyanate have greater absorption in the blue and green regions. As the sensitivity of the human eye and the sensitivity of most photo-electric cells rises towards yellow-green, it is more convenient to measure a red coloured solution than a yellow one. In addition, the absorption of the ferric thiocyanate solutions is greater and covers a larger scale of extinction values than does the absorption of ceric solutions alone (Fig. 1), even though the latter are of a higher concentration.

It was found that the solution of ferrous ammonium sulphate, acidified with sulphuric acid, was sufficiently stable for some weeks. A slight increase in extinction values, up to 0·10, caused by the gradual oxidation of this reagent by atmospheric oxygen must be taken into account.

No change in activity of the other reagents was observed. Standard solutions of potassium iodide, prepared several months before, showed the same catalytic effect as freshly prepared solutions.

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The Determination of Potassium and Traces of Sodium in Some Potassium Salts

By C. Jackson

For determining sodium in certain potassium salts of weak acids the sample is titrated with 0·2 *N* perchloric acid in glacial acetic acid, a small controlled excess being added. After the precipitated potassium perchlorate has been removed by filtration, the filtrate is evaporated to dryness and the sodium is determined as sodium zinc uranyl acetate. It is thus possible to estimate volumetrically the total equivalent alkali metal and gravimetrically both sodium and potassium on one sample.

The method can be applied to the determination of sodium in most potassium salts.

The determination of sodium as sodium zinc uranyl acetate has received much attention.[1,2,3] Although the method is applicable in the presence of appreciable amounts of potassium,[4,5] it cannot be applied when the potassium is in large excess, as, for example, in the determination of sodium in potassium salts, owing to precipitation of potassium zinc uranyl acetate. Moreover, the reagents commonly used for precipitating potassium produce a filtrate unsuitable for sodium determinations; sodium cobaltinitrite is obviously ruled out and perchloric acid in an alcoholic medium leaves a filtrate that cannot be evaporated without danger. Flame photometry[6,7,8,9] is rapid and convenient, but in many laboratories the necessary facilities for it are not available.

In these laboratories sodium and potassium salts of weak acids, such as acetic acid, are usually titrated with perchloric acid in glacial acetic acid.[10] During the titrations a heavy precipitate formed in the presence of potassium, whereas with sodium salts the titrated liquid remained clear. The solubility of potassium perchlorate has been shown[11,12] to be less in glacial acetic acid than in the conventional alcoholic medium, the values being 0·027 g per litre in the former and 0·15 g per litre in the latter.

Preliminary experiments showed that potassium could be quantitatively precipitated by the use of a small measured excess of perchloric acid and that the filtrate from the

precipitated potassium perchlorate could be evaporated to dryness without danger. The zinc uranyl acetate determination of sodium in the residue presented no difficulty.

METHOD

REAGENTS—

Perchloric acid, 0·2 N—To 1 litre of glacial acetic acid add 46 ml of 60 per cent. perchloric acid (analytical reagent grade) and then 500 ml of acetic anhydride (analytical reagent grade), stirring and cooling the solution continuously. Dilute to 2 litres with glacial acetic acid. Standardise this solution against anhydrous sodium carbonate dissolved in acetic acid, using methyl violet as indicator.

Methyl violet—A 0·2 per cent. solution in ethanol.

Ethanol saturated with potassium perchlorate.

Zinc uranyl acetate solution—Prepare this as described in the British Pharmacopoeia, 1953, p. 687.

Ethanol saturated with sodium zinc uranyl acetate.

Acetone—Dry.

PROCEDURE—

Weigh sufficient of the potassium salt to give a titration of about 40 ml of 0·2 *N* perchloric acid, transfer it to a dry beaker and dissolve it in 50 ml of glacial acetic acid. Add 6 drops of methyl violet solution and titrate with the standard perchloric acid until the indicator becomes emerald green. If a volumetric assay is required, note the volume added; then add a further 0·5 ml. Filter and collect the precipitated potassium perchlorate on a tared sintered-glass crucible of porosity 3, transferring and washing the precipitate with glacial acetic acid (a flexible polythene wash-bottle is useful for this). Finally wash the precipitate with a little ethanol saturated with potassium perchlorate, collecting the ethanolic washes separately and rejecting them. Dry the precipitate at 160° C and weigh it.

1 g of potassium perchlorate ≡ 0·28217 g of potassium.

TABLE I

RECOVERY OF SODIUM AND POTASSIUM

Potassium carbonate			Sodium carbonate			
taken, g	found,* g	found, %	taken, g	found, g	recovered, g	recovered, %
0·5892	0·5883	99·85	—	0·0029	—	—
0·5913	0·5899	99·76	—	0·0033	—	—
0·5876	0·5886	100·17	—	0·0029	—	—
0·5895	0·5910	100·25	—	0·0029	—	—
0·5899	0·5899	100·00	0·0292	0·0319	0·0289	99·0
0·5899	0·5907	100·13	0·0292	0·0310	0·0280	95·9
0·5898	0·5898	100·00	0·0293	0·0321	0·0291	99·3
0·5903	0·5909	100·10	0·0057	0·0087	0·0057	100·0
0·5900	0·5912	100·20	0·0057	0·0089	0·0059	103·5
Mean (9)		100·05	Mean (5)			99·5
Standard error of mean ..		±0·05	Standard error of mean			±1·2

* The potassium perchlorate was dried at 105° C.

Evaporate the filtrate to dryness on an electric hot-plate in a fume cupboard. Add about 5 ml of water and, with the aid of a rubber-tipped rod, loosen the slightly tarry residue. (The use of redistilled acetic acid and acetic anhydride slightly reduces the amount of this residue, but analytical grade reagents are satisfactory and usually free from sodium). Filter the solution through a Whatman No. 41 filter-paper into a small beaker, washing the original beaker and the filter-paper thoroughly with water. Evaporate the filtrate to about 2 ml, add 25 ml of zinc uranyl acetate reagent and set the beaker aside for 30 minutes.

Filter on a tared sintered-glass crucible of porosity 3, transferring the precipitate with the aid of a small amount of reagent. Wash with three 2-ml portions of ethanol saturated

with sodium zinc uranyl acetate and then three 2-ml portions of dry acetone. Dry the precipitate at 100° C for 30 minutes, cool and weigh it.

1 g of sodium zinc uranyl acetate ≡ 0·01495 g of sodium.

Results

The method was tested on potassium carbonate and sodium carbonate of analytical reagent grade. The dried carbonates were dissolved in a weighed amount of glacial acetic acid, weighed aliquots being taken for test. The recoveries are shown in Table I.

Recoveries of potassium were erratic and higher than was consistent with the presence of the amounts of sodium found. The high results appeared to be due to residual acetic acid, as the precipitates had been dried at 105° C, and a higher temperature was subsequently found necessary to remove the last traces.

The tests were therefore repeated with a drying temperature of 160° C; as shown in Table II (first five results), recoveries were then more consistent and in agreement with the presence of traces of sodium. Further replicate tests were also made (Table II, second five results), and the figures found for sodium agree with the value of 0·2 per cent. (0·46 per cent. as sodium carbonate) found by flame photometry.

Table II

Recovery of sodium and potassium

Potassium perchlorate dried at 160° C

Potassium carbonate			Sodium carbonate		
taken, g	found, g	found, %	taken, g	found, g	found, %
0·6877	0·6853	99·65			
0·6879	0·6859	99·71			
0·6876	0·6855	99·69	Test for sodium omitted		
0·6880	0·6856	99·65			
0·6874	0·6859	99·78			
0·5907	0·5872	99·41	nil	0·0028	0·47
0·5900	0·5868	99·46	nil	0·0028	0·47
0·5923	0·5896	99·54	nil	0·0026	0·44
0·5930	0·5895	99·41	nil	0·0030	0·51
0·5926	0·5891	99·41	nil	0·0028	0·47
Mean (10) ..		99·57	Mean (5) ..		0·47
Standard error of mean ..		±0·04	Standard error of mean ..		±0·011

Scope of the method

If it is required to determine sodium alone in a neutral potassium salt, this can be done by adding the calculated amount of perchloric acid to precipitate, say, 98 per cent. of the anticipated amount of potassium. Most of the potassium can then be removed by filtration, and the sodium can be determined in the filtrate as described above.

If the potassium salt is difficult to dissolve in glacial acetic acid, the sample can be dissolved in a small amount of water before the acetic acid is added. After the perchloric acid solution has been added, sufficient acetic anhydride is added to convert the water into acetic acid. Heat is evolved in the course of this addition and the solution should be cooled before filtering.

With this technique, tests of which the results are shown in Table III were carried out on potassium nitrate (analytical reagent grade) with additions of sodium nitrate and on potassium chloride (analytical reagent grade) with additions of sodium chloride.

The results show that the method could be extended to the determination of sodium in neutral potassium salts, but the greatest advantage appears in determinations of sodium in the potassium salts of weak acids. It is not considered that the distribution of results as between sodium nitrate and sodium chloride is indicative of a difference in behaviour between the two salts. The chances against the two samples of nitrate and the two samples of chloride giving results significantly different from 100·0 per cent. and in opposite directions

are only 8 to 1, and the discrepancies in any event depend upon differences of not more than 0·5 to 1·0 mg.

TABLE III

RECOVERIES OF SODIUM FROM NEUTRAL POTASSIUM SALTS

Potassium salt taken, g	Sodium salt added, g	Sodium salt found, g	Recovery of sodium salt g	Recovery of sodium salt %
Potassium nitrate—				
0·7980	nil	0·0004	Blank experiment	
0·8012	nil	0·0004	Blank experiment	
0·8012	0·0341	0·0341	0·0337	98·8
0·7986	0·0372	0·0369	0·0365	98·1
Potassium chloride—				
0·5970	nil	0·0007	Blank experiment	
0·5990	nil	0·0007	Blank experiment	
0·5988	0·0322	0·0340	0·0333	103·4
0·5978	0·0274	0·0287	0·0280	102·2
			Mean (4) ..	100·6

The novel points of this technique are the use of the acetic acid medium for precipitating potassium perchlorate and of a standard solution of perchloric acid. It is to be expected that the potassium and sodium estimations will still be subject to the usual interferences; for example, ammonium salts will form an insoluble perchlorate and lithium will give an insoluble lithium zinc uranyl acetate; phosphate or other anions that react with zinc or uranyl salts must be absent. Therefore a volumetric assay and a gravimetric potassium determination could probably be made on potassium phosphates, of which all the potassium is titratable in the medium specified. No sodium determination would be possible here. The full procedure can be used on potassium carbonate, bicarbonate, hydroxide, acetate and, it would seem probable, other salts of organic acids. Although we have not tried a wide range of compounds, the technique appears suitable for sodium or potassium estimations on a variety of potassium salts.

I am indebted to British Drug Houses Ltd. for determining by flame photometry the sodium in the sample of potassium carbonate used for the results shown in Table II.

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The Determination of Small Amounts of *m*-Dinitrobenzene in Nitrobenzene

By F. G. ANGELL

Small amounts of *m*-dinitrobenzene in nitrobenzene can be concentrated by chromatographic adsorption on alumina and elution with a mixture of benzene and light petroleum. The adsorbed *m*-dinitrobenzene is extracted with ethanol from the dried extruded alumina column and determined polarographically for concentrations greater than 0·02 per cent. or absorptiometrically with acetone and sodium hydroxide for concentrations less than 0·02 per cent.

The well-known colour reaction between *m*-dinitrobenzene and simple ketones in sodium hydroxide solution has often been used for its detection and determination in small quantities, but this method is unsuitable for quantitative measurement in dark-coloured samples of nitrobenzene.

Adsorption chromatography is a possible means of overcoming this difficulty. By percolation of a light petroleum solution of 2 to 3 g of crude nitrobenzene through an alumina column, followed by elution with 10 per cent. v/v of benzene in light petroleum, nitrobenzene was removed, leaving *m*-dinitrobenzene on the column. Development with the mixture of benzene and light petroleum was continued until no odour of nitrobenzene was detectable in the eluate. A small amount, about 1 or 2 mg, of nitrobenzene remaining did not interfere with the subsequent polarographic or absorptiometric determination of the *m*-dinitrobenzene. The alumina column was allowed to drain and was extruded in sections, which were individually extracted with ethanol and the extracts tested for *m*-dinitrobenzene by the acetone - sodium hydroxide test. If the colour produced was pink, the *m*-dinitrobenzene was determined absorptiometrically, but with extracts of a deeper red colour, polarographic reduction was found to be a more convenient method of determination.

Cruse and Harl[1] showed that small concentrations of trinitrobenzene in *m*-dinitrobenzene could be determined polarographically in a base solution buffered at pH 12·0. They found that the two approximately equal reduction steps of *m*-dinitrobenzene occurred at the same half-wave potential as the second and third steps of trinitrobenzene. Shikata's results[2] indicated that the reduction potential for nitrobenzene coincided with that for the second step for *m*-dinitrobenzene. Cruse and Harl's results for the values of $E_{\frac{1}{2}}$ measured against the normal calomel electrode, *i.e.*, nitrobenzene, first step −0·62 volt; *m*-dinitrobenzene, first step −0·44 volt, second step −0·61 volt; and trinitrobenzene, first step −0·315 volt, second step −0·44 volt, third step −0·609 volt, have been confirmed, and unpublished work in these laboratories indicates that similar relationships exist between *m*-nitroaniline and *m*-dinitrobenzene, mixtures of which may be quantitatively analysed by a similar procedure to that outlined for mixtures of *m*-dinitrobenzene and nitrobenzene.

