THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

Ordinary Members

Lawrence Thomas Coleman; Leslie Arthur Cook, B.Sc. (Lond.), Dip. Chem. (Wigan Mining and Tech. Coll.); Denis Crowther, B.Sc. (Manc.); Sydney Farthing; Peter David Foulkes, B.Sc. (Reading); Hendrik Jan Hardon, D.Sc. (Utrecht); Douglas Frederick Inkpin; John Charles Kerridge, B.Sc. (Lond.); Kenneth Geoffrey Langley, B.Sc. (Lond.), A.R.I.C.; Colin Macfarlane, A.R.I.C.; Haydn Alonzo Morris; Herbert Guy Nicholl, B.Sc. (Lond.), A.R.I.C.; Irmgard Karla Helmtraud Otter, Dr.Phil. (Graz); Michael Allan Owen; John Arthur Paine.

Junior Members

Terence Brian Featherstone; Grieves Harnby, B.Sc. (Birm.), A.R.I.C.; Raymond Arthur Jones.

DEATHS

We record with regret the deaths of

Andrew Dargie
Henry John Davis
Thomas Macara
Archibald Steele Whamond.

NORTH OF ENGLAND SECTION

The thirty-second Annual General Meeting of the Section was held at 2.15 p.m. on Saturday, January 26th, 1957, at the Engineers' Club, Albert Square, Manchester. The President was among the 35 members present, over whom the Chairman of the Section, Mr. J. R. Walmsley, A.M.C.T., F.R.I.C., F.P.S., presided. The following appointments were made for the ensuing year:—*Chairman*—Mr. A. N. Leather. *Vice-Chairman*—Dr. J. R. Edisbury. *Hon. Secretary and Treasurer*—Mr. A. C. Wiggins, J. Lyons & Co. Ltd., 5 Laurel Road, Liverpool, 7. *Members of Committee*—Messrs. A. C. Bushnell, J. F. Clark, A. A. D. Comrie, C. J. House, B. Hulme and R. Mallinder. Messrs. F. Dixon and T. W. Lovett were appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which a paper entitled "Recent Advances in the Analysis of Fertilisers" was given by H. N. Wilson, F.R.I.C.

SCOTTISH SECTION

The twenty-second Annual General Meeting of the Section was held at 1.30 p.m. on Thursday, January 17th, 1957, at the Rhul Restaurant, 123 Sauchiehall Street, Glasgow. The Chairman of the Section, Dr. F. J. Elliott, F.R.I.C., F.R.S.E., presided. The following office bearers were elected for the forthcoming year:—*Chairman*—Dr. Magnus Pyke. *Vice-Chairman*—Mr. A. N. Harrow. *Hon. Secretary and Treasurer*—Mr. J. A. Eggleston, Boots Pure Drug Co. Ltd., Airdrie Works, Airdrie, Lanarkshire. *Members of Committee*—Messrs. D. M. W. Anderson, J. Brooks, R. A. Chalmers, J. W. Gray, H. C. Moir and J. W. Murfin. Messrs. J. Andrews and J. McL. Malcolm were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which Dr. F. J. Elliott, F.R.I.C., F.R.S.E., reviewed the activities of the Section during his two year term of office. Dr. Magnus Pyke, F.R.I.C., F.R.S.E., gave an address on present conditions in the analytical world, the developments leading up to them and the changes that are likely to occur in the future.

WESTERN SECTION

THE Section participated in a meeting of the South-Western Counties Section of the Royal Institute of Chemistry at 5.30 p.m. on Friday, January 18th, 1957, in the Technical College, Tavistock Road, Plymouth. The Chair was taken by the Chairman of the South-Western Counties Section, Dr. F. D. M. Hocking, L.R.C.P., M.R.C.S., A.C.G.F.C., F.R.I.C.

A lecture on "Silicosis" was given by Professor E. J. King, M.A., Ph.D., D.Sc., F.R.I.C.

MIDLANDS SECTION

THE second Annual General Meeting of the Section was held at 7 p.m. on Thursday, January 24th, 1957, in the Gas Showrooms, Nottingham. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P. The following appointments were made for the ensuing year:—*Chairman*—Dr. R. Belcher. *Vice-Chairman*—Dr. S. H. Jenkins. *Hon. Secretary*—Mr. G. W. Cherry, 48 George Frederick Road, Sutton Coldfield, Warwickshire. *Hon. Treasurer*—Mr. F. C. J. Poulton. *Members of Committee*—Dr. Bella B. Bauminger, Messrs. H. E. Brookes, H. J. G. Challis, J. R. Leech, R. Sinar, J. H. Thompson, W. H. Stephenson and T. S. West. Miss M. E. Tunnicliffe and Mr. H. J. Alcock were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which the Chair was taken by the Vice-Chairman, Dr. S. H. Jenkins, F.R.I.C.

A talk on "Some Contradictions and Discrepancies Concerning a Classical Method of Analysis" was given by R. Belcher, Ph.D., D.Sc., F.R.I.C.

BIOLOGICAL METHODS GROUP

THE twelfth Annual General Meeting of the Group was held at 6.30 p.m. on Wednesday, January 23rd, 1957, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Vice-Chairman of the Group, Mr. S. A. Price, B.Sc., presided. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Dr. S. K. Kon. *Vice-Chairman*—Dr. J. I. M. Jones. *Hon. Secretary and Treasurer*—Mr. K. L. Smith, Standards Department, Boots Pure Drug Co. Ltd., Nottingham. *Members of Committee*—Miss A. Jones, Miss J. Stephens, Messrs. L. J. Harris, S. A. Price, K. C. Sellers and J. Simpson. Mr. J. W. Lightbown was re-appointed Hon. Recorder and Messrs. D. M. Freeland and J. H. Hamence were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Group, at which the Chair was taken by Mr. S. A. Price. A discussion on "The Relationship Between Statistics and Microbiological Assay" was opened by J. P. R. Tootill, B.Sc.

JOINT COMMITTEE ON METHODS OF ASSAY OF CRUDE DRUGS

THE Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry, which, as reported in *The Analyst*, 1956, **81**, 322, has been appointed to prepare standard methods of assay for crude drugs and kindred materials used in commerce for which there are no official methods, has appointed a number of small working panels. Three panels are already engaged in collaborative experimental work; their consitutions and terms of reference are given below.

PANEL 1: *Digitalis purpurea:* CHEMICAL METHOD—

Constitution: Professor H. Brindle (*Chairman*), Dr. G. E. Foster, Mr. G. J. Rigby, Dr. J. M. Rowson, Mr. K. L. Smith and Professor J. P. Todd.

Terms of reference: "To investigate chemical methods for the assay of digitalis and its preparations and to attempt to correlate them with biological methods of assay."

PANEL 2: CAPSICUM: CAPSAICIN CONTENT—

Constitution: Mr. H. B. Heath (*Chairman*), Mr. E. A. Elsbury, Mr. C. A. MacDonald, Mr. G. R. A. Short and Mr. D. O. Singleton.

Terms of reference: "To investigate methods of assay of capsicum and capsicum products with particular reference to the determination of the capsaicin content."

PANEL 3: ANTHRAQUINONE DRUGS—

Constitution: Dr. J. M. Rowson (*Chairman*), Dr. J. W. Fairbairn, Mr. C. A. Johnson, Dr. W. Mitchell, Mr. H. A. Ryan and Mr. W. Smith.

Terms of reference: "To investigate methods for estimating the purgative activity of drugs and of preparations of drugs containing anthraquinone derivatives with a view to recommending standard methods of assay."

Analytical Applications of the Barker Square-wave Polarograph

Part III.* Orthophosphoric Acid as a Solvent and Base Electrolyte in Direct Inorganic Polarographic Analysis

BY G. W. C. MILNER AND L. J. SLEE

Orthophosphoric acid is a versatile solvent for diverse materials and the resulting solutions may be analysed polarographically. The polarographic behaviour of several elements in a *M* phosphoric acid base solution has therefore been investigated, and half-wave potentials and diffusion-current constants are reported.

With use of the square-wave polarograph, simple and direct procedures are described for the determination of small amounts of lead in copper, tin, zinc and aluminium-base alloys, respectively. The determination of lead (a few tenths of a per cent.) in monazite and in various miscellaneous materials is also described. With no samples were chemical separations necessary after their dissolution in phosphoric acid. Direct procedures are also described for the determination of the copper content of lead, tin, and aluminium-base alloys, the zinc content of copper, tin, and aluminium-base alloys, and the cadmium content of tin and lead-base alloys. All the procedures outlined provide a considerable improvement on those in which use is made of conventional polarography.

ALTHOUGH the polarographic behaviour of many elements has been investigated in several mineral-acid base electrolytes, including hydrochloric, sulphuric, nitric and perchloric acids, very little consideration has been given to the use of orthophosphoric acid as a base electrolyte.[1] It appears that molybdenum is the only element to have been studied in such a base solution, Höltje and Geyer[2] reporting a doublet wave with half-wave potential values of −0·33 and −0·90 V against the saturated-calomel electrode (S.C.E.) in *M* phosphoric acid.

The use of phosphoric acid in analytical chemistry has assumed greater importance since the realisation of its ability to effect the complete solution of materials that dissolve only with difficulty by conventional procedures. It is, for example, very convenient for effecting the complete solution of several types of minerals,[3] and its powerful solvent properties are most probably due to the formation of soluble complexes at the high temperatures that can be attained by heating. In conjunction with other mineral acids, such as hydrochloric, nitric and hydrofluoric, the solution of a wide range of materials can be effected, with subsequent removal of the other acids, simply by evaporation until only phosphoric acid remains. After such a solution procedure, phosphoric acid is potentially useful as a base electrolyte for the direct determination of constituents in these solutions. Consequently

* Presented at the XVth International Congress on Pure and Applied Chemistry (Analytical Chemistry), Lisbon, September 8th to 16th, 1956. For particulars of Parts I and II of this series, see reference list, p. 151.

the polarographic characteristics of several elements have been determined in this base solution. Values are given for half-wave potentials in volts against the saturated-calomel electrode and for diffusion-current constants (I). With use of the square-wave polarograph, the phosphoric acid base solution has been applied to the direct determination of copper, lead, cadmium and zinc in metallurgical and other types of sample.

Experimental

Apparatus—

Conventional polarograms, used for the determination of diffusion-current-constant data, were recorded with an automatic pen-recording polarograph incorporating a Brown Electronik potentiometer constructed in this laboratory.[4] The equipment used for recording square-wave polarograms was designed by Dr. G. C. Barker and has already been described.[5]

A study of the polarographic behaviour of various elements in orthophosphoric acid—

Initially the polarographic behaviour of several elements of importance in inorganic and metallurgical analysis was studied in a M phosphoric acid base solution prepared from the reagent of specific gravity of 1·75. The elements included iron, copper, bismuth, lead, thallium, cadmium, zinc, nickel, cobalt, antimony, tin and indium.

Suitable solutions were prepared by taking convenient aliquots of a solution of the metal in hydrochloric or nitric acids and evaporating almost to dryness in a 50-ml beaker. Then 6·8 ml of the phosphoric acid were added and the solution was warmed gently at first to remove water and the remainder of the more volatile acids. The solution was then heated to the maximum temperature of an efficient electric hot-plate for about 15 minutes; the temperature reached at this stage will be in the region of 220° C. No fuming takes place and these conditions can be realised only by experience. After cooling, each solution was diluted with distilled water to a volume of 100 ml in a calibrated flask. Suitable aliquots of the solutions were then de-aerated with nitrogen, and the polarograms were recorded with the square-wave polarograph. For ease of comparison a concentration of 50 μg per ml was used for each element in conjunction with a selected low sensitivity of the instrument. Details of the half-wave potential values and peak heights for these elements are given in Table I.

Table I

Polarographic data for the reduction of metal ions in a M phosphoric acid base solution

Metal ion	Peak height, mm	Reversibility	$E_{\frac{1}{2}}$,* volts against S.C.E.
Fe^{3+}	55	reversible	+0·10
Cu^{2+}	188	reversible	+0·01
Bi^{3+}	8·5	reversible	−0·085
Pb^{2+}	60	reversible	−0·395
Tl^{+}	28	reversible	−0·455
Cd^{2+}	105	reversible	−0·585
Zn^{2+}	111	reversible	−0·995
Ni^{2+}	—	irreversible	~ −1·01
Co^{2+}	—	no apparent reduction	—
Sb^{5+}	—	no apparent reduction	—
Sn^{4+}	—	no apparent reduction	—
In^{3+}	—	no apparent reduction	—

* Half-wave potential values against the S.C.E. were obtained with the square-wave polarograph by measuring the potential against the mercury - mercurous phosphate anode and applying a correction amounting to +0·404 V against S.C.E. for the potential of the mercury - mercurous phosphate electrode against the S.C.E. Several of these figures were checked by the accurate method of plotting $\log \frac{i}{i_d - i}$ against the potential of the dropping electrode.

From the results in Table I it can be seen that the lowest limit of detection is obtained for copper. Iron, lead, thallium, cadmium and zinc are also reversibly reduced and the peaks should be suitable for analytical purposes. The reduction of bismuth is less reversible,

but the peak is usable. Elements that are very irreversibly reduced include antimony, tin, indium and cobalt. The peak for copper cannot be used indiscriminately in the presence of iron, however, because of the fairly close proximity of the peaks for these two elements. The copper determination is limited to samples in which the ratio of ferric iron to copper is about 1 to 1 or less. A hydrochloric acid base solution is more suitable for the determination of copper in the presence of ferric iron. Nevertheless, there are some instances in analytical chemistry when the copper peak in a phosphoric acid base solution could be used with advantage, especially, for example, in several types of non-ferrous alloy systems.

The zinc peak likewise cannot be used indiscriminately, owing to the irreversible reduction of nickel at a slightly more negative potential. The interference from nickel is, however, negligible for ratios of nickel to zinc of 2 to 1 or less. As cobalt is not reduced in this base solution, there is no interference from this element in the determination of zinc. Similarly, the extreme irreversibility of indium permits the direct determination of cadmium in the presence of indium, a determination that has presented difficulty to polarographic workers for many years. It is possible to determine cadmium in the presence of a hundredfold excess of indium without interference. For example, a *M* phosphoric acid solution containing 200 μg of indium per ml and 2 μg of cadmium per ml gave a peak for cadmium identical with that for a solution containing 2 μg of cadmium per ml. The extremely irreversible reduction of stannic ions in phosphoric acid solutions permits the determination of lead to be accomplished directly in the presence of tin. The procedure previously adopted for this determination involved the removal of the tin as its volatile halide before the lead peak is recorded from a chloride base solution.[5]

Although the separation of the tin is unnecessary with a phosphoric acid base electrolyte, a slight disadvantage arises from the smaller value of the diffusion-current constant for lead. The value is 3·03 in this medium compared with a value of 3·86 in *M* hydrochloric acid (see Table II). Nevertheless, there is sufficient sensitivity with a square-wave polarograph for the determination of lead concentrations down to 0·025 μg per ml.

EVALUATION OF DIFFUSION-CURRENT CONSTANTS—

In order that a quantitative correlation of the behaviour of various elements in phosphoric acid solutions could be made with reference to other base electrolytes, a knowledge of the diffusion-current constants for the various elements was required. This constant is based on the Ilkovič equation and is defined by the expression—

$$I = \frac{i_d}{cm^{\frac{2}{3}} t^{\frac{1}{2}}},$$

where i_d is the diffusion current in μA, c is the concentration of the reducible ion in millimoles per litre, m is the mass of mercury flowing in mg per second and t is the drop time in seconds. All these variables are easily measured by using conventional polarographic equipment. Unfortunately the Ilkovič equation is not strictly applicable in square-wave polarography by virtue of the dependence of the diffusion current on the capacity of the mercury - solution interface. Nevertheless, a knowledge of diffusion-current constants is extremely useful as an approximate quantitative guide to the magnitude of diffusion currents obtained with the square-wave polarograph.

The polarographic behaviour of several elements in a *M* phosphoric acid base electrolyte was investigated with a conventional polarograph. The elements included most of those examined with the square-wave polarograph, namely copper, lead, thallium, cadmium, zinc, antimony, indium and tin, and the results obtained showed similar behaviour. For example, copper, lead, thallium, cadmium and zinc were reversibly reduced and, with the exception of zinc, the polarographic steps were well developed. Antimony, tin and indium did not produce reduction steps, which also confirms the results obtained with the square-wave polarograph. As the reversibly reduced ions appeared to possess favourable characteristics in the phosphoric acid solutions, the diffusion-current constants for these elements were determined by using millimolar solutions in the presence and absence of a maximum suppressor. Diffusion currents were measured with a calibrated microammeter and the capillary characteristics "*m*" and "*h*" were determined by the usual procedures. The results obtained are given in Table II.

The diffusion-current constant values in column 4 of Table II are taken from Kolthoff and Lingane.[1] These figures were checked and our results agreed to within $\pm$ 2 per cent.

The values in column 3 for the phosphoric acid base solution should be accurate to ± 2 per cent., apart from that for zinc. With some elements in the phosphoric acid solution, the limiting current developed more slowly in the presence of gelatin and this caused a rounding off at the top of the polarographic step. This effect was particularly noticeable in the reduction of cadmium and zinc, making it difficult to obtain an accurate measurement of the diffusion current. Therefore any procedure for these two elements, in which phosphoric acid is used as a base electrolyte precludes the addition of gelatin. As copper is the only element among those studied producing a maximum in this base solution, the presence of gelatin would only be necessary for its determination.

TABLE II

DIFFUSION-CURRENT CONSTANTS FOR METALLIC IONS IN *M* PHOSPHORIC ACID

Capillary characteristics: $m = 1{\cdot}510$ mg per second; $h = 50{\cdot}6$ cm

Metal ion	Gelatin concentration, %	$\frac{i_d}{cm^{\frac{1}{2}}\,t^{\frac{1}{2}}}$	Value in *M* hydrochloric acid + 0·01 per cent. of gelatin, for comparison
Cu^{2+}	nil	maximum produced	—
	0·002	2·97	3·39
Pb^{2+}	nil	3·03	3·86
	0·01	3·03	—
Tl^{+}	nil	2·59	not quoted
	0·01	2·59	—
Cd^{2+}	nil	2·86	3·58
	0·01	poorly developed step	—
Zn^{2+}	nil	2·8*	Not quoted, owing to masking by discharge of H^{+}
	0·01	poorly developed step	

* Diffusion current not well developed owing to H^{+} discharge.

A comparison of diffusion-current constants in *M* phosphoric acid and *M* hydrochloric acid shows higher values in the latter medium. This difference is probably due to the higher viscosity of phosphoric acid, since an increase in viscosity results in a decrease of the diffusion coefficient for the element in solution. However, the difference is never greater than 20 per cent., which indicates a loss in sensitivity of this order for determinations in phosphoric acid solutions. The diffusion current for the reduction of zinc could not be measured accurately, because of interference from the discharge of hydrogen ions. With the square-wave polarograph, however, the zinc peak is well formed before the increase in current due to the reduction of hydrogen ions takes place and it is possible to use this peak for analytical purposes.

Although an investigation of the analytical applications of phosphoric acid in conventional polarography was not carried out in this work, it would appear to be potentially useful for the determination of copper, lead, thallium and cadmium. Also many of the advantages of the phosphoric acid solution in analysis, which are illustrated later with reference to square-wave polarography, should apply in conventional polarography.

ANALYTICAL APPLICATIONS OF THE ORTHOPHOSPHORIC ACID BASE ELECTROLYTE WITH THE SQUARE-WAVE POLAROGRAPH

DETERMINATION OF LEAD—

The value of the square-wave polarograph for the determination of lead in ferrous and non-ferrous metallurgical alloys has already been shown by Ferrett and Milner.[5,6] These workers used a chloride base solution and effected the removal of the interfering element tin by volatilisation as its volatile bromide. As previously mentioned, the very irreversible behaviour of stannic tin in the phosphoric acid base solution obviates the need for its removal. This improvement is slightly offset, however, by the decrease in sensitivity for lead compared with the chloride media, but this is only of importance for the determination of trace amounts of this element. Details of the procedures developed for the determination of the lead content of various materials and the results obtained follow.

Lead in tin-base materials—Although the tin peak is completely suppressed in a *M* phosphoric acid base solution, this is not so with solutions containing trace amounts of chloride ions. Under such conditions chlorostannic complex ions are present in solution and they produce an interfering peak. This behaviour unfortunately precluded the use of hydrochloric acid for effecting the initial solution of the sample, as, although most of the hydrochloric

acid is removed on strong heating with phosphoric acid, its complete removal is not easy to ensure. In experiments with prepared solutions to examine the possibility of determining small amounts of lead in the presence of tin, quantities of Specpure tin metal were attacked with lead-free nitric acid and the resulting solutions were carefully evaporated almost to dryness. Phosphoric acid was then added and the solution was heated at the maximum temperature of an efficient hot-plate to dissolve the metastannic acid, followed by heating for a further 10 minutes. After cooling, the solution was diluted with cold water and made up to volume to give a final solution molar in phosphoric acid. Polarograms obtained with solutions containing 0·1 and 1·0 μg of lead per ml, respectively, both in the absence and presence of a thousandfold excess of tin, showed negligible interference from tin. The values for the peak heights are given in Table III, and a typical polarogram is shown in Fig. 1.

TABLE III

PEAK HEIGHTS FOR LEAD IN THE PRESENCE AND ABSENCE OF TIN

Lead concentration		Tin concentration		Peak height, divisions	Instrument sensitivity
M	μg per ml	*M*	μg per ml		
5×10^{-7}	0·1	nil	nil	19	maximum
5×10^{-7}	0·1	1×10^{-3}	100	22	maximum
5×10^{-6}	1·0	nil	nil	20	1/10 maximum
5×10^{-6}	1·0	1×10^{-2}	1000	20	1/10 maximum

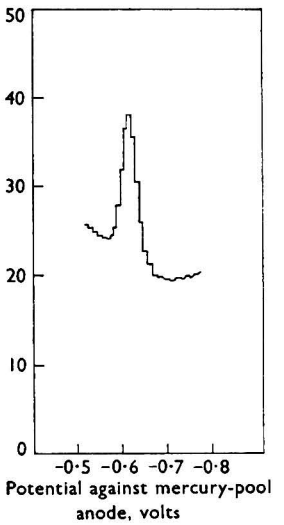

Fig. 1. Square-wave polarogram for a 1 μg per ml solution of lead in the presence of tin; the ratio of tin to lead is 1000 to 1

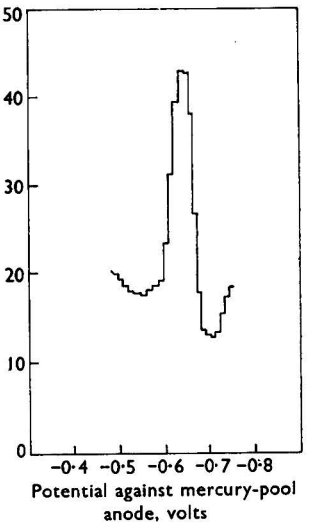

Fig. 2. Square-wave polarogram for a 1 μg per ml solution of lead in the presence of copper; the ratio of copper to lead is 1000 to 1

The satisfactory results in Table III indicated the possibility of being able to determine directly the lead content of pure tin metal and tin-base alloys. No difficulties were envisaged in alloy analysis, since of the other constituents antimony is very irreversibly reduced and the peak for copper is well separated from that for lead. Details of the procedure finally used in alloy analysis are as follows—

Attack 100 mg of sample (white metal, Babbitt metal or refined tin) with 3 ml of lead-free nitric acid in a small beaker and evaporate the solution almost to dryness. Add 6·8 ml of phosphoric acid; sp.gr. 1·75, and heat strongly on an efficient hot-plate to dissolve metastannic acid, and then heat for about 10 minutes more. Cool the solution and dilute it to 100 ml with cold water. Place a 5-ml aliquot in a polarograph cell, de-aerate for a few minutes and then record the lead peak. Finally determine the lead content of the sample by the standard-addition technique.

Typical results obtained in the analysis of tin-base samples are given in Table IV, the agreement between the polarographic and the chemical values being reasonably satisfactory. The chemical results were obtained by taking several grams of sample and precipitating the lead as sulphate after volatilisation of tin and antimony as their bromides. The determinations were completed gravimetrically in the usual way as lead molybdate. In the analysis of the pure tin sample, there was no interference from the major constituent and this corresponded to a tin to lead ratio as high as about 2000 to 1. However, this is probably the limiting ratio for such a determination. The concentration of lead in the solution from this sample was about 0·5 μg per ml, but it is possible to analyse solutions containing 0·1 μg of lead per ml, provided that the tin to lead ratio is not greater than 1000 to 1. The results for the Babbitt and white-metal samples confirmed the non-interference of both antimony and copper. The cadmium in the white metals produced a peak, following, but well separated from, the lead peak.

TABLE IV

DETERMINATION OF LEAD IN TIN-BASE ALLOYS

Alloy type	Composition, %	Lead	
		Chemical value, %	Square-wave polarographic value, %
White metal	Cu, 3·12; Sb, 7·3; Cd, 0·9; remainder Sn	0·11	0·09
White metal	Cu, 1·27; Sb, 7·9; Cd, 0·44; remainder Sn	0·39	0·42
Babbitt metal	Cu, 3·75; Sb, 8·5; remainder Sn	0·14	0·14
Babbitt metal	Cu, 3·6; Sb, 8·6; remainder Sn	0·22	0·22
Refined tin	Cu, 0·03; Bi, 0·03; In, < 0·005	0·055	0·055

Lead in copper-base alloys—Although no interference is incurred from copper in the determination of lead for moderate ratios of copper to lead, Ferrett and Milner[6] were unable to determine directly small amounts of lead in copper-base axe-head samples with use of a chloride base solution. When the applicability of the phosphoric acid base solution to this problem was investigated, gelatin was included in the final solution at the suggestion of Dr. G. C. Barker.[7] The addition of gelatin did not affect the square-wave polarogram for lead and under these conditions a well defined peak was obtained for a ratio of copper to lead of 1000 to 1, spurious instrumental responses obtained in the absence of gelatin being eliminated. In this work polarograms were recorded on solutions molar in phosphoric acid containing 1 μg of lead per ml both in the absence and presence of 100 μg of copper per ml. To 5-ml aliquots of solution, 0·2 ml of 0·05 per cent. gelatin solution was added, and the lead peak was recorded with the square-wave polarograph. The peak for lead in the presence of a thousandfold excess of copper is given in Fig. 2, a peak height of 30 divisions being obtained for 1 μg per ml solution of lead on a sensitivity setting of 1/10 of the maximum, both in the absence and in the presence of copper.

Several types of copper-base alloys contain tin as an alloying constituent in amounts up to approximately 10 per cent. The ratio of tin to lead in these materials is therefore much lower than the ratios encountered in the analysis of tin-base alloys. Zinc may be another major constituent, but should cause no difficulty in the determination of lead, since its reduction occurs at a more negative potential. Further considerations indicated that none of the usual minor alloying constituents of this type of alloy should cause interference and the square-wave polarograph should be applicable to the direct determination of the lead content of all types of copper-base alloys.

In the analysis of representative samples, the solution of 100 mg of material was effected by the same method as that employed for tin-base alloys. A 5-ml aliquot from the 100 ml of solution was transferred to the polarograph cell, and 0·2 ml of a 0·05 per cent. gelatin solution was added. After the solution had been de-aerated for a few minutes, the lead peak was recorded and the lead content of the sample was determined by the standard-addition technique.