The height of the wave at −0·45 volt measured against the saturated calomel electrode was found to be directly proportional to the *m*-dinitrobenzene concentration and was unaffected by small concentrations of nitrobenzene. It was therefore used as the basis of measurement, which is described in the experimental section.

Experimental

Chromatographic separation of *m*-dinitrobenzene—

A glass tube 60 cm long and of 1·5 cm internal diameter, fitted at the lower end with a tight-fitting cork carrying a short glass outlet-tube, was used for the chromatographic separation. A plug of cotton wool was inserted above the cork to retain the adsorbent in position. The column was packed with Peter Spence's grade O dry alumina, gentle suction being applied at the lower end of the tube during the packing operation. The tube was tapped vigorously to ensure even packing and the upper surface of the adsorbent was maintained as nearly horizontal as possible. In all experiments the depth of alumina was 25 cm.

The alumina was wetted with 30 to 40 ml of light petroleum of boiling range 60° to 80° C until the solvent was within 1 cm of the upper surface of the adsorbent, when 50 ml

of a light petroleum solution containing 2 g of nitrobenzene and 0·02 g of *m*-dinitrobenzene was introduced. The chromatogram was developed with a mixture of benzene and light petroleum (1 + 9, v/v) and the eluate was collected in 10-ml fractions, which were examined qualitatively for nitrobenzene (characteristic odour) and *m*-dinitrobenzene by the acetone - sodium hydroxide colour reaction. Fractions 1 to 3 contained only light petroleum; nitrobenzene was present in the fractions that followed, but was not detected in fractions 15 to 17. None of these fractions contained any *m*-dinitrobenzene. Further development with the mixture of benzene and light petroleum to which 2 per cent. of ethanol had been added displaced the *m*-dinitrobenzene from the column as a sharp-fronted band. Dinitrobenzene was found in fractions 27 to 38, and fraction 27 contained a little nitrobenzene. Fig. 1 shows diagrammatically the qualitative conditions in the column (*a*) after elution of most

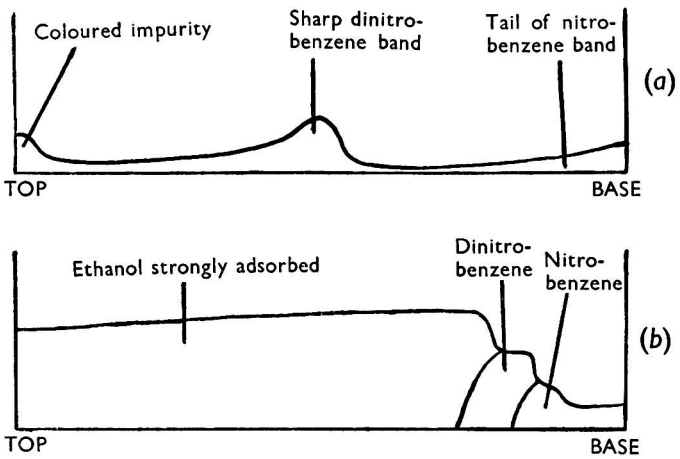

Fig. 1. Essential features of adsorption chromatogram (*a*) after elution with a mixture of benzene and light petroleum, and (*b*) after displacement development with a 2 per cent. solution of ethanol in a mixture of benzene and light petroleum

of the nitrobenzene with the mixture of benzene and light petroleum, and (*b*) after partial development with 2 per cent. of ethanol in the mixture of benzene and light petroleum. The solvent was removed from fractions 27 to 38 by careful evaporation on a water-bath and the residue was extracted with ethanol and analysed polarographically for *m*-dinitrobenzene. The recovery of *m*-dinitrobenzene was invariably low. The following modified procedure was therefore adopted.

After removal of nitrobenzene with the mixture of benzene and light petroleum, the column was allowed to drain overnight and placed horizontally over a sheet of paper. Slight pressure was applied to the top of the column so that by gentle tapping the alumina could be loosened and extruded in 10 sections, each approximately 2·5 cm in length. The individual sections were transferred to small beakers, dried at 100° C, and the dried portions of alumina were transferred to centrifuge tubes. Ten millilitres of ethanol were added to each tube, the contents were shaken vigorously, the alumina was allowed to settle and 0·5 ml of the supernatant liquid was withdrawn and tested for the presence of *m*-dinitrobenzene by means of the acetone - sodium hydroxide reaction. Those sections that gave a positive reaction were centrifuged and a 5·0-ml aliquot was withdrawn for quantitative analysis, either by the polarographic or by the absorptiometric method. In practice, *m*-dinitrobenzene was found to be concentrated in sections 6, 7 and 8. Sections 1 to 4 and section 10 could, therefore, be discarded. The polarographic examination of these sections showed that all the nitrobenzene was eluted from the chromatogram by treatment with the mixture of benzene and light petroleum.

POLAROGRAPHIC DETERMINATION OF *m*-DINITROBENZENE—

Polarographic base solution (*pH* 12)—Mix together 100 ml of *N* sodium hydroxide, 500 ml of 2 *N* ammonium chloride, 20 ml of 5 per cent. gelatin solution and 280 ml of distilled water.

Procedure—Solutions of *m*-dinitrobenzene (0·1 to 2·0 mg) in 5·0 ml of ethanol were diluted to 12·5 ml with the above base solution and polarograms recorded on the Cambridge instrument with a saturated calomel electrode as reference electrode and a dropping-mercury electrode with a drop-time of 3 seconds at 25° C (±0·5° C). At a sensitivity of 1/100 a diffusion current of 0·122 μA was found to be equivalent to 5·0 mg of *m*-dinitrobenzene per 25 ml of solution. The step height of the wave at −0·45 volt was directly proportional to the dinitrobenzene concentration for solutions in the range 5×10^{-5} *M* to 10^{-3} *M*, and was unaffected by ethanol concentrations up to 50 per cent. by volume. The precision of the polarographic method was found to be ±2 per cent. of the amount present for amounts of nitrobenzene between 0·5 and 2·0 mg, and ±5 per cent. of the amount present for amounts of dinitrobenzene between 0·1 and 0·5 mg. To cover this range the sensitivity was varied between 1/20 and 1/100, but at higher sensitivities the use of counter-current to oppose the *iR* current did not yield well-defined steps. For this reason such solutions (*i.e.*, those yielding only a pink coloration in the acetone - sodium hydroxide test) containing less than 0·1 mg of dinitrobenzene were, preferably, examined absorptiometrically.

ABSORPTIOMETRIC DETERMINATION OF *m*-DINITROBENZENE—

Five millilitres of ethanol extract, obtained after extrusion of the alumina, were added to 0·25 ml of 2·5 per cent. aqueous sodium hydroxide solution and the mixture was diluted to 25 ml with acetone to secure a homogeneous solution. The extinction was measured in a 4-cm cell with a Spekker photo-electric absorptiometer fitted with Ilford 605 and Calorex H 503 filters. The following figures show that maximum development of colour was attained after 5 minutes, and this time was used in the preparation of the calibration graph for amounts of *m*-dinitrobenzene not greater than 0·1 mg.

Time, minutes	1	3	5	7	8	10
Extinction	0·802	1·207	1·296	1·290	1·262	1·218

Beer's law was obeyed for concentrations not greater than 0·1 mg per 25 ml, for which the extinction × 0·073 = milligrams of dinitrobenzene per 25 ml, and certain triplicated points (0·02 and 0·07 mg) showed that an accuracy of ±3 per cent. is attainable in this range.

Finally, it is unnecessary to use both polarographic and absorptiometric techniques in the analysis of any given sample; for if an aliquot from one section of the chromatogram contained 0·5 mg and the adjacent section contained 0·05 mg, then even if the lesser amount can only be determined to ±10 per cent., the accuracy of the determination of the whole sample (*e.g.*, 2 g of nitrobenzene) will be adequate for most purposes. Table I summarises the results obtained polarographically for mixtures of 2·4 g of nitrobenzene with small amounts of *m*-dinitrobenzene.

TABLE I

POLAROGRAPHIC DETERMINATION OF *m*-DINITROBENZENE IN SYNTHETIC MIXTURES CONTAINING 2·4 g OF NITROBENZENE

Weight of DNB taken, mg	Section No.	Weight of DNB found in section, mg	Total weight of DNB recovered, mg
2·00	8	0·94	
	7	0·81	1·87
	6	0·12	
0·50	8	0·25	
	7	0·21	0·46
0·20	8	0·16	
	7	0·04	0·20
0·10	8	0·07	
	7	0·03	0·10

A dark brown sample of nitrobenzene gave, for sections 8 and 7, 1·01 and 0·35 mg of dinitrobenzene, respectively, *i.e.*, a total of 1·36 mg, and a duplicate determination on the same sample gave for sections 8, 7 and 6, 0·39, 0·79 and 0·15, *i.e.*, a total of 1·33 mg, or 0·057 and 0·055 per cent. for the duplicate determinations.

Method

Reagents—

Alumina—Peter Spence, grade O.
Sodium hydroxide solution, 2·5 per cent. w/v.
Benzene in light petroleum (boiling range 60° to 80° C), 20 per cent. v/v.
Polarographic base solution (pH 12)—Mix together 100 ml of *N* sodium hydroxide, 500 ml of 2 *N* ammonium chloride solution, 20 ml of 5 per cent. gelatin solution and 280 ml of distilled water.

Procedure—

Prepare an alumina column 25 cm long by 1·5 cm in diameter, clamp it vertically and wet the adsorbent with 25 ml of light petroleum (60° to 80° C). Add to the top of the column a weighed nitrobenzene sample (2 to 3 g) dissolved in 50 ml of light petroleum. Develop the chromatogram with 350 ml of the mixture of 20 per cent. of benzene in light petroleum and allow it to drain overnight. Hold the column over a sheet of white paper, apply slight pressure to the top and extrude it in 10 sections, each 2·5 cm in length. Transfer sections 5, 6, 7, 8 and 9 to small beakers and dry at 100° C for 15 minutes.

Place each dried section in a 20-ml test tube, cover with 10·0 ml of ethanol, stopper the tube, shake vigorously by hand and allow the alumina to settle. Transfer 0·5 ml of the supernatant ethanol from each section by pipette to another test tube, add 0·5 ml of 2·5 per cent. sodium hydroxide solution and 3·0 ml of acetone. Reject any sections that do not give a positive reaction and decide on the appropriate analytical procedure, *viz.*, polarographic if a distinct pink or magenta colour is produced, or absorptiometric if only a pale pink colour develops.

Polarographic procedure for more than 0·02 per cent. of *m*-dinitrobenzene—

Prepare a calibration graph from standard alcoholic solutions containing from 0·2 to 1·0 mg of *m*-dinitrobenzene per 5 ml diluted to 12·5 ml with the standard base solution. Record polarograms at 25° C, with a saturated calomel electrode as reference electrode and a dropping-mercury electrode with a drop time of 3 seconds and an appropriate galvanometer sensitivity, 1/100 or 1/20, with a Cambridge polarograph.

Procedure for the alcoholic extract—Centrifuge the alcoholic extracts that give a distinct pink colour. Take 5 ml of the clear extract by means of a pipette and dilute it to 12·5 ml with base solution. Record a polarogram in a manner identical to that described in the preceding paragraph and calculate the amount of nitrobenzene from the calibration graph.

Absorptiometric procedure for less than 0·02 per cent. of *m*-dinitrobenzene—

Prepare a calibration graph from alcoholic solutions containing from 0·02 to 0·1 mg of *m*-dinitrobenzene per 5 ml. To 5 ml of the solution add 0·25 ml of 2·5 per cent. w/v sodium hydroxide. Five minutes after mixing, measure the extinction through Ilford 605 and Calorex H 503 filters.

Procedure for the alcoholic extract—Transfer 5·0 ml of the clear extract by pipette to a stoppered 25-ml measuring cylinder, add 0·25 ml of 2·5 per cent. sodium hydroxide solution and dilute to 25 ml with acetone. Mix well and measure the extinction at 605 mμ in a 4-cm cell exactly 5 minutes after mixing. If $(a + b + c + d \ldots)$ mg of *m*-dinitrobenzene is found in successive extracts, then the combined alumina sections contained $2(a + b + c + d \ldots)$ mg. Hence, if the weight of sample taken is *W* g, the percentage of

$$m\text{-dinitrobenzene} = \frac{2(a + b + c + d \ldots)}{10W}.$$

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An Apparatus for Simplifying Titration in a Controlled Atmosphere

Application to the Determination of Moisture in Transformer Oil with Fischer Reagent

By R. H. PRINCE

The construction and use of equipment for carrying out titrations in controlled atmospheres is described in detail. It is especially suited to Fischer determinations of moisture, and such a determination of moisture in transformer oil is described; the apparatus is simpler than any other assembly for this determination. A comparison between titrations of moisture in *n*-butanol to the visual end-point in the apparatus described and to the dead-stop end-point in an apparatus of the type described by Bonner shows good agreement.

It is often necessary to carry out titrations in a "controlled" atmosphere, by which is meant that the atmosphere in the titration vessel must be free from some component that causes interference. For example, in titrations with chromous salts, which have recently been shown to be applicable to the rapid determination of oxygen in water and oxygen in gas mixtures,[1] oxygen must be rigidly excluded. Carbon dioxide must be excluded in the titration of concentrated solutions of strong bases with acids and also in the titration of weak acids. Procedures for estimations of this kind are simplified by use of the apparatus described below.

The apparatus was developed primarily for the estimation of moisture with Fischer reagent on a semi-micro and macro scale, but it should be applicable with slight modification to any titrations that must be made in a controlled atmosphere.

The advantages of the apparatus are that it is simple to construct, that the titration vessel and burette assemblies are not rigidly attached to one another, so that no special stand is required, and that sample volumes of from 10 to 250 ml may be titrated simply by interchanging titration vessels. For the Fischer estimation in particular, further advantages are that the titration vessels may be oven-baked free from moisture before a determination, that protection of the burette tip ensures that no decomposition of Fischer reagent occurs at the tip and that the apparatus can be used for both direct (visual) and dead-stop back-titrations; for these two procedures on the semi-micro scale, and with large liquid volumes, the apparatus described is simpler than that now in common use.

In the procedure, self-sealing vaccine cap closures (sometimes referred to as "serum-bottle sleeved caps") are used as seals for the titration vessels, in conjunction with a simple burette-tip shield. Pipettes for introducing liquid into the vessels are constructed with hypodermic syringe needles ground on to the tips.

Vaccine caps have been used extensively by Jenkins and Quick[2] for moisture estimations with the Fischer reagent. These caps were also used on the micro scale by Levy, Murtaugh and Rosenblatt[3] for the determination of moisture in penicillin sodium salt.

Recently, after the present work had been completed, Smith, Mitchell, and Billmeyer[4] have described the use of vaccine caps in the determination of organic acids, bases and esters on the semi-micro scale. Weight titrations were used by these authors; all reagents and samples were added from hypodermic syringes and weighed. Their paper also contains extensive references to the use of hypodermic equipment in analysis.*

The apparatus described below is particularly useful for the determination of moisture in liquid samples of very low moisture content—transformer oil, for example—when it is advisable to use as large a sample as possible.

*Menville and Henderson[5] have also recently described a procedure similar to the one outlined in this paper.

Apparatus

Titration assembly—

General—The apparatus is shown in Fig. 1 (*ii*); *d* is a 5-ml burette graduated in 0·01-ml divisions and calibrated by the British Standard Procedure.[6] The burette has an elongated tip with a shield, *e*, connected to a source of dry nitrogen at *h* (see "Drying of Nitrogen," p. 610). The contents of the burette are protected from atmospheric moisture by the guard tube, *c*, containing anhydrous magnesium perchlorate (anhydrone).

Fischer reagent (or standard water solution) can be transferred from the 1-litre reagent bottle, *g*, to the burette by applying pressure at *a* through a drying tube containing magnesium

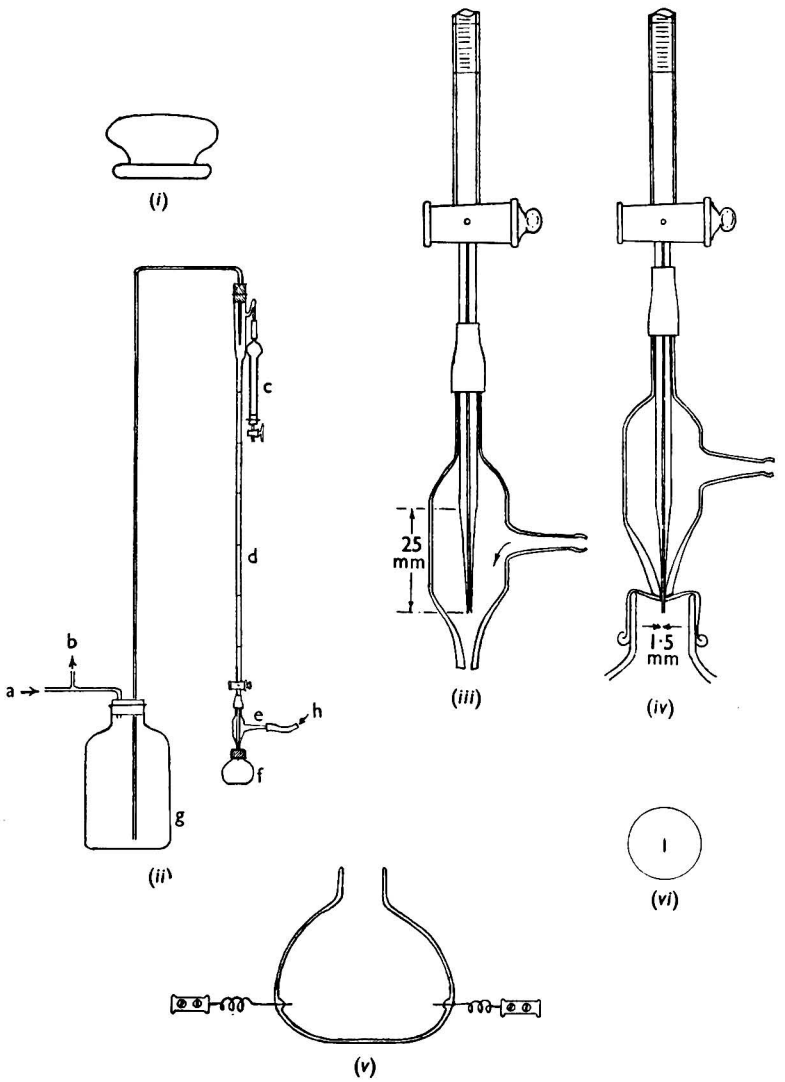

Fig. 1. Apparatus: (*i*) and (*vi*) vaccine cap; (*ii*) titration assembly; (*iii*) and (*iv*) details of burette tip shield; (*v*) titration vessel

perchlorate. When sufficient reagent has been transferred, pressure can be released by opening a tap connected to a similar drying tube at *b*. The titration vessel, *f*, is described below. The contents of the vessel can be stirred either by manual agitation, which is suitable for visual titrations, or by means of a magnetic stirrer for dead-stop electrometric titrations.

For back-titrations two burette assemblies are used, one for Fischer reagent, the other for standard water solution.

The burette tip assembly—Protection of the burette tip is necessary because of the extreme sensitivity of Fischer reagent to moisture. The importance of this is stressed in Mitchell and Smith's discussion[7] of visual macro methods for moisture determination with Fischer reagent. It is particularly important on the micro and semi-micro scale.

The tip assembly is shown in Fig. 1 (*iii*) and (*iv*), which include dimensions of the jet. Although the jet is more fragile than one with the conventional taper, the tip shield affords adequate protection. The shield is a sliding fit on the elongated stem of the jet, to which it is attached by a piece of rubber tubing. A small amount of silicone grease applied to the

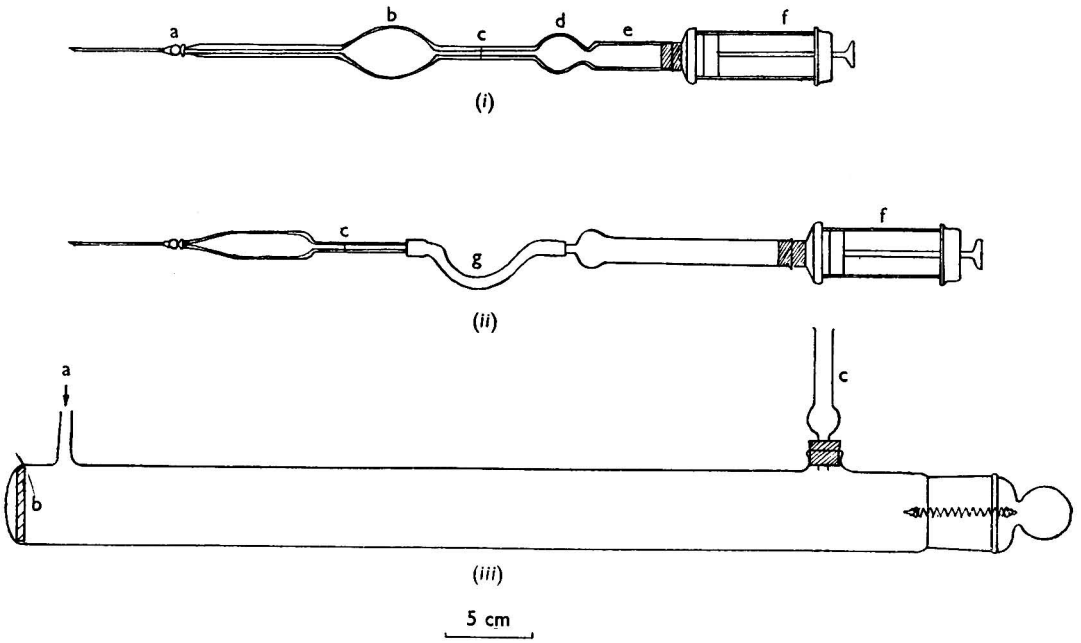

Fig. 2. Pipettes, (*i*) rigid type and (*ii*) non-rigid type; (*iii*) tubular desiccator for rigid type of pipette

stem facilitates operation. The dimensions of the shield are not critical, but the wall of the lower portion is thickened to about 2 mm so that when the burette is in use, as in Fig. 1 (*iv*), an effective seal is formed. During titration, as in Fig. 3 (*vi*), the flow of dry nitrogen through the side-arm, *e*, is stopped.

The liquid in the burette can, when necessary, be adjusted to the zero mark either by allowing liquid to flow from the shield when in the position shown in Fig. 1 (*iii*), when the nitrogen carries the liquid away and rapidly dries the orifice, or by raising the shield to the position shown in Fig. 1 (*iv*) and immediately lowering the shield after adjusting the liquid volume.

The titration vessels—Titration vessels are of the squat shape shown in Fig. 1 (*v*), chosen because it minimises the amount of splashing when the reagent is added and holds the maximum amount of liquid for its height. Vessels with volumes ranging from 80 to 280 ml, with and without sealed-in platinum electrodes, were constructed. They may be dried by heating in an oven at 120° C and cooling in a desiccator over anhydrone.

Vaccine caps—The vessels are closed with vaccine caps of the type shown in Fig. 1 (*i*). These must be made of unfilled, translucent latex, as filled latex does not form a good seal after penetration. The cap is prepared for use by making an incision about 2 mm long, as in Fig. 1 (*vi*); this can be done with a razor blade or scalpel. If an unfilled latex cap is used the incision in no way impairs the efficiency of the cap as a seal. The caps may be used for many titrations and are inexpensive.

Pipettes—Two types of pipette have been used, as shown in Fig. 2. Type (*i*) is rigid and consists of a hypodermic syringe needle, *a*, ground on to the stem of a pipette constructed

from 2-mm capillary tubing. Capillary tubing is used to improve accuracy and to avoid the need for keeping the pipette vertical when measuring a volume of liquid. A mark, *c*, is etched at the required volume calibration. The bulb, *d*, is necessary to prevent methanol accidentally reaching the desiccant (anhydrone) in *e*. The pipette is connected to a 20-ml hypodermic syringe, *f*, by means of a rubber bung. Pipettes to deliver 10 or 15 ml were constructed and calibrated, and deliveries were found to be reproducible to $\pm 0{\cdot}01$ ml. A pipette is shown in use in Fig. 3 (*iii*).

Type (*ii*) is a modification of a pipette developed by Jenkins and Quick[2]; the body is connected by rubber tubing to the drying tube containing anhydrone. The pipette is operated by withdrawing the piston of the syringe, *f*, when liquid rises into the bulb and into the capillary stem. When the liquid rises above the calibration mark, *c*, the rubber tubing, *g*, is pinched with the fingers and the syringe, *f*, is removed. The liquid meniscus is allowed to fall to the calibration mark; the liquid in the pipette is then introduced via the vaccine cap into the titration vessel by releasing the rubber tubing. Air displaced from the vessel passes through a drying tube connected to a hypodermic needle in the manner shown in Fig. 3 (*iv*).

The second type of pipette has the advantage that it is readily accommodated in a conventional desiccator when not in use. The pipette shown in Fig. 2 (*i*) is kept in the desiccator shown in Fig. 2 (*iii*); dry nitrogen passes into the desiccator at *a* and out through the drying tube *c*. A cork pad *b* is kept in the desiccator to prevent possible breakage of the bottom of the desiccator when inserting the pipette.

NOTE—All items of apparatus including titration vessels, pipettes, vaccine caps, magnetic stirrer rotor and syringes are kept in a desiccator over magnesium perchlorate when not in use.

DRYING OF NITROGEN—

It was observed in titrations with a double-burette apparatus based on that described by Bonner[8] and with a phosphorus pentoxide drying train, that the initial results were erratic when the apparatus had been out of use for several hours with the nitrogen passing. After two or three titrations in the vessel, results again become normal. This behaviour may have been due to slow volatilisation of phosphorus trioxide usually present in small amounts in the pentoxide. This trioxide can be removed only with difficulty.[9] When nitrogen has been passing for some time, the trioxide could accumulate in the titration vessel and interfere in the moisture estimation. For this reason it is advisable to use magnesium perchlorate as the drying agent.

ELECTRICAL EQUIPMENT USED FOR OBSERVATION OF THE DEAD-STOP END-POINT—

A Pye "Scalamp" moving-coil galvanometer (92 scale divisions per micro-ampere; coil resistance, 360 ohms; scale with centre zero $\pm$ 75 divisions) modified by the inclusion of a 0·1-megohm resistor in series with the coil was found satisfactory. A potential of 20 millivolts was applied to the electrodes by means of a Cambridge potential divider, termed by the manufacturers[10] a "potential source."