The results obtained in the analysis of several typical alloys are shown in Table V. Good agreement was obtained between the polarographic figures and those by standard chemical methods on much larger sample weights. It can be seen from the results that no

difficulty was encountered in the determination of lead, even for copper to lead ratios as high as 3000 to 1. The lowest concentration of lead was encountered with the standard brass samples and amounted to about 0·2 μg of lead per ml. This value represents the lowest limit for this determination and the accuracy here is only approximately 30 per cent.

TABLE V

DETERMINATION OF LEAD IN COPPER-BASE ALLOYS

Alloy type	Composition, %	Lead	
		Chemical value, %	Square-wave polarographic value, %
B.C.S. bronze A	Sn, 9·96; Sb, 0·24; Zn, 1·86; remainder Cu	1·83	1·78
B.C.S. bronze C	Sn, 9·80; Zn, 2·53; Ni, 0·09; Fe, 0·06; remainder Cu	0·41	0·40
Phosphor bronze	Sn, 7·95; Zn, 4·2; Ni, 0·02; remainder Cu	0·28	0·31
B.C.S. manganese brass	Zn, 33·9; Mn, 1·03; Al, 1·62; Sn, 1·75; Ni, 1·01; Fe, 0·91; remainder Cu	0·78	0·80
Cartridge brass	Bi, 0·015; Sb, 0·01; As, 0·018; Ni, 0·02; Sn, 0·03; Fe, 0·025; Zn, 30; remainder Cu	0·06	0·058
Standard brass	Sn, 1·34; Ni, 2·85; Fe, 0·95; Al, 0·46; Cu, 55·45; remainder Zn	0·02*	0·013

* Spectrographic result.

Aluminium-base alloys—The lead and tin contents of aluminium alloys are generally quite small, but nevertheless a knowledge of the lead content is frequently required. The polarographic method was applied to solutions prepared from typical alloy samples, the final solutions containing 1 mg of sample per ml as before. The solution procedure was the same as that used for the tin-base alloys, with the exception that 5 ml of nitric acid were required. No interferences were expected in the analysis of this type of sample and this is substantiated by the good agreement between the chemical and polarographic figures for the lead contents reported in Table VI. The lowest concentration of lead determined in this work was in the B.C.S. alloy and amounted to 0·15 μg of lead per ml.

TABLE VI

DETERMINATION OF LEAD IN ALUMINIUM ALLOYS

Alloy type	Composition, %	Lead	
		Chemical value, %	Square-wave polarographic value, %
B.C.S. aluminium alloy No. 216	Cu, 4·15; Fe, 0·81; Sn, 0·02; Ni, 0·22	0·01	0·015
N.B.S. aluminium alloy No. 86C	Cu, 7·92; Zn, 1·50; Fe, 0·90	0·031	0·027
Duralumin	Cu, 4·07; Fe, 0·45; Mn, 0·54; Si, 0·51; Mg, 0·58; Ni, 0·05; Zn, 0·06	0·03	0·039
N.B.S. aluminium - silicon alloy No. 87	Si, 6·21; Ni, 0·59; Fe, 0·46; Cu, 0·30; Mn, 0·30; Sn, 0·063	0·068	0·076
Pure aluminium	Cu, 0·02; Fe, 0·37; Si, 0·030; other elements, < 0·03	0·02	0·026

Zinc-base alloy—The Mazak type of alloy contains several elements in trace amounts, including lead, tin and copper. In earlier work on the investigation of a chloride base electrolyte, the copper and cadmium contents of these alloys could be determined directly, but the direct determination of the lead content proved impossible because of interference from tin. With use of the phosphoric acid base solution for the determination of the lead content, the interference from tin was eliminated, but a decreased sensitivity resulted. For this reason the lowest lead content determinable in Mazak alloys was about 0·005 per cent. In the analysis of suitable alloy samples, solutions were prepared as previously described on p. 143, and the standard-addition technique was employed to determine the lead content.

The results on several standard samples are reported in Table VII, and for all samples reasonable agreement was obtained between the polarographic and chemical results. The lowest concentration of lead determined here was 0·06 μg per ml in the National Bureau of Standards sample and the accuracy of such a determination is better than 10 per cent.

TABLE VII

DETERMINATION OF LEAD IN ZINC ALLOYS

Alloy type	Composition, %	Lead	
		Chemical value, %	Square-wave polarographic value, %
N.B.S. alloy No. 94B	Al, 4·07; Cu, 1·01; Fe, 0·017; Mn, 0·014; Mg, 0·042; Sn, 0·005	0·006	0·007
Standard Mazak alloy, A9	Cd, 0·0067; Sn, 0·0045; Cu, 0·05; Mg, 0·04	0·007	0·008
Standard Mazak alloy, A10	Cd, 0·01; Sn, 0·0065; Cu, 0·095; Mg, 0·04	0·010	0·010
B.C.S. zinc metal No. 194b	Fe, 0·001; Cd, 0·006; Cu, 0·002; Zn, 99·964 (by difference)	0·027	0·023

Ferrous alloys—Although the potentialities of the square-wave polarograph have already been shown for the determination of the lead content of steels with use of a chloride base solution,[6] the application of the phosphoric acid base solution should result in the extension of the method to samples containing tin in addition to lead. The procedure used in the analysis of samples involved the solution of 100 mg in 7 to 8 ml of lead-free nitric acid, diluted with a little water. After the evaporation of the solution almost to dryness, 6·8 ml of phosphoric acid were added and the solution was strongly heated. Then the determination was completed as described previously. Experimental work was confined to the B.C.S. steel No. 212 to study the effect of tin. Sufficient tin was added to a solution of the sample to make it approximately 12 per cent. in tin. The square-wave polarographic value for the lead content of this solution was found to be 0·265 per cent., which compares favourably with the chemical value of 0·28 per cent. and confirms the non-interference of tin.

Minerals—A knowledge of the lead content of a monazite mineral is very important in age-determination work on the earths crust, lead being the end-product of the decay scheme of the uranium and thorium in this type of mineral. A polarographic method is available for this determination, involving a preliminary separation of the lead as sulphate from other sample constituents, strontium sulphate acting as a carrier for the small lead sulphate precipitate.[8] Unfortunately a fairly long standing period is needed in this method to ensure complete precipitation of the lead sulphate. An absorptiometric procedure employed at the Chemical Research Laboratory, Teddington, is based on the coloured complex formed by lead with dithizone. Again some preliminary separation of the lead is necessary and it is precipitated as its insoluble sulphide in the presence of silver sulphide as a carrier. When the applicability of the phosphoric acid base solution to this determination was examined, it was found that solution of the sample could be effected in hot phosphoric acid and the lead content could be determined directly on the resulting solution. Further details of the method are as follows—

> Attack 100 mg of the finely ground sample in a small platinum dish with 10 ml of hot lead-free nitric acid and 2 ml of hydrofluoric acid. Digest hot for a period up to about 60 minutes, when a large portion of the sample should have gone into solution. Then evaporate just to dryness, add 6·8 ml of phosphoric acid and heat strongly on an efficient hot-plate for about 30 minutes to obtain complete solution of the sample. After cooling, dilute the solution with water and make up to a volume of 100 ml with water. Record the lead peak on a 5-ml aliquot of this solution in the usual way and determine the lead content of the sample by the standard-addition technique.

Results obtained by the above method on various samples of monazite are given in Table VIII, together with the chemical or conventional polarographic values or both. The agreement between the results for the various methods is good, if account is taken of the complex nature of the sample material. There is a tendency for the square-wave polarographic results to be slightly higher than those by the other methods, and this is possibly

explained by the fact that this new method is the only one that avoids any chemical separation of the lead. The precision of the square-wave polarographic method was determined on the sample of monazite CRL181/54 shown in Table VIII and found to be quite high. Six determinations by two different operators gave an average value for the lead content of 0·237 ± 0·007 per cent. This method takes about 2 hours for a single determination, compared with 2 days at least by the conventional polarographic method.

TABLE VIII

RESULTS FOR LEAD IN MONAZITE SAMPLES

Sample type	Composition, %	Lead		
		Chemical value, %	Conventional polarographic value, %	Square-wave polarographic value, %
Monazite N.B.S. 2601	ThO_2, 9·65; U_3O_8, 0·38; remainder rare-earth oxides and phosphates	0·23	0·225	0·25 (± 0·006)
Monazite CRL 181/54	ThO_2, 10·38; U_3O_8, 0·17	0·26	—	0·237 (± 0·007)
Monazite CRL 398/53	ThO_2, 6·04; U_3O_8, 0·23	0·17	—	0·19 (± 0·005)
Monazite CRL 399/53	ThO_2, 5·24; U_3O_8, 0·18	—	0·22	0·24 (± 0·005)
Monazite (ebonite)	—	—	0·22	0·22 (± 0·003)

Miscellaneous samples—Miscellaneous materials successfully examined by this procedure include an opal glass and a sample of fluorspar. With each material 100 mg of sample were attacked with lead-free nitric acid and 2 ml of hydrofluoric acid in a small platinum dish. After evaporation of the solution to dryness, 6·8 ml of phosphoric acid were added, and the mixture was heated strongly to effect complete solution of the sample. Then the lead peak was recorded and evaluated as before. Results for National Bureau of Standards samples are given in Table IX. The satisfactory agreement between the polarographic and chemical figures serve as an illustration of the possibilities for the direct determination of lead in materials other than alloys.

TABLE IX

DETERMINATION OF LEAD IN MISCELLANEOUS MATERIALS

Sample	Composition, %	Lead	
		Chemical value, %	Square-wave polarographic value, %
Opal glass N.B.S. No. 19	Al_2O_3, 6·01; CaO, 10·48; F, 5·72; As, 0·1; remainder SiO_2	0·097 (PbO)	0·099 (PbO)
Fluorspar N.B.S. No. 79	SiO_2, 1·88; Zn, 0·35; Fe_2O_3, 0·15; remainder CaF_2	0·23	0·25

DETERMINATION OF COPPER—

The determination of copper with the square-wave polarograph has previously been studied in a chloride base solution and found to possess many advantages in the analysis of such materials as steels, zinc, magnesium and aluminium-base alloys.[5,6] With samples containing antimony the chloride base electrolyte could not be used, however, without the previous removal of the antimony, which gives an interfering peak. For example, in the analysis of tin-base white-metal samples containing 7 to 8 per cent. of antimony, the antimony and tin were removed as their volatile bromides before the copper peak was recorded from the chloride base solution. As antimony is not reduced at the dropping-mercury electrode from a phosphoric acid base solution, this type of base solution should be more satisfactory for the determination of copper in the presence of antimony. Unfortunately, iron can cause difficulties in this base solution in certain circumstances. Direct methods have, however,

been developed for determining the copper content (up to about 4 per cent.) of lead, tin and aluminium-base alloys, provided that the ferric iron to copper ratio in the sample is not greater than 1 to 1.

In experiments to investigate the behaviour of copper in the *M* phosphoric acid base electrolyte, solutions were prepared by evaporating suitable aliquots of a standard copper solution in nitric acid with 6·8 ml of phosphoric acid, sp.gr. 1·75. On cooling, each solution was diluted to a volume of approximately 50 ml with water and then boiled for a few minutes. After the solution had cooled to room temperature, it was diluted to 100 ml with water. A linear relationship was observed between peak height and concentration for the range of copper concentrations up to about 30 μg per ml. For higher concentrations, however, this relationship was not followed. It was found that slight variations in the anode-pool potential from sample to sample sometimes resulted in difficulty in the measurement of the height of the copper peak, owing to the incomplete formation of the peak near zero applied potential. To circumvent this the anode was covered with a thin layer of mercurous phosphate, thereby fixing the potential of the mercury-pool anode at + 0·404 V against the S.C.E. For the analysis of the alloys, sample weights were taken to give a final copper concentration in the range 0 to 30 μg per ml. Several determinations were carried out on each sample to ascertain the precision of the method, which was usually found to be better than 2 per cent. Details of the application to alloys follow.

Lead-base alloys—In the analysis of this type of alloy, 100 mg of sample were dissolved in nitric acid. Then the solution was evaporated almost to dryness and heated as before in the presence of 6·8 ml of phosphoric acid. After cooling slightly, the solution was diluted to about 50 ml with water and then boiled for a few minutes. After cooling to room temperature, the solution was diluted to 100 ml with water, and the copper peak was recorded on a small aliquot. Some typical results obtained by this method are given in Table X. Only one B.C.S. sample was available for analysis and for this excellent agreement was obtained with the chemical value. Two lead-base standards prepared for spectrographic work were available and the polarographic results for copper were found to be in reasonable agreement with the known value for this constituent. In this work the lowest concentration of copper determined in the presence of a 7500-fold excess of lead was 0·13 μg per ml, and this represents about the limiting concentration for the determination.

TABLE X

DETERMINATION OF COPPER IN LEAD-BASE ALLOYS

Alloy type	Composition, %		Copper: Chemical value, %	Copper: Square-wave polarographic value, %
B.C.S. white-metal A	Sb, 12·04; Sn, 4·64; Pb, 82·6; Bi, 0·03; Fe, 0·06; As, 0·06; Zn, 0·08		0·33	0·32
Lead-base standard (1)	Bi, Zn, Sb, Cd, As, Mg, Al, Cu, Ag, Sn, Fe	each 0·10	0·10	0·080 0·078
Lead-base standard (2)	Bi, Zn, Sb, Cd, As, Mg, Al, Cu, Ag, Sn, Fe	each 0·01	0·01*	0·013

* Known from preparation of alloy.

Tin-base and aluminium-base alloys—The solution procedure described above for lead-base alloys was employed for these alloys, but the weight of sample taken was such as to give a final concentration of copper in the range 0 to 30 μg per ml. The peak for copper in a white-metal alloy ("Babbitt metal" in Table XI) is shown in Fig. 3. The results for various alloys are given in Table XI and for all there was good agreement with the chemical values. With the tin-base samples the precision of the determination was better than 2 per cent. for copper contents ranging from 0·03 to 4 per cent.

DETERMINATION OF ZINC—

Ferrett and Milner[6] have already applied the square-wave polarograph to the direct determination of the zinc content of copper-base alloys by using an ammonia - ammonium chloride base electrolyte. This base solution is clearly limited to alloys in which the major

constituent is not precipitated by ammonia. An acidic base electrolyte is potentially more useful for the analysis of several different types of alloys, but has so far proved useless in conventional polarographic analysis, owing to the masking of the zinc step by the diffusion current from the reduction of hydrogen ions. With the square-wave polarograph this interference is less troublesome; for example, a well developed peak is produced for zinc in a *M* phosphoric acid base solution before the discharge of the hydrogen ions (see Fig. 4). The possibility of employing this base electrolyte for the determination of zinc was therefore examined in some detail.

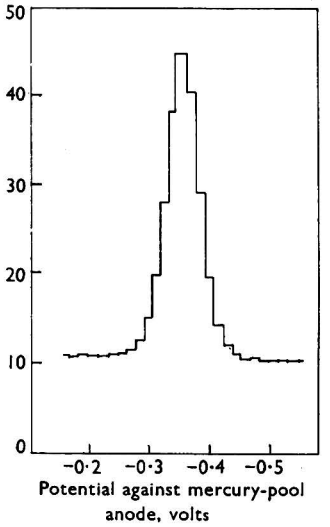

Fig. 3. Square-wave polarogram for copper in a white-metal sample

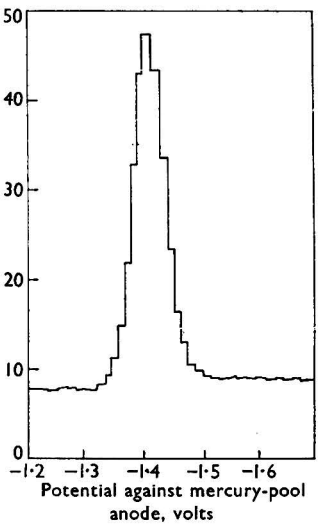

Fig. 4. Square-wave polarogram for zinc in *M* phosphoric acid

TABLE XI

DETERMINATION OF COPPER IN TIN-BASE AND ALUMINIUM-BASE ALLOYS

Alloy type	Composition, %	Copper	
		Chemical value, %	Square-wave polarographic value, %
White metal	Cu, 1·27; Sb, 7·9; Cd, 0·44; remainder Sn	1·27	1·25*
White metal	Cu, 3·12; Sb, 7·3; Cd, 0·9; remainder Sn	3·12	3·20
Babbitt metal	Cu, 3·75; Sb, 8·5; remainder Sn	3·75	3·75*
Refined tin	Cu, 0·03; Bi, 0·03; In, < 0·005	0·03	0·029
B.C.S. aluminium alloy No. 216	Fe, 0·81; Sn, 0·02; Ni, 0·22; Pb, 0·01	4·15	4·19*

* These results are the mean of four separate analyses, with a precision of better than ± 2 per cent.

Difficulties occurred at once when calibration graphs were produced with solutions prepared by heating with phosphoric acid, and then diluting with water in the cold. The peak height - concentration relationship was not linear, and it proved impossible to reproduce the peak height for a given concentration of zinc. It was observed that the peak height was dependent on the time taken for heating in the presence of phosphoric acid, the height being smaller the longer the heating time. These results indicated that quantitative reduction of zinc ions was not occurring, owing to complex formation with solvent molecules, and also that the extent of complex formation with the solvent depended on the time of heating. However, the complex was easily decomposed by boiling the sample solution before recording the polarogram. The procedure finally adopted involved heating with phosphoric acid for about 15 minutes, and then dilution with about 50 ml of water; the solution was boiled for about 10 minutes, cooled to room temperature and finally diluted to 100 ml with water. With use of this technique, reproducible peak heights were produced and linear calibration

graphs were obtained in the concentration range from 0 to 30 μg of zinc per ml. Higher concentrations were not investigated.

The fact that nickel is reduced at only a slightly more negative potential than zinc in a phosphoric acid base electrolyte presents a limitation of this method applied to the determination of zinc in alloy systems. Fortunately the reduction of nickel is irreversible and causes no interference in the determination of zinc for nickel to zinc ratios of 2 to 1 or less. For the analysis of copper, tin, lead and aluminium-base alloys 100 mg of sample were dissolved in the minimum amount of Polaritan nitric acid and heated in the presence of 6·8 ml of phosphoric acid in the usual way. After slight cooling, 50 ml of water were added and the solution was boiled for 10 minutes. Then the solution was cooled to room temperature and diluted to 100 ml with water. The zinc peak was recorded on a 5-ml aliquot of this solution.

Results obtained by this procedure for the determination of the zinc content of several types of analysed metallurgical samples are given in Table XII. The agreement with the reported chemical values is good for all the alloy systems studied with the exception of the white-metal samples. The chemical values for zinc in the B.C.S. white-metal samples A and B has now been shown to be in error by different workers. Hunter and Miller[9] obtained figures of 0·03 per cent. for standard A and 0·44 per cent. for standard B. They used an ion-exchange method for the separation of the zinc, followed by titration with ethylenediaminetetra-acetic acid. In earlier work Milner[10] reported figures of 0·023 and 0·43 per cent., respectively, for these standard samples by a procedure involving the separation of the zinc as its sulphide, followed by titration with potassium ferrocyanide, with naphthidine as the indicator. The agreement between the polarographic results and the above revised chemical values is reasonably satisfactory and the precision is normally better than 2 per cent. The lowest concentration of zinc determined in the analysis of these samples occurred with the lead-base standard and amounted to 0·10 μg per ml. However, it is estimated that it would be possible to detect 0·02 μg of zinc per ml if use is made of the maximum sensitivity of the instrument.

TABLE XII

DETERMINATION OF ZINC IN VARIOUS TYPES OF NON-FERROUS ALLOYS

Alloy type	Composition, %	Zinc	
		Chemical value, %	Square-wave polarographic value, %
B.C.S. aluminium alloy No. 216	Cu, 4·15; Fe, 0·81; Sn, 0·02; Ni, 0·22	0·26	0·26 (± 0·008)
N.B.S. aluminium alloy No. 86C	Cu, 7·92; Fe, 0·90; Si, 0·68; Ni, 0·03; Mn, 0·04; Cr, 0·0 29	1·50	1·45 (± 0·01)
Duralumin	Cu, 4·07; Fe, 0·45; Mn, 0·54; Si, 0·51; Mg, 0·58; Ni, 0·05; Zn, 0·06	0·06	0·052 (± 0·002)
B.C.S. white-metal A	Sb, 12·04; Sn, 4·64; Pb, 82·6; Bi, 0·03; Fe, 0·06; As, 0·06; Zn, 0·08	0·08	0·018 (± 0·002)
B.C.S. white-metal B	Pb, 3·86; Sn, 84·0; Sb, 7·51; Cu, 4·08; Fe, 0·05; As, 0·04	0·40	0·44 (± 0·015)
Lead-base standard (1)	Bi, Zn, Sb, Cd, As, Mg, Al, Cu, Ag, Sn, Fe { each 0·10	0·014	0·011 (± 0·000)
B.C.S. bronze A	Sn, 9·96; Sb, 0·24; Zn, 1·86; remainder Cu	1·86	1·83 (± 0·015)

DETERMINATION OF CADMIUM—

Although cadmium possesses reasonably favourable polarographic characteristics in a phosphoric acid base solution ($I = 2·86$ compared with 3·41 in M hydrochloric acid), analytical applications in metallurgical analysis are limited to the very few alloys requiring an analysis for this constituent. The determination of cadmium is sometimes needed in lead, tin and copper-base alloys, and the possibilities of using the square-wave polarograph for this type of determination were therefore examined. Solutions containing 1 μg of cadmium per ml and 500 μg of lead, tin or copper per ml, respectively, in M phosphoric acid were prepared. Well developed peaks were obtained for cadmium in all experiments, although the peak in the presence of lead was only just resolved.

Some typical lead and tin-base alloys were analysed for cadmium with use of the square-wave polarograph, the solution procedure employed being identical with that for the determination of lead in white metals. The cadmium results for the various samples are reported in Table XIII. Good agreement was obtained with the chemical values for all alloys. No difficulties occurred with the tin-base samples owing to the apparent non-reducibility of the major constituent. However, with lead-base alloys, although the determination of the cadmium content (0·07 per cent.) of the lead-base standard was accomplished without difficulty, the interference from lead became quite serious for samples with lower cadmium contents, and the limiting ratio of lead to cadmium is about 1500 to 1.

TABLE XIII

DETERMINATION OF CADMIUM IN TIN AND LEAD-BASE ALLOYS

Alloy type	Composition, %	Cadmium: Chemical value, %	Cadmium: Square-wave polarographic value, %
White metal	Cu, 1·27; Sb, 7·9; Cd, 0·44; Pb, 0·39; remainder Sn	0·44	0·44
White metal	Cu, 3·12; Sb, 7·3; Cd, 0·9; Pb, 0·11; remainder Sn	0·90	0·92
Lead-base standard (1)	Bi, Zn, Sb, Cd, As, Mg, Al, Cu, Ag, Sn, Fe { each 0·10	0·07	0·071

CONCLUSIONS

The use of a phosphoric acid base electrolyte in conjunction with the square-wave polarograph has resulted in a considerable improvement in the analytical methods for the determination of copper, lead, cadmium and zinc contents of various materials, particularly metallurgical alloys, including copper, tin, lead, aluminium and zinc-base alloys. The major advantages of this new base electrolyte in polarographic analysis arises from such factors as the non-interference from tin in the determination of lead and similarly of indium in the determination of cadmium. Further, the polarographic methods described represent a considerable improvement on existing methods in simplicity and speed. For example, the polarographic determination of a single constituent of an alloy takes approximately 30 minutes and it is possible to determine the copper, lead, cadmium and zinc contents of a tin-base alloy in less than 2 hours. This new base solution, however, results in a slight loss of sensitivity compared with a *M* hydrochloric acid solution. The diffusion-current constants of the elements studied were found to be approximately 20 per cent. smaller in the phosphoric acid solution.

The applicability of phosphoric acid as a solvent has been shown by the variety of materials that can be dissolved by it and maintained in solution. Monazites were the only mineral samples examined in this work. Phosphoric acid should, however, be applicable to the solution of a wide range of rocks and minerals, which may then be examined polarographically for various constituents.

Several of the samples of monazite were kindly supplied by the Chemical Research Laboratory, Teddington.

REFERENCES

1. Kolthoff, I. M., and Lingane, J. J., "Polarography," Second Edition, Interscience Publishers Inc., New York, 1952.
2. Höltje, R., and Geyer, R., *Z. anorg. Chem.*, 1941, **246**, 258.
3. Strickland, E. H., *Analyst*, 1955, **80**, 548.
4. Whittem, R. N., and Milner, G. W. C., Atomic Energy Research Establishment, Memorandum C/M 185, 1953.
5. Ferrett, D. J., and Milner, G. W. C., *Analyst*, 1955, **80**, 132.
6. ——, ——, *Ibid.*, 1956, **81**, 193.
7. Barker, G. C., private communication.
8. Smales, A. A., *Proc. Roy. Soc. Edinb.*, 1948, **63**, 125.
9. Hunter, J. A., and Miller, C. C., *Analyst*, 1956, **81**, 79.
10. Milner, G. W. C., *Anal. Chim. Acta*, 1952, **6**, 226.

NOTE—References 5 and 6 constitute Parts I and II, respectively, of this series.

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The Determination of Lead and Copper in Organic Materials (Foodstuffs) by a Dry-ashing Procedure

By D. ABSON and A. G. LIPSCOMB

A method of dry-ashing described has been found suitable for the preliminary destruction of the organic matter of a wide range of food materials before the determination of trace amounts of lead and copper. The sample material is sulphated, which permits ashing to be carried out up to a temperature of 550° C without loss of lead by volatilisation. An aqueous suspension of light magnesium carbonate is used as an ashing-aid when the amount of ash is small, being added to the sulphated sample after it has been charred and crushed to a powder. After solution of the ash, lead and copper are determined by measurement of the colours of the complexes formed in chloroform solution with diphenylthiocarbazone and diethylammonium diethyldithiocarbamate, respectively.

When ashing is used as a means of destroying organic matter before the determination of lead, a temperature below 550° C is usually recommended in order to prevent losses due to volatilisation,[1,2,3] and frequently a temperature not greater than 500° C is preferred.[4] Various workers recommend additions to the material before ashing in order to improve recovery. Sulphuric acid is added by Schmidt[5] to prevent the loss of volatile lead compounds. Seiser, Necke and Muller[6] consider that blood can be ashed without loss of lead only if sulphuric acid is first added. They recommend a temperature between 500° and 530° C and state that lead sulphate is not volatile below 550° C. Tompsett and Anderson[7] favour the addition of phosphate on similar grounds. Complete recovery of lead may also be prevented by the formation of insoluble compounds during ashing; for example, the formation of lead silicates owing to reaction with silicates in the sample or with the silica basin in which the sample is ashed. In the latter event the losses can be reduced by mixing with the sample a solution of a suitable metal salt so that on ashing the bulk of the ash is increased and the proportion of lead in contact with the dish is decreased. Lockwood[8] mixes the sample with calcium nitrate solution before ashing it at 500° C and finds an 80 to 95 per cent. recovery of added lead by this technique. Other ashing-aids frequently used include solutions of calcium acetate, calcium hydroxide, magnesium acetate and magnesium nitrate.