PREPARATION OF REAGENTS

Chloroform—Dry chloroform is required in the procedure described below for estimating moisture in transformer oil; this procedure is based on that of Acker and Frediani.[11] The chloroform is dried as follows. Place about 1½ litres of chloroform, B.P., in a 2-litre flask and add 50 g of anhydrone. Tightly stopper the flask and set it aside for 2 days with occasional shaking. After this treatment the chloroform should contain less than 15 p.p.m. of water. Decant the chloroform into a dry 1-litre flask fitted with a B24 curved adaptor, like that shown in Fig. 3 (*ii*), and sealed with a vaccine cap to permit withdrawal of chloroform with the pipettes described.

Methanol—Methanol was dried with freshly cut magnesium ribbon and iodine. Place 4 to 5 g of magnesium ribbon cut into lengths of about 1 cm in a flask and add 100 ml of methanol and about 0·5 g of iodine. Heat the flask under a reflux condenser until the iodine dissolves. Add 1 litre of analytical reagent grade methanol and continue to heat under the reflux condenser for a further half hour. Distil the methanol through an 18-inch vacuum-jacketed column packed with Fenske rings and collect the fraction boiling at 64° to 65° C.

Methanol prepared in this way contained less than 0·03 per cent. of water. It was collected and kept for use in a flask as shown in Fig. 3 (*i*). When portions were required

for titrations, the flask was placed in the position shown in Fig. 3 (*iii*), in which *b* is a piece of wood with a U-shaped groove to support the neck of the flask.

Pyridine—Impurities in pyridine accelerate decomposition of Fisher reagent.[12] Impurities were removed by distillation through a Widmer column and collection of the fraction

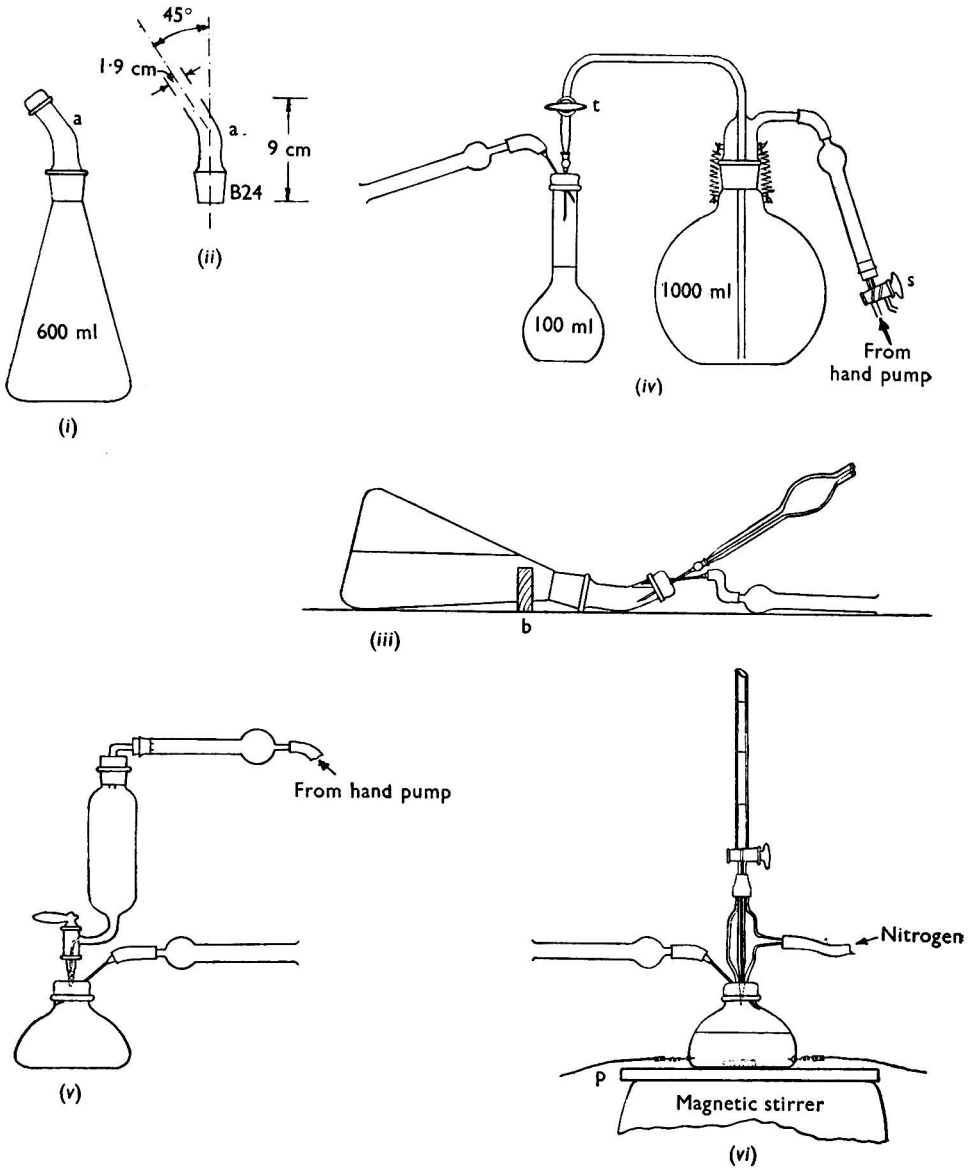

Fig. 3. Apparatus in use for Fischer determination of moisture: (*i*) and (*ii*) flask and adaptor for containing dried distilled methanol; (*iii*) removal of methanol without ingress of moisture; (*iv*) preparation of a standard solution of water; (*v*) injection of transformer oil into titration vessel; (*vi*) titration assembly in use

boiling in the range 114° to 116° C. After a single fractionation the sample contained less than 0·3 per cent. of water.

Fischer reagent—This was prepared from methanol and pyridine purified as described above, sulphur dioxide and resublimed analytical reagent grade iodine. It had the following composition: 196 g of resublimed iodine; 533 ml of pyridine; 1330 ml of dry methanol;

45 ml of liquid sulphur dioxide. This reagent has a water equivalent of about 2 mg of water per ml.

Standard water solution—This solution, used for the back-titration of Fischer reagent, was prepared by adding about 1·75 ml of distilled water to 1 litre of dried methanol.

Procedure

Standardisation of standard water solution—

The water solution is standardised in the usual manner with the aid of a portion of methanol of known titre against Fischer reagent to which has been added a weighed amount of water. The apparatus for preparing this water-in-methanol standardisation solution is shown in Fig. 3 (*iv*). A quantity of water is weighed into a dried 100-ml flask, sealed with a vaccine cap, from a Lunge-Rey pipette equipped with a ground-on hypodermic needle. The flask is then filled to the graduation mark from the litre flask containing methanol dried as described above. Pressure is applied by means of a hand-pump: when the methanol reaches the mark, tap *t* is turned off and the flask (A) is removed.

A second flask (B), into which no water has been introduced, is filled with dried methanol in the same way. The pressure in the litre flask is then released by turning tap *s* to connect the flask with the atmosphere via the drying tube.

The water equivalent of the standard water solution is then determined in the conventional way[13] by taking 15-ml aliquots of the solutions from flasks A and B by the method illustrated in Fig. 3 (*iii*). The standard water solution so prepared should have a water equivalent of 2 to 2·5 mg of water per ml.

Determination of moisture in transformer oil—

The procedure is based on the work of Acker and Frediani,[11] who found that in presence of chloroform, transformer oil is miscible with methanol over a wide range of composition. The solvent composition suggested by them, a mixture of 4 volumes of chloroform with 1 volume of methanol, has been found satisfactory.

Using one of the titration vessels closed with a cap and titrating to the "dead-stop" end-point, titrate about 4 ml (*p* ml) of Fischer reagent with standard water solution (S.W.S.). Let *a* ml of S.W.S. $\equiv$ *p* ml of Fischer reagent. Refill the burettes.

Fix a vaccine cap, in which an incision has already been made, to a titration vessel of about 280 ml capacity equipped with platinum electrodes and containing the stirrer rotor. Transfer to the flask, with a 20-ml hypodermic syringe, about 100 ml of dried methanol and 25 ml of dried chloroform. Mix thoroughly by means of the magnetic stirrer. Place a hypodermic needle carrying a guard tube through the vaccine cap, rest the top of the vaccine cap against the orifice of the burette tip shield and raise the vessel into the position shown in Fig. 1 (*iv*). Add an excess of Fischer reagent and lower the vessel and shield to the original position.

In the same way, transfer the vessel to the burette containing the standard water solution. This burette is fixed above the magnetic stirrer as in Fig. 3 (*vi*): the vessel is kept raised in position by placing the porcelain plate, *p*, in the position shown.

Connect the electrodes and the galvanometer to the potential source in the usual way.[14] Set the galvanometer selector switch to the most sensitive position, switch on the stirrer and add standard water solution until the solution in the vessel is a pale orange-brown colour. Continue the addition dropwise until the light spot of the galvanometer just returns to the "1" position on the scale. No readings need be taken up to this stage. Remove the titration vessel while lowering the tip shield as described above.

Add 30 to 40 g (*W* grams) of the oil sample from a weighed Ripper burette by inserting the tip through the slit in the vaccine cap and expelling the oil by means of a hand pump connected to the burette through a drying tube, as in Fig. 3 (*v*).

Stir the mixture with the magnetic stirrer until the transformer oil has dissolved. Then, by the procedure just described, add a measured volume (*b* ml) of Fischer reagent, stir, and again titrate with standard water solution (*c* ml) until the light spot returns to the "1" position on the galvanometer scale.

Calculation—

Let the water equivalent of the standard water solution be *Z* mg per ml.

Then the proportion of water in the transformer oil $= \left(\frac{ba}{p} - c\right)\frac{Z}{W} \times 100.$

Results

A typical set of results is shown in Table I. Determinations 1 to 3 were made on the same sample of transformer oil. Determination 4 was made on a portion of the same sample which had been transferred to another bottle and had been exposed to the atmosphere for a considerable time.

Table I

Moisture content of transformer oil

Determination	1	2	3	4
Weight of sample, g	29·2	38·4	25·3	39·8
Water, per cent. w/w	0·0033	0·0034	0·0038	0·0051

Water in four samples of *n*-butanol was determined by direct titration with the apparatus described, the end-point being observed visually, and also in an apparatus of the type described by Bonner,[8] the dead-stop end-point being used. The reagents used were as described above, and 5-ml portions of the alcohol were taken with the pipettes described in this paper and titrated in presence of 15 ml of dried methanol of known moisture content. The two methods give similar results, the moisture contents of the four samples being determined as 0·48, 0·42, 0·44 and 0·48 per cent. w/v by direct titration in the apparatus described, and 0·47, 0·42, 0·43 and 0·47 per cent. w/v, respectively, by the dead-stop end-point titration.

Conclusions

The apparatus is simple to use for visual titration on a semi-micro scale, and is simpler than any other assembly for the determination of moisture in transformer oil. It is suitable for general use in determinations with Fischer reagent both by direct and by back-titration, being simpler and more flexible than the visual micro apparatus described by Mitchell and Smith[15] and simpler, for back-titration, than the double-burette apparatus based on that described by Bonner.[8]

The author wishes to express his thanks to Mr. G. C. Goode for carrying out experimental work with the apparatus, to Mr. F. Bailey for construction of the various items of glassware, and to the management of British Nylon Spinners Limited for permission to publish this paper.

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February 18th, 1953

The Determination of Copper in Plant Material

By W. A. FORSTER

In the presence of the ammonium salt of ethylenediaminetetra-acetic acid, copper can be determined colorimetrically as the diethyldithiocarbamate complex without interference from aluminium, cobalt, iron, manganese, nickel, zinc, calcium, magnesium and phosphorus. The method is rapid, and analyses of 24 plant digest solutions by the new method and by a dithizone procedure were in close agreement.

Šedivec and Vašák[1] have described the use of disodium ethylenediaminetetra-acetate (sodium salt of EDTA) to obviate interference from cobalt, iron, nickel and manganese in the determination of copper by means of sodium diethyldithiocarbamate. In their method two extractions of the copper diethyldithiocarbamate complex with ethyl acetate are required, and the extract, made up to a definite volume, has to be filtered before the colour is measured.

In the method described below, the ammonium salt instead of the sodium salt of EDTA is used to obviate interferences and carbon tetrachloride is used as extractant. A single extraction gives reproducible results and the extract can be clarified by inserting a plug of cotton wool in the stem of the filter funnel. The ammonium salt of EDTA has the added advantage of buffering the solution.

Method

As EDTA forms complexes with copper and the other metals in the solution, a fairly large excess of sodium diethyldithiocarbamate must be added. Table I shows Spekker absorptiometer readings for 40-μg copper standards treated with a solution of the ammonium salt of EDTA and various amounts of sodium diethyldithiocarbamate and extracted with carbon tetrachloride.

Table I

Effect of excess of sodium diethyldithiocarbamate on absorptiometer readings for 40 μg of copper

1-cm cells, Ilford No. 601 violet filters and a water setting of 0·7

Amount of 1 per cent. sodium diethyldithiocarbamate solution added, ml	0·5*	1	2	2·5	3
Spekker absorptiometer readings (corrected)	0·282, 0·237	0·087	0·068	0·072	0·073

* Added as 5 ml of 0·1 per cent. solution.