Dry-ashing as a means of destroying organic matter has found wider acceptance for copper determinations than for lead. If the ash is sulphated and the silica dishes used are not worn, there is no loss of copper on ashing.[9] When old worn dishes are used, the copper may be tenaciously held at the surface of the dish and give rise to low results. Comrie[10] uses magnesium nitrate to keep the ash bulky and to reduce contact with the dish.

The method of dry-ashing described below is the outcome of an investigation into the suitability of ashing as an alternative to wet-oxidation with sulphuric and nitric acids before the colorimetric determination of lead and copper. It has been successfully used for some time in our laboratory for routine determinations of metals in a wide variety of raw materials and finished confectionery goods, and possesses certain advantages over the wet-oxidation procedure previously used, notably that there is a considerable saving of reagents, especially nitric and sulphuric acids, and in that dry-ashing does not require constant attention, as does wet-oxidation. The procedures for the extraction of the metals as coloured complexes (lead with dithizone from alkaline citrate - cyanide solution and copper with diethyldithiocarbamate from acid solution) are adapted from the scheme presented by Strafford, Wyatt and Kershaw.[11] The dry-ashing stage can, of course, be linked with other existing methods for the determination of these metals.

Experimental

Preliminary ashing experiments—

Recovery experiments were initially confined to lead, as it was considered that losses during ashing would be more likely with this metal than with copper. For the purpose of these preliminary experiments a bulk sample of granulated sugar was used to provide the basic material, this substance being chosen for its uniform nature and because it is easily

destroyed by ashing to yield a light soluble ash virtually free from trace metals, calcium, phosphates and other substances liable to interfere with the subsequent extraction processes.

Influence of various additions before ashing on the recovery of lead—Amounts of lead up to the equivalent of 4 p.p.m. were added as a standard solution to a series of 5-g samples, which, after various additions had been made, were ashed at a temperature not greater than 500 °C. The ash so obtained was dissolved in boiling dilute hydrochloric acid, 4 ml of 50 per cent. citric acid solution were added, the solution was neutralised with ammonium hydroxide, sp.gr. 0·880, and made alkaline by adding 0·5 ml in excess. Then 5 ml of 10 per cent. potassium cyanide solution were added and, when cool, the solution was transferred to a separating funnel and extracted with dithizone solution and the lead determined as described under "Method" (p. 157).

Recovery of lead was incomplete when the sugar was simply charred over a burner and ashed. Sulphating the ash, by adding 1 ml of sulphuric acid to the samples before ashing, and increasing the bulk of the ash, by adding an ashing-aid (achieved by dissolving the samples in 5 ml of 5 per cent. magnesium nitrate solution before charring), both led to improved recovery of lead, as shown in Table I. It was thought that by adding to the material both sulphuric acid and the ashing-aid recovery should be further improved. In practice, however, this procedure proved unsatisfactory owing to the retention of an unoxidised residue of carbon, which gave a greyish brown colour to the ash. The same difficulty was encountered when other ashing-aids (solutions of magnesium acetate, calcium nitrate and calcium acetate) were tried in conjunction with sulphuric acid. In any event calcium salts were unsuitable, owing to the formation of insoluble calcium sulphate. It was possible to get rid of the residual unoxidised carbon by moistening the ash with water or nitric acid and re-ashing, but this had the effect of breaking down the bulk of the ash and thereby largely vitiated the efficacy of the ashing-aid. The recovery of added lead from samples subjected to this treatment was found to be variable and incomplete.

It was assumed that the incomplete oxidation of carbon that was observed, especially when magnesium nitrate solution was added to the sulphated material, was probably due to the formation of a protective layer of magnesium sulphate around some of the organic matter, and it was thought that this could probably be overcome by first sulphating the material and then removing excess of sulphuric acid by heating before adding the ashing-aid. A sample of sugar was therefore sulphated as before and heated over a burner until the black spongy mass first formed became dry and brittle, when it was powdered by being crushed with a pestle. After being heated further, the crushed char was saturated with 5 ml of the magnesium nitrate solution, dried and ashed. The material so treated ashed quickly to give a carbon-free ash, which was, however, only small in bulk, as the magnesium nitrate solution had largely dried out in a layer on the bottom of the dish, giving little protection to the ash formed. From these observations it appeared that a more satisfactory result could be obtained if the ashing-aid were added in such a form that it entirely covered and supported the crushed char and for this purpose an undissolved powder was considered. Light magnesium carbonate was selected as being potentially suitable, as it is extremely light in relation to bulk and being insoluble could be added as a suspension in water. Preliminary trials showed that 7 g of light magnesium carbonate shaken with 100 ml of water gave a suitable suspension and 10 ml of this suspension gave adequate coverage of the crushed char obtained from 5 or 10 g samples. On drying the mixture before ashing, it was observed that the individual particles of charred material were dispersed in a matrix of magnesium

TABLE I

INFLUENCE OF SULPHURIC ACID AND MAGNESIUM NITRATE SOLUTION ON THE RECOVERY OF LEAD FROM SUGAR SAMPLES ASHED BELOW 500° C

	Sample alone		Sample with sulphuric acid added		Sample with magnesium nitrate added	
Lead added, p.p.m.	Average lead found, p.p.m.	Average recovery, %	Average lead found, p.p.m.	Average recovery, %	Average lead found, p.p.m.	Average recovery, %
0·5	0·47	94	0·52	104	0·48	96
1·0	0·95	95	0·97	97	0·93	93
2·0	1·56	78	1·86	93	1·78	89
4·0	3·15	79	3·82	95	3·58	89

carbonate, which also formed a coating on the inside of the dish. The material, being in a finely divided state, ashed quickly to give a bulky carbon-free ash.

TABLE II

RECOVERIES OF ADDED LEAD AND COPPER FROM SUGAR SAMPLES TREATED WITH SULPHURIC ACID AND MAGNESIUM CARBONATE AND ASHED BELOW 550° C

Amount of metal added, p.p.m.	Average metal found, p.p.m.	Average recovery, %
Lead—		
0·5	0·50	100
2·0	2·01	101
3·0	3·04	101
4·0	3·93	98
Copper—		
5·0	5·0	100
20·0	19·8	99

A series of sugar samples containing known amounts of added lead were treated in this manner and ashed at a temperature between 500° and 550° C (it had been established in a separate experiment that lead was not lost from sulphated material ashed below approximately 550° C). Full recovery of the added lead was attained under these conditions, as shown by the results in Table II. The procedure was then checked for the recovery of copper, which was added to a separate series of test samples, the copper content of the ashes being determined by extraction of the acid solution with diethylammonium diethyldithiocarbamate as described under "Method" (see p. 157). Recovery was also complete with this metal, as indicated in Table II.

Effect of temperature of ashing on recovery of lead—In order that routine determinations of lead and copper by an ashing procedure could be conducted as rapidly as possible, it was important to know the effect of the temperature of ashing on the recovery.

The equivalent of 1 p.p.m. of lead was added to a series of sugar samples, which were sulphated, charred and treated with magnesium carbonate suspension and then ashed at various temperatures between 470° and 675° C. The samples were kept in a muffle furnace for a fixed time (2 hours) and the temperature was kept at a steady value (within ± 5° C) by adjusting the flue of the furnace. At each particular temperature similar samples without the addition of sulphuric acid were ashed, in order to compare the recovery of lead with that from sulphated material. Duplicate sulphated and non-sulphated samples were placed alternately in the chamber of the muffle. Table III shows the results of this experiment. They confirm that materials to which sulphuric acid has been added can be ashed without loss of lead at higher temperatures than can materials that have not been sulphated. The approximate temperatures above which lead is lost from sulphated and non-sulphated materials are 550° C and 500° C, respectively.

TABLE III

EFFECT OF TEMPERATURE OF ASHING ON THE RECOVERY OF 1 p.p.m. OF LEAD FROM SUGAR SAMPLES, IN PRESENCE AND IN ABSENCE OF SULPHURIC ACID

	Sulphated samples		Non-sulphated samples	
Temperature of muffle furnace, °C	Average lead found, p.p.m.	Average recovery, %	Average lead found, p.p.m.	Average recovery, %
470	1·04	104	1·05	105
520	1·05	105	0·72	72
560	1·00	100	0·71	71
580	0·60	60	0·25	25
610	0·38	38	0·30	30
675	0·27	27	0·14	14

APPLICATION OF ASHING METHOD TO VARIOUS MATERIALS—

Having established an ashing method that gave good recoveries of added lead and copper from test samples, we applied the procedure to a variety of food materials. The lead and

copper contents of the raw materials listed in Table IV were determined after destruction of organic matter by the following methods—

(*a*) sulphating and charring the sample, crushing the char, adding 10 ml of magnesium carbonate suspension and ashing at 500° to 550° C,
(*b*) as (*a*), but with omission of the ashing-aid, and
(*c*) wet oxidation with sulphuric and nitric acids.

Duplicate determinations were made on 10-g samples, the solutions obtained being divided into two portions, each equivalent to 5 g of material, for separate lead and copper determinations. To prevent possible interference from iron, hydroxylamine solution was added to the lead aliquot before extraction.

TABLE IV

RESULTS OF DUPLICATE DETERMINATIONS OF LEAD AND COPPER CONTENTS OF VARIOUS FOODSTUFFS BY DRY-ASHING AND WET-OXIDATION

Material	Lead found with—			Copper found with—		
	Sulphated sample, p.p.m.	Sulphated, then magnesium carbonate added, p.p.m.	Wet-oxidation, p.p.m.	Sulphated sample, p.p.m.	Sulphated, then magnesium carbonate added, p.p.m.	Wet-oxidation, p.p.m.
Honey	0·25	0·23	0·23	0·4	0·5	0·5
Malt	0·20	0·15	0·17	8·6	8·9	8·7
Glacé cherries	0·65	0·75	0·70	1·6	1·5	1·5
Jam	0·20	0·17	0·17	0·4	0·4	0·4
Milk powder	0·10	—	0·10	3·6	—	3·7
Condensed milk	0·13	0·13	0·15	0·7	0·7	0·8
Milk crumb	0·25	0·30	0·33	2·1	2·3	2·1
Cocoa powder	0·27	0·23	0·25	33·0	32·5	32·5
Gelatin	0·35	0·43	0·38	6·7	6·8	6·7
Glyceryl monostearate	1·05	1·10	1·15	0·5	0·7	0·9
Wax	0·48	0·55	0·55	0·5	0·6	0·6
Chewing-gum base—						
Material A	1·10	1·45	1·45	1·4	1·8	1·8
Material B	1·68	1·78	1·83	0·3	0·3	0·3
Material C	0·63	0·95	1·00	0·1	0·1	0·1

Effect of size of ash—Reference to Table IV shows good agreement between the lead and copper contents determined with the different methods of destruction (ashing with and without ashing-aid and wet-oxidation) with the exception of certain materials having much smaller ashes than the other materials examined, namely the chewing-gum base-materials and wax. With these the lead and copper recovery was significantly lower when the materials were ashed without an ashing-aid than when ashed with an ashing-aid or wet-oxidised. Ash-aided ashing and wet-oxidation showed good agreement for lead and copper contents for all materials regardless of ash size. These results confirm that an ashing-aid is necessary for full recoveries of lead and copper with materials having a low ash content, but is not necessary otherwise.

COMPARISON OF SPEED AND CONVENIENCE OF ASHING AND WET-OXIDATION—

Although the over-all time taken for ashing of any particular material was usually somewhat longer than the time taken for wet-oxidation, constant attention was not necessary, and so it was possible to carry on with other operations such as extracting one batch of solutions while another series of samples was being ashed. The majority of materials examined were ashed to apparent completion in 1 to 2 hours and some were completely ashed in a much shorter time than this. It was observed generally that the most difficult materials to destroy by wet-oxidation were those that were completely ashed in the shortest time.

INTERFERENCE FROM ALKALINE-EARTH METALS AND PHOSPHATES—

(*a*) *Precipitation of phosphates from alkaline solution*—When the acid solution derived from materials containing appreciable amounts of alkaline-earth phosphates is made alkaline with ammonium hydroxide before the extraction of lead with dithizone solution, a precipitation of calcium or magnesium ammonium phosphate occurs, which makes the subsequent

extraction procedure difficult or impossible and gives rise to errors due to the occlusion of lead in the precipitate. Various means of overcoming this difficulty have been put forward, including the following—

(*a*) the addition of excessively large amounts of citrate before the solution is made alkaline,[12]

(*b*) the separation of the calcium and phosphorus components by chemical manipulation, and their separate extraction with dithizone,[2] and

(*c*) the separation of the lead by extraction from acid solution with sodium diethyldithiocarbamate[7] or with diethylammonium diethyldithiocarbamate,[13] followed by evaporation of the extract, wet-oxidation of the residue and dithizone extraction of the clear solution so obtained.

Recently, Johnson and Polhill[14] have introduced the use of sodium hexametaphosphate, which, added to the acid solution before it is made alkaline prevents or delays phosphate precipitation.

Of the raw materials listed in Table IV the acid solutions of the ashes from the milk products, cocoa powder and gelatin showed the formation of a phosphate precipitate when neutralised with ammonium hydroxide. With the solution derived from milk powder the precipitation was immediate and heavy even when no magnesium carbonate was present in the ash, whereas with the other materials the extent of precipitation was considerable when the ashing-aid was present, but only gradual and slight when it was not present. Increasing the amount of citrate normally added before neutralisation to 10 ml (5 g) had some temporary effect on keeping the phosphate in solution, but a gradual precipitation occurred. To investigate the effect of sodium hexametaphosphate on solutions of ashes containing phosphate and magnesium, 5 samples of condensed milk, milk crumb, cocoa powder and gelatin were ashed with the incorporation of the magnesium carbonate ashing-aid, and the ashes were dissolved as for the determination of lead, including the addition of citrate, but before neutralisation with ammonium hydroxide 10 ml of 10 per cent. sodium hexametaphosphate solution were added. In each case immediate precipitation of phosphates was prevented, but a granular deposit formed slowly as the solutions cooled. A rapid extraction of such solutions was possible, but unsatisfactory, as precipitation tended to occur in the separating funnel before extraction was complete. The precipitation of phosphates from the solution of the unaugmented ash of milk powder was completely prevented by the addition of 10 ml of sodium hexametaphosphate solution. It was concluded from these observations that the addition of sodium hexametaphosphate to the solutions obtained from food materials rich in phosphates satisfactorily prevented precipitation on neutralisation, provided the magnesium ashing-aid had not been incorporated. (As materials containing appreciable amounts of phosphates generally have a sufficiently bulky ash, the addition of an ashing-aid to such materials is not necessary.)

TABLE V

INFLUENCE OF CALCIUM SULPHATE ON RECOVERIES OF LEAD AND COPPER

Calcium content of sample	Metal added, p.p.m.-	Recovery of metal after ash had been treated with— Cold acid, %	Warm acid, %	Boiling acid, %
High (≡ 2% of CaO)	1·0 of lead	64	68	80
	2·5 of lead	62	78	87
	4·0 of copper	49	63	91
	10·0 of copper	50	64	86
Moderate (≡ 1% of CaO)	1·0 of lead	74	85	100
	2·5 of lead	89	89	97
	4·0 of copper	79	79	95
	10·0 of copper	78	95	97
Nil	2·5 of lead	103	99	103
	10·0 of copper	98	101	99
High; ash treated with sodium carbonate solution	2·5 of lead		Mean = 98	
	10·0 of copper		Mean = 103	

(*b*) *Formation of calcium sulphate*—When materials containing calcium salts are wet oxidised, or ashed by a method involving the addition of sulphuric acid, a residue of calcium sulphate is formed that is generally held to adsorb traces of lead and copper from solution,

so that its formation during ashing is likely to lead to low recoveries of these metals. To investigate the extent to which such adsorption might occur a series of experiments was carried out in which lead and copper were added to sugar samples containing added calcium, the samples were sulphated with 1 ml of sulphuric acid and ashed at 500° to 550° C, and the proportion of the metals recovered from the filtered solutions of the ashes was determined. Calcium was added as 4 per cent. calcium chloride solution and the amount added was varied to give the equivalent of a high calcium content (10 ml, equivalent to 2 per cent. of CaO in sample) or a moderate calcium content (5 ml), in relation to the calcium contents of the materials we deal with, of which milk powder (maximum CaO content about 1·6 per cent.) is richest in calcium. The temperature of the dilute acid in which the ashes were dissolved was also varied, being either cold (room temperature), warm (10 minutes in an oven at 70°C) or boiling. Recoveries of the metals were calculated as percentages of the colorimeter readings for control solutions prepared by ashing samples as for the reagent blank determinations and afterwards adding the same amounts of lead and copper as originally added to the test samples.

From the results, shown in Table V, it is evident that losses of lead and copper by adsorption are of importance only when relatively large amounts of calcium sulphate are formed, provided the ash is taken up in boiling hydrochloric acid solution. The results indicate that low recoveries might be experienced with certain food materials rich in calcium.

Preliminary trials with the ash of milk powder showed that by digesting it with hot sodium carbonate solution a decomposition was effected, with the formation of calcium carbonate, so that on subsequent acidification the ash was completely soluble. When a further series of ashes, prepared from samples of sugar containing 10 ml of calcium chloride solution and added lead and copper, were digested with 20 ml of hot 5 per cent. sodium carbonate solution for about 1½ hours before acidification, the recoveries of lead and copper from the solutions obtained were complete, as is shown in Table V. After this treatment had been applied to the ash of milk powder, the solution, when prepared for the determination of lead, deposited considerable amounts of phosphates on neutralisation, despite the addition of citrate and 10 ml of 10 per cent. sodium hexametaphosphate solution, and it was found necessary to increase the amount of sodium hexametaphosphate solution to 20 ml in order to prevent precipitation completely.

METHOD

REAGENTS—

All reagents used should be obtained substantially free from lead and copper by selection or by purification as described.

Sulphuric acid, 20 per cent. v/v—Add 1 volume of sulphuric acid, sp.gr. 1·84, to 4 volumes of water.

Hydrochloric acid, diluted (1 + 1)—Dilute concentrated hydrochloric acid with an equal volume of water.

Magnesium carbonate suspension—Well wash 80 g of light magnesium carbonate with boiling water and then add water to a total volume of 1 litre. Shake well before use.

Potassium cyanide solution, 10 per cent. w/v—Dissolve 50 g of potassium cyanide in water and dilute to 100 ml. Extract with successive small portions of dithizone solution until the last extract is green. Remove the excess of dithizone from the aqueous solution by repeated extractions with chloroform, add 10 ml of 20-volume hydrogen peroxide and set aside for at least 1 day before diluting to a volume of 500 ml.

Citric acid solution—A 50 per cent. w/v solution in water.

Hydroxylamine hydrochloride solution—A 20 per cent. w/v solution in water.

Sodium hexametaphosphate solution, 10 per cent. w/v—Dissolve 50 g of sodium hexametaphosphate in water and dilute to 500 ml. Add thymol blue indicator and then ammonium hydroxide, sp.gr. 0·880, to a blue-green colour. Extract with successive portions of dithizone solution until the extracts are green. Make slightly acid with dilute hydrochloric acid, remove excess of dithizone by extraction with chloroform and finally make alkaline as before with ammonium hydroxide.

Ammonium hydroxide, sp.gr. 0·880.

Dithizone solution, 0·02 per cent. w/v—Dissolve 0·01 g of dithizone in 50 ml of chloroform. Transfer to a 100-ml separating funnel and shake vigorously for 1 minute with 50 ml of water containing 1 ml of ammonium hydroxide, sp.gr. 0·880. Reject the chloroform layer, make

slightly acid with hydrochloric acid and extract with two successive 25-ml portions of chloroform. Combine the chloroform extracts and reject the acid layer. Wash the combined chloroform extracts with two successive 10-ml portions of water. Prepare freshly as required.

Ammonium hydroxide - cyanide wash solution—Dilute 40 ml of ammonium hydroxide, sp.gr. 0·880, and 20 ml of 10 per cent. potassium cyanide solution to 1 litre with water.

Standard lead solution, 1 ml ≡ 10 μg of lead—Dissolve 1·60 g of lead nitrate in water, add 10 ml of concentrated nitric acid and dilute to 1 litre. Dilute 1 ml of this solution to 100 ml with water when required.

Potassium iodide solution, 20 per cent. w/v—Dissolve 100 g of potassium iodide in water and dilute to 500 ml. Add 1 ml of ammonium hydroxide, sp.gr. 0·880, transfer to a separating funnel and shake vigorously for 30 seconds with 25 ml of diethylammonium diethyldithiocarbamate working solution. Reject the chloroform layer and wash the aqueous layer with two successive 10-ml portions of chloroform, rejecting the washings.

Sodium metabisulphite solution—A 5 per cent. filtered solution in water.

Diethylammonium diethyldithiocarbamate solutions—(*a*) *Stock solution*, prepared weekly. Dilute 3·0 ml of redistilled diethylamine to 10·0 ml with chloroform, and add slowly with stirring 1·0 ml of redistilled carbon disulphide previously diluted to 10·0 ml with chloroform. Cool and preserve in a dark coloured, glass-stoppered bottle. (*b*) *Working solution*, prepared daily, as required. Dilute 5·0 ml of stock solution to 100 ml with chloroform.

Standard copper solution, 1 ml ≡ 10 μg of copper—Dissolve 3·93 g of crystalline copper sulphate, $CuSO_4.5H_2O$, in water and dilute to 1 litre. Dilute 1 ml of this solution to 100 ml with water when required.

Sodium carbonate solution—A 5 per cent. solution in water.

Chloroform—Redistil over lime in an all-glass apparatus.

Water—Prepare by distillation in all-glass apparatus. (NOTE—We have recently found that the use of a proprietary ion-exchange purifier provides suitable water without distillation.)

PROCEDURE FOR THE DESTRUCTION OF ORGANIC MATTER—

Materials having low ash contents—Well mix the finely divided sample (10 g) with 5 ml of 20 per cent. sulphuric acid in a silica dish having a diameter of 10 cm, and heat over an Argand burner, gently at first to avoid possible spurting or frothing, and then more strongly until a brittle char remains. (With certain materials that do not mix well with the sulphuric acid, it is often advantageous to add one drop of Teepol before heating; the Teepol should also be added to the blank.) The charring stage is speeded up by breaking and turning over the partly charred cake of material with a platinum wire. Crush the dry char to a powder with a clean dry porcelain pestle, and heat again over the burner to remove traces of free sulphuric acid. If the char is particularly hard, transfer it to a porcelain mortar to crush it and then brush it back into the dish. Add by pipette 10 ml of the suspension of magnesium carbonate, covering all the particles of charred material, and dry the contents of the dish over the burner at a low heat. Transfer the dish to an electrically heated muffle furnace at a temperature not exceeding 550° C and ash the contents as completely as possible.

Other materials—Proceed as described above, but omit the magnesium carbonate suspension.

PROCEDURE FOR EFFECTING SOLUTION OF THE ASH—

When the magnesium carbonate ashing-aid has been used, allow the dish to cool and then moisten the ash with water to reduce effervescence on the addition of acid. To the ash in the silica dish add 10 ml of diluted hydrochloric acid (1 + 1), bring the solution to boiling point over a gauze, and filter it through an acid-washed filter-paper into a 50-ml graduated cylinder. Add a further 10 ml of dilute hydrochloric acid to the dish, boil, swirl round and filter into the cylinder and repeat this step with about 10 ml of water. Dilute the volume of filtrate in the cylinder to 50 ml with water, drain into a 100-ml conical flask, swirl round to ensure thorough mixing of the contents, pour back into the cylinder, then back into the flask and mix again. Divide the solution into two equal portions by returning 25 ml to the cylinder and thence to another 100-ml conical flask, washing out the cylinder into the second flask with a little water. Use one portion for the determination of lead and the other for the determination of copper.

Considerable amount of calcium sulphate present—Transfer the ash as completely as possible to a 100-ml beaker, using a glass rod to loosen the ash. Wash traces of ash remaining in the dish into the beaker with two 10-ml portions of 5 per cent. sodium carbonate solution, each being brought to boiling point before being poured into the beaker. Cover the beaker with a clock-glass and gently simmer the contents on a gauze over a low flame or on a hot-plate for at least 1½ hours, stirring frequently with the glass rod. Then add a small piece of litmus paper and make just acid by the careful addition of dilute hydrochloric acid. Add a further 10 ml of acid, boil the solution and filter it through an acid washed filter-paper into a 50-ml graduated cylinder. Rinse out the beaker with 10 ml of dilute hydrochloric acid and finally with water, filtering into the cylinder, and dilute the filtrate to 50 ml with water. Divide the solution into two equal portions, as described above, for the separate determinations of lead and copper.

PROCEDURE FOR DETERMINING LEAD—

To the lead aliquot of the ash solution add 4 ml of citric acid solution and 10 ml of 10 per cent. sodium hexametaphosphate solution. (After treating the ash with sodium carbonate, it may be necessary to increase the amount of sodium hexametaphosphate solution to 20 ml.) Add a small piece of litmus paper to the solution and neutralise by adding ammonium hydroxide, sp.gr. 0·880, from a burette, adding 0·5 ml in excess. Add 5 ml of 10 per cent. potassium cyanide solution and 1 ml of 20 per cent. hydroxylamine hydrochloride solution, cool the solution and transfer it to a 100-ml separating funnel, washing in with 10 ml of water.

Add 2 ml of chloroform and 1 ml of 0·02 per cent. dithizone solution, shake vigorously for 30 seconds and allow the layers to separate. If the chloroform layer is coloured pink, add further 0·5-ml increments of dithizone solution, shaking for 30 seconds after each addition, until the lower layer becomes purple or blue, indicating the presence of excess of dithizone. Run off the chloroform layer into a second separating funnel containing 50 ml of ammonium hydroxide - cyanide wash solution, washing through with 2 ml of chloroform. Add 2 ml of chloroform and 0·5 ml of dithizone solution to the solution in the first separating funnel, shake for 30 seconds, separate and run the lower layer into the second funnel, again washing through with 2 ml of chloroform. Shake the second funnel containing the combined extracts for 30 seconds and allow the layers to separate. Run off the lower layer into a dry 25-ml graduated cylinder and wash through with one or two small amounts of chloroform. Make the volume of extract in the cylinder to 15 ml with chloroform, drain into a 50-ml conical flask, swirl to mix and stopper the flask.

Measure the optical density of the solution against chloroform, using an EEL colorimeter (Evans Electroselenium Ltd.) or other suitable instrument with a green filter. Determine also the optical density of the reagent blank, subtract it from the test reading and find the lead content of the sample by reference to a calibration curve.

PROCEDURE FOR CONSTRUCTING A CALIBRATION CURVE FOR LEAD—

Measure 0, 0·5, 1·0, 1·5, 2·0 and 2·5-ml portions of standard lead solution (1 ml ≡ 2 p.p.m. on 5 g) into 100-ml conical flasks, add 15 ml of dilute hydrochloric acid and 10 ml of water and continue as described for the determination of lead in the test solution. Determine the optical density of each extract against that containing no added lead as solution blank and plot the readings against amount of lead in p.p.m. in 5 g of sample.

PROCEDURE FOR DETERMINING COPPER—

To the copper aliquot of the ash solution add 10 ml of dilute hydrochloric acid and 5 ml of 20 per cent. potassium iodide solution and warm to about 40° C. Reduce the liberated iodine by adding 2 ml of 5 per cent. sodium metabisulphite solution, cool and transfer to a 100-ml separating funnel, washing in with 10 ml of water. Add 10 ml of diethylammonium diethyldithiocarbamate working solution, shake vigorously for 30 seconds and allow the layers to separate. Run off the lower layer into a second separating funnel containing 50 ml of water and wash through with 2 ml of chloroform. Repeat the extraction with 10 ml of diethylammonium diethyldithiocarbamate working solution, shaking for 30 seconds and transfer the extract to the second funnel, washing through with 2 ml of chloroform. Shake the second funnel vigorously for 30 seconds, allow the layers to separate and run the lower layer into a dry 50-ml graduated cylinder, washing through with one or two small volumes of chloroform. Dilute the extract to 30 ml with chloroform and pour through a dry filter-paper into a 100-ml conical flask.

Measure the optical density of the solution against chloroform, using a blue filter with the EEL colorimeter, and find the copper content of the sample by reference to a calibration curve, allowing for the reading of the blank solution.

PROCEDURE FOR CONSTRUCTING A CALIBRATION CURVE FOR COPPER—

Measure 0, 2·5, 5·0, 7·5, 10·0 and 12·5-ml portions of standard copper solution (1 ml ≡ 2 p.p.m. on 5 g) into 100-ml conical flasks and add sufficient water to make the volume in each flask approximately 15 ml. Add 25 ml of dilute hydrochloric acid and proceed as described for the determination of copper in the test solution.

Determine the optical densities of the extracts against that containing no added copper and plot the instrument readings against amount of copper in p.p.m. in 5 g of sample.

BLANK DETERMINATIONS—

Carry out blank determinations exactly as described throughout, omitting only the sample.

PRECAUTIONS TO BE OBSERVED IN THE METHOD—

Care should be taken to prevent the adventitious gain of lead and copper. Contamination of samples by air-borne dust during ashing can be reduced by covering the dishes with clock-glasses between stages in the procedure. Apparatus should be thoroughly cleaned with hot dilute hydrochloric acid and water before use. Glassware and reagent bottles should be of Pyrex or similar glass. Filled etchings on glassware may give up lead and should not be allowed to come into contact with solutions.

SUMMARY

A method is described for the dry ashing of food materials and the colorimetric determination of lead and copper in the solution of the ash obtained. Initial sulphation of the sample before ashing permits the ashing to be carried out up to a temperature of 550° C without loss of lead or copper. Ashing is further speeded up by crushing the charred material to a powder, and a suspension of magnesium carbonate is used to increase the bulk of small ashes. Interference during the determination of lead caused by the alkaline-earth phosphates normally present in food materials is overcome by the addition of sodium hexametaphosphate solution before neutralisation. When large amounts of calcium sulphate are formed, the ash is rendered completely soluble by a preliminary treatment with hot sodium carbonate solution.

REFERENCES

1. Fairhall, L. T., *J. Ind. Hyg.*, 1922-23, **4**, 9.
2. Roche-Lynch, G., Slater, R. H., and Osler, T. G., *Analyst*, 1934, **59**, 787.
3. Weyrauch, F., and Muller, H., *Z. Hyg. Infekt Kr.*, 1933, **115**, 216.
4. "Official Methods of Analysis," Eighth Edition, The Association of Official Agricultural Chemists, Washington, D.C., 1955, p. 427.
5. Schmidt, P., *Dtsch. Med. Wschr.*, 1928, **54**, 520.
6. Seiser, A., Necke, A., and Muller, H., *Z. angew. Chem.*, 1929, **42**, 96.
7. Tompsett, S. L., and Anderson, A. B., *Biochem. J.*, 1935, **29**, 1851.
8. Lockwood, H. C., *Analyst*, 1954, **79**, 143.
9. Monier-Williams, G. W., "Trace Elements in Food," Chapman & Hall Ltd., London, 1949, p. 38.
10. Comrie, A. A. D., *Analyst*, 1935, **60**, 532.
11. Strafford, N., Wyatt, P. F., and Kershaw, F. G., *Ibid.*, 1945, **70**, 232.
12. Kent-Jones, D. W., and Herd, C. W., *Ibid.*, 1933, **58**, 152.
13. Analytical Methods Committee, "The Determination of Lead in Foodstuffs," *Ibid.*, 1954, **79**, 397.
14. Johnson, E. I., and Polhill, R. D. A., *Ibid.*, 1955, **80**, 364.

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Polarographic Determination of Arsenic in Zinc-smelting Residuals and Zinc Metal

By R. E. COULSON

A simple and rapid method has been developed for the determination of 0·1 to 5 per cent. of arsenic in residuals arising from the production of zinc from sulphide ores. With use of a base solution of *N* sulphuric acid containing 0·01 per cent. w/v of gelatin, the only metal that interfered was cadmium. Arsenic was separated when required by co-precipitation as arsenate with ferric hydroxide. The absolute accuracies, with and without separating the arsenic, were ± 0·01 and ± 0·04 per cent., respectively.

Arsenic in zinc can be determined down to 0·0001 per cent. after separation by the iron-collection technique.

ARSENIC is a normal impurity in the sulphide ores used for the production of zinc, and the elimination of this impurity is an important part of the process. The residuals produced at various stages in the process, which must be treated to recover the lead and zinc that they contain, have arsenic contents in the range 0·1 to 5 per cent. Determination of arsenic in these residuals by the usual chemical methods is a lengthy process compared with the determination of other constituents such as lead or zinc, and a reduction in the time required was sought by the application of polarographic methods.

EXPERIMENTAL

The most useful base solution described for the determination of arsenic was *N* sulphuric acid containing 0·01 per cent. of gelatin as a maximum suppressor,[1] in which tervalent arsenic gives a well defined reduction wave. Use of such a base solution makes possible a method with a small number of steps, as will be seen below. During the progress of this work, it was reported[2] that gelatin was not a suitable maximum suppressor, as the height of the arsenic wave is reduced by the addition of gelatin. This effect had been observed, but with a nominal gelatin concentration of 0·01 per cent. no variation in step height was caused by the small differences in gelatin concentration that occurred in practice.

All measurements were made with a Tinsley pen-recording polarograph, with open cells 2 inches high and 1 inch in diameter. The solutions were de-aerated with cylinder nitrogen and the temperature was maintained at 25° ± 0·1° C. All voltage measurements were made with reference to the anode pool.

In the base solution used, satisfactory polarograms were recorded with arsenic concentrations from 0·002 to 0·15 g per litre with no significant change in the diffusion-current constant. Above 0·15 g per litre the diffusion-current constant decreases. Below 0·002 g per litre the slope of the top of the wave becomes excessive and the accuracy of the results is greatly reduced.

Variations of ± 10 per cent. in the sulphuric acid concentration in the base solution produced no measurable change in the wave height obtained, while similar variations in the gelatin concentration produced changes of 4 per cent., the wave height decreasing with a rise in gelatin concentration. Variations occurring in practice do not cause any measurable errors in the wave height produced.

The composition range of the residuals analysed was as follows: arsenic, 0·1 to 5%; cadmium, 0·1 to 0·5%; calcium, 1 to 5%; iron, 0·1 to 4%; lead, 15 to 60%; zinc, 20 to 70%.

EFFECT OF CADMIUM—

In *N* sulphuric acid, cadmium and arsenic give a combined wave. The wave height for cadmium is only one-fifth that of an equal concentration (g per litre) of arsenic. As the accuracy aimed at in the determination of arsenic was ± 0·05 per cent. absolute, and in most samples the cadmium content was less than 0·1 per cent., it was expected that, for the majority, it would be unnecessary to separate the arsenic from the cadmium.

For samples containing more than 0·1 per cent. of cadmium, the arsenic was separated by co-precipitation with ferric hydroxide.[3]

EFFECT OF ZINC AND AMMONIUM SULPHATES—

To bring the arsenic in the drosses into solution, an initial attack by nitric acid, followed by fuming with sulphuric acid is satisfactory. The lead can then be removed as lead sulphate. Zinc is then the only metal present in major quantities. In concentrations up to 40 g per litre of zinc, zinc sulphate has no effect on the wave height. Ammonium sulphate may be present in considerable amounts from the neutralisation of sulphuric acid. The wave height of arsenic is decreased by ammonium sulphate concentrations in excess of 20 g per litre, and therefore concentrations of this salt must be kept low.

METHOD FOR DETERMINING ARSENIC IN RESIDUALS

REAGENTS—

All reagents should be of recognised analytical quality.

Sulphuric acid, approximately 5 N—Add 140 ml of concentrated sulphuric acid to 800 ml of water, cool, and dilute to 1 litre.

Gelatin solution, 0·25 per cent. w/v—Dissolve 2·5 g of leaf gelatin plus 1 g of phenol in hot water, cool, and dilute to 1 litre.

Standard arsenic solution, 0·4 g per litre—Dissolve 0·528 g of arsenious oxide in the minimum amount of dilute sodium hydroxide solution and dilute to about 500 ml. Add a small piece of litmus paper and just acidify with 5 *N* sulphuric acid. Cool, and dilute to 1 litre.

Ferric ammonium sulphate solution, 20 per cent. w/v—Dissolve 20 g of the hydrated salt in water and dilute to 100 ml.

PROCEDURE—

Digest 10 g of sample (see note) with 20 ml of concentrated nitric acid and then evaporate the solution to a small bulk. Add 20 ml of concentrated sulphuric acid, evaporate to fumes of this acid, and continue fuming for 5 minutes. Cool, and wash down the sides of the beaker with a jet of water; then repeat the fuming for 5 minutes. Cool, add about 150 ml of water, boil for 5 minutes and cool to room temperature. Filter the solution through a Postlip 633B or equivalent filter-paper into a 250-ml calibrated flask. Wash the residue well with water and discard it. Dilute the filtrate to volume and mix well. By pipette put 10 ml of this solution into a 400-ml beaker and dilute to a volume of about 200 ml. For samples containing less than 0·1 per cent. of cadmium continue by Procedure A. For other samples continue by Procedure B.

NOTE—The samples, which contain both oxidised and metallic material, cannot be reduced to a fine state of division; 10 g is the minimum weight required to obtain a representative sample.

Procedure A: no separation of arsenic—Pass sulphur dioxide through the solution for 10 minutes and then boil off excess and cool. Transfer the solution to a 250-ml calibrated flask. Add ammonia solution until a permanent precipitate of ferrous hydroxide is formed, and then immediately add 50 ml of 5 *N* sulphuric acid. Add 10 ml of 0·25 per cent. gelatin solution, dilute the solution to volume and mix well.

Prepare a standard solution from 10 ml of standard arsenic solution with 50 ml of 5 *N* sulphuric acid and 10 ml of 0·25 per cent. w/v gelatin diluted to 250 ml.

Transfer about 15 ml of each solution to a polarograph cell, pass nitrogen for 5 minutes and then record polarograms, commencing at — 0·5 V. For samples containing less than 1·4 per cent. of arsenic a sensitivity of 10 μA full scale is suitable.

Procedure B: separation of arsenic—Add 5 ml of 20 per cent. w/v ferric ammonium sulphate solution. Bring the solution to the boil and add ammonia solution slowly while the solution is gently boiled, until the iron is completely precipitated, and there is a slight excess of ammonia present. Collect the precipitate on a Whatman No. 54 or equivalent filter-paper and wash it once with water containing one or two drops of ammonia solution. Discard the filtrate.

Wash the precipitate back into the precipitation beaker with hot water. Wash the paper with 10 ml of diluted sulphuric acid (1 + 9) and then with hot water. Dilute the solution to a volume of about 200 ml and continue as described above.

CHEMICAL METHOD—

The results obtained by the polarographic methods were compared with results obtained by the chemical method previously employed, which was as follows—

A 10-g sample was brought into solution, and lead sulphate was removed as in the polarographic method. The arsenic in the solution was then reduced with sulphur dioxide, separated as arsenious sulphide from 8 *N* hydrochloric acid, and then converted to arsenite and titrated with standard iodine solution in the usual way.

RESULTS—

Typical results for arsenic obtained by Procedure A on residuals containing less than 0·1 per cent. of cadmium were as follows—

Arsenic by chemical method, % ..	1·32	1·40	1·44	1·63	2·21	3·10
Arsenic by Procedure A, %	1·30	1·40	1·40	1·56	2·14	3·15

The following results are for arsenic with and without its separation, in residuals containing more than 0·1 per cent. of cadmium—

Cadmium content, %	0·19	0·33	0·22	0·43	0·17
Arsenic by chemical method, % ..	0·14	0·18	0·19	0·28	0·52
Arsenic by Procedure A, %	0·13	0·17	0·15	0·33	0·50
Arsenic by Procedure B, %	0·13	0·18	0·18	0·29	0·52

The mean absolute deviations for the results obtained by Procedure A, *i.e.*, no separation of cadmium, given above, are ± 0·04 and ± 0·03 per cent.; therefore the higher cadmium content does not introduce any appreciable error. The polarograms obtained with the latter samples showed irregularities in the slope at the foot of the arsenic wave, but as the results obtained showed a maximum absolute deviation of ± 0·07 per cent. compared with that sought of ± 0·05 per cent., these irregularities were not investigated. They are apparently due to a shift in half-wave potentials. When a greater accuracy is required in the lower range of arsenic contents Procedure B gives results with a mean absolute deviation of less than 0·01 per cent.

The time required for the polarographic method is about 3 hours compared with about 8 hours for the chemical method.

METHOD FOR DETERMINING ARSENIC IN ZINC

The method was mainly required for the determination of 0·01 per cent. or less of arsenic, but occasionally for samples encountered in experimental work in which arsenic contents of 0·1 per cent. or more occurred.

The arsenic content of zinc can be determined directly by dissolving a sample in nitric acid, fuming with sulphuric acid and reducing the arsenic with sulphur dioxide. The whole solution may then be treated as already described. The limit imposed by the maximum tolerable zinc content of the final solution, 40 g per litre, restricts this method to arsenic contents of not less than 0·05 per cent.

By applying the iron-collection technique already described, as much as 25 g of zinc may be taken and the final volume be as little as 25 ml, which extends the range down to 0·0001 per cent. of arsenic. Appropriate sample weights and final volumes of solution for various arsenic contents are as follows—

Arsenic content, %	0·0001 to 0·001	0·001 to 0·01	0·01 to 0·1
Sample weight, g	25	5	5
Final volume, ml	25	25	250

PROCEDURE—

Dissolve a suitable sample weight in nitric acid and dilute to a volume of about 600 ml. Add 5 ml of 20 per cent. w/v ferric ammonium sulphate. Bring to the boil; continue boiling gently and add ammonia solution until a permanent precipitate forms. Then add more ammonia solution, a few drops at a time until all the iron is precipitated and any zinc precipitate has re-dissolved. This can be judged by the colour of the precipitate. Allow the precipitate to settle and collect it on a Whatman No. 54 or equivalent filter-paper. Wash the precipitate twice with hot water containing a few drops of ammonia solution. Discard the filtrate.

Wash the precipitate back into the precipitation beaker with hot water. Wash the paper with 10 ml of diluted sulphuric acid (1 + 9) and then with hot water. Dilute the solution to about 150 ml and pass in sulphur dioxide for 10 minutes. Remove the excess of sulphur dioxide by boiling the solution. If a final volume of 25 ml is required, evaporate the filtrate to a volume of less than 20 ml. Cool the solution and then transfer it to a calibrated flask of appropriate size. Add the requisite volumes of 5 *N* sulphuric acid and 0·25 per cent. w/v gelatin solution to give a final solution of *N* sulphuric acid and 0·01 per cent. w/v of gelatin. Dilute the solution to volume and mix well. De-aerate the solution and record polarograms as already described, the same standard solution being required.

RESULTS—

Results within the range 0·1 to 0·001 per cent. of arsenic showed a mean relative deviation of ± 2 per cent. Below 0·001 per cent. the accuracy of the method diminishes as the arsenic content approaches 0·0001 per cent., at which concentration an absolute accuracy of ± 0·00002 per cent. was the best that could be obtained.

REFERENCES

1. Lingane, J. J., *Ind. Eng. Chem., Anal. Ed.*, **1943**, **15**, 583.
2. Everest, D. A., and Finch, G. W., *J. Chem. Soc.*, **1955**, 704.
3. Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffmann, J. I., "Applied Inorganic Analysis," Second Edition, Chapman & Hall Ltd., London, 1953, p. 265.

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Determination of Uranium by Ammonium Thiosulphate and Sodium Hypophosphite

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Uranium is determined gravimetrically by precipitation as the greenish phosphate from dilute mineral-acid solution by means of sodium hypophosphite and ammonium thiosulphate; the precipitate is ignited at 900° C, the sulphur present being removed during the ignition. Elements forming sulphides insoluble in dilute mineral acids are removed by treatment with hydrogen sulphide, and zirconium and titanium, the only interfering elements, are removed by treatment with sodium hypophosphite alone. The method has been successfully applied to the determination of uranium in steel.

MOLYBDENUM has been determined by precipitation as the sulphide from acid solution by treatment with ammonium thiosulphate and sodium hypophosphite, which react in presence of acid to produce hydrogen sulphide.[1] Copper, mercury, lead, bismuth, arsenic, tin, antimony and cadmium, whose sulphides are insoluble in acid, interfere, whereas those elements with sulphides that are soluble in acid or water do not.

Uranium, however, is an exception; although its sulphide is soluble, when a solution of a sexavalent uranium salt is boiled with ammonium thiosulphate or sulphurous acid and sodium hypophosphite in presence of dilute mineral acid, a precipitate is formed. This precipitate is a phosphate, although it is not uranyl ammonium phosphate, $UO_2NH_4PO_4$, which is precipitated from neutral solutions of uranium salts, or uranyl hydrogen phosphate, UO_2HPO_4, a white precipitate formed in acetic acid solutions of uranium salts in presence of a reducing agent, such as sodium thiosulphate or sulphurous acid. This reaction only occurs when the uranium is in the sexavalent state, uranium^{IV} and lower valency forms being unaffected; hence, if the uranium solution is treated in a Jones reductor and then oxidised to the quadrivalent state by exposure to air, treatment with ammonium thiosulphate and sodium hypophosphite gives a precipitate of sulphur only.

The only interfering elements are zirconium and titanium, but they can be removed by modifying the procedure. If the acid solution is first boiled with sodium hypophosphite,

these elements are separated as white crystalline precipitates, the uranium remaining in solution, from which it can be precipitated by boiling with ammonium thiosulphate or sulphurous acid.

This reaction may, to some extent, be considered as specific for uranium. Uranium can be easily separated from vanadium and other elements that generally interfere in the ordinary method of determination. Very few elements form phosphates that are insoluble in dilute mineral acids. Those that would interfere, *e.g.*, zirconium, phosphate, can be easily removed. Elements forming sulphides insoluble in dilute mineral acids are removed by treatment with hydrogen sulphide before addition of the hypophosphite and ammonium thiosulphate. Moreover, this characteristic green precipitate is a sure indication of the presence of uranium in the solution, as no other element produces this type of precipitate in acid solution.

Method

Reagents—

Ammonium thiosulphate solution—A 20 per cent. filtered aqueous solution of ammonium thiosulphate.

Sodium hypophosphite solution—A 20 per cent. solution of crystalline sodium hypophosphite, acidified with 2 ml of concentrated hydrochloric acid and allowed to stand for 2 to 3 minutes before use.

Procedure—

Place a suitable aliquot (say, 50 ml) of the uranium solution in a 600-ml beaker and add about 150 ml of distilled water and 8 to 10 ml of concentrated hydrochloric acid. Warm the solution, add 20 ml of sodium hypophosphite solution, boil, and then add 20 to 25 ml of ammonium thiosulphate solution. Stir the solution vigorously for about 2 minutes; after a short time a greenish precipitate of uranium phosphate will separate, together with sulphur from the thiosulphate. Set the beaker aside in a warm place, so that the precipitate settles. The supernatant liquid should be colourless, except for some colloidal sulphur, but any green colour indicates incomplete separation of uranium. If this is so, add more sodium hypophosphite solution and more ammonium thiosulphate, with vigorous stirring. Collect the precipitate on a Whatman No. 41 filter-paper, wash it thoroughly with warm 1 per cent. hydrochloric acid and then with hot water until it is free from chloride. Dry the paper and precipitate in a tared crucible and then ignite them in a muffle furnace, initially at a low temperature until the paper and sulphur have burned away and then at 900° C for $\frac{1}{2}$ hour. Weigh the residual white ash.

Results—

Extra-pure uranium nitrate (obtained from Chemical & Dye Corporation, New York) was dried in a vacuum-desiccator. A solution containing 1 g of solid in 250 ml of water was prepared, aliquots of this solution were taken and uranium phosphate was precipitated and ignited as described above; it contains 53·60 per cent. of uranium, corresponding to the formula $UO_2P_2O_7$. The results were as follows—

Uranium nitrate solution taken, ml	..	..	10	20	20	30	50
Uranium phosphate found, g	..	..	0·0355	0·0712	0·0709	0·1058	0·1764
Theoretical amount of uranium phosphate, g	..		0·0359	0·07078	0·07078	0·10617	0·17695

Determination of Uranium in Steel

Methods for the determination of uranium in steel have been described by Kelly, Meyers and Illingworth,[2] by Johnson[3] and by Little.[4] As the percentage of uranium present is small, there is a possibility of a large error in separating it from the iron. The usual procedure involves precipitating the iron as ferric hydroxide and extracting the precipitate with ammonium carbonate solution, a troublesome procedure, owing to the gelatinous nature of the hydroxide. In the proposed method the precipitation is quickly carried out, the precipitate being compact and coagulating very easily. The determination can be made in acid medium in presence of a large amount of iron.

A slightly modified procedure was applied to determine the recovery of uranium from samples of various steels, as follows—

About 1 to 2 g of steel drillings were dissolved in diluted hydrochloric acid (1 + 1) and a suitable volume of standard uranium solution (4 g of uranium nitrate per litre)

was added. The solution was diluted to about 200 ml and the acidity was adjusted so that it contained about 3 per cent. of hydrochloric acid. The solution was treated with hydrogen sulphide to precipitate sulphides of copper, molybdenum, etc., which were filtered off, and the filtrate was boiled to remove hydrogen sulphide, a few drops of nitric acid being added to oxidise any ferrous iron. An acidified solution of sodium hypophosphite containing 3 to 4 g was added, with vigorous stirring, and any precipitate formed was removed by filtration. About 5 g of ammonium thiosulphate were added to the filtrate, which was then warmed and stirred vigorously for a few minutes. The precipitate was allowed to settle for ½ hour and then collected on a Whatman No. 41 filter-paper, washed free from iron and chloride and ignited as described under "Procedure." The recoveries of uranium, added as uranium nitrate, for a number of steels are shown in Table I.

TABLE I

RECOVERY OF URANIUM FROM STEEL

Sample	Uranium nitrate solution added, ml	Uranium phosphate found, g	Theoretical amount of uranium phosphate, g
B.C.S. steel No. 215	10	0·0356	0·0353
	15	0·0520	0·0529
Plain carbon steel	20	0·0709	0·0707
	40	0·1405	0·1415
	50	0·1770	0·1769
Nickel - chromium - molybdenum steel	30	0·1063	0·1061

REFERENCES

1. Rây, H. N., *Analyst*, 1953, **78**, 217.
2. Kelly, G. L., Meyers, F. B., and Illingworth, C. B., *J. Ind. Eng. Chem.*, 1919, **11**, 316.
3. Johnson, C. M., *Chem. & Met. Eng.*, 1919, **20**, 523 and 588.
4. Little, S., *Chemist Analyst*, 1922, **38**, 22.

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A Systematic Scheme of Semi-micro Qualitative Analysis for Anionic Surface-active Agents

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The systematic scheme of semi-micro qualitative analysis presented allows the identification of an anionic surface-active agent as a member of one of the twenty-one groups of commoner anionic surface-active agents commercially available.

Two novel reagents are described; they may be used to indicate the presence of fatty acids, sulphated or sulphonated anionic surface-active agents, quaternary ammonium or pyridinium salts and cation-active amines when present as single substances.

THE development in recent years of a large variety of materials that are used either as wetting agents or detergents has created a need for a systematic scheme for their qualitative analysis. During the past fifteen years, several schemes have been published[1 to 11], but all of these have been designed for the macro scale of working, requiring substantial quantities of the test material and a considerable time for their operation. The work described in this paper requires only milligram quantities of the active material and the analysis is often completed in less than an hour.

Many of the published schemes[4,6,7,8,10,11] rely on the precipitation of the anion-active compound by a cation-active compound as a means of differentiation, but this use of amphiphilic ions of opposite charge to bring about precipitation is open to criticism on the grounds that solubilisation may occur. The formation of chloroform-soluble complexes of anionic

and cationic surface-active agents with dye-stuffs of opposite charge has often received mention in the literature in both the quantitative[12,13,14,15,16,17,18] and qualitative[8,9,11,19] analysis of surface-active agents. Gobel[20] reported the use of an aqueous solution containing equal amounts of methylene blue and fluorescein as an indicator for anionic and cationic materials, the change in colour and fluorescence of the solution on the addition of a surface-active agent indicating the type of material present. This method can be criticised in that with aqueous solutions a certain minimum concentration of surface-active agent is required for the formation of micelles, with the consequent change in colour of the dye-stuff solution.

In the work described below use is made of a mixture of two dye-stuffs, which, when used at two different pH values, give coloured chloroform extracts in accordance with the type of surface-active agent present. Successful results were obtained with individual samples drawn from some fifty different members of the twenty-one classes of anion-active compounds covered by this scheme.

As yet, the scheme has not been extended to deal with mixtures of differing classes of detergents, but preliminary work suggests that it can be applied to some mixtures with little or no modification.

Many of the commercial products used as surface-active agents contain much "inactive" material, partly owing to the method of manufacture and partly to the addition of "builders." In either event it is advisable to extract the active material from a commercial product before attempting its analysis.

Miller, Bann and Ponsford[21] have used *n*-butanol in a continuous liquid - liquid extraction process for the extraction and concentration of detergents from their aqueous solutions. In the proposed semi-micro scheme it was found that three successive extractions with *n*-butanol gave too small a yield of the active material to be of value, and an alternative extractant involving use of *tert.*-butanol was devised.

Anionic materials are often marketed as salts of nitrogen-containing cations, and it is therefore necessary to be able to differentiate between nitrogen in the anion or in the associated cation. Bergeron, Derenemesnil, Ripert and Monier[6] precipitated the anionic material from solution as the insoluble benzidine salt, which was removed and converted to the sodium salt by treatment with sodium hydroxide solution. The free benzidine base was filtered off and a semi-micro Kjeldahl determination was carried out on the prepared sodium salt. Bergeron *et al.*[6] found that it is not possible to obtain a clean separation by this method and that a nitrogen content of less than 0·5 per cent. should be regarded as a negative test.

A semi-micro Kjeldahl determination was too time-consuming to find place in a scheme of qualitative analysis and so a simpler procedure was evolved. This entailed the precipitation of the anionic material with a phosphonium salt, followed by its isolation and examination for nitrogen.

Experimental

Apparatus and technique—

The procedure closely resembled that previously described by one of us (H.H.).[22] The entire analysis was carried out with use only of 3-inch × ⅜-inch test-tubes, 2-inch × ½-inch boiling-tubes and 1-inch × ¾-inch beakers. All of these held the same volume of liquid, roughly 3 ml. Separations were made by using a centrifuge designed to carry the test-tubes, and the required layer was removed by means of a pipette with a rubber teat. All vessels were heated in a heating block and those whose contents required prolonged periods of boiling were equipped with a length of capillary tubing sealed about 5 mm from its lower end to prevent bumping.