In subsequent experiments, 2·5 ml of 1 per cent. diethyldithiocarbamate were used.

Reagents—

Standard copper solution—Dissolve 0·1964 g of copper sulphate ($CuSO_4.5H_2O$) in water. Acidify with 1 ml of 98 per cent. sulphuric acid and dilute to 250 ml. This solution contains 200 μg of copper per ml. Prepare a working standard containing 8 μg of copper per ml daily as required.

Ammonium ethylenediaminetetra-acetate—Suspend 125 g of ethylenediaminetetra-acetic acid in water, add 100 ml of concentrated ammonia solution, sp.gr. 0·880, and when dissolved filter and dilute to 500 ml.

Concentrated ammonia solution, about 14 N—Pass ammonia gas into glass-distilled water.

Sodium diethyldithiocarbamate solution—A filtered, freshly prepared, 1 per cent. w/v solution.

Carbon tetrachloride—Redistilled B.P. reagent.

PROCEDURE—

Digest the dried plant material with nitric and perchloric acids[2] and, by means of a pipette, transfer a suitable aliquot into a 125-ml pear-shaped Pyrex-glass separating funnel (round separating funnels are unsuitable as efficient extraction cannot be attained). Add 5 ml of the EDTA solution and 3 drops of phenolphthalein, and then concentrated ammonia solution to give a distinct pink colour. Dilute with water to approximately 60 ml, add 2·5 ml of diethyldithiocarbamate solution and then 10 ml of carbon tetrachloride. Dry the stem of the funnel with filter-paper, insert a small plug of absorbent cotton wool, and shake the funnel vigorously for 2 minutes. As the colour of a solution of the copper diethyldithiocarbamate complex in carbon tetrachloride fades rapidly in sunlight,[3] it is advisable to transfer the extract at once to a 1-cm cell and measure the colour on the absorptiometer, with violet Ilford No. 601 filters and a water setting of 0·7. Calibration of a Spekker absorptiometer used for these analyses gave results conforming to the specifications suggested by Taylor, Williams and Dreblow.[4]

As reference method, Sandell's procedure for the determination of copper in biological materials[5] is recommended, with the exception that 5 ml instead of 1 ml of ammonium citrate should be added to the final solution from which the copper diethyldithiocarbamate is extracted.

In both the EDTA and Sandell's methods, the range is from 0 to 40 μg of copper and the graph shows slight deviation from linearity. Curvature is slightly less in the EDTA method, and the same amounts of copper produce slightly lower absorptiometer readings in this method than in Sandell's method.

For the amounts of copper for which the method was devised, 0·0 to 40 μg, a single extraction with carbon tetrachloride was found to be sufficient. For larger amounts of copper, more extractions may be necessary.

RESULTS

Table II shows the results obtained when various metals and phosphorus (as potassium dihydrogen phosphate) were added to 40 μg of copper standards analysed by the EDTA method. It is evident that none of the usual constituents of plant ash interferes in the determination of copper by the proposed method.

TABLE II

EFFECT OF PHOSPHORUS AND METALS ON THE DETERMINATION OF 40 μg OF COPPER BY THE PROPOSED METHOD

Constituents added to solution	Copper recovered, μg	Copper recovered when 10 mg of phosphorus was also present, μg
Aluminium, 600 μg	40·3	39·9
Cobalt, 600 μg	39·7	39·8
Iron, 600 μg	39·9	39·9
Manganese, 600 μg	40·2, 39·9	40·0, 39·9
Nickel, 600 μg	40·1	39·9
Zinc, 600 μg	40·1	40·2
Calcium, 25 mg	39·6	39·6
Magnesium, 25 mg	40·2	39·9
Calcium and magnesium, 25 mg of each	39·6	39·7
Aluminium, iron, manganese, nickel and zinc, 600 μg of each	40·3	—
Aluminium, cobalt, iron, manganese, nickel, zinc, 600 μg of each + 25 mg of both calcium and magnesium	—	39·8

Three aliquots were taken of each of 24 plant digest solutions (solutions 1 to 6 were tomato and 7 to 24 oat). One aliquot (*a*) was analysed by Sandell's method, the other two by the EDTA method—(*b*) without added copper, and (*c*) with the addition of 20 μg of copper. The results, shown in Table III, indicate that analyses by the two methods are in close agreement, and that added copper is recovered quantitatively.

TABLE III

DETERMINATION OF COPPER IN PLANT DIGEST SOLUTIONS

Solution	Copper content (a) Sandell's method, μg	(b) EDTA method, μg	(c) EDTA method, μg	Copper recovered, μg
1	9·8	9·8	29·8	20·0
2	11·0	11·0	31·0	20·0
3	11·0	11·0	30·8	19·8
4	14·7	14·7	34·9	20·2
5	11·7	11·6	31·7	20·1
6	16·5	16·5	36·7	20·2
7	12·7	12·8	32·8	20·0
8	12·8	13·0	33·3	20·3
9	14·3	14·3	34·3	20·0
10	13·5	13·5	33·8	20·3
11	13·7	13·8	33·8	20·0
12	14·2	14·3	34·3	20·0
13	28·8	29·2	—	—
14	10·8	11·0	30·8	19·8
15	11·2	11·3	31·3	20·0
16	11·5	11·6	31·8	20·2
17	13·5	13·9	34·0	20·1
18	12·3	12·8	32·5	19·7
19	13·8	13·8	33·9	20·1
20	13·8	13·8	33·8	20·0
21	12·8	12·8	32·6	19·8
22	12·3	12·3	32·3	20·0
23	12·7	12·6	32·8	20·2
24	16·8	16·3	36·7	20·4

Mean difference (a) − (b) = −0·06 μg of copper (Standard Error 0·04)
Mean recovery of added copper (c) − (b) = 20·05 μg of copper (Standard Error 0·04)

The author is indebted to Dr. J. T. Martin for his criticism of this paper, and to Mr. D. Akenhead, Director, Commonwealth Bureau of Horticulture and Plantation Crops, for very kindly supplying a translation of the paper by Šedivec and Vašák.

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AGRICULTURAL RESEARCH COUNCIL UNIT OF
PLANT NUTRITION (MICRONUTRIENTS)
LONG ASHTON RESEARCH STATION
BRISTOL

February 16th, 1953

ERRATUM: September (1953) issue, p. 551, title and lines 13 and 17 of synopsis; p. 552, line 9. *For* "esters" *read* "ethers."

Notes

THE DETERMINATION OF ADDED HEXAMETHYLENETETRAMINE IN TWO-STAGE PHENOL - FORMALDEHYDE RESINS

In the preparation of two-stage phenol - formaldehyde resins (Novolaks), a molecular excess of phenol over formaldehyde is used, the condensation reaction being stopped when the resin is fusible and completely soluble in alcohol. Such resins are not cured by application of heat, as they are deficient in formaldehyde. It is usual to introduce the formaldehyde into the resin by incorporating hexamethylenetetramine (hexamine), and then, on application of heat, the resin is converted to the infusible and insoluble state. The proportion of hexamine present can affect the degree of cure, and its determination is an essential preliminary to utilisation of the resin.

Methods for the determination of hexamine have been reported[1,2,3] that depend on the decomposition of hexamine and the subsequent determination of the liberated ammonia or formaldehyde. It was considered that a more rapid titrimetric method could be devised.

Experimental

Hexamine, which is a weak base, can be titrated with a strong acid. In one method the hexamine is extracted with water, filtered to remove the resin and determined in the aqueous solution with acid. Owing to the finely divided nature of the resin, filtration is protracted. A conductimetric method of titration affords more rapid estimation, as filtration can be avoided.

Substances used—

Hexamine of 99·9 per cent. purity and two phenol - formaldehyde resin powders, which we shall call I and II, at different degrees of condensation and not containing added hexamine, were used for testing. Resin I was more highly condensed than resin II. Of resin I, 97·8 per cent. passed through a 200-mesh sieve and of resin II, 98·2 per cent. The average particle sizes of resins I and II were 21·7 and 19·2 μ, respectively, as measured by a light absorption method. The amount of "free phenol" in resin I was 3·73 per cent. and in II was 5·56 per cent. These values were obtained by bromination of the water extract of the resins and so must be regarded as upper limits for the "free phenol" content. The hexamine all passed through a 100-mesh sieve.

Procedure—

Weighed quantities of hexamine were added to given amounts of resin to give concentrations ranging from 9 to 4 per cent. approximately; the amount of hexamine was measured conductimetrically.

Powdered resin, 2·5 g for hexamine contents greater than 6 per cent. and 5·0 g for contents less than 6 per cent., was mixed with a weighed quantity of hexamine and made up to 500 ml with water in a calibrated flask. An additional 2·0 ml of water for 2·5-g and 4·0 ml for 5·0-g amounts of resin was run into the flask from a pipette to allow for the volume occupied by the resin. The flask was then shaken and the solution set aside for 10 minutes, to enable the resin fines to settle; consequently very little solid was transferred to the titration beaker. Hexamine dissolves readily in the cold. The conductimetric circuit consisted of two platinum electrodes, 5 mm square, placed about 1 cm apart in a 250-ml beaker and connected through a milliameter to the secondary of a mains transformer giving 6·3 volts at 50 cycles per second. The distance apart of the electrodes, although not critical, must not be altered during the course of a titration. The use of alternating current and the limiting of current flowing to between 0 and 10·0 milliamperes prevented electrolysis of the solution and polarisation of electrodes. Current readings were easily estimated to within 0·01 of a milliampere with the A.C. milliammeter used. The paddle-type glass stirrer used was run continuously during titrations. The solution was titrated with 0·01 *N* acid previously standardised against alkali that had been standardised, in turn, against potassium hydrogen phthalate. A 50-ml aliquot of the hexamine solution was transferred to the beaker by means of a pipette. The stirrer was started, the current switched on and a zero reading taken on the milliammeter. A value of 0 to 0·4 mA was usually observed. Successive amounts of acid were added—2-ml portions before the end-point and 0·5 ml after the end-point were found to be satisfactory—and the current reading was recorded after each addition. The current was only switched on, momentarily, whilst a reading was taken. The observed value of the current had to be corrected each time owing to the change in volume of the solution. The observed value of

the current was multiplied by $(V + v)/V$, where V is the initial volume (50 ml) and v the amount of 0·01 N acid added. A typical set of readings is shown in Table I.

TABLE I

CONDUCTIMETRIC TITRATION OF HEXAMINE WITH ACID

Volume (v) of 0·01 N acid added, ml	Current (i) observed, mA	Corrected current $i(V + v)/V$, mA	Volume (v) of 0·01 N acid added, ml	Current (i) observed, mA	Corrected current $i(V + v)/V$, mA
0	0·26	0·26	16·00	3·30	4·35
1·00	0·44	0·45	16·50	3·48	4·56
3·00	0·85	0·90	17·00	3·70	4·96
5·00	1·23	1·35	17·50	3·90	5·27
7·00	1·60	1·82	18·00	4·09	5·56
9·00	1·95	2·30	18·50	4·28	5·86
11·00	2·25	2·76	19·00	4·50	6·21
13·00	2·63	3·22	19·50	4·72	6·56
15·00	3·05	3·97	20·00	4·92	6·88

The graph of corrected current against amount of titrant was plotted and the end-point of the titration determined from it. Fig. 1 is a typical graph for a strong-acid and weak-base system.

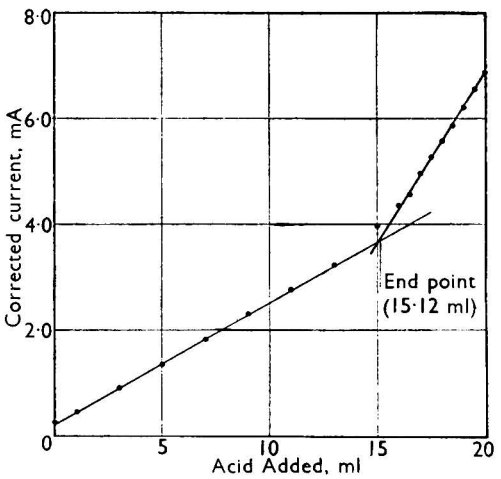

Fig. 1. Typical graph of conductimetric titration of hexamine

Points plotted near the end-point show a tendency to deviate from the linear relationship governing the alkaline and the acid part of the graph, *e.g.*, at 15·00 ml. With the titre found for the hexamine solution, the percentage recovery was calculated. One millilitre of 0·01 N acid is equivalent to 0·001402 g of hexamine. The results are shown in Table II. Each result is the average of three titrations.

TABLE II

RECOVERY OF HEXAMINE

Resin	Amount of resin plus hexamine, g	Amount of hexamine used, g	Amount of hexamine recovered, g	Hexamine in resin, %	Hexamine recovered, %
I	2·7487	0·2487	0·2493	9·05	9·07
I	2·7474	0·2474	0·2476	9·00	9·01
I	2·7169	0·2169	0·2166	7·98	7·97
I	2·7023	0·2023	0·2023	7·49	7·49
II	5·2561	0·2561	0·2553	4·88	4·86
II	5·2676	0·2676	0·2674	5·08	5·08
II	5·1984	0·1984	0·1983	3·82	3·82
II	5·1946	0·1946	0·1941	3·75	3·74

EFFECT OF "FREE PHENOL"—

It was considered that "free phenol" in the resin might lead to inaccuracies in the titration, so a solution of hexamine of known strength was made up and the titration results investigated for 25-ml aliquots containing various added amounts of a phenol solution. These amounts gave phenol contents corresponding to 1 to 6 per cent. of phenol in the powdered resins used. The solution to be titrated was each time made up to 50 ml with a measured amount of water. The results in Table III show that "free phenol," present at percentages covering the range experienced in the resins, does not affect the titration of the hexamine. The agreement found in Table II between percentage hexamine added and recovered also leads to the same conclusion.

TABLE III

EFFECT OF FREE PHENOL ON TITRATION RESULTS

Titre*, ml	Phenol, %
15·15	0
15·15	1
15·13	2
15·15	3
15·10	4
15·10	5
15·15	6

* Average of three titrations.

An interesting comparison of methods was afforded on applying the Kjeldahl method with selenium powder as catalyst. Digestions could be completed in 1½ to 2 hours. The results gave a 99·9 per cent. recovery for hexamine from resin at the 10 per cent. level and 99·8 per cent. at the 6 per cent. level. This is of the same order of accuracy as the conductimetric method, but it takes at least 2 hours more for a duplicate determination.

We wish to thank Brotherton & Co Ltd. for the gift of a sample of 99·9 per cent. hexamine and the Directors of Norton Grinding Wheel Company Limited for permission to publish this note.

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March 10th, 1953

A MODIFIED PROCEDURE FOR THE QUANTITATIVE BROMINE ABSORPTION OF OILS AND FATS

DURING an investigation into the properties, identification and estimation of stearolic acid, attention was paid to the direct gravimetric determination of the bromine absorption of oils and fats. This determination was first suggested by Becker,[1] and was later studied and modified by other workers.[2,3,4,5,6] As they have shown, the method is to be recommended for its speed and simplicity.

The modification suggested by Toms[3] is the one most widely used, but in our investigation it was found to suffer from the disadvantages (*a*) that only 0·02 to 0·03 g of the oil is taken for the determination, so that an error of ±0·0001 g in any one weighing would produce an error of about ±0·75 in the calculated iodine value and (*b*) that the thin film sometimes agglomerates into droplets on exposure to bromine, so extending the time required for the subsequent removal of bromine by warming to 2 to 3 hours, during which time the possibility of substitution occurring is greatly increased, also prolonging the time taken for the determination. These disadvantages make the method unsuitable for routine work in most laboratories.

It was desirable, therefore, (*a*) to take a larger quantity of oil for the determination to reduce the effect of weighing errors, (*b*) to stir the oil during the removal of bromine to assist its expulsion,

and (*c*) to avoid heating during the determination in order to reduce the risk of substitution. The following method was evolved.

METHOD—

Reagent—Bromine, A.R.

Procedure—Weigh accurately 0·2 to 0·3 g of the oil in a 2½-inch watch-glass containing a short glass rod, spreading the oil over the surface of the watch-glass. Place the watch-glass and its contents under an inverted glass dish upon a glass or porcelain base. Also place under the dish a small crucible containing 3 to 4 ml of bromine. After 30 minutes, remove the watch-glass, stir its contents at room temperature (in our laboratories 25° to 35° C) until the bromine has evaporated, and re-weigh the watch-glass and contents. The increase in weight is due to the bromine absorbed.

$$\text{Hence, the calculated iodine value} = \frac{\text{Increase in weight}}{\text{Weight of sample}} \times \frac{126{\cdot}9}{79{\cdot}9}.$$

Mostly the calculated iodine values agree well with those found by Wijs' method. However, as Toms showed,[3] the bromometric method gives much higher results for tung oils, owing to the presence of conjugated double bonds. A comparison of results by the above method and by Wijs' method is shown in Table I.

TABLE I

CALCULATED IODINE VALUE

	Bromometric method	Wijs' method
Oleic acid	88·7	89·1
Elaidic acid	88·2	89·3
Olive oil	87·3	86·4
Cottonseed oil	104·5, 105·8	109·5
Sesame oil	113·2, 110·3	108·2

STEAROLIC ACID ($CH_3(CH_2)_7C{:}C(CH_2)_7COOH$)—

Our investigations have shown that for stearolic acid the bromometric method also gives a much higher calculated iodine value than is given by Wijs' method. However, when the method described above is used, the bromine absorption is incomplete and the results are variable. There is a rapid absorption of two equivalents of bromine, followed by a much slower absorption of a further two equivalents of bromine. After this, further absorption of bromine occurs with the liberation of hydrogen bromide and separation into two phases. The weight then decreases and finally becomes constant at a value corresponding to the absorption of five equivalents of bromine, after which no further absorption of bromine occurs.

The following modifications of the bromometric procedure were found necessary to attain a reproducible calculated iodine value in the presence of stearolic acid—

(*i*) The time of exposure to bromine vapour must be increased to 24 hours.

(*ii*) After removal of the bromine by stirring, the watch-glass and contents must be placed in an oven at 110° C for 30 minutes, after which the bromination product should be a clear oil.

Stearolic acid gave the following figures—

Calculated iodine value (bromometric method): 219·1, 221·9 (theoretical value for absorption of five equivalents: 226·3).

Calculated iodine value (Wijs' method): 89·3 (theoretical value for absorption of two equivalents: 90·6).

Comparisons of the calculated iodine values of mixtures containing stearolic acid by Wijs' method and the bromometric method are made in Table II.

It can be seen that results by the bromometric method are lower than would have been expected, but in the absence of a better method, the results give a rough guide to the amounts of stearolic acid present.

It should be remembered, however, that this method does not specifically indicate the presence of stearolic acid, and care should be taken not to confuse the acid with acids containing conjugated double bonds[3] or "oxidised" acids,[3,6] which also react with bromine vapour.

It has been suggested[6] that the "oxidised" acids might contain hydroxyl groups. To test the effect of hydroxyl groups on the bromine vapour method, the bromine absorption of tetrahydroxystearic acid (m.p. 173° C) was determined after exposure to bromine for 30 minutes and for 24 hours. The iodine value of tetrahydroxystearic acid calculated from these bromine

TABLE II

ESTIMATION OF STEAROLIC ACID BY A COMBINATION OF WIJS' AND BROMOMETRIC METHODS

		Mixture		
		1	2	3
Composition of mixture	Stearolic acid, per cent. ..	25	30	50
	Oleic acid, per cent.	25	60	30
	Stearic acid, per cent.	50	10	20
Iodine value by bromometric method	Found	73·2	114·8	134·2
	Calculated*	77·2	119·2	136·6
Iodine value by Wijs' method	Found	45·3	80·2	71·5
	Calculated*	44·6	80·3	71·3
Estimated amount of stearolic acid†, per cent.		21·5	26·6	48·2

* The calculated figures are those expected from determinations of the iodine values of the component acids.

† The estimated amount of stearolic acid $= 100(B - W)/(B_s - W_s)$ per cent., where B = iodine value (bromometric method), W = iodine value (Wijs' method), B_s = value of B for stearolic acid = 220, W_s = value of W for stearolic acid = 90.

absorptions was 1·4 after 30 minutes and 70·2 after 24 hours. During the reaction, hydrogen bromide was liberated and separation into two phases occurred.

It is evident that this particular compound interferes with the analysis, although with the shorter reacting time this interference could be neglected.

We are indebted to the Director, Medical Services, Ministry of Health, for permission to publish this note.

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WELLCOME CHEMICAL LABORATORIES
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April 7th, 1953

A MODIFICATION OF THE VAN GULIK METHOD FOR THE DETERMINATION OF FAT IN SOFT CHEESE

THE van Gulik method[1] for the determination of the fat content of cheese has been accepted by some workers as satisfactory for routine laboratory procedure.[2,3,4] Fort[5] suggested the general adoption of this technique at the XIIth International Dairy Congress. It has, however, been found unsatisfactory in this laboratory for the determination of the fat content of the white cheese produced in Israel. Duplicates differed appreciably and the meniscus could be read only with difficulty. This white cheese is a soft skim milk cheese made from milk powder to which rendered fat or margarine is added to bring the fat content to 9 per cent. (on a fresh weight basis).

The fat determination was carried out according to the original van Gulik method as described by Bernaerts.[1] Three grams of cheese were weighed into a small beaker with perforated walls. The beaker was placed in a butyrometer, which was half filled with sulphuric acid of specific

gravity 1·50. The casein was dissolved at 65° C with frequent shaking. After the addition of 1 ml of amyl alcohol, the butyrometer was filled with sulphuric acid of the same specific gravity as above. The upper hole was stoppered and after vigorous shaking the butyrometer was spun in a centrifuge at 1000 r.p.m. and a radius of 25 cm for 5 minutes. The fat percentage was read at 65° C. The cheese mass dissolved very slowly in the acid recommended, taking several hours. When amyl alcohol was added, the fat did not separate distinctly on centrifugation and appeared to contain opaque white masses. A microscopic examination of the opaque masses showed that these were particles, which were insoluble in the fat and which did not take up a fat stain (Sudan III). It appeared that the opaque masses were from the non-fatty part of the cheese and apparently were not dissolved by the acid.

In order to overcome these difficulties, experiments were made with higher concentrations of sulphuric acid as dissolving agent. When the specific gravity of the sulphurc acid was raised to 1·70 and all other details of the van Gulik test were kept as originally specified, the cheese mass was dissolved in 1 hour and, when amyl alcohol was added, the fat column separated clearly on centrifugation and gave good agreement between duplicates. The fat column was then transparent with no turbid portions.

The above variation of the van Gulik fat test was compared with the Schmid - Bondzynski and Ratzlaff[6,7] gravimetric fat determination and good agreement was found within the limits of experimental error. The results are shown in Table I.

TABLE I

THE MODIFIED VAN GULIK FAT DETERMINATION IN WHITE CHEESE COMPARED WITH THE SCHMID - BONDZYNSKI - RATZLAFF METHOD

Sample No.	Number of replicates	Fat on fresh weight basis, %			Fat by Schmid - Bondzynski - Ratzlaff method, %	Difference, %
		Range of values	Mean	Standard deviation		
1	6	6·25 to 6·75	6·50	0·160	6·33	+0·17
2	5	9·50 to 10·00	9·75	0·177	9·74	+0·01
3	7	7·95 to 8·25	8·01	0·104	8·60	−0·59
4	5	7·00 to 7·56	7·27	0·278	7·50	−0·23
6	5	8·00 to 8·50	8·25	0·250	8·61	−0·36

Apparently, with a soft cheese containing much more water than the hard varieties, a more concentrated sulphuric acid solution is needed for the complete solution of the cheese mass. Sulphuric acid of specific gravity 1·70 is apparently concentrated enough to dissolve the protein satisfactorily without burning the fat.

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January 15th, 1953

A MODIFIED PROCEDURE FOR DISSOLVING CHROMITE ORES

VARIOUS materials have been used from time to time for fusion of chromite ore; they include sodium carbonate,[1] a mixture of sodium carbonate and potassium nitrate,[2] sodium peroxide[3] and a mixture of sodium peroxide and sodium hydroxide.[4] The procedure is generally laborious. Moreover, the material of the vessel used, even when this is platinum, is attacked. A recent suggestion[5] to fuse the ore with sodium peroxide in a test tube made from stout glass largely solves these difficulties, but it is inapplicable in a silica determination. Solution with acidic reagents has also been investigated. A phosphoric - sulphuric acid mixture,[6] sulphuric acid in presence of manganese dioxide,[7] hydrochloric acid under pressure[8] and hydrofluoric acid[9] have all been used. Fusion with potassium bifluoride[10] or potassium pyrosulphate[11] has also been found suitable.

In this laboratory, fusion of chromite ore with potassium acid sulphate or, better still, with a mixture of sulphuric acid and potassium sulphate yielded highly satisfactory results. The solution can be effected in a stout Hysil-glass beaker. The procedure, which is rapid and gives subsequent determinations that are entirely reliable, compares well in simplicity with a similar fusion with sodium peroxide[5] in glass test tubes, and has the added advantage that determination of silica is also possible; reduction of chromate is not necessary.

Procedure—Place 1 g of the finely powdered dry ore, all of which passes a 100-mesh sieve, in a 100-ml dry beaker. Add 5 to 6 ml of sulphuric acid, sp.gr. 1·82, cover the beaker with a clock glass and gently heat over a hot-plate, gradually adding 15 to 17 g of anhydrous potassium sulphate. Slowly heat externally with a bunsen flame until a homogeneous melt is obtained and the entire mass is uniformly red all through. Overheating should be avoided. Cool the mass, extract with dilute sulphuric acid (1 + 20), evaporate to dryness, take up with water, and filter to remove the silica. Determine the various constituents in the solution in the usual way.

The decomposition of the chromite by acid potassium sulphate is not fundamentally different from pyrosulphate fusion.[11] It has been shown[12] that the potassium acid sulphate decomposes above its melting point according to—

$$2KHSO_4 \rightarrow K_2S_2O_7 + H_2O.$$

We believe that the enhanced efficiency of the acid potassium sulphate is due to the joint action of the acid sulphate and the pyrosulphate formed *in situ*.

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March 27th, 1953

THE QUALITATIVE DETECTION OF TELLURIUM IN TELLURIUM - LEAD ALLOYS

IN their comprehensive scheme for the detection of alloying elements in lead-based alloys, Evans and Higgs[1] were unable to recommend a spot-test for tellurium in tellurium - lead alloys[2] containing 0·04 to 0·05 per cent. of tellurium and 0·06 to 0·07 per cent. of copper; they considered the amount present to be below the limit of such tests. In connection with work on the quantitative determination of tellurium in such alloys,[3] a suitable test has now been devised.