In all this work great care was taken to clean all apparatus thoroughly after use. The sensitivity of many of the tests and the small quantities of material used made the risk of contamination very real.

Reagents—

In addition to the usual reagents normally found in the laboratory the following special solutions are needed.

Mixed indicator solution at pH 1·99—Dissolve 0·01 g of Disulphine blue V 200 (obtainable from Imperial Chemical Industries Ltd.), 0·04 g of Dimidium bromide (obtainable from Burroughs Wellcome Ltd.) and 16·4 g of sodium acetate in distilled water, add 210 ml of *N* hydrochloric acid and dilute to 1 litre.

Mixed indicator solution at pH 8·6—Dissolve 0·01 g of Disulphine blue V 200, 0·04 g of Dimidium bromide, 3·09 g of boric acid and 3·72 g of potassium chloride in distilled water, add 12 ml of *N* sodium hydroxide and dilute to 1 litre.

Ammonium cobaltothiocyanate reagent[23]—Dissolve 200 g of ammonium thiocyanate and 30 g of cobalt nitrate in distilled water and dilute to 1 litre.

Phenol test reagents—(*a*) Dissolve 0·1 g of Genochrome (obtainable from May & Baker Ltd.) and 6·0 g of sodium metabisulphite in distilled water and dilute to 100 ml. (*b*) A 0·5 per cent. aqueous solution of sodium periodate. (*c*) A 15 per cent. aqueous solution of sodium carbonate.

Diazotised sulphanilic acid reagents[24]—(*a*) A 0·5 per cent. solution of sulphanilic acid in 2 per cent. hydrochloric acid. (*b*) A 0·5 per cent. aqueous solution of sodium nitrite.

Nitrogen test reagent—A saturated aqueous solution of Eulan NK (obtainable from M. W. Hardy & Co.).

Buffer solution at pH 7·0—Mix 250 ml of 0·2 *M* potassium dihydrogen phosphate and 147·7 ml of 0·2 *M* sodium hydroxide.

EXTRACTION OF THE ACTIVE MATERIAL—

Dissolve sufficient of the test sample estimated to contain 20 mg of the active material in 1 ml of water in a test-tube. Make just acid to Congo red paper, add 0·5 ml of light petroleum, boiling range 40° to 60° C, and diethyl ether mixture (1 + 1), shake well and spin in a centrifuge. If difficulty is experienced owing to the formation of an emulsion, add a few drops of *tert.*-butanol and then mix gently to ensure a clean separation on centrifuging. Remove the ether layer with a pipette. Repeat this extraction twice more and combine the extracts.

Wash the combined ether extracts three times with 50 per cent. ethanol, combine the washings, evaporate to dryness in a beaker and unite with the original aqueous solution. The washed ether extracts are evaporated to dryness in a beaker and examined separately as indicated in the qualitative scheme.

Make the extracted aqueous solution alkaline to litmus with 15 per cent. sodium carbonate solution, add 0·5 ml of *tert.*-butanol, shake well and then add sufficient sodium chloride to saturate the solution. Spin in a centrifuge after shaking thoroughly. Remove the alcohol layer with a pipette and repeat the extraction with two further 0·5-ml portions of *tert.*-butanol. Combine the alcohol extracts, dry by shaking with anhydrous sodium sulphate, and evaporate to dryness in a beaker. Remove the final traces of alcohol by the addition of a little *n*-hexane, followed by evaporation. The residue is the extracted active material used in the systematic scheme described later.

SPECIAL TESTS—

The tests described below are used several times in the systematic scheme and to avoid repetition they are described in detail here and reference is then made back to this section.

1. *Test with mixed indicator solution at pH* 1·99—Put into a test-tube 0·5 ml of the mixed indicator solution at pH 1·99, add 0·2 ml of chloroform and mix thoroughly by means of a clean pipette. Spin in a centrifuge, and examine the chloroform layer for the complete absence of colour, which indicates that the test-tube, reagents and pipette are free from contamination. Add the specified amount of the test solution or material to the tube and again mix the contents and spin it in a centrifuge. Note the colour of the chloroform layer.

2. *Test with mixed indicator solution at pH* 8·6—Repeat the test as described above, but using mixed indicator solution at pH 8·6 in place of mixed indicator solution at pH 1·99.

3. *Test with ammonium cobaltothiocyanate reagent*—Carry out the test as described above for mixed indicator solution at pH 1·99, but using the ammonium cobaltothiocyanate reagent in place of the indicator solution.

4. *Hydrolysis with* 2 N *sodium hydroxide*—In a boiling-tube place 5 mg of the extracted active material, add 1·5 ml of 2 *N* sodium hydroxide solution and a capillary tube sealed near its lower end, and place the tube in the heating block. Bring the solution to the boil and continue boiling for exactly 10 minutes. Cool, make the solution just acid to litmus with diluted hydrochloric acid (1 + 1) and then just alkaline with 15 per cent. sodium carbonate solution. Add 0·5 ml of *tert.*-butanol, shake well and then add sufficient sodium chloride to saturate the solution. Spin in a centrifuge after shaking thoroughly. Repeat

this extraction with two further portions of *tert.*-butanol (compare with "Extraction of the active material," p. 168). The indicated amounts of the combined extracts are then used to carry out the tests specified.

5. *Hydrolysis with diluted hydrochloric acid* (1 + 1)—Put 5 mg of the active material into a boiling-tube, add 1·5 ml of diluted hydrochloric acid (1 + 1) and a piece of capillary tube sealed near its lower end, and place the boiling-tube in the heating block. Bring to the boil and continue boiling for 10 minutes. Cool, make the solution just acid to Congo red paper, using 40 per cent. sodium hydroxide solution and diluted hydrochloric acid (1 + 1) as necessary, and then extract three times with 0·5-ml portions of light petroleum - diethyl ether mixture (1 + 1). Combine the solvent extracts, wash three times with 50 per cent. ethanol, unite the ethanol extracts, evaporate to dryness and combine with the aqueous solution from the hydrolysis. The combined solvent extracts are evaporated to dryness and the residue is taken up in 0·2 ml of chloroform and used for the tests specified in Tables III, IV and V. The aqueous solution is extracted with *tert.*-butanol after saturation with sodium chloride (compare with "Extraction of the active material," p. 168) and the extract is examined as indicated in Tables III, IV and V.

6. *Guerbet test*[4,9,10,11,25]—Put 3 mg of the active material at the bottom of a test-tube and add 4 drops of concentrated nitric acid, then place the tube in the heating block and evaporate to dryness. Cool, dissolve the residue in 0·2 ml of 50 per cent. ethanol, and add two drops of concentrated hydrochloric acid and 15 mg of zinc dust. Warm the mixture gently for 2 minutes, spin in a centrifuge and remove the clear liquid by means of a pipette to a clean test-tube. Cool thoroughly in running water and add two drops of 0·5 per cent. sodium nitrite solution. After a few seconds 10 mg of urea are added to destroy any excess of nitrous acid, and then 3 mg of 2-naphthol dissolved in 0·1 ml of 50 per cent. ethanol. Then 20 per cent. sodium acetate solution is added until the solution is faintly alkaline to litmus. Note the colour of the solution immediately.

7. *Brentamine fast red AL test*[4,9,10,11]—Dissolve 6 mg of Brentamine fast red AL salt in 0·3 ml of buffer solution at pH 7 and spin the solution in a centrifuge. Place 3 mg of the active material in a test-tube and dissolve it in 0·2 ml of buffer solution at pH 7, and then add 0·2 ml of the prepared clear solution of Brentamine fast red AL. Note the formation of any colour. Then add 0·1 ml of chloroform to the solution, and shake the tube and spin it in a centrifuge. Note the colour of the chloroform layer.

As an additional confirmation of this test, place 3 mg of the active material in a test-tube and dissolve it in 4 drops of 2 *N* sodium hydroxide. Evaporate the contents to dryness and gently heat the tube over a semi-micro burner until the white melt becomes tinged with grey. Cool the tube, dissolve the melt in two drops of water, and make the solution acid with diluted hydrochloric acid (1 + 1). Mix two drops of 0·5 per cent. sulphanilic acid solution and two drops of 0·5 per cent. sodium nitrite solution in a separate test-tube, adding 5 mg of urea within a few seconds. Add the resulting mixture to the solution from the sodium hydroxide fusion, and then add 20 per cent. sodium acetate solution until the solution is faintly alkaline to litmus. Alkylnaphthalenesulphonates give a red colour, whereas alkylbenzenesulphonates and alkyltoluenesulphonates give a yellow to orange colour.

8. *Test for presence of monoglyceride*—Place 3 mg of the active material at the bottom of an ignition tube and cover it with 10 mg of potassium bisulphate powder. Cover the end of the tube with a small circle of filter-paper moistened with a freshly prepared solution of equal volumes of 1 per cent. sodium nitroprusside and diethanolamine. Invert a small glass cup over the paper and gently heat the base of the tube until the contents have become molten and signs of charring appear. Set the tube aside and examine the filter-paper for the formation of a strong cornflower-blue colour within 5 minutes.

CLASSIFICATION OF THE TYPE OF ACTIVE MATERIAL PRESENT—

Types of anion-active materials capable of identification in the present scheme are—

1. Fatty acids and their salts.
2. Primary and secondary alkyl sulphates.
3. Alkanesulphonates.
4. "Sulphonated" castor oil.
5. Highly sulphated oil.
6. Fatty-acid monoglyceride sulphate.
7. Fatty-acid isethionate.

8. Dialkyl esters of sulphosuccinic acid.
9. Sulphated alkylphenol - ethylene oxide condensates.
10. Alkylphenylphenolsulphonate.
11. Alkyldiphenylsulphonate.
12. Alkylbenzenesulphonates and alkyltoluenesulphonates.
13. Alkylnaphthalenesulphonates.
14. Water-soluble petroleum sulphonates.
15. Oil-soluble petroleum sulphonates.
16. Fatty-acid monoethanolamide sulphate.
17. Fatty-acid amide of methyltaurine.
18. Fatty-acid arylamidesulphonate.
19. Substituted (long-chain) benzimidazolesulphonate.
20. Monosulphostearylsuccinamide.
21. Mono- and di-benzylsulphanilates.

In addition, the use of the indicator reagents will confirm the presence of cation-active amines, amidoamines, quaternary cationic salts and non-ionic surface-active agents when present as single substances, but as yet the qualitative scheme of analysis has not been extended to include these products.

TABLE I

The light petroleum - diethyl ether extract from the initial extraction (p. 168) is evaporated to dryness in a beaker and any residue is dissolved in 0·2 ml of chloroform. This solution is used to carry out the following tests.

Apply the indicator test at pH 1·99 (test 1), using two drops of the above solution. Note the colour of the chloroform layer.

<table>
<tr><td rowspan="3">Pink. Extract still contains sulphated or sulphonated anionic. Evaporate to dryness. Dissolve in light petroleum - diethyl ether mixture and wash three times with 50 per cent. ethanol. Repeat test. If still pink, strong indication of presence of petroleum sulphonates.</td><td colspan="2">Green. Carry out test at pH 8·6, using 4 drops of solution.</td><td colspan="3">Colourless. Carry out the test at pH 8·6 (test 2), using 4 drops of the solution.</td></tr>
<tr><td>Pink or colourless. Cation-active amine or amide.</td><td>Green. Quaternary cationic</td><td>Pink. Free fatty acid present.</td><td colspan="2">Colourless. Carry out test for non-ionic (test 3), using 4 drops of solution.</td></tr>
<tr><td colspan="3"></td><td>Blue-green. Non-ionic present.</td><td>Colourless. Surface-active agent not present, or only those of low molecular weight.</td></tr>
</table>

TABLE II

Dissolve 4 mg of the extracted active material (p. 168) in 0·2 ml of water and use this solution to test for the type of surface-active agent present. Apply the indicator test at pH 8·6 (test 2), using two drops of the solution and note the colour of the chloroform layer.

<table>
<tr><td rowspan="3">Pink. Anionic present.</td><td rowspan="3">Green. Quaternary cationic salt present.</td><td colspan="3">Colourless. Apply test at pH 1·99 (test 1), using 4 drops of the solution.</td></tr>
<tr><td>Green. Cation-active amines or amides.</td><td colspan="2">Colourless. Apply test for non-ionic (test 3), using 4 drops of the solution.</td></tr>
<tr><td>Blue-green. Non-ionic present.</td><td>Colourless. Surface-active agent absent, or only those of low molecular weight.</td></tr>
</table>

EXAMINATION OF ANION-ACTIVE AGENTS—

For the sake of convenience this group of materials is first divided into two parts, those containing nitrogen in the anion and those that do not. Before this division can be made, however, it is necessary to remove interference from the possible presence of nitrogen in the associated cation, *e.g.*, from ammonium, ethanolamine and similar nitrogenous bases.

To accomplish this separation dissolve 3 mg of the extracted active material (p. 168) in 0·5 ml of water. Add a solution of Eulan NK until no further precipitation occurs (on shaking the precipitate should flocculate). Extract the solution three times with 0·5-ml portions of chloroform, combine the extracts and wash them three times with 0·3-ml portions of water. Dry the chloroform extracts containing the "anion - Eulan NK" complex with anhydrous sodium sulphate and evaporate to dryness in a 2-inch × $\frac{3}{8}$-inch ignition tube.

Carry out a sodium fusion on this residue in the normal way and test for the presence or absence of nitrogen, using the copper acetate - benzidine acetate method.[26] The result determines whether the method in Table III, IV or V is to be followed.

Material not containing nitrogen in the anion—Carry out the Guerbet test as indicated (test 6). If the resultant colour is yellow, indicating the absence of aromatic nuclei in the molecule, refer to Table III. The formation of an orange to red colour indicates the presence of aromatic nuclei in the molecule and reference should be made to Table IV.

TABLE III

Anion-active material not containing an aromatic nucleus in the molecule. Hydrolyse 5 mg of the active material with 2 *N* sodium hydroxide as in test 4 (p. 168). Apply the indicator test at pH 1·99 (test 1) to two drops of the alcohol extract.

<table>
<tr><td colspan="2">*Colourless.* Apply indicator test at pH 8·6 (test 2) to 4 drops of the alcohol extract.</td><td colspan="3">*Pink.* Hydrolyse 5 mg of the active material with diluted hydrochloric acid (1 + 1) as in test 5. Apply the indicator test at pH 8·6 (test 2), using 4 drops of the solution from the light petroleum - diethyl ether extract.</td></tr>
<tr><td>*Colourless.* Esters of sulphosuccinic acid.</td><td>*Pink.* Apply test for glyceride (test 8).
Negative result, acyl isethionate.
Positive result, Fatty-acid monoglyceride sulphate.</td><td>*Pink.* Dissolve 3 mg of active material in 0·1 ml of water and add 1 ml of 2 *N* acetic acid. Heat in water bath for 5 minutes.
Cloudy result, "Sulphonated" castor oil.
Clear solution, Highly sulphated oil.</td><td>*Colourless.* Apply indicator test at pH 1·99 (test 1) to two drops of the *tert.*-butanol extract.</td><td></td></tr>
<tr><td></td><td></td><td></td><td>*Colourless.* Primary and secondary alkyl sulphates.</td><td>*Pink.* Alkanesulphonates.</td></tr>
</table>

DISCUSSION OF THE PROCEDURES

EXTRACTION PROCEDURE—

The extraction procedure used permits the active material to be obtained relatively free from inactive organic and inorganic materials. It affords a convenient means of concentrating the more dilute powders, pastes and liquids without having to resort to drying. It gives the active material in a standard form convenient for analysis.

The initial extraction from acid solution with light petroleum - diethyl ether mixture (1 + 1) removes inactive fatty material, fatty acids, perfumes and so on, when present. A subsequent extraction with *tert.*-butanol after making alkaline with sodium carbonate solution and saturation with sodium chloride effects the final separation of the active material.

Although *tert.*-butanol is normally completely miscible with water in all proportions, it has only a limited solubility in saturated sodium chloride solutions. It has been found to give satisfactory extractions of the active material from aqueous solutions saturated with sodium chloride. As a solvent, it has the advantage of a low boiling-point, 82·5° C, which permits the extract to be evaporated to dryness rapidly without the decomposition sometimes experienced with the higher boiling solvents; also, the final traces of alcohol may be removed by the addition of a small quantity of *n*-hexane, with which it forms a low-boiling azeotrope (63·7° C).

Extractions with *tert.*-butanol are carried out from solutions that have been brought from acidity to alkalinity with a sodium carbonate solution and then saturated with sodium chloride. This procedure eliminates the possibility of mineral acids or caustic alkalis being extracted by the alcohol, with the resulting interference in the indicator tests subsequently applied.

TABLE IV

Apply the ammonium cobaltothiocyanate test (test 3), using 4 drops of the solution of the active agent (p. 168). Note the appearance of the chloroform layer.

<table>
<tr><td rowspan="5">Green colour. Apply the diluted hydrochloric acid (1 + 1) hydrolysis (test 5) to 3 mg of the active material. Apply indicator test at pH 1·99 (test 1) to 4 drops of the alcohol extract. A colourless chloroform layer confirms the presence of a sulphated alkylphenol - polyglycol ether.</td><td colspan="4">Colourless. Place 2 mg of the active material in a test-tube and dissolve it in 0·1 ml of Genochrome solution, add an excess of 15 per cent. sodium carbonate solution followed by 0·1 ml of 0·5 per cent. sodium periodate solution. Observe the formation of a colour within 1 minute.</td></tr>
<tr><td>Blue-green. Alkylphenylphenolsulphonate.</td><td colspan="3">Red to brown. Refer to Table I for behaviour of the light petroleum - diethyl ether extract on original material.</td></tr>
<tr><td rowspan="3"></td><td rowspan="3">Extract gives positive indicator test at pH 1·99 (test 1) after 6 washes. Take 2 mg of the active material and dissolve it in 0·1 ml of water. Add 0·2 ml of 5 per cent. calcium chloride solution and extract with 0·2 ml of diethyl ether. Apply indicator test at pH 1·99 to extract (test 1).

Colourless or very faint pink, water soluble petroleum sulphonate.

Strongly pink, oil soluble petroleum sulphonate.</td><td colspan="2">Extract gives negative indicator test at pH 1·99 (test 1) after 6 washes. Apply test 7 (p. 169).</td></tr>
<tr><td>Orange to red. Alkylnaphthalenesulphonate.</td><td>Pale yellow. Dissolve 3 mg of the active material in 0·1 ml of chloroform in a test-tube. Sprinkle a few crystals of fused aluminium chloride on the side of the tube above the liquid, tilt the tube to just moisten the chloride and observe the formation of colour on the crystals.

Red-brown, alkyldiphenylsulphonate.

Yellow, alkylbenzenesulphonate or alkyltoluenesulphonate.</td></tr>
</table>

INDICATOR REAGENTS—

In combining two dye-stuffs of differing colour and opposite charge in solution at two values of pH, one acid and the other alkaline, a pair of reagents is produced that between them are capable of differentiating between four classes of active material when present as single substances. These represent the main four classes of the scheme, namely, fatty-acid soaps, sulphated and sulphonated anionics, cation-active amines, amides and amidoamines and, lastly, quaternary ammonium, pyridinium and quinolinium salts.

In preparing these solutions it was necessary to select a pair of dye-stuffs that, when shaken with chloroform, were not themselves extracted at either pH value in the absence of active material. Of the cationic dye-stuffs as yet mentioned in the literature, none proved entirely satisfactory, and it seemed probable that only dyes containing a true quaternary nitrogen atom in the molecule would prove to be of value. Samples were therefore obtained of Alcian blue 8GS, pinacyanol chloride, pinacryptol green, pinacryptol yellow, pinaverdol and Acronol yellow TC180. Some of these showed promise, but were not satisfactory on all counts. Owing to the large number of sulphonated dye-stuffs of all colours commercially available, no difficulty was experienced in the selection of a suitable anionic dye-stuff. The success of the indicator method therefore centred round the search for a suitable cationic dye-stuff.

The formula of Dimidium bromide (2:7-diamino-10-methyl-9-phenylphenanthridinium bromide) as given in a Note on its analysis[27] clearly indicated the presence of a true quaternary nitrogen atom in the molecule and in addition the text stated that the colour of its aqueous solution was pink. A sample of Dimidium bromide was obtained and when made up in solution with Disulphine blue V200 at two values of pH it was found in the absence of active

material to give a colourless extract at both pH 1·99 and pH 8·6. In the presence of active material it gave the expected coloured chloroform extracts, correctly indicating the type of active material present. The solutions at both pH values had very good stability and no decomposition took place over a period of 6 months.

Active material with nitrogen in the anion—

TABLE V

Place 3 mg of the extracted active material in a test-tube and dissolve it in 0·2 ml of water, warming if necessary. The solution is cooled and should remain clear. Add two drops of concentrated hydrochloric acid and look for the formation of a strong cloud or precipitate.

<table>
<tr><td rowspan="6">Immediate cloud or precipitate. Substituted benzimidazolesulphonate.</td><td colspan="4">Solution remains clear. Carry out the phosphoric acid hydrolysis (test 9) on the original extracted material. Apply the indicator test at pH 1·99 (test 1) to 4 drops of the ether extract.</td></tr>
<tr><td>Green. Sulphostearylsuccinamide.</td><td colspan="3">Colourless. Make the aqueous solution from the above hydrolysis acid to litmus, add three drops of 0·5 per cent. sodium nitrite solution, followed after a few seconds by 10 mg of urea. Then add 3 drops of a solution of 10 mg of 2-naphthol in 0·1 ml of 50 per cent. ethanol. Make just alkaline to litmus with 20 per cent. sodium acetate solution and observe the colour.</td></tr>
<tr><td></td><td>Orange-red. Acylarylaminesulphonate.</td><td colspan="2">Yellow colour only. Gently fuse 2 mg of the active material with 5 mg of potassium bisulphate and examine for the odour of benzaldehyde.</td></tr>
<tr><td></td><td></td><td>Odour of benzaldehyde. Benzylsulphanilates.</td><td>No odour of benzaldehyde. Carry out diluted hydrochloric acid (1 + 1) hydrolysis (test 5) on original material. Apply indicator test at pH 1·99 (test 1) to whole of evaporated light petroleum - diethyl ether extract.</td></tr>
<tr><td></td><td></td><td></td><td>Green. Fatty-acid ethanolamide sulphate.</td><td>Colourless. Acylamidesulphonate.</td></tr>
</table>

At pH 1·99 fatty-acid soaps gave a colourless extract, whereas sulphated and sulphonated anionics gave a pink colour. Cation-active amines, amides and quaternary nitrogen salts gave a blue-green colour. At pH 8·6 fatty-acid soaps, sulphated and sulphonated anionics gave a pink colour, but only quaternary nitrogen salts gave a blue-green extract. The cation-active amines and amides gave colourless extracts at pH 8·6 (but pink in the presence of fatty acid). Non-ionic amphiphilic compounds gave colourless extracts at both pH values.

The use of the ammonium cobaltothiocyanate reagent as a confirmatory test for the presence of polyethylene glycol condensates has received mention in previous schemes of detergent analysis.[4,7,8,10] This reagent also gives a positive test with quaternary nitrogen salts. When single substances are being examined, the prior application of the other indicator tests that are not prone to interference by non-ionic surface-active agents allows the cobaltothiocyanate to be used in this scheme.

A recent application[23] of the ammonium cobaltothiocyanate reagent in the colorimetric determination of non-ionic esters makes use of an extraction procedure in which the photometric absorption of a chloroform extract is related to the concentration of non-ionic material in the solution tested. This forms the basis of the qualitative test for the presence of non-ionic surface-active agents.

TEST FOR ALKYLPHENYLPHENOLSULPHONATES—

p-Diethylaminoaniline reacts with phenols and naphthols possessing an unsubstituted *para* position in alkaline solution in the presence of an oxidising agent with the formation of a blue to green colour.[28,29] This reagent is commercially available as its crystalline complex with sulphur dioxide under the name of Genochrome.[30] Aqueous solutions of this material were found to have only moderate stability. It was found that the addition of a small

amount of sodium metabisulphite gave a reagent of considerably improved stability. It is advisable to carry out a blank test if the reagent has been prepared for some time.

TEST FOR THE PRESENCE OF NITROGEN IN THE ANION—

A method was sought whereby the anion could be precipitated by a non-nitrogenous cation and separated from the possible nitrogen-containing cations in solution. Attention was directed to sulphonium and phosphonium compounds, which, by analogy with the quaternary ammonium salts, could be expected to give precipitates with anion-active materials. Of these two types of material only a sample of Eulan NK (triphenyl-3:4-dichlorobenzyl-phosphonium chloride),[31] a phosphonium salt manufactured as a moth-proofing agent was available. This compound precipitates anion-active material from aqueous solution and the resulting "anion - Eulan NK" complex can be extracted by chloroform. The extract is washed with water to remove any nitrogenous cations (ethanolamine, etc.), which may also have been extracted, it is dried with anhydrous sodium sulphate and evaporated to dryness in an ignition tube. A sodium fusion carried out on the residue is then used to test for nitrogen.

The procedure is simple and can be completed within 10 minutes. The use of an extraction procedure for the separation of the precipitated material eliminates the possibility of solubilisation by the surface-active agent, which might otherwise give incomplete separation from nitrogenous cations. It is interesting to note that the Eulan NK as marketed and used in the reagent contained urea as a diluent, but in spite of this no nitrogen was found in a blank test carried out as described.

TEST FOR ESTERS OF GLYCEROL—

One class of material included in this scheme is an ester of glycerol and its presence may be confirmed by utilising a test for glycerol in the molecule. The test used is a modification of that given by Feigl[32] and by Gilby and Hodgson,[5] in which the active material is heated with potassium bisulphate and the acrolein liberated is identified by means of filter-paper moistened with a freshly prepared mixture of equal volumes of 1 per cent. sodium nitroprusside and piperidine. In the present application, improved results were obtained by replacing the piperidine in the reagent by diethanolamine. This may well be due to the low volatility and rather hygroscopic nature of diethanolamine.

HYDROLYSIS WITH 2 *N* SODIUM HYDROXIDE—

Boiling 5 mg of the active material with 1·5 ml of 2 *N* sodium hydroxide for 10 minutes is effective in bringing about the hydrolysis of any completely organic ester groups that may be present in the molecule, although it will not completely hydrolyse organic sulphuric acid monoesters.

It is found that, when the long chain of the molecule is linked with the sulphate or sulphonate group by means of an intermediate organic ester group, the sodium hydroxide hydrolysis results in the loss of anionic properties conferred by the sulphate or sulphonate group. In some instances, however, this loss is replaced by the anionic properties of the fatty acids resulting from the hydrolysis.

Loss of anionic properties due to sulphate or sulphonate groups is indicated by colourless chloroform extracts in the indicator tests at pH 1·99 and pH 8·6, while the presence of fatty acid is indicated by a colourless extract at pH 1·99 and a pink extract at pH 8·6.

HYDROLYSIS WITH DILUTED HYDROCHLORIC ACID (1 + 1)—

Boiling with diluted hydrochloric acid (1 + 1) effects the hydrolysis of sulphuric acid monoesters without leading to the complete hydrolysis of true sulphonates or fatty-acid amides. Thus fatty-alcohol sulphates are hydrolysed with the complete loss of anionic properties as is shown by negative indicator tests at both pH values. Fatty-acid ester sulphates in which the sulphate group is attached to the oleophilic acid portion of the molecule give rise to free fatty acid, which is indicated by a colourless test at pH 1·99 and a pink colour at pH 8·6.

Fatty-acid ethanolamide sulphates are mainly hydrolysed to the free fatty ethanolamide, which gives a blue-green indicator test at pH 1·99 and generally a pink colour at pH 8·6. This pink colour is probably due to the partial hydrolysis of the fatty ethanolamide with the resultant liberation of fatty acid. True sulphonates and fatty amidesulphonates retain

the anionic properties of the sulphonate group as shown by the pink colour produced in the indicator test at pH 1·99.

HYDROLYSIS WITH SYRUPY PHOSPHORIC ACID—

Hydrolysis with syrupy phosphoric acid is used in the scheme to effect the conversion of amidosulphates and amidosulphonates to the corresponding free acid and amine. In carrying out this test it is of great importance to make certain that none of the active material adheres to the side of the tube and so avoids contact with the acid. Should this happen, an incorrect result will be obtained on applying the indicator tests. In the hydrolysis tests previously described the solution is actively boiling while hydrolysis procedes and any active material on the sides of the tube is continually returned to the solution by vapour condensation on the side of the tube. With phosphoric acid, however, the liquid is not actively boiling during the hydrolysis and any active material adhering to the side of the tube and not initially covered by the acid remains unattacked. It is also advisable to transfer the solution to a second clean test-tube by means of a pipette before dilution.

This treatment is effective in hydrolysing an amide linkage with the liberation of free acid and amine. The presence of free fatty acid is indicated by a negative indicator test at pH 1·99 and a pink colour at pH 8·6. If a fatty amine is liberated in the hydrolysis, then the indicator test at pH 1·99 gives a blue-green colour. An aromatic primary amine giving a negative indicator test at pH 1·99 can be detected by diazotising and coupling with 2-naphthol to form a coloured dye-stuff.

TEST FOR STABILITY TO 2 *N* ACETIC ACID—

This test has found place in several previous schemes[1,5,6] as a means of differentiating between classes of anionic materials. In the present scheme it is used to differentiate between "sulphonated" castor oil and acyl isethionates.

THE GUERBET TEST—

This test was originally described as a means of detecting the presence of the benzoyl group in cocaine and other alkaloids that give rise to benzoic acid on oxidation. It has been used[4,9,10,11,25] to indicate the presence of aromatic nuclei in surface-active agents. We found it to give satisfactory results with all the alkylarylsulphonates tested and also with sulphated alkylphenol - polyglycol condensates. As the quantity of material used is small, care should be taken to adhere to the volume of solution stated.

BRENTAMINE FAST RED AL TEST—

Brentamine fast red AL is a stabilised diazonium salt of 1-aminoanthraquinone (Imperial Chemical Industries Ltd.). It is used in the present scheme to differentiate between the presence of benzene and naphthalene rings in alkylarylsulphonates. The procedure of van der Hoeve has been modified for use on the semi-micro scale of working. As the extracted active material should be essentially neutral in reaction, we have found that the correct conditions of pH can be simply obtained by dissolving both the active material under test and the diazonium salt in a buffer solution at pH 7. In the presence of alkylnaphthalene-sulphonates an orange-red colour or precipitate is formed. Alkylarylsulphonates not containing a naphthalene nucleus give no more than a yellow colour. The colour produced can be extracted with chloroform.

SODIUM HYDROXIDE FUSION TEST—

It is of value to have additional confirmation of the nature of the alkylarylsulphonate when present, and for this purpose use is made of the conversion of the alkylarylsulphonate to alkylphenol or alkylnaphthol on evaporation with 2 *N* sodium hydroxide followed by gentle fusion. The melt is dissolved in two drops of water, made acid and then coupled with the diazotised sulphanilic acid reagent. The colour formed depends on the nature of the aromatic nucleus in the original sulphonate. It is strongly advised that the analyst should become familiar with the colours obtained in this test by carrying out the procedure with materials of known composition.

THE ALUMINIUM CHLORIDE TEST—

This test is used to differentiate between alkyldiphenylsulphonates and alkylbenzene-sulphonates or alkyltoluenesulphonates. The procedure is that given by Bergeron *et al.*[6]

adapted to the semi-micro scale. It is advisable to dry the chloroform over anhydrous calcium chloride.

DIFFERENTIATION OF PETROLEUM SULPHONATES—

The relative solubility of the calcium salts of petroleum sulphonates in diethyl ether is used as a means of differentiating between oil and water-soluble products of this nature. The test is based on the original observation of Von Pilat and Sereda.[33]

We thank the following Firms for their co-operation in supplying samples of their products, together with details of their composition: Allied Colloids (Manufacturing) Co. Ltd., The Clayton Aniline Co. Ltd., Fine Dyestuffs & Chemicals Ltd., The Geigy Co. Ltd., The Imperial Chemical Industries Ltd., The Manchester Oil Refinery Ltd., Lankro Chemicals Ltd., Leda Chemicals Ltd., Messrs. M. W. Hardy & Co. and T. Swan & Co. Ltd.

REFERENCES

1. Linsenmeier, K., *Melliand Textilber.*, 1940, **21**, 468.
2. Skinkle, J. H., *Amer. Dyestuff Reporter*, 1946, **35**, 449.
3. Goldstein, H. B., *Ibid.*, 1947, **36**, 629.
4. Van der Hoeve, J. A., *Rec. Trav. Chim. Pays-Bas*, 1948, **67**, 649.
5. Gilby, J. A., and Hodgson, H. W., *Mfg Chem.*, 1950, **21**, 371 and 423.
6. Bergeron, J., Derenemesnil, R., Ripert, J., and Monier, G., *Bull. Mens. ITERG*, 1950, **4**, 118.
7. Wurzschmidt, B., *Z. anal. Chem.*, 1950, **130**, 8.
8. Simmons, W. H., *Analyst*, 1951, **76**, 279.
9. Kortland, C., and Dammers, H. F., *Chem. Weekblad*, 1953, **49**, 341.
10. Van der Hoeve, J. A., *J. Soc. Dyers & Col.*, 1954, **70**, 145.
11. Kortland, C., and Dammers, H. F., *J. Amer. Oil Chem. Soc.*, 1955, **32**, 58.
12. Auerbach, M. E., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 492.
13. Epton, S. R., *Nature*, 1947, **160**, 795.
14. Barr, T., Oliver, J., and Stubbings, W. V., *J. Soc. Chem. Ind.*, 1948, **67**, 45.
15. Epton, S. R., *Trans. Faraday Soc.*, 1948, **44**, 226.
16. Wijga, P. W. O., *Chem. Weekblad*, 1949, **45**, 447.
17. Klevens, H. B., *Anal. Chem.*, 1950, **22**, 175 and 1141.
18. Miller, D. D., and Elliker, P. R., *J. Dairy Sci.*, 1951, **34**, 273.
19. Koot, W., *Ing.-chim.*, 1954, **36**, 12.
20. Gobel, E. F., *Rev. Quim. Ind., Rio de Janeiro*, 1946, **15**, 16.
21. Miller, M. A., Bann, B., and Ponsford, A. P., *J. Appl. Chem.*, 1951, **1**, 523.
22. Holness, H., "Inorganic Qualitative Analysis by Semi-Micro Methods," Sir Isaac Pitman & Sons Ltd., London, 1954.
23. Brown, E. G., and Hayes, T. J., *Analyst*, 1955, **80**, 755.
24. Feigl, F., "Spot Tests," Elsevier Publishing Co. Inc., Amsterdam and New York, 1954, Volume II, p. 108.
25. Guerbet, M. M., *Compt. Rend.*, 1920, **171**, 40.
26. Feigl, F., *op. cit.*, p. 76.
27. Foster, G. E., and Grove, W. F., *Analyst*, 1946, **71**, 287.
28. Warfield, P. F., *Anal. Chem.*, 1952, **24**, 890.
29. Camber, B., *Nature*, 1955, **175**, 1085.
30. Anon., *May & Baker Lab. Bull.*, 1956, **2**, 9.
31. B.I.O.S. Final Report No. 259, H.M. Stationery Office, London.
32. Feigl, F., *op. cit.*, p. 283.
33. Von Pilat, S., and Sereda, J., *Petrol. Z.*, 1933, **29**, 1.

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o-Dithiols in Analysis

Part III.* Reactions of Toluene-3:4-dithiol in Acetate Buffer and Alkaline Solutions: Toluene-3:4-dithiol as a reagent for Copper, Cobalt, Iron^{II}, Antimony^{V} and Thallium

By ROBERT E. D. CLARK

Many metals do not form coloured complexes with toluene-3:4-dithiol in sodium hydroxide, aqueous pyridine or acetate buffer solution. Brilliant colours are, however, given by copper, cobalt, iron^{II}, thallium, nickel and manganese; also in the presence of pyridine by vanadium^{IV} and vanadium^{V}, ruthenium^{III} (bleached by excess of reagent) and antimony^{V} (under special conditions). The order of ease of formation of the first seven complexes is copper, cobalt, iron^{II}, thallium, nickel, manganese and vanadium. A number of the reactions appeared to be specific or readily rendered so by the choice of suitable conditions. Any one of the elements copper, cobalt, iron^{II}, thallium and antimony^{V} could be detected in the presence of the others and of all the more common metallic cations; no less common cations were encountered that were likely to interfere.

TOLUENE-3:4-DITHIOL (dithiol), described as a reagent for tin in earlier papers,[1,2] has hitherto been used as an analytical reagent in a strongly acid medium. It seemed of interest, therefore, to determine how it reacted in alkaline or weakly acid solutions. Under these conditions it was found that many cations that react with dithiol in mineral acids did not give coloured products. On the other hand, several hitherto unrecorded colour reactions were observed and the colours produced were found to be stable in aqueous pyridine solution. With an excess of dithiol in aqueous pyridine solution, only palladium, mercury and silver gave precipitates resembling those obtained in mineral-acid solution, and these settled readily on warming. Gold gave a pale yellow coloration or a precipitate, depending upon the concentration of pyridine, and tin^{IV} gave a pale yellow colour. The colour reactions of the metals investigated are given in Table I. Reactions in sodium hydroxide, ammonia or acetate buffer solutions were found to be similar, unless otherwise stated.

TABLE I

COLOUR REACTIONS OF CERTAIN METALS IN A PYRIDINE - DITHIOL MIXTURE

Metal present	Colour produced	Remarks
Copper	Greenish yellow with green fluorescence	Orange in sodium hydroxide solution. Reactions given with copper^{I} or copper^{II}
Cobalt	Sky-blue	Sky-blue colour in sodium hydroxide solution, discharged when shaken in air
Iron^{II}	Magenta	—
Thallium ..	Orange	Colour only in hot solution; orange precipitate formed when cooled
Nickel	Greenish yellow	Yellow in sodium hydroxide solution with an excess of dithiol
Manganese ..	Brownish yellow	—
Vanadium^{V} ..	Sea-green	Blue in acetate buffer solution
Antimony^{V} ..	Red	Only under special conditions
Ruthenium^{III} ..	Sea-green	Only under special conditions. Colour produced only when pyridine was present. Colour bleached by excess of dithiol. Reaction not given by ruthenium^{II}

While investigating the reactions of the first seven cations in Table I, it was found that the presence of cyanide prevented the development of colour in all instances except for thallium and antimony, and the presence of fluoride interfered only with antimony, manganese and vanadium. Coloured precipitates formed by some heavy metals did not cause interference, as the colour formed by the cation under investigation was easily visible in the supernatant liquid.

* For particulars of Parts I and II of this series, see reference list, p. 182.

When an aqueous pyridine solution containing any two of the first seven cations listed in Table I was treated dropwise with dithiol reagent, the colour produced was that of the first mentioned of the two cations in the Table. In this way the order of ease of formation of the complexes was determined.

Similar colours were in most instances observed when 1 : 8-dimercaptonaphthalene[3,4] was used in place of dithiol, but the purity and distinctiveness of the colours given by metals in sodium hydroxide, aqueous pyridine, acetate buffer and mineral-acid solutions were inferior to those produced with dithiol. The colours produced when 1 : 8-dimercaptonaphthalene was used in aqueous pyridine solution were the same as for dithiol, except for antimony^{V} (orange), nickel (orange-red) and vanadate (green). The order of ease of formation was copper, cobalt, iron and nickel.

Owing to its particular interest, the formation of the blue colour with cobalt was investigated more fully than the other reactions, but it seems that dithiol is a specific reagent for at least five of the cations investigated. Difficulty was encountered in finding ions that interfere with some of the colour reactions; even hydrogen sulphide fails to decompose the copper and cobalt complexes in aqueous pyridine solution.

Application of dithiol for detecting various metals

Copper—

A solution of aqueous pyridine containing 1 to 6 μg of copper per ml gives a yellow colour with dithiol, a green fluorescence appearing when the concentration of copper is greater than 6 μg per ml. The optical density of a solution containing 1 μg per ml was 0·05,* an Ilford No. 622 blue filter being used, but was not proportional to the copper content. The colour was produced either by dissolving in pyridine the black precipitate formed with dithiol in acid solution, or by dissolving metallic copper, copper oxide or copper sulphide in a pyridine-dithiol mixture. It was still visible in a 10^{-3} M copper solution containing a 100-fold molar excess of cyanide ion and seemed little affected by a 20-fold excess. With the exception of the cyanide ion, no instance of interference was encountered.

When sodium hydroxide or ammonia solution was used in place of pyridine, the solution had a moderately stable orange or orange-red colour, this being visible with concentrations of copper as low as 0·5 μg per ml, but the black precipitate obtained in acid solutions was insoluble in alkali unless an excess of dithiol was present.

Tests for copper—(*a*) To 1 ml of test solution add 0·5 to 1 ml of pyridine and then one drop of dithiol. A greenish yellow colour, unchanged by the addition of a drop of ferrous sulphate solution (distinction from nickel), indicates the presence of copper. If the colour does not appear with the first drop of dithiol but a precipitate forms, an oxidising agent or one of the metals gold, mercury^{II}, silver or palladium is probably present. Boil to coagulate the precipitate and continue to add dithiol; the characteristic colour of copper, if present, will then appear.

(*b*) To 1 ml of test solution add 2 N sodium hydroxide in excess and a little zinc dithiol.[5] Warm the solution; the presence of copper is indicated by the immediate formation of the characteristic orange or orange-red colour. Iron gives no colour, nickel a pale yellow colour and cobalt a blue colour, discharged on shaking. By using zinc dithiol, the manganese colour is not elicited.

Cobalt—

A solution of aqueous pyridine containing cobalt gives a brilliant blue colour with dithiol, this colour being visible at concentrations as low as 0·1 to 0·2 μg per ml. The optical density of a solution containing 1 μg per ml was 0·125, an Ilford No. 608 red filter being used, and this was proportional to the concentration of cobalt up to 3 μg per ml at least.

The blue complex was produced when the black precipitate formed in dilute acid solution was treated with an excess of dithiol. It is probably derived from cobalt^{III}, since, if a strong reducing agent is present (stannous chloride and sodium dithionite were effective in aqueous pyridine), the solution becomes yellow, but the blue colour re-appears on the surface where the solution is in contact with air.

* The optical densities quoted were determined by means of a photo-electric colorimeter on a solution obtained by mixing 2 ml of 0·03 M ethanolic diacetyldithiol[5] with 3 ml of pyridine, heating, cooling, adding a known amount of the metal and then diluting the solution to 6 ml with water. The thickness of the light cell was 1 cm.

Tests for cobalt were applied (*a*) in an acetate buffer solution, in the presence of *iso*amyl acetate to extract the coloured product (measurement of the colour intensity showed that the colour was never fully developed under these conditions), and (*b*) in aqueous pyridine. In each test dithiol was added drop by drop. No interference was observed in the presence of at least 10-fold and often several thousandfold excesses of each of the following compounds and ions (those marked with an asterisk interfered in acetate buffer but not in aqueous pyridine solutions)—

Organic compounds—Acetaldehyde, acetates, ethanol, catechol, citric acid, dimethylglyoxime*, 8-hydroxyquinoline, oxalic acid, phenol, resorcinol, tartrates and thiourea.

Anions—Azide, borate, bromide, chlorate, chloride, fluoroborate, fluoride, hypophosphite, iodide, nitrate, phosphate, sulphate and thiocyanate.

Cations and metals—(When necessary, a citrate was added to keep the metals in solution.) Arsenic^{III}, arsenic^{V}, antimony^{III}, antimony^{V}, bismuth*, cadmium, gallium, germanium, indium, iridium^{III}, lead (in acid solution the insoluble yellow lead complex collects at the interface, but the blue colour of cobalt is visible in the upper layer), manganese, molybdenum^{VI}*, osmium* (as osmic acid), platinum^{II}, platinum^{IV}, rhenium^{VII}, rhodium^{III}, ruthenium^{III}* (interferes unless an excess of dithiol is used), selenium^{IV}, tellurium^{IV}, thallium, tin^{II}, tin^{IV}, tungsten^{VI}, uranium^{VI}, vanadium^{IV}, vanadium^{V}, zinc and the common metals not precipitated as sulphides by hydrogen sulphide. Gold, mercury^{II}, palladium^{II} and silver reacted but did not interfere.

The only cation found to interfere in aqueous pyridine solution was copper when present in a large excess. The colour produced by 1 μg of cobalt per ml was detected in the presence of a 100-fold excess of copper, but not with a 1200-fold excess. When large amounts of nickel were present (20 mg per ml) in a solution containing cobalt and an excess of dithiol, the yellow colour of the nickel complex and the blue colour of the cobalt complex combined to give a green solution.

When large amounts of iron^{II} were present in a solution containing cobalt and an excess of dithiol, the brilliant red colour of the iron^{II} complex masked the blue colour of the cobalt. It was found, however, that a cobalt content of up to 2 to 3 μg per ml, in a solution of aqueous pyridine containing 2 μg per ml of iron, could be measured to within $\pm$ 2 per cent. by means of a photo-electric colorimeter with an Ilford No. 608 red filter.

Although, in aqueous pyridine solution, cyanide discharges the cobalt colour, the solution becoming orange and then fading, this is not so in acetate buffer solution. The sensitivity of the reaction is lowered, but the reaction of dithiol with nickel is inhibited. It was found that 15 μg of cobalt per ml were easily detected in the presence of 30 mg of nickel in approximately 15 per cent. potassium cyanide solution. Similarly, in acetate buffer solution, a large excess of fluoride inhibits the iron reaction; a few micrograms of cobalt were readily detected in 20 per cent. potassium fluoride solution in the presence of a 50,000-fold excess of iron^{III}. Fluoride in large excess (addition of an equal volume of saturated potassium fluoride solution) also partly suppressed the nickel reaction (5 μg of cobalt were detected in the presence of 0·8 mg of nickel in 1 ml of solution).

Tests for cobalt—(*a*) To 1 ml of test solution containing mineral acid, add an excess of sodium acetate crystals and 0·5 ml of *iso*amyl acetate. Add dithiol dropwise and shake, when, if cobalt is present, the upper layer becomes blue.

(*b*) To 1 ml of test solution add 0·5 ml of pyridine and then dithiol dropwise. An immediate blue colour indicates the presence of cobalt. Alkali may be present in excess. If the blue colour does not form immediately, an oxidising agent, gold, mercury^{II}, palladium^{II} or silver may be present and can be dealt with as for test (*a*) for copper. Cyanides and copper interfere. The colour caused by copper can be bleached with dithionite.

IRON—

In acetate buffer solution iron^{III} gives a violet colour, easily extracted by solvents. Colour does not appear immediately when the reaction is carried out in aqueous pyridine solution, but the red colour of iron^{II} develops slowly, more quickly if warmed. When ferric chloride solution is made more than 5 *N* with respect to hydrochloric acid and an equal volume of pyridine is added, the addition of dithiol causes iron^{III} to be reduced to iron^{II}, but the red colour does not appear until the reaction is almost complete.

In aqueous pyridine solution the red colour of iron^{II} is visible with concentrations as low as 0·2 μg per ml and the optical density for 1 μg per ml is 0·103. The optical density was found to be proportional to the iron concentration up to 3 μg per ml at least.

Copper interferes, but the copper colour is easily bleached with dithionite, which does not bleach the iron colour. If the reaction is allowed to proceed in cold solution, cobalt does not interfere unless present in large amounts (when dithionite may again be used), although the red solution formed becomes blue when warmed and shaken with air. Very large amounts of nitrogenous bases (atropine and strychnine) lower the sensitivity of the reaction.

Test for iron^{II}—To 0·5 ml of cold test solution add 0·5 ml of pyridine and then dithiol dropwise. A magenta colour appearing immediately indicates iron^{II}. Copper interferes and cobalt also if in large quantity. For procedure suitable for use in their presence, see p. 181. A slow development of a red colour, especially on warming, may indicate iron^{III}, but there is risk of confusion with antimony^{V}. The solution should therefore first be reduced.

THALLIUM—

The yellow precipitate formed when dithiol is added to thallium in an acetate buffer solution is unchanged in colour by the addition of pyridine, in which it readily dissolves on heating. This provides a distinction from the yellow complex of lead, which is almost decolorised by pyridine. If dithiol is added to an aqueous pyridine solution containing thallium, an orange-yellow colour forms immediately, 20 to 30 μg per ml being readily visible. The colour is produced immediately in cold solution in the presence of moderate quantities of cobalt or iron; this colour is discharged when the solution is set aside or warmed, a blue or red colour developing. If a solution is made more than 5 *N* with respect to hydrochloric acid before pyridine is added, the thallium reaction is inhibited. The reaction is unaffected by cyanide and can, therefore, be made specific or at least highly selective for thallium.

Test for thallium—To 1 ml of test solution acidified (0·1 to 2 *N*) with mineral acid, add 0·5 to 1 ml of pyridine solution. Add *M* potassium cyanide dropwise until the solution is colourless, or nearly so, and then add dithiol. A yellow or orange colour forming immediately in cold solution and rapidly becoming paler with the formation of a precipitate that re-dissolves when heated indicates thallium.

NICKEL—

A solution of aqueous pyridine containing 2·5 to 10 μg of nickel per ml gives a yellow colour with dithiol; a greenish yellow colour is formed when the concentration of nickel is greater than 10 μg per ml. The optical density of a solution containing 1 μg per ml was 0·021, an Ilford No. 622 blue filter being used. The optical density was found to be proportional to the concentration of nickel up to 10 μg per ml at least.

ANTIMONY—

In aqueous pyridine solution antimony^{III} does not give a coloured complex, but antimony^{V} gives a red or pink colour of varying intensity. It seems likely that dithiol is oxidised in the reaction and that some of the antimony^{V} is reduced to antimony^{III}.

If a solution containing antimony^{V} is made more than 5 *N* with respect to hydrochloric acid and an equal volume of pyridine is added, the addition of dithiol instantly produces a brilliant red colour that is unchanged by the addition of sodium hydroxide; 0·5 μg of antimony^{V} per ml is easily detected. The solution does not remain clear on standing, but the coloured complex is soluble in *iso*amyl acetate. A brilliant red solution was produced by dissolving antimony pentoxide in a pyridine - dithiol mixture, but not in alkaline dithiol in the absence of pyridine.

Only copper, cobalt, iron and nickel interfere in this reaction. The presence of hydrochloric acid in excess prevents interference by thallium. In the presence of cyanide the reaction appeared to be specific for antimony^{V}. No interference was caused by molybdate, tungstate, vanadate or any of the more common metals (except silver) dissolved together in dilute hydrochloric acid. Silver interferes, but cannot normally be present except in very strong acid.

Test for antimony^{V}—To 0·5 ml of test solution previously oxidised with bromine water, add *M* potassium cyanide until any colours formed are bleached. Add an equal

volume of concentrated hydrochloric acid and about 1 ml of pyridine, and then add dithiol dropwise. A brilliant red or orange-red colour forming instantly in cold solution indicates antimony^{V}.

MANGANESE—

Dithiol or diacetyldithiol (but not zinc dithiol) give an intense yellowish green precipitate, soluble in aqueous pyridine solution and also in ethylene dichloride to give a yellow or green solution. With an excess of dithiol the colour becomes an intense dark greenish brown, which can be detected with 2 μg of manganese per ml in aqueous pyridine solution. Many cations interfere.

VANADIUM—

When dithiol is added to a solution of a vanadate in aqueous pyridine, a yellow colour is at first produced. On addition of more than 0·5 mole of dithiol this changes to an intense sea-green colour; if alkali is then added the solution turns brown. The green colour was easily visible with 1 μg of vanadium per ml.

In acetate buffer the colour is blue, but fades rapidly. The colour is soluble in and stabilised by methyl acetate or *iso*amyl alcohol. In acetate buffer solution vanadyl salts give an intense red coloration, which, in cold solution, changes to blue and then fades in 1 to 2 minutes. Molybdate and many cations interfere.

APPLICATIONS TO QUALITATIVE ANALYSIS

It was noted in Part I[1] that dithiol complexes may be formed in preference to sulphides. Dithiol can therefore be used to dissolve sulphides. Copper sulphide dissolves in a pyridine - dithiol mixture or in a solution of zinc dithiol in sodium hydroxide or ammonia solution to give characteristic colour reactions. Iron, cobalt and manganese sulphides also dissolve in a pyridine - dithiol mixture to give their characteristic colours, but nickel sulphide dissolves more slowly. The colour characteristic of manganese is developed with diacetyldithiol in pyridine but not with zinc dithiol. The best solvent found for cobalt sulphide was a solution containing 0·2 g of potassium hydroxide, a few drops of pyridine and a few milligrams of zinc dithiol in 0·5 ml of water. On warming, the upper pyridine layer becomes blue in the presence of cobalt.

A common test for cyanides depends upon their ability to discharge the black colour of a suspension of copper sulphide.[6] In the same way traces of cyanide will discharge the blue colour of a pyridine - dithiol mixture containing a trace of cobalt.

DETECTION OF COPPER, COBALT AND IRON IN THE PRESENCE OF ONE ANOTHER AND OF NICKEL—

Interference between these metals can be eliminated by reduction. At temperatures below 40° to 50° C sodium dithionite reduces the copper complex to a colourless compound and the addition of more dithiol causes the blue colour of cobalt to appear. Further reduction causes the blue colour to be replaced by a yellow colour, after which the red colour of iron^{II} appears.

Tests were made to determine the lower limits of detection of these metals in the presence of each other and of other cations. These tests were carried out with 1 to 2 ml of a solution containing 50 to 100 mg (as element) of the chlorides of lead, mercury^{II}, bismuth, cadmium, arsenic^{III}, antimony^{III}, tin^{IV}, chromium, aluminium, zinc, manganese, calcium, strontium, barium, magnesium, sodium, potassium and ammonium dissolved in 100 ml of water with sufficient hydrochloric acid to keep the solution clear. For each test 1 ml of a solution containing a small quantity of copper, cobalt or iron and 0·5 to 1 mg of the other two and nickel, either separately or in various combinations, were added together with 1 ml of pyridine. A solution of dithiol reagent, prepared by dissolving 0·1 to 0·2 g of dithiol in cold 95 per cent. ethanol, was added dropwise until the copper colour appeared. Two droppers were used to add, alternately, freshly prepared sodium dithionite solution to bleach the solution and dithiol reagent to re-develop the colour. When cobalt was present, the solution, at a certain stage, became blue, and when iron^{II} was present the procedure was continued until a red colour appeared.

The lower limits of detection were found by using various amounts of copper, cobalt and iron, and the results are shown in Table II. Satisfactory results were also obtained with solutions prepared as described in Part IV[5] instead of the dithiol reagent. When testing for traces of cobalt in the presence of nickel, it was found advantageous to add an excess of sodium hydroxide after the final addition of dithionite.