METHOD

REAGENTS—

Acetic acid - hydrogen peroxide mixture[4]—A mixture of 2 volumes of 30 per cent. w/v hydrogen peroxide solution, 1 volume of glacial acetic acid and 2 volumes of water.

Sodium chloride solution—A saturated solution in distilled water.

Stannous chloride solution—A *M* solution in concentrated hydrochloric acid, sp.gr. 1·18.

Hydrochloric acid, sp.gr. 1·18.

PROCEDURE—

Clean the surface of the metal as recommended by Evans and Higgs.[1] Make a small hole in the lead and add 4 drops of acetic acid - hydrogen peroxide mixture. Allow the reaction to proceed for a minute, then transfer the turbid solution to a micro test tube by means of a capillary tube. Add 10 drops of sodium chloride solution and then 2 drops of hydrochloric acid. Heat the test tube in a bath of boiling water until a clear solution is obtained and keep the solution hot for about 2 minutes. Add 4 drops of stannous chloride solution and stir well with a thin glass rod. Set the mixture aside in the water-bath for a few minutes. All drops must be delivered by capillary tubes.

If tellurium is present, a greyish-black coloration of the colloidal metal is produced. Comparison with a similar volume of water against a white tile is advisable. Lead alloys containing no tellurium did not give a coloration. Selenium will give a similar coloration, which depends on the amount present. The proposed test does not distinguish between selenium and tellurium, but selenium is not normally a constituent of lead alloys. An attempt to apply in the above investigation the test of Feigl and Uzel,[5] which utilises a tervalent copper complex, proved unsuccessful.

Thanks are due to the Directors of British Enka Ltd., for permission to publish this note.

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March 3rd, 1953

THE SEPARATION OF SMALL AMOUNTS OF ARSENIC, COPPER AND BISMUTH FROM LEAD AND ZINC BY MEANS OF DIETHYLAMMONIUM DIETHYLDITHIOCARBAMATE

IN 1945[1] we described a scheme of analysis for trace metals, which depends upon separation of arsenic, copper and bismuth from lead, zinc, and so on, by extraction from strongly acid solution with a solution of diethylammonium diethyldithiocarbamate in chloroform. The extraction solution is the sulphuric acid solution remaining after wet decomposition of the organic material, to which has been added hydrochloric acid. The dilution and the acidity are adjusted so that the solution is at least 3 *N* in sulphuric acid and 2 *N* in hydrochloric acid before extraction. Even at this acidity, we find that a small proportion of the lead may sometimes be extracted with the copper and arsenic, and we now prefer an acidity of 3 *N* in sulphuric acid and 3 *N* in hydrochloric acid.

Subsequently, it appears to have been assumed that this separation is effective from a solution that is 2 *N* in hydrochloric acid alone, and extraction under such conditions has been suggested particularly as a means of separating bismuth from lead.[2] This is incorrect, and it is necessary to remove this impression, since at this acidity almost the whole of the lead is extracted with the copper and bismuth. In a solution containing hydrochloric acid alone, the acidity must be raised to 4 to 6 *N* for quantitative separation of bismuth and the other elements from lead.

Hydrochloric acid is essential to the success of the method; from sulphuric acid solutions even as highly acid as 10 *N* about 85 per cent. of the lead is extracted by the dithiocarbamate reagent, along with the copper and bismuth.

Experience has shown that to ensure quantitative recoveries diethylammonium diethyldithiocarbamate of the highest purity must be used. Satisfactory reagent is now available

commercially as a "fine chemical," in the form of a creamy-white crystalline solid, which gives a clear, colourless, stable solution in chloroform. We now prefer to use a 1 per cent. solution of this material, in place of a reagent prepared *in situ* from carbon disulphide and diethylamine,[1] which rapidly discolours on keeping.

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THE DETERMINATION OF ZINC BY MEANS OF SODIUM DIETHYLDITHIOCARBAMATE

ATKINS suggested the use of sodium diethyldithiocarbamate for the determination of zinc in fresh water[1] and in sea-water.[2] The reaction was carried out in Nessler tubes and the opacity of the solution was compared visually with standards. Attempts have now been made to use this method in conjunction with a Spekker photo-electric absorptiometer, as measurement of turbidity by this instrument is more accurate than is visual comparison of standards.

The accuracy of the method was examined by repeated determinations of the turbidity produced by a solution containing a known amount, 5 p.p.m., of zinc. Large variations in turbidity were observed, as measured by the drum readings of the absorptiometer, and these variations were eliminated by carrying out the precipitation at constant temperature in the presence of a protective colloid.

In the early experiments a 1 per cent. solution of gum arabic was used as the protective colloid. It was found that similar solutions of gum arabic did not have the same protective properties; this occurred with different samples of gum, or even with samples taken from the same bottle. Furthermore, the solutions aged. Satisfactory results were given by a 1 per cent. solution of polyvinyl alcohol, which was prepared from commercial polyvinyl acetate (Gelva **25**, supplied by Shawinigan Ltd.) in the following way. The stoicheiometric amount of *N* alcoholic sodium hydroxide solution was added to a 5 per cent. alcoholic solution of the polymer and the mixture was boiled for a few minutes. The polyvinyl alcohol was then filtered off, washed with alcohol until free from sodium acetate, washed with xylene and dried at 105° C. The material prepared in this way had an ash of 1·5 per cent. and a saponification value of less than 10. It was found that a freshly made aqueous solution aged, but this difficulty was overcome by boiling it for half an hour.

In carrying out a determination, the distilled water, solutions and glassware were brought to a temperature of 25° C by means of a thermostat. The zinc solution was run into a 50-ml flask and diluted to 40 ml, 1 ml of a 1 per cent. solution of polyvinyl alcohol was added and then 5 ml of a 0·1 per cent. solution of sodium diethyldithiocarbamate; the solution was then diluted to 50 ml and its turbidity determined by means of an absorptiometer, a 4-cm cell and a pair of neutral filters. A linear relationship was established, over a pH range 4·8 to 6·3, between the drum reading and the concentration of zinc in solution for 0 to 10 parts of zinc per million; duplicate determinations indicated that the accuracy was of the order of $\pm 0{\cdot}05$ p.p.m. The substitution of violet filters for the neutral filters widened the scale and was of value when small amounts of zinc were determined. The linear relationship was first established with standard solutions of zinc chloride; when zinc acetate solutions were used the relationship was still linear, but the slope of the line was altered. Thus the calibration is affected by the anions present, and the method can be used only when the composition of the solution is known, or when it is possible to carry out the calibration under the same conditions as the zinc determination.

Recently Evans and Davies[3] have used this method in an investigation of the corrosion of zinc in distilled water, and it was used by one of the authors (G. H. N.) in a study of the corrosion of zinc by potassium chloride solution.

REFERENCES

1. Atkins, W. R. G., *Analyst*, 1935, **60**, 400.
2. ——, *J. Marine Biol. Soc.*, 1936, **20**, 625.
3. Evans, U. R., and Davies, D. E., *J. Chem. Soc.*, 1951, 2607.

DEPARTMENT OF METALLURGY
UNIVERSITY OF CAMBRIDGE

J. E. O. MAYNE
G. H. NOORDHOF

March 5th, 1953

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1953, **78**, 444).

Public Analyst	*Appointment*
ELVIDGE, William Farrand (Deputy) ..	County of Westmorland.
HARRIS, Tennyson	County Borough of Birkenhead.
HARRIS, Tennyson	Borough of Crosby.
HARRIS, Tennyson	Borough of Swinton and Pendlebury.
SHERRATT, John Graham	County Borough of Blackpool.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1953, **78**, 445).

Agricultural Analyst	*Appointment*
ELVIDGE, William Farrand (Deputy) ..	County of Westmorland.
HARRIS, Tennyson	County Borough of Birkenhead.

Ministry of Food

STATUTORY INSTRUMENTS*

1953—No. 1307. The Food Standards (Preserves) (Amendment) Order, 1953. Price 2d.

*This Order, which came into operation on September 1st, 1953, provides that the revised standards for damson jam, gooseberry and raspberry jam, gooseberry and strawberry jam and any jam packed in a hermetically sealed container prescribed by the Food Standards (Preserves) Order, 1953 (S.I., 1953, No. 691; Analyst, 1953, **78**, 324), shall have effect as respects all sales on and after September 1st, 1953.*

It also prescribes revised standards for fig and lemon jam and for rhubarb and ginger jam, as follows—

Description of Jam	*Minimum fruit content* Fruit content, per cent.
Fig and Lemon	40 (8)
Rhubarb and Ginger	40 (1)

The figures in brackets denote the minimum quantity by weight of the second named fruit to be contained in the finished jam expressed as a percentage based on the number of parts by weight of the second named fruit required to be present in 100 *parts by weight of finished jam.*

1953—No. 1310. The Food Standards (Saccharin Tablets) Order, 1953. Price 2d.

*This Order, which came into operation on September 1st, 1953, and should be read with the Food Standards (General Provisions) Order, 1944 (S.R. & O., 1944, No. 42; Analyst, 1944, **69**, 49), as amended (S.R. & O., 1944, No. 654; Analyst, 1944, **69**, 247), revises the standard for Saccharin Tablets previously prescribed in the Saccharin Order, 1949 (which is now revoked), and applies the standard to all sweetening tablets containing saccharin. Article 3 of the Order states—*

3. A saccharin tablet or other sweetening tablet containing saccharin
   - (*i*) shall contain not less than 0·18 grain and not more than 0·22 grain of saccharin or the equivalent weight of soluble saccharin;
   - (*ii*) may contain as excipient sodium bicarbonate with or without other suitable substances, the total amount of excipient not to exceed four times the maximum quantity of saccharin;
   - (*iii*) shall not contain more than 5 per cent. water-insoluble matter nor less bicarbonate than that required to render the saccharin completely soluble.

1953—No. 1311. The Artificial Sweeteners in Food Order, 1953. Price 3d.

This Order, which came into operation on September 1st, 1953, prohibits the use of artificial sweeteners, other than saccharin, in the composition or preparation of any food sold or intended for sale for human consumption. The following definition is included in the Order—

"Artificial sweetener" means any chemical compound which is sweet to the taste, but does not include saccharin, any sugar or other carbohydrate or polyhydric alcohols.

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

British Standards Institution

NEW SPECIFICATIONS*

B.S. 615:1953. Kohlrausch Flasks. Price 2s. 6d.
B.S. 1969:1953. Tests for Performance Characteristics of Sintered Filters. Price 2s. 6d.
B.S. 1992:1953. Butyl Acetylricinoleate. Price 2s. 6d.
B.S. 1993:1953. *sec*Butyl Alcohol. Price 2s.
B.S. 1994:1953. Dichloromethane (Methylene Chloride). Price 2s.
B.S. 1995:1953. Di-2-ethylhexyl Phthalate. Price 2s. 6d.
B.S. 1996:1953. Dimethyl Phthalate. Price 2s. 6d.
B.S. 1997:1953. Glycerol Triacetate (Triacetin). Price 2s. 6d.
B.S. 1998:1953. Triphenyl Phosphate. Price 2s. 6d.
B.S. 1999:1953. Tritolyl Phosphate (Tricresyl Phosphate). Price 2s. 6d.
B.S. 2005:1953. Glossary of Terms Applicable to Fillings and Stuffings. Price 2s. 6d.

DRAFT SPECIFICATIONS

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

Draft Specification prepared by Technical Committee DNC/1—Denture Base Materials and Teeth.
CR(DNC)4564—Draft B.S. for Acrylic Resin Denture Base.
Draft Specification prepared by Technical Committee DAC/5—Sampling of Dairy Products.
CR(DAC)4651—Draft Addendum to B.S. 809—Sampling Milk and Milk Products. F. Sampling of Ice-Cream.
Draft Specification prepared by Technical Committee ISE/18—Sampling and Analysis of Iron and Steel.
CR(ISE)5139—Draft B.S. Methods for the Determination of Silica in Blast Furnace Slag and Aluminium in Iron, Steel and Ferro-alloys after Mercury Cathode Separation.
Draft Specification prepared by Sub-Committee NFE/–/3/– —Sampling and Analysis of Aluminium and Aluminium Alloys.
CR(NFE)5205—Draft B.S. Method for the Determination of Iron in Aluminium (Volumetric - Titanous Chloride Method).

Book Reviews

BRITISH PHARMACOPOEIA 1953. PUBLISHED UNDER THE DIRECTION OF THE GENERAL MEDICAL COUNCIL. Pp. xxiv + 894. London: The Pharmaceutical Press. 1953. Price 50s.

The eighth edition of the British Pharmacopoeia, which came into official use on September 1st, 1953, is a completely revised version of the "B.P., 1948" and its Appendix of 1951, set in a new style, in which the monograph headings have been translated into English and the use of metric weights and measures has been largely extended.

The exclusion of drugs of botanical origin, and their preparations, a marked feature of the seventh edition, has been extended almost to completion.

New monographs, to the number of 63, deal with recently introduced synthetics. The exclusions number 158, leaving a total of nearly 750 monographs.

The revision has entailed many alterations to the monographs carried over from the 1948 edition.

The chief interest for analysts in the new pharmacopoeia will lie in the 25 appendixes, which occupy about one-third of the book, and have taken their share of the revision and re-writing of the whole work. The part dealing with biological assays and tests now includes a section on the design and accuracy of biological assays.