TABLE II

LIMITS OF DETECTION OF COPPER, COBALT, AND IRON WITH A PYRIDINE - DITHIOL MIXTURE IN THE PRESENCE OF EACH OTHER AND OF NICKEL

Tests were carried out with 1 to 2 ml of a solution containing 50 to 100 mg (as element) of the chlorides of Pb, Hg^{II}, Bi, Cd, As^{III}, Sb^{III}, Sn^{IV}, Cr, Al, Zn, Mn, Ca, Sr, Ba, Mg, Na, K and NH_4^+ dissolved in 100 ml of water

Limit of detection of copper, μg per ml ..	10	< 30	< 30	10
Cobalt added, mg per ml	—	0·5 to 1	0·5 to 1	—
Iron added, mg per ml	—	—	0·5 to 1	0·5 to 1
Nickel added, mg per ml	—	—	0·5 to 1	0·5 to 1
Limit of detection of cobalt, μg per ml ..	< 1	< 10	50 to 100	< 10
Copper added, mg per ml	—	0·5 to 1	0·5 to 1	—
Iron added, mg per ml	—	—	0·5 to 1	0·5 to 1
Nickel added, mg per ml	—	—	0·5 to 1	0·5 to 1
Limit of detection of iron, μg per ml ..	< 1	10 to 20	< 10	< 100
Copper added, mg per ml	—	—	0·5 to 1	0·5 to 1
Cobalt added, mg per ml	—	—	—	0·5 to 1
Nickel added, mg per ml	—	0·5 to 1	—	0·5 to 1

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REFERENCES

1. Clark, R. E. D., *Analyst*, 1936, **61**, 242.
2. ——, *Ibid.*, 1937, **62**, 661.
3. Price, W. B., and Smiles, S., *J. Chem. Soc.*, 1928, 2372.
4. Obellianne, P., *Bull. Soc. Chim. France*, 1952, 147.
5. Clark, R. E. D., *Analyst*, 1957, **82**, 182.
6. Vogel, A. I., "A Text-book of Macro and Semimicro Qualitative Inorganic Analysis," Fourth Edition, Longmans, Green & Co. Ltd., London, New York and Toronto, 1954, p. 343.

NOTE—References 1 and 2 constitute Parts I and II, respectively, of this series.

DEPARTMENT OF SCIENCE AND TECHNOLOGY
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o-Dithiols in Analysis

Part IV*. Diacetyltoluene-3:4-dithiol, Dibenzoyltoluene-3:4-dithiol and the Zinc Complex of Toluene-3:4-dithiol

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Diacetyltoluene-3:4-dithiol, dibenzoyltoluene-3:4-dithiol and the zinc complex of toluene-3:4-dithiol provide a stable source of toluene-3:4-dithiol. The first two are hydrolysed by alkalis to give toluene-3:4-dithiol, but are stable in acids; diacetyltoluene-3:4-dithiol is hydrolysed slowly by warm aqueous pyridine and almost immediately by ethanolic potassium ethoxide. Diacetyltoluene-3:4-dithiol gives colour reactions with palladium^{II}, rhenium^{VII}, tellurium^{IV} selenium^{IV}, and iridium^{IV} in concentrated hydrochloric acid, the complexes being formed in the order given. Dibenzoyltoluene-3:4-dithiol similarly reacts with palladium^{II} and selenium^{IV}. Reactions for palladium^{II}, rhenium^{VII} and tellurium^{IV} are apparently specific or readily rendered so. The zinc complex of toluene-3:4-dithiol may often be used directly in place of toluene-3:4-dithiol in acid solution. A solution of toluene-3:4-dithiol suitable for analysis may be prepared from the zinc complex by dissolving it either in ethanol containing a small amount of aqueous hydrochloric acid or in aqueous sodium hydroxide solution.

THE keeping qualities of pure toluene-3:4-dithiol (dithiol) are so poor that it is necessary to store it in sealed ampoules. Solutions in organic solvents are unstable and alkaline solutions rapidly deposit the disulphide. In earlier papers[1,2] it was suggested that the

* For particulars of Parts I, II and III of this series, see reference list, p. 185.

reagent, dissolved in sodium hydroxide solution, should be preserved in an atmosphere of hydrogen or that an excess of thioglycollic acid should be added. Stone[3] suggested a solution containing some thioglycollic acid in 95 per cent. ethanol, but the keeping qualities of this solution still leave much to be desired. Moreover, the presence of thioglycollic acid is often undesirable, especially as it interferes with the reactions described in Part III.[4]

These difficulties can be overcome by generating dithiol, as and when required, from the derivatives described in this paper.

DIACETYLTOLUENE-3:4-DITHIOL AND DIBENZOYLTOLUENE-3:4-DITHIOL

Diacetyltoluene-3:4-dithiol (diacetyldithiol) and dibenzoyltoluene-3:4-dithiol (dibenzoyldithiol) are colourless stable crystalline compounds, hydrolysed by alkalis, but stable to acids. Diacetyldithiol is hydrolysed almost instantly in hot ethanolic potassium ethoxide solution, but dibenzoyldithiol only when heated under reflux for some minutes. Diacetyldithiol is hydrolysed rapidly, even in warm aqueous pyridine, and so elicits the colour reactions in this medium described in Part III.[4] Dibenzoyldithiol gives several of the same colour reactions if a small amount of sodium hydroxide is present.

Diacetyldithiol is readily soluble in most organic solvents. Its solutions in alcohols (methanol, ethanol, *n*-propanol, ethylene glycol), acetone, acetic acid and in solvents liable to peroxidation are somewhat unstable, as the disulphide is precipitated and then a yellow and ultimately a red colour is produced. Solutions can be kept without apparent change for a year in ethyl acetate or ethyl Cellosolve. Dibenzoyldithiol solutions are much more stable; a 0·01 *M* solution in 95 per cent. ethanol is permanent.

Of the two reagents, it would appear that diacetyldithiol is, for purposes of qualitative analysis, the more generally useful and that it is most conveniently stored in the solid state or, if frequently required, as a 1 per cent. solution in ethanol, which will remain stable for 3 to 4 weeks.

PREPARATION OF DITHIOL DERIVATIVES—

Diacetyldithiol—A solution of 1 g of dithiol in 2 to 3 parts by weight of acetic anhydride was heated on a water bath for 30 minutes. The products when poured into water gave the theoretical yield of an oil that did not readily crystallise. Colourless crystals of diacetyldithiol were recrystallised from *cyclo*hexane and had m.p. 48° C. By titration the composition of the product was found to be: $C_7H_6S_2$,* 64·5 per cent.; CH_3CO, 35·2 per cent. (calculated values for $C_{11}H_{12}O_2S_2$ are: $C_7H_6S_2$, 64·3 per cent.; CH_3CO, 35·8 per cent.).

Diacetyl-4-chloro-1:2-dimercaptobenzene—4-Chloro-1:2-dimercaptobenzene, prepared by the method of Mills and Clark,[5] was heated with acetic anhydride on a water bath for 30 minutes. The product was recrystallised from ethanol and had m.p. 68° C. Found on analysis: C, 46·3 per cent.; H, 3·8 per cent. (calculated values for $C_{10}H_9O_2S_2Cl$ are: C, 46·1 per cent.; H, 3·5 per cent.).

Dibenzoyldithiol—Dithiol (1 mole) was dissolved in 5 per cent. aqueous sodium hydroxide solution (2 moles) and shaken with benzoyl chloride (2 moles). Colourless crystals of dibenzoyldithiol were recrystallised from ethanol and had m.p. 92° C. By titration with mercuric chloride the product was found to contain: $C_7H_6S_2$, 41·2 per cent. (calculated value for $C_{21}H_{16}O_2S_2$ is 41·3 per cent.).

PREPARATION OF DITHIOL SOLUTION FROM ITS DERIVATIVES—

A suitable dithiol solution for use in analysis can be prepared as follows—

Add one drop of water to 0·1 g of potassium hydroxide and heat until it has dissolved. Add 1 ml of 95 per cent. ethanol and 20 to 100 mg of diacetyldithiol. Boil for 10 to 15 seconds and then dilute with ethanol to the concentration required. Alternatively, use 2 to 3 ml of 1 per cent. ethanolic solution and proceed as before. The solution should be used within 1 to 3 hours. If the presence of alkali is undesirable, add 2 *N* hydrochloric acid until a permanent precipitate begins to form.

* The dithiol contents of the compounds (as a percentage of $C_7H_6S_2$) were determined by dissolving a weighed amount of the compound (10 to 20 mg) in 5 ml of pyridine containing 1 drop of 0·01 *M* cobalt chloride. The brilliant blue solution produced was warmed and titrated with 0·01 *M* mercuric chloride solution from a microburette, the end-point being read to 0·01 ml. With dibenzoyldithiol one drop of 2 *N* sodium hydroxide was added.

REACTIONS OF DITHIOL DERIVATIVES WITH CATIONS—

When ethanolic diacetyldithiol was added to mineral-acid solutions containing the more common heavy metals, the reagent was precipitated unchanged as a white emulsion. In acetic acid or acetate buffer solution, precipitates formed slowly when the solution was boiled. These precipitates generally resembled the precipitates given by dithiol, except that the white cadmium complex slowly turned pink, whereas the dithiol complex remained white under the same conditions. A few preliminary analyses of these precipitates, which could not be purified, appeared to indicate that the lead and cadmium complexes formed in this way retained one acetyl group.

When ethanolic diacetyldithiol was added to 0·002 *M* solutions of palladium^{II}, rhenium^{VII}, tellurium^{IV} and selenium^{IV} in 9 to 10 *N* hydrochloric acid, coloured products were instantly produced, which could all be extracted with ethylene dichloride. The colour reactions of these metals were—

Palladium^{II}—Brick-red complex formed instantly in cold solution (2 to 3 μg per ml could be detected).

Rhenium^{VII}—Intense green colour developed rapidly when the solution was warmed (0·5 μg per ml could be detected).

Tellurium^{IV}—Intense yellow precipitate formed in hot solution with an excess of reagent (5 μg per ml could be detected). In 5 *N* hydrochloric acid a yellow precipitate formed after a few seconds and then rapidly darkened, possibly owing to the presence of free tellurium.

Selenium^{IV}—Evanescent red colour produced that changed to a yellow colour (20 μg per ml would produce the red colour). The colour of the solvent extract was yellowish green.

Iridium^{IV} caused the solution to darken slowly, the solvent extract being brown in colour. The presence of iridium did not interfere with the selenium reaction. In more dilute acid (5 *N*) osmium^{VIII} also gave a black precipitate, but other metallic ions did not give intensely coloured precipitates or colours at acidities above 0·5 *N*.

Dibenzoyldithiol reacted with palladium^{II} under similar conditions to give a brick-red complex, but rhenium^{VII} and tellurium^{IV} did not give colours. Selenium^{IV} did not give a colour immediately, but a red colour developed within 30 to 60 seconds and remained for 1 to 2 minutes. This was not always observed in mixtures containing other elements. Colours were not observed with other cations. Dibenzoyldithiol would, therefore, appear to be specific for palladium^{II}. The reaction was at once obtained with palladium^{II} in a solution containing all the more common cations.

When 2 per cent. ethanolic diacetyldithiol was added dropwise with shaking to 2 to 4 ml of hot solution containing a mixture of metals in 8 *N* hydrochloric acid in the presence of ethylene dichloride, the complexes were formed in the order palladium^{II}, rhenium^{VII} and tellurium^{VI}. By this means, it was possible to "titrate" the elements one by one with no noticeable interference; a sharp change in colour of the aqueous layer indicated that the previous element in the order of formation had been removed from solution. With the quantities investigated no interference was observed and 10 μg per ml of palladium^{II}, rhenium^{VII} and tellurium^{IV} were each detected without difficulty in a 10-fold excess of each of the other two and also of selenium, these elements being present in a solution containing 0·05 to 0·1 per cent. of each of the more common cations. Diacetyldithiol would therefore appear to be a specific or at least highly selective reagent for palladium^{II}, rhenium^{VII} and tellurium^{IV}.

THE ZINC COMPLEX OF DITHIOL

A second approach to the problem was made by investigating the metallic derivatives of the *o*-dithiols, a number of which have been obtained in an analytically pure state. Of those investigated the zinc complex of dithiol (zinc dithiol) appears to be the best for providing a stable source of dithiol. It is a colourless, odourless and water-repellent stable compound from which dithiol is instantly liberated by the action of acids or alkalis.

Zinc dithiol can often be used directly. When added to acid solutions of the heavy metals, the zinc is replaced and the familiar dithiol precipitates are obtained. Zinc dithiol is soluble in cold pyridine and in aqueous sodium hydroxide solution and at once elicits most of the reactions given by dithiol in these media. Hence, in aqueous pyridine, the reactions

given by copper, cobalt, iron and nickel were produced at once, that for thallium seemed to be poorly developed, while those for antimony and vanadium were not given. When dithiol was first liberated by means of hydrochloric acid, however, the usual reactions were given.

Zinc dithiol gives cleaner solutions than those produced with diacetyldithiol. It cannot, however, be used in certain reactions (see "Manganese," Part III, p. 181), or when the addition of zinc is undesirable.

PREPARATION—

A solution of 1 g of dithiol in 5 ml of chloroform was added to a hot solution containing 2 g of zinc acetate in 50 ml of water and the resulting solution was shaken. The precipitate formed was filtered off, washed with chloroform and dried at 80° C. The dried precipitate was a white bulky powder, easily charged, and the yield was almost the theoretical. Found on analysis: S, 28·6 per cent.; Zn, 29·9 per cent.; $C_7H_6S_2$, 68·3 per cent. (calculated values for $C_7H_6S_2Zn$ are: S, 29·2 per cent.; Zn, 29·8 per cent.; $C_7H_6S_2$, 70·3 per cent.).

PREPARATION OF DITHIOL SOLUTION FROM ZINC DITHIOL—

A suitable dithiol solution for use in analysis can be prepared from zinc dithiol as follows—

To 3 to 5 ml of 95 per cent. ethanol add a suitable amount of zinc dithiol and then a few drops of concentrated hydrochloric acid or 5 *N* sulphuric acid and shake the solution. A clear solution is produced within a few seconds in the cold. Alternatively, add a suitable amount of zinc dithiol to 2 *N* sodium hydroxide solution together with one drop of ethanol and shake the solution.

I express my gratitude to Dr. F. G. Mann, F.R.S., and to Mr. P. S. Jewell, for their interest, help and encouragement, and to Hopkin & Williams Ltd., Carnegies of Welwyn Ltd. and the Clayton Aniline Co. Ltd. for gifts of chemicals.

REFERENCES

1. Clark, R. E. D., *Analyst*, 1936, **61**, 242.
2. ——, *Ibid.*, 1937, **62**, 661.
3. Stone, I., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 791.
4. Clark, R. E. D., *Analyst*, 1957, **82**, 177.
5. Mills, W. H., and Clark, R. E. D., *J. Chem. Soc.*, 1936, 175.

NOTE—References 1, 2 and 4 constitute Parts I, II and III, respectively, of this series.

DEPARTMENT OF SCIENCE AND TECHNOLOGY
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June 25th, 1956

A Rapid, Simple and Accurate Method for the Determination of Sulphonamides

BY LEE KUM-TATT*

The present investigation was prompted chiefly by the lack of a rapid, accurate and general method for the routine determination of sulphonamides. The silver nitrate method, which appeared to be the most promising, was studied, and a volumetric method has been developed for the determination of seven of the most common sulphonamides. This method is only applicable to sulphonamides that form insoluble silver salts with silver nitrate. The proposed method, when applicable, is superior in speed and precision to other chemical methods and is also applicable to the determination of sulphonamides in simple mixtures.

THE widespread use of sulphonamides has necessitated the search for a simple, rapid and accurate analytical method for their determination. The British Pharmacopoeia[1] method,

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which involves a volumetric titration with sodium nitrite, with starch - iodide paper as the external indicator, is widely used, but this and the bromimetric methods suffer from several inconveniences and limitations.[2,3,4,5] Fritz and Keen[6] titrated certain sulphonamides in non-aqueous media with sodium methoxide, with thymol blue or azo violet as the indicator. The standardisation of the sodium methoxide by titration with benzoic acid in butylamine often gives erratic results, owing to the formation of a gel. Further, perfectly anhydrous conditions[7] and complete freedom from carbon dioxide are absolutely essential for accurate results.

The silver nitrate method for the determination of sulphonamides has been the subject of several papers.[8,9,10,11] Several methods have been proposed, but each has some drawback. A gravimetric method for the determination of sulphonamides as the silver salt was described by Stainier and Lapière.[8] Another method involving the determination of the excess of silver nitrate used for the precipitation of the sulphonamides was also described in the same paper. The main disadvantages of these procedures are the time needed for the analysis (at least 1 hour) and the care needed to avoid having an excess of sulphuric acid present, which will affect the results, as the silver salts are soluble in this acid. Further, the gravimetric method is not suitable for the determination of sulphonamides in tablets. Perel'man and Kozlova[9] claimed that sulphadiazine and sulphathiazole could be determined by direct titration with silver nitrate, in the presence of an excess of potassium chromate, with an accuracy of within 1 per cent.

A modified method has now been developed. It was found that, when the sulphonamide was dissolved in acetone and magnesium oxide was added, a direct titration could be carried out with silver nitrate, potassium dichromate being used as the indicator. The end-point was sharp and excellent results were obtained with both pure sulphonamides and tablets containing sulphonamides. A modified gravimetric method and a back-titration method were also studied, and the results obtained were satisfactory for most purposes.

Method

Reagents—

Acetone—Analytical-reagent grade.
Silver nitrate solutions, 0·05 N *and* 0·10 N.
Sodium dichromate solution, 0·5 *per cent.*
Ammonium thiocyanate solution, 0·1 N.
Ferric ammonium sulphate solution, 10 *per cent.*
Magnesium oxide.
Nitric acid, 10 *per cent.*

Direct titration procedure—

Weigh about 200 mg of sample into a 250-ml conical flask, add 50 ml of acetone and warm the flask until the solid has dissolved. Add 1 ml of sodium dichromate solution and approximately 1 g of magnesium oxide, and titrate with 0·05 *N* silver nitrate solution until a permanent red colour appears. Carry out a blank determination; 0·1 ml or less of the 0·05 *N* silver nitrate should be required.

Gravimetric procedure, with a back-titration procedure—

Weigh about 200 mg of sample into a 150-ml beaker and dissolve it in 25 ml of acetone, with warming. Add 50 ml of 0·10 *N* silver nitrate solution, and place the beaker on a water bath for ½ hour. Collect the precipitated silver salt on a suitable filter, wash it well with distilled water and dry it to constant weight.

To the filtrate and washings add 4 ml of ferric ammonium sulphate solution and 10 ml of 10 per cent. nitric acid, and titrate with 0·1 *N* ammonium thiocyanate solution until a red colour appears.

With the gravimetric method it was found necessary to add a large excess of silver nitrate solution.

Discussion of results

The sulphonamides were determined by the proposed procedures. The hydrogen atom present in the $-SO_2NH-$ linkage in sulphanilamide, sulphaguanidine and sulphacetamide does not appear to be sufficiently activated either by the other hydrogen atom in sulphanilamide or by the adjacent group in the other two compounds to be replaced by the silver atom from silver nitrate. Table I shows the results of these determinations.

TABLE I

RECOVERY OF SULPHONAMIDES BY PROPOSED METHODS, 200-mg AMOUNTS BEING TAKEN FOR EACH TEST

Sulphonamide tested	Volumetric method: Volume of 0·05 *N* silver nitrate used, ml	Volumetric method: Amount of sulphonamide recovered, mg	Gravimetric method: Weight of silver salt found, mg	Gravimetric method: Amount of sulphonamide recovered, mg	Back-titration method: Volume of 0·1 *N* silver nitrate used, ml	Back-titration method: Amount of sulphonamide recovered, mg
Sulphapyridine	16·0	199	285·2	200	8·0	199
Sulphamerazine	15·1	200	281·2	200	7·5	198
Sulphadiazine	15·9	199	285·2	200	8·0	199
Sulphadimidine	14·3	199	275·6	199	7·2	200
Sulphathiazole	15·6	199	284·4	201	7·3	198
Phthalylsulphathiazole ..	9·90	200	252·8	200	5·0	202
Succinylsulphathiazole ..	10·7	200	256·5	199	5·4	202

Some differential titrations are possible. Mixtures such as that of sulphapyridine and sulphanilamide, or any one of sulphapyridine, sulphadiazine, sulphadimidine, sulphamerazine, sulphathiazole, phthalylsulphathiazole or succinylsulphathiazole ("acid sulphonamides") with either sulphanilamide or sulphaguanidine can be analysed by first titrating with silver nitrate, to give the "acid sulphonamide" content, and then determining the total sulphonamide content by the British Pharmacopoeia method. The volumetric method cannot be applied to mixtures of sulphacetamide and any one of the "acid sulphonamides," because it was found that sulphacetamide interferes with the titration. However, the "acid sulphonamides" present in such a mixture can be accurately determined by both the gravimetric and back-titration methods. Table II gives results of the analyses of some mixtures by the various methods.

TABLE II

RECOVERY OF "ACID SULPHONAMIDES" IN MIXTURES WITH SULPHANILAMIDE, SULPHAGUANIDINE AND SULPHACETAMIDE, 200-mg AMOUNTS OF EACH SULPHONAMIDE BEING TAKEN FOR EACH TEST

"Acid sulphonamide" present	Sulphanilamide present—: by volumetric method, mg	by gravimetric method, mg	by back-titration method, mg	Sulphaguanidine present—: by volumetric method, mg	by gravimetric method, mg	by back-titration method, mg	Sulphacetamide present—: by gravimetric method, mg	by back-titration method, mg
Sulphapyridine	200	201	198	200	199	199	199	199
Sulphadiazine	200	200	199	200	200	199	199	200
Sulphadimidine	199	199	199	199	200	200	199	199
Sulphamerazine	200	200	200	200	199	198	200	199
Sulphathiazole	200	199	200	200	200	200	199	198
Phthalylsulphathiazole ..	200	200	201	200	201	199	200	199
Succinylsulphathiazole ..	199	199	198	200	199	199	201	200

Certain sulphonamide tablets and sulphonamides with known excipients added were also determined by the volumetric method. It was found that the normal tablet excipients do not interfere with the titration. Table III shows the results obtained in the analysis of three different types of sulphonamides from different sources by both the volumetric method and the British Pharmacopoeia method. Table IV contains the results for some sulphonamides that have had excipients such as starch, alginic acid and lactose added to them.

CONCLUSIONS

The proposed volumetric method fulfils the requirements for a general method for the routine determination of sulphonamides. The procedures for carrying out the determination are very simple and general. The method does not require the use of elaborate

apparatus, neither is there a necessity for temperature control nor need to have absolutely anhydrous conditions. Tedious filtration or washing procedures are not involved, and the method is suitable for the determination of both pure sulphonamides and sulphonamides in tablets, as the common tablet excipients do not interfere. It is known that the presence of lactose interferes in the determination of succinylsulphathiazole by the sodium nitrite method[12]; this is not so, however, with the proposed method nor is a preliminary hydrolysis of the succinylsulphathiazole or phthalylsulphathiazole to sulphathiazole necessary.

TABLE III

SULPHONAMIDE CONTENT OF TABLETS BY VOLUMETRIC AND BRITISH PHARMACOPOEIA METHODS

Sulphonamide present	Sulphonamide found by volumetric method, mg per tablet	Sulphonamide found by B.P. method, mg per tablet
Sulphapyridine	480	482
	494	490
	482	478
Sulphadimidine	459	460
	493	490
	486	486
Sulphathiazole	500	495
	485	488
	492	496

TABLE IV

RECOVERY OF SULPHONAMIDES BY PROPOSED VOLUMETRIC METHOD IN THE PRESENCE OF 40-mg AMOUNTS OF ADDED EXCIPIENTS, 200-mg AMOUNTS OF THE SULPHONAMIDES BEING TAKEN FOR EACH TEST

Excipient added	Sulphapyridine recovered, mg	Sulphathiazole recovered, mg	Phthalyl-sulphathiazole recovered, mg	Sulpha-merazine recovered, mg
Starch	200	199	201	199
Alginic acid ..	201	200	202	199
Lactose	199	200	200	200
Calcium phosphate	201	201	202	201

A limitation to the present method is that in general it can be applied only to sulphonamides that form an insoluble salt with silver nitrate. When this method is applicable, it offers very real advantages in speed and precision over any other chemical methods known.

I thank Dr. A. Jackson and Dr. D. A. Frye (Director and Chief Chemist, respectively, of the Government Chemistry Department) for their interest, encouragement and criticism in this work. I also gratefully acknowledge the assistance of Mr. Thng Soon Tee, who helped carry out the experiments.

REFERENCES

1. "British Pharmacopoeia," The Pharmaceutical Press, London, 1953.
2. Garratt, D. C., "The Quantitative Analysis of Drugs," Second Edition, Chapman & Hall Ltd., London, 1955, p. 469.
3. Schurman, I., *J. Ass. Off. Agric. Chem.*, 1941, **24**, 810.
4. Conway, H. S., *J. Amer. Pharm. Ass., Sci. Ed.*, 1945, **34**, 236.
5. Towne, R. W., and Hitchens, R. M., *J. Amer. Pharm. Ass.*, 1939, **28**, 585.
6. Fritz, J. S., and Keen, R. T., *Anal. Chem.*, 1952, **24**, 308.
7. Vespe, V., and Fritz, J. S., *J. Amer. Pharm. Ass., Sci. Ed.*, 1952, **41**, 197.
8. Stainier, C., and Lapière, C., *Anal. Chim. Acta*, 1947, **1**, 178.
9. Perel'man, Ya., and Kozlova, V. I., *Farmatsiya*, 1947, **10**, 22; *Chem. Abstr.*, 1947, **41**, 7666.
10. De Reeder, P. L., *Anal. Chim. Acta*, 1954, **10**, 413.
11. Marques Leal, A., and Silipe, M. A. R., *J. Farm., Lisbon*, 1949, **8**, 85; *Chem. Abstr.*, 1951, **45**, 4885E.
12. Andrade, J. L., Baumucker, J., and Marquez, V. M., *J. Amer. Pharm. Ass., Sci. Ed*,. 1956, **65**, 446.

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A Direct Colorimetric Determination of Elementary Sulphur

By HORACE A. ORY, VIRGIL L. WARREN and HULEN B. WILLIAMS

Use has been made of the Schoenberg reagent, N-(4:4′-dimethoxybenzohydrilidene)benzylamine, in a direct colorimetric quantitative test for free sulphur. The reaction between sulphur and this reagent was studied by means of infra-red spectroscopy; phenyl cyanide was shown to be one of the reaction products, and the results suggest that the reaction proceeds through a series of possible intermediates.

The results show that sulphur in milligram quantities can be determined with an accuracy of 3 per cent., or better, by this method. It was necessary that the mole ratio of sulphur to reagent should be less than 0·75 in order that the amount of blue thioketone produced should be determined by the amount of sulphur present and not by the amount of reagent.

In the course of our efforts to characterise chemically the sediments from marsh lands of a western portion of LaFourche Parish, Louisiana, difficulties arose in the analyses for total sulphur, sulphate and free sulphur in the sediments. It soon became apparent that further research and development would be necessary for the determination of free sulphur.