Although the Pharmacopoeia of 1953 is an indispensable book of reference for all who have dealings with drugs and medicines, it would seem that, by excluding many substances with long-established pharmaceutical and other uses, the previous editions will not be rendered completely obsolete.

F. L. OKELL

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

A TEXTBOOK OF PHARMACOGNOSY. By GEORGE EDWARD TREASE, B.Pharm., Ph.C., F.R.I.C., F.L.S. Sixth Edition. Pp. viii + 821. London: Baillière, Tindall and Cox. 1952. Price 37s. 6d.

The sixth edition of Trease's "Textbook of Pharmacognosy" shows every sign of careful revision. Whilst retaining the general characteristics familiar to many generations of pharmaceutical students the text has been rearranged, pruned and brought up to date by the inclusion of new material. Some of these additional chapters are perhaps a little unexpected in a textbook of pharmacognosy, yet they are not altogether illogical. There are two short chapters, for instance, on bacteria and antibiotics, which serve to draw the student's attention to these related fields from which so many important drugs are now derived, although they hardly fall within the scope of pharmacognosy. There are also chapters on the chemical constituents of drugs and on chromatographic analysis with special reference to the separation of drug mixtures. These chapters again are sketchy and are obviously intended merely to draw the attention of students to the subjects discussed. The bibliographies at the ends of these chapters will be most useful to those wanting to know where further information can be found.

"A Textbook of Pharmacognosy" is still primarily concerned with the nature of vegetable (and some animal) drugs and with their diagnostic characters, and it may come as a surprise to non-pharmacists in this synthetic—and micro-biosynthetic—age that there should still exist a demand for a textbook of pharmacognosy and that it should still be necessary to revise such a textbook, except perhaps by way of making deletions. Yet it is only in the last year or two that the source of curare has been shown to be *Chondrodendron tomentosum* and not a species of *Strychnos*, whilst the recent revival of interest in the *Veratrum* alkaloids and the official recognition of picrotoxin indicate that vegetable drugs may still occupy an important place in medicine. It is indeed considerations of this kind that justify the author in adopting the conservative attitude he has elected to take in deciding what to include in, and what to exclude from, the latest edition of his textbook. He is even bold enough to predict that further additions to the list of vegetable drugs in clinical use may be expected in the future.

The book under review has now held the field for nearly thirty years and the sixth edition will be found just as essential by the present generation of pharmacists as was the first edition by their fathers; it will continue to be the standard book of reference for the non-pharmacist who may be called upon from time to time to examine and report on crude drugs. The subject-matter appears to be thoroughly reliable and up to date, and there are singularly few errors. Those noted by the reviewer were confined to the mis-spelling of Sir Alexander Fleming's name and to the maps of Europe, S.W. Asia and the East Indies, which fail to take into account the political changes that have taken place there since the 1939–45 war. F. A. ROBINSON

Publications Received

MÉTHODES D'ANALYSE ET DE CONTROLE INDUSTRIEL DES MATIÈRES GRASSES. By G. WOLFF and J. P. WOLFF. Pp. viii + 262. Paris: Dunod. 1953. Price 1480 fr.

THE MANUFACTURE OF COMPRESSED YEAST. By F. G. WALTER, A.A.C.I. Second Edition. Pp. x + 318. London: Chapman & Hall Ltd. 1953. Price 37s. 6d.

ANNUAL REPORTS ON THE PROGRESS OF CHEMISTRY FOR 1952. Pp. 429. London: The Chemical Society. 1953. Price 30s.

ORGANIC CHEMISTRY. By P. B. SARKAR, D.Sc., F.N.I., and P. C. RAKSHIT, M.Sc., Ph.D. Seventh Edition by P. B. SARKAR, D.Sc., F.N.I. Pp. vi + 598. Calcutta: H. Chatterjee & Co. Ltd. 1953. Price Rs. 8.

EXCERPTA MEDICA. Section XVI. CANCER (EXPERIMENTAL AND CLINICAL). Volume I, No. 1, July, 1953. Pp. 96. Amsterdam: Excerpta Medica, N.V. Subscription $10.00 per annum; Volume I, July to December, six issues only, $5.00.

Medical Abstracts. A new section of the International Medical Abstracting Service.

CHEMICAL TISSUE TESTS FOR DETERMINING THE MINERAL STATUS OF PLANTS IN THE FIELD. By D. J. D. NICHOLAS, B.Sc., Ph.D., A.K.C. Pp. 34. Salisbury: The Tintometer Ltd. 1953. Price 8s. 6d.

SUGAR BEET CULTIVATION. Ministry of Agriculture and Fisheries, Bulletin No. 153. Pp. iv + 98. London: H. M. Stationery Office. 1953. Price 5s.

A SPECTROPHOTOMETRIC ATLAS OF THE $^2\Sigma^+$—$^2\Pi$ TRANSITION OF OH. National Bureau of Standards Circular 541. By ARNOLD M. BASS and HERBERT P. BROIDA. Pp. ii + 22. Washington: U.S. Government Printing Office. 1953. Price 20 cents.

CHIEF CHEMIST. The Newcastle Breweries Ltd., Haymarket, Newcastle upon Tyne, invite applications for the post of Chief Chemist. Candidates should have a first class honours degree in biochemistry, or another recognised qualification in analytical chemistry. Starting salary will be within the range of £1350 to £1500 according to experience and qualifications. Non-contributory pension scheme. Write, in confidence, to the Secretary, stating age, experience and qualifications.

A METALLURGIST is required by MARCONI'S WIRELESS TELEGRAPH CO., LTD., for work in their new factory at BASILDON (new town), Essex. Applicants should have H.N.C. or equivalent, in chemistry; 3 years' experience in electronics or electrical engineering works laboratory; knowledge of metal finishing. Job involves tests of ferrous and non-ferrous metals, insulating liquids and materials. House available to rent. Please reply, giving full details of qualifications and experience and quoting ref. 1165, to Dept. C.P.S., Marconi House, 336/7, Strand, London, W.C.2.

GOLD COAST LOCAL CIVIL SERVICE

A VACANCY exists for a Chemist-Assayer in the Local Civil Service of the Gold Coast. Candidates must possess an Honours degree in Chemistry with (*a*) Certificate from University of having reached good standard in practical inorganic chemistry; two-year course of post-graduate training at Imperial College of Science and Technology *or* (*b*) at least two years' post-graduate experience in silicate analysis and assaying. Duties will be to take charge of the Geological Survey Chemical Laboratory; assay ore of all kinds; carry out silicate analyses of rocks and minerals; participate in research of inter-relation of rocks and Geochemistry.

Appointment is on contract for two tours in first instance with consolidated salary in the scale of £1090 to £1950 per annum. Gratuity at rate of £37 10s. 0d. for each three months' service. Outfit allowance of £60 if initial salary is below £1530, £30 if above. Income tax at low local rates. Quarters provided, when available, at rental of £60 to £90 per annum. Free first class passages. Leave at rate of 7 days for each completed month of resident service.

Apply in writing to the Director of Recruitment, Colonial Office, Great Smith Street, London, S.W.1, giving briefly age, qualifications and experience. Mention the reference number (CDE.105/13/06).

A MODERN LABORATORY, situated in the City of London, E.C.1 postal area, fully equipped for dealing with food analysis and bacteriological investigations, can offer accommodation and facilities for another consultant, on a basis to be mutually agreed. Write Box No. 3847, THE ANALYST, 47, Gresham Street, London, E.C.2.

ADMIRALTY VICTUALLING DEPARTMENT require Assistant Technical Examining Officers at Risley, nr. Warrington, or in London, but must be willing to serve elsewhere in U.K. Must be British born subjects and have either 1st or 2nd class honours degree (with Chemistry as principal subject) or hold A.R.I.C. Post-graduate experience with textiles or food an advantage. Salary according to age and qualification, commencing between £390 at 21 and £810 at 34 or above, and rising to £900 (London rates: provinces slightly less) PLUS Pay Addition at present 10 % on first £500 of salary, 5 % on balance. Appointment is unestablished, but successful candidates may be eligible to compete later for establishment. Forms from M.L.N.S. Technical and Scientific Register (K), Almack House, 26, King Street, London, S.W.1, quoting F.406/53A.

SOUTHERN RHODESIA GOVERNMENT
VACANCY: ANALYTICAL CHEMIST: HEALTH DEPARTMENT

APPLICANTS, under 40, must have at least B.Sc. degree, or equivalent qualification, in Chemistry.

Starting salary varies between £624 to £984 p.a. depending on qualifications and experience, on scale rising to £1482 p.a. Marriage allowance, £50 p.a., Childrens' £60 p.a. first, £24 p.a. each other dependant child also payable.

The successful applicant will carry out, *i.a.*, the analysis of waters, food and drugs, dairy produce and certain chemical specimens; toxicology and forensic chemistry; general miscellaneous analyses.

Application forms and further details from the Secretary, Rhodesia House, 429, Strand, London, W.C.2. Completed forms to be returned by October 31st.

ANALYST REQUIRED for research Laboratory. Wide range of work with opportunities for initiative. Several years experience on analysis of steels and aluminium alloys required. A.R.I.C. or equivalent an advantage. Full details and salary required to Box 3850, THE ANALYST, 47, Gresham Street, London, E.C.2.

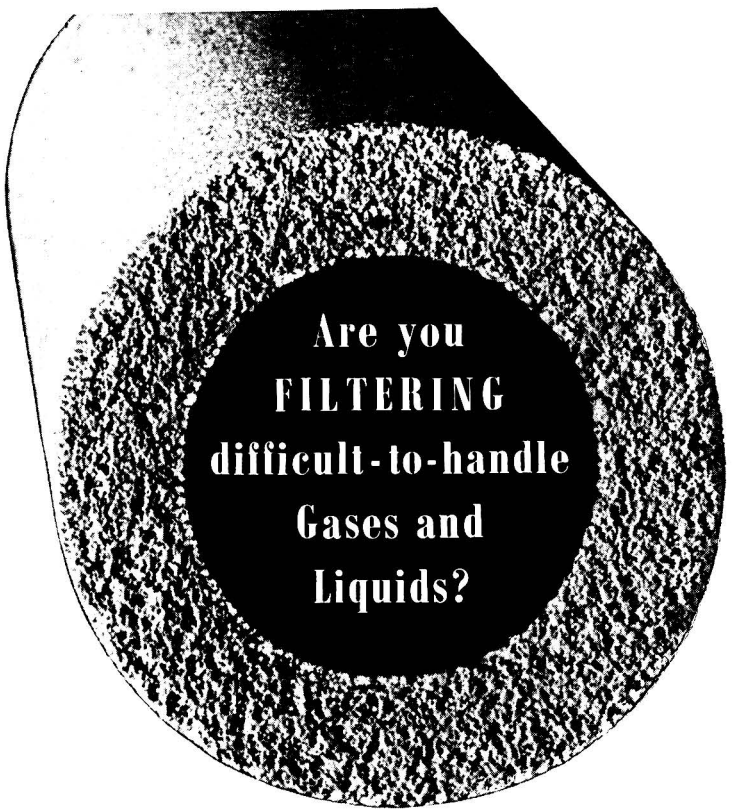
C.A.V. Limited Research Laboratory requires a qualified metallurgist for fundamental metallurgical and physical investigations. Applicants should have a degree in metallurgy and two years industrial or university research would be an advantage. Some knowledge of electron and X-ray diffraction methods desirable. Candidates must have completed military service and should be between the ages of 24-30. Salary according to age, experience and qualifications. The appointment will include participation in a pension scheme, and affords a considerable opportunity for advancement. Applications should be addressed to the personel manager, C.A.V. Limited, Acton, London, W.3.

A VACANCY occurs for a Chemist with B.Sc. or equivalent qualification on the staff of the laboratories of Vine Products Ltd. of Villiers Road, Kingston-on-Thames, Surrey. Some experience in the analysis of foods and beverages is essential. The salary offered is £750 but would vary according to qualifications and experience. A superannuation scheme is in operation. Please communicate with the Assistant General Manager by letter or phone (Kingston 5641).

ANALYST for rapidly expanding International Pharmaceutical Company. B.Sc., P.Pharm. or equivalent qualifications. Previous experience of general Pharmaceutical Control work necessary and of antibiotics an advantage. The Factory is in a non-industrial area, and possibility of assistance with accommodation exists. Good starting salary and exceptional prospects. Write Box No. 3848, THE ANALYST, 47, Gresham Street, London, E.C.2.

MINISTRY OF SUPPLY requires PRINCIPAL SCIENTIFIC OFFICER at Culcheth, near Warrington, to take charge of staff providing analytical services to Metallurgical Research and Development Division. Qualifications—1st or 2nd class honours degree in Chemistry, but A.R.I.C. with background of study and experience in metallurgy, or suitable degree in metallurgy with experience in metallurgical analysis may also be considered. Good experience in modern metallurgical analytical techniques essential. Minimum age 31. Salary within range £1,033–£1,377. Women somewhat less. Appointment unestablished. F.S.S.U. benefits may be available. Application forms from M.L.N.S., Technical and Scientific Register (K), 26, King Street, London, S.W.1, quoting F 473/53A. Closing date 31 October, 1953.

FOR SALE. Analytical Edition, Ind. Eng. Chem., vols 1 to 24; Brit. Abstracts from commencement; unbound best offer. Box No. 3849, THE ANALYST, 47, Gresham Street London, E.C.2.



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