The determination of free sulphur is usually accomplished by indirect methods depending on the extraction or sublimation of elementary sulphur from the material investigated, the amount of free sulphur being indicated by the weight of material extracted or by chemical procedures applied to the isolated sulphur. Since the solvents used extract not only free sulphur but also other material that happens to be soluble in the solvent, and since amorphous sulphur is insoluble in the solvents ordinarily used (except pyridine), the indirect methods are generally unsatisfactory, and particularly unsatisfactory in the analysis of complex mixtures, such as the coastal muds under investigation.

Although the literature provided no direct method of analysis, two promising colour reactions of free sulphur were found to have been reported. The first of these[1,2] is the production of a blue colour by the addition of free sulphur and pyridine to sodium hydroxide solution. The reaction is very sensitive, but the colour produced changes through green to brown in the presence of more than a trace of sulphur. Sommer[3] found that the quantity of free sulphur could be crudely estimated colorimetrically by a method (involving extraction with pyridine) based on this reaction, although the progressive and rapid fading of colour, caused by oxidation of sulphur to thiosulphate, was a major obstacle to an accurate determination. Further, the method depended on a colloidal dispersion of the sulphur in the aqueous pyridine solution, which assumes a blue colour in the presence of alkali. This type of reaction, and this reaction in particular, would appear to offer little promise as the basis of an accurate colorimetric determination of free sulphur, since the colloidal dispersion would be quite sensitive to the effects of other substances also extracted by the pyridine.

The second colour reaction[4,5] was the production of blue *p*:*p*′-dianisylthioketone from a mixture of sulphur and Schoenberg's reagent, N-(4:4′-dimethoxybenzohydrilidene)benzylamine, heated to 210° C for several minutes. This reaction was studied by Schoenberg and is the basis of the first direct test for free sulphur. It is sensitive to 40 μg of free sulphur and the reagent was found not to react with selenium or combined sulphur to produce a blue thioketone. The products of the reaction were not completely identified, although Schoenberg detected hydrogen sulphide by the lead acetate test and proved the identity of the *p*:*p*′-dianisylthioketone.

It is the essential purpose of this paper to report the results of our investigations of the Schoenberg reaction and its development into a quantitative colorimetric method for the determination of elemental sulphur.

Experimental

Absorbance measurements—

Absorption spectra, in the visible and near ultra-violet regions, were obtained for the reagent and a benzene extract of a mixture of the reagent and sulphur that had been heated

at a temperature of 210° C for 10 minutes. The reagent showed no significant absorption at wavelengths longer than 400 mμ and no peaks above 270 mμ. The thioketone, on the other hand, showed a strong absorption at 590 mμ and other peaks in the ultra-violet region. Interfering absorption by excess of reagent would cause errors in the determination of thioketone concentration if measurement were made in the ultra-violet region; however, determination of thioketone concentration at 590 mμ would not be hampered by interference due to reagent absorption. Further, the use of the peak at 590 mμ for absorption measurement would make possible visible measurements by comparison, as well as measurements by spectrophotometers and colorimeters with suitable filters. The absorption spectrum obtained from *p*:*p*'-dianisylthioketone agreed well with that reported by Donle and Volkert.[6]

Several dilutions were made of a benzene solution of the thioketone having an absorbance of about 1. The absorbances of these solutions were measured and plotted against the concentration of the thioketone relative to that of the original solution. A precise linear equation of zero intercept relating absorbance and concentration of thioketone was indicated. As no absolute determination of the thioketone concentration was made, the extinction coefficient could not be evaluated numerically; however, the excellent linearity obtained was indicative of close adherence to Beer's law.

Samples of sulphur for preparation of a standard curve were obtained from measured volumes of a standard solution of sulphur in carbon disulphide. The carbon disulphide was evaporated and the samples then treated as described below under "Procedure." The standard curve may be represented by the equation $S = 11{\cdot}00\,A + 0{\cdot}40$, where S is the amount of sulphur in the sample in mg and A is its absorbance.

The relative error in the quantity of sulphur calculated from the absorbance with use of the above equation was 3 per cent. when compared with the actual quantity taken, in the range from 1 to 22 mg.

It was observed that the mole ratio of sulphur to reagent must be less than 0·75 in order that the amount of thioketone produced should be controlled by the amount of sulphur present. If the ratio is greater than this value, the amount of thioketone produced is linearly related to the amount of reagent present, in accordance with the equation $R = 150{\cdot}0\,A + 5{\cdot}0$, where R is the amount of reagent present in mg and A is the absorbance. The relative error in the quantity of reagent calculated from the absorbance according to the above equation, compared with the quantity taken, was 1·6 per cent. The accuracy of sulphur determination is probably equally good, but the weights of samples taken were so small in the case of sulphur, that small experimental errors would appear to be large errors relative to the quantities of sulphur taken. If evenly distributed weights of sulphur were determined, the relative error observed would probably be within 2 per cent.

Method

Apparatus and reagents—

A Beckman DK spectrophotometer was used for recording the spectra of the reagent and the thioketone solution and for preliminary measurements of absorbance. Measurements of absorbance reported here were made with a Beckman DU spectrophotometer with matched 1·00-cm quartz cells. Comparable results were obtained with a Klett - Summerson photo-electric colorimeter with a 600-mμ filter.

Infra-red spectral data were recorded with a Perkin - Elmer model 21 infra-red spectrograph.

Commercial N-(4:4'-dimethoxybenzohydrilidene)benzylamine (obtained from Eastman Kodak Co.) was used as the reagent without further purification.

Reagent quality benzene was used for extraction.

A standard solution of reprecipitated sulphur dissolved in reagent quality carbon disulphide, from which measured volumes were taken and evaporated, was used for recording the standard curve.

The phenyl cyanide used for recording the infra-red spectrum was the commercial grade.

Procedure—

A measured quantity of sample (large enough to contain milligram quantities of free sulphur) is mixed well with a portion of Schoenberg's reagent weighing fifteen times as much as the sample and placed in a small test-tube, and then heated at 210° C for 10 minutes. The sample is cooled and extracted with benzene until no more blue colour is taken up. The

extract is filtered into a 25-ml calibrated flask and diluted to the mark. The absorbance of this solution is measured. Those solutions having an absorbance greater than about 1 are diluted before measurement and the absorbance found is multiplied by the appropriate factor.

It is necessary that the reaction mixture be fluid at 210° C, and sometimes in order to achieve this it may be necessary to add a greater excess of reagent.

Infra-red spectral studies

Since the products of the reaction had not been completely identified previously, and since the mixtures after reaction had an odour similar to that of phenyl cyanide, the following experiment was performed. A mixture of sulphur and reagent was placed in a distillation flask with a condenser and receiving flask attached, and this system was placed under reduced pressure. The flask and contents were heated to 210° C and kept there until a small amount of material distilled over. An infra-red spectrum of this distillate was recorded and the corresponding spectrum of an authentic sample of phenyl cyanide was recorded on the same chart paper. The spectra of the reaction product and phenyl cyanide were identical, with the exception of two extra bands in the spectrum obtained from the reaction product. Further, except for the two extra bands, both spectra agreed well with the spectrum of phenyl cyanide reported in the A.P.I. Tables.[7] Of the two extra bands, at 1244 and 1512 cm^{-1}, the former could arise from some hydrogen sulphide trapped in the liquid, since the second fundamental of hydrogen sulphide has been reported[8,9] in this region.

Five samples of sulphur - reagent mixture containing an excess of free sulphur were heated at 210° C for 2, 4, 6, 8 and 10 minutes, respectively. The disappearance of early forming bands, as yet unidentified, suggests the formation of intermediates.

Discussion of results

Mathematical relationships—

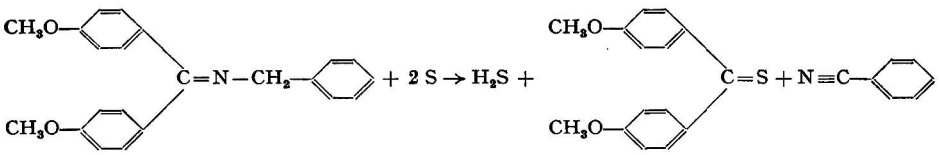
Although the straight line representing the relation between absorbance and concentration of various dilutions of a concentrated thioketone solution passes through the origin, the straight line representing the relation between absorbance of extract and quantity of sulphur present does not. The implied loss of 0·40 mg of sulphur in the process of reaction may be caused by reaction between sulphur and impurities in the reagent.

Stability of thioketone solution—

The thioketone solution is so stable that no problems are to be expected from its decomposition before measurement of absorbance. A solution kept in the light for 5 hours showed no change in its absorbance in that time. Another solution kept in the dark for 5 days showed no change in its absorbance in that time. Schoenberg and Mostafa[10] report that the thioketone is stable to oxygen (passed through the system) in the dark, but is readily converted to the corresponding ketone in sunlight.

Mechanism of the reaction—

The reaction can probably be summarised by the following equation—



Feigl[11] suggests that at elevated temperatures the double bond between nitrogen and carbon is loosened to such an extent that there is partial dissociation into free radicals, followed by the addition of sulphur to the radical containing bivalent carbon, which leads to the formation of *p*:*p*′-dianisylthioketone. He does not speculate on the fate of the radical containing nitrogen.

The identification of phenyl cyanide as one product of the reaction suggests that sulphur also attacks the radical containing nitrogen, perhaps forming an intermediate, but ultimately removing two hydrogen atoms homolytically from the methylene group as hydrogen sulphide, while the unpaired electrons form two more C–N bonds to yield phenyl cyanide.

REFERENCES

1. Itallie, L. van, *Pharm. Weekblad*, **1938**, **75**, 258.
2. ——, *Ibid.*, **1938**, **75**, 1445.
3. Sommer, H., *Ind. Eng. Chem., Anal. Ed.*, **1940**, **12**, 368.
4. Schoenberg, A., *Nature*, **1934**, **134**, 628.
5. Schoenberg, A., and Urban, W., *Ber.*, **1934**, **67B**, 1999.
6. Donle, H. L., and Volkert, G., *Z. phys. Chem., Abt. B*, **1930**, **8**, 60.
7. American Petroleum Institute Research Project **44**, Infrared Spectral Data, Serial No. 541, Benzonitrile, contributed by the Socony-Vacuum Oil Company, Paulsboro, New Jersey.
8. Bailey, C. R., Thompson, J. W., and Hale, J. B., *J. Chem. Phys.*, **1936**, **4**, 625.
9. Sprague, A. D., and Nielsen, H. H., *Ibid.*, **1937**, **5**, 85.
10. Schoenberg, A., and Mostafa, A., *J. Chem. Soc.*, **1943**, 275.
11. Feigl, F., "Chemistry of Specific, Selective and Sensitive Reactions," Academic Press Inc., New York, **1949**, pp. **366** to **369**.

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A Method for the Detection and Determination of Iodide in the Presence of Chloride

BY M. Z. BARAKAT, M. F. ABD EL-WAHAB AND M. M. EL-SADR

The use of bismuth - 2-mercaptothiazoline reagent is proposed for the micro-detection of iodide, even in the presence of bromide, chloride and fluoride. The sensitivity of the reaction is about 20 μg. The fact that iodide, but not chloride, reacts quantitatively with the proposed reagent to form an insoluble red complex iodide provides a new and easy gravimetric method for the determination of iodide in solution in the presence of chloride. The experimental error does not exceed 0·6 per cent.

The use of antimony - 2-mercaptothiazoline reagent is also proposed for the detection of iodide, in presence of bromide, chloride or fluoride.

THE reaction of bismuth salts with thiourea to form an intense yellow colour in the presence of dilute nitric acid is a well known qualitative test for bismuth.[1]

Naiman[2] first reported the detection of bismuth with a mixture of 2-methylbenzothiazole and potassium iodide, a coloured complex iodide being formed. Later, the use of such a reaction for the detection of iodides was suggested by McAllister,[3] who found that the yellow metallic complex formed by the interaction of bismuth sulphate (acid) and 1-methyl-2-mercaptoimidazole reacted with iodide ions to form an intensely coloured complex iodide. The sensitivity of this reaction was in the region of 100 μg.

The mercaptoimidazole ring was considered as being essential for colour formation. Other thio compounds, such as thiourea, did not give the reaction. Similarly, other mercapto-imidazoles were tested for the detection of iodides, *e.g.*, 2-mercaptoimidazole, which on reaction with bismuth salts did not give such a sensitive colour for iodides. Likewise, 4-aminomethyl-2-mercaptoimidazole reacted with bismuth to give a yellow compound, but this did not react with iodides.

The present investigation is concerned with the detection and gravimetric determination of iodide (even in the presence of chloride) by means of a new reagent based on the same principle; it appears to be more sensitive than the bismuth - mercaptoimidazole reagent previously suggested by McAllister as a specific colour reagent for iodides.

An antimony - 2-mercaptothiazoline reagent may also be used for the detection of iodide, but not chloride, bromide or fluoride. With iodide an orange precipitate is formed, whereas with iodate a yellow precipitate is obtained. Other halogen radicals do not react.

EXPERIMENTAL

THE BISMUTH - 2-MERCAPTOTHIAZOLINE REAGENT—

The "bismuth" reagent is prepared by dissolving 0·305 g of bismuth oxynitrate in 3·6 ml of cold concentrated sulphuric acid, with shaking to effect solution. Distilled water is added

gradually in small portions (about 5 ml) with shaking and the solution is diluted to 100 ml. To the clear acid solution is added 0·238 g of crystalline 2-mercaptothiazoline, and the mixture is stirred well with a glass rod until the solid has almost disappeared. The solution is filtered and the clear colourless filtrate is used for the detection and determination of iodides.

DETECTION OF IODIDES—

The "bismuth" reagent reacts with iodide ions to give an intensely red insoluble complex iodide. To 0·5 ml of a 0·1 per cent. potassium iodide solution were added 2 ml of "bismuth" reagent, when a red precipitate was formed. With smaller volumes of iodide solution, a red tint and a slight turbidity were observed. When 2 ml of "bismuth" reagent were added to crystalline potassium iodide, the sensitivity of the test was reduced to 1 mg. Free iodine reacts similarly, but the red precipitate dissolves in excess of the reagent.

A spot-test was carried out as follows—

> To 0·1 ml of 0·1 per cent. potassium iodide solution, *i.e.*, 100 μg of potassium iodide, on a white porcelain slab was added 0·1 ml of "bismuth" reagent; a red precipitate was deposited. Similarly 0·08, 0·06, 0·04 and 0·02-ml amounts of the potassium iodide solution gave red precipitates, but 0·01 ml did not give a red colour. Hence the sensitivity is 1 mg of potassium iodide in 1 ml of solution.

Although the complex iodide formed in the reaction is insoluble in excess of the reagent, it is soluble in excess of iodide solution. It is practically insoluble in water, acetic acid and dilute mineral acids, but decomposes in concentrated hydrochloric and sulphuric acids, with the liberation of iodine. In alkalis, such as 20 per cent. sodium hydroxide solution, the complex iodide dissolves to give a colourless solution. Oxidising agents such as hydrogen peroxide liberate iodine from it.

EFFECT OF VARIOUS IONS—

Fluoride, chloride, chlorate and perchlorate do not react with the "bismuth" reagent, but bromide gives a yellow precipitate soluble in excess of the bromide solution. Iodate and periodate give a purplish violet precipitate after shaking for a while. With bromate the solution is colourless at the beginning, but develops an orange-yellow colour on standing, owing to liberation of hydrogen bromide.

Metals that form insoluble sulphates give a white precipitate, while others, *e.g.*, sodium, potassium and ammonium, do not react.

No interference was observed in testing for iodide with the "bismuth" reagent in the presence of such acid radicals as carbonate, bicarbonate, chloride, bromide, sulphate, bisulphate, nitrate, phosphate, hypochlorite, chlorate, perchlorate, acetate, oxalate, tartrate, citrate, cyanide, thiocyanate, ferricyanide and permanganate. Although bromide alone gives a yellow precipitate, in the presence of iodide the red colour or precipitate predominates.

Interference was only observed in the presence of nitrite, sulphide, sulphite, thiosulphate, iodate, periodate, bromate and chromate.

DETERMINATION OF IODIDE IN PRESENCE OF CHLORIDE—

In the oxidation of iodide to iodate for iodimetric titration, aqueous bromine is used in a number of methods. There is some disagreement about the pH range suitable for the oxidation of iodide, and for the removal of excess of bromine by boiling.[4] A simple method[5] for the detection of iodide in the presence of chloride involves the oxidation of iodide to iodate in neutral solution with potassium permanganate, in presence of manganous sulphate; this test is suitable for determining small amounts of iodide.

The Mohr method cannot be applied to iodides, and Volhard's method cannot be applied in the presence of chloride. It is possible to determine iodide and chloride in admixtures by titration with silver nitrate with use of adsorption indicators such as di-iododimethylfluorescein, but if chloride is present in excess the result for iodide may be as much as 1 per cent. high.

Since iodide and not chloride ions are precipitated quantitatively as an insoluble red complex iodide by the "bismuth" reagent, it should be practicable to determine iodide gravimetrically, even in the presence of chloride. This has been found to be possible with an experimental error not exceeding 0·5 per cent.

DETECTION OF BROMIDE—

When 50, 40, 30, 20, 10 and 5-mg amounts of solid potassium bromide were treated with 2 ml of the "bismuth" reagent, a heavy yellow precipitate was formed; with 2 and 1 mg of solid potassium bromide and 2 ml of the "bismuth" reagent there was only a slight yellow colour, and no precipitate was observed.

To 1 ml and 0·5 ml of a 0·1 per cent. potassium bromide solution were added 2 ml of the "bismuth" reagent. A yellow colour was observed, but at lower concentrations the test was negative.

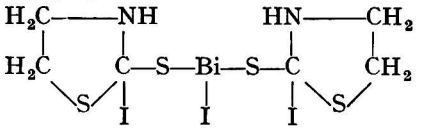
DETECTION OF IODATE AND BROMATE—

Tests were carried out in which 50, 20 and 10-mg amounts of solid potassium iodate were treated with 2 ml of "bismuth" reagent, when a violet precipitate was produced. With smaller quantities of solid iodate, *e.g.*, 2 or 1 mg, the solution turned orange-red, a red precipitate forming on standing. When 0·5 and 0·2-ml portions of 0·1 per cent. potassium iodate solution were treated with 2 ml of "bismuth" reagent, an orange colour appeared, but with 0·1 ml of iodate the solution remained colourless and only a slight turbidity was produced.

With 50, 20 and 10-mg amounts of solid potassium bromate and 2 ml of "bismuth" reagent, after 1-minute's shaking, an orange colour was observed, hydrobromic acid and bromine being liberated. On heating, the colour disappeared gradually. When 5 mg of solid bromate were treated with 2 ml of "bismuth" reagent, a yellowish white precipitate was formed, and there was no orange colour on shaking.

STRUCTURE OF THE COMPLEX IODIDE—

The following formula is proposed for the insoluble red complex iodide—



The complex was made by treating an iodide solution with bismuth - 2-mercaptothiazoline reagent; the precipitate was filtered off, washed with excess of reagent, then with distilled water and finally with ethanol. It was dried in a vacuum-desiccator over concentrated sulphuric acid.

The solid was shown to contain nitrogen, sulphur, iodine and bismuth. The iodine content was quantitatively determined by the following method. An accurately weighed quantity of the dry solid was mixed with an excess of potassium carbonate to minimise loss of iodine, and ignited in a muffle furnace at 400° C for 4 hours.[7] The iodide was extracted from the residue with 93 per cent. ethanol,[8] which was removed by evaporation on a water bath at 80° C. The residue was dissolved in distilled water, acidified, and hydrogen peroxide was added to liberate iodine, which was extracted with chloroform and titrated with standard sodium thiosulphate. The bismuth content of the complex was determined by the bismuth oxide method.

The average of five determinations was 46·03 per cent. of iodine (theory 46·01 per cent.); for three determinations of bismuth the average was 25·25 per cent. (theory 25·24 per cent.). The theoretical values are based on the formula $C_6H_{10}N_2I_3S_4Bi$.

Moreover, 2-mercaptothiazoline exists in the solid state as hydrogen-bonded thioamide complexes,[9] which probably break down in carbon tetrachloride solution. Also 2-mercaptothiazoline is known to give the mercury derivative $O_2N{\cdot}HgC_3H_4NS_2$, which reacts with ethyl iodide to form an amorphous yellowish brown complex[10] of the composition $C_8H_{13}N_2I_3S_4Hg$.

DETECTION OF IODIDE IN VARIOUS MATERIALS—

Some 20 litres of tap water were evaporated to a small volume, which was treated with the "bismuth" reagent; the result of the test was negative.

Two litres of Mediterranean sea water were evaporated to dryness, and the test for iodide in the residue was positive. Sea-water contains 17 to 18 μg of iodine per litre, according to Orr and Leitch, and 50 μg per litre, according to Goldschmidt.

To a 100-ml sample of urine were added 654·01 mg of potassium iodide, *i.e.*, the equivalent of 500 mg of iodine. A 1-ml portion of this urine (containing 5 mg of iodine) was treated

with 2 ml of reagent, when a red precipitate of the complex iodide was formed. On dilution of the urine, iodide could be determined at concentrations down to 0·25 mg per ml.

Iodide in pharmaceutical preparations official in the Egyptian Pharmacopia was easily detected. A typical preparation, Lobelia compound mixture, had the following composition—

Potassium iodide	0·30 g
Tinct. Lobelia Etheris	0·50 ml
Tinct. Camphor Co...	1·00 ml
Syrup Tolu	2·00 ml
Aqua ad	30·00 ml

A 1-ml portion of this preparation was treated with 2 ml of "bismuth" reagent, when a red precipitate formed. The test could be carried out on 0·1 ml of sample.

THE ANTIMONY - 2-MERCAPTOTHIAZOLINE REAGENT—

The "antimony" reagent is prepared by dissolving 0·5 g of antimony trichloride in 85 ml of distilled water and 15 ml of concentrated hydrochloric acid, and to the solution adding 0·5 g of 2-mercaptothiazoline; after being well shaken, the solution is filtered.

When tested, the "antimony" reagent did not react with fluoride, chloride and bromide, but with iodide an orange precipitate was formed, and iodate gave a yellow precipitate, which on being shaken became violet and finally dissolved to give a yellow solution. With antimony trichloride solution alone, only a yellow solution was formed.

GRAVIMETRIC DETERMINATION OF IODIDE—

Determinations were carried out on a standard solution prepared by dissolving 654·01 mg of potassium iodide, equivalent to 500 mg of iodine, in water and diluting to 100 ml in a calibrated flask. Aliquots were treated with excess of "bismuth" reagent and shaken well. The precipitate was collected in a tared Gooch crucible containing a pad of asbestos or Whatman No. 50 filter-paper, and washed with reagent solution, water and finally ethanol. The solid was dried in a vacuum-desiccator over concentrated sulphuric acid. Results were based on the assumption that the complex has the formula $C_6H_{10}N_2I_3S_4Bi$.

Results—Typical results for the gravimetric determination of iodide are shown in Table I.

TABLE I

GRAVIMETRIC DETERMINATION OF IODIDE

Volume of iodide solution taken, ml	Iodine content, mg	Weight of iodide complex, mg	Iodine found, mg	Error, %
1	5	10·8	4·97	−0·60
4	20	43·8	19·92	−0·40
10	50	108·0	49·70	−0·60
20	100	216·0	99·39	−0·61
40	200	433·0	199·24	−0·38
100	500	1080·0	496·95	−0·61

DETERMINATION OF IODIDE IN THE PRESENCE OF A FIXED AMOUNT OF CHLORIDE—

Determinations were carried out on a standard solution prepared by dissolving 654·01 mg of potassium iodide and 100 mg of sodium chloride in water and diluting to 100 ml in a calibrated flask. The iodide was determined gravimetrically, as described above.

Results—Typical results are shown in Table II, from which it is seen that the maximum error was 0·6 per cent.

TABLE II

DETERMINATION OF IODIDE IN THE PRESENCE OF A FIXED CONCENTRATION OF CHLORIDE

Volume of iodide - chloride solution taken, ml	Iodine content, mg	Weight of iodide complex, mg	Iodine found, mg	Error, %
1	5	10·8	4·97	−0·60
10	50	108·0	49·70	−0·60
20	100	217·0	99·85	−0·15
40	200	434·0	199·70	−0·15

DETERMINATION OF IODIDE IN THE PRESENCE OF DIFFERING AMOUNTS OF CHLORIDE—

A standard iodide solution was prepared by dissolving 6·5401 g of solid potassium iodide, corresponding to 5 g of iodine, in water and diluting with water to 100 ml in a calibrated flask. Similarly, a standard chloride solution was prepared from 10 g of solid sodium chloride in 100 ml of water. Measured volumes of the solutions were mixed, and the iodide was determined gravimetrically as described above.

Results—The results for various mixtures are shown in Table III.

TABLE III

DETERMINATION OF IODIDE IN THE PRESENCE OF VARIOUS AMOUNTS OF CHLORIDE

Volume of iodide solution taken, ml	Iodine content, mg	Volume of chloride solution taken, ml	Sodium chloride content, g	Weight of complex iodide, mg	Iodine found, mg	Error, %
4	200	15	1·5	433	199·24	−0·38
		20	2	433	199·24	−0·38
		5	0·5	433·2	199·33	−0·34
6	300	1	0·1	651	299·55	−0·15
		3	0·3	650	299·09	−0·30
		5	0·5	652	300·01	+0·00
8	400	7	0·7	868	398·19	−0·45
		9	0·9	870	400·33	+0·08
		15	1·5	869	399·87	−0·03
10	500	1	0·1	1082	497·88	−0·42
		5	0·5	1081	497·42	−0·52
		10	1	1083	498·34	−0·33
12	600	10	1	1303	599·57	−0·07
		30	3	1300	598·19	−0·30
		50	5	1298	597·27	−0·46
14	700	30	3	1520	699·42	−0·08
		50	5	1520	699·42	−0·08
		70	7	1518	698·50	−0·02
16	800	20	2	1736	798·81	−0·15
		40	4	1738	799·73	−0·03
		80	8	1740	800·65	+0·08
18	900	25	2·5	1953	898·66	−0·15
		50	5	1952	898·20	−0·20
		100	10	1950	897·28	−0·30
20	1000	5	0·5	2172	999·43	−0·06
		10	1	2164	995·75	−0·43
		100	10	2167	997·25	−0·28

REFERENCES

1. Vogel, A. I., "A Text-Book of Qualitative Chemical Analysis," Third Edition, Longmans, Green & Co. Ltd., London, New York and Toronto, 1945, p. 152.
2. Naiman, B., *J. Chem. Educ.*, 1937, **14**, 484.
3. McAllister, R. A., *Nature*, 1952, **169**, 708.
4. Jensen, P. W., and Crittenden, A. L., *Anal. Chem.*, 1954, **26**, 1373.
5. D'Ans, J., and Kanakowsky, T., *Z. angew. Chem.*, 1950, **62**, 168.
6. Vogel, A. I., "A Text-Book of Quantitative Inorganic Analysis," Longmans, Green & Co. Ltd., London, New York and Toronto, 1942, pp. 316 and 317.
7. El-Sadr, M. M., and Barakat, M. Z., *Bull. Clin. & Sci. Soc., Abbassia, Faculty of Medicine*, 1954, **5**, 1.
8. Reith, J. F., and Van Dijk, C. P., *Biochem. J.*, 1937, **31**, 2128.
9. Flett, M. St. C., *J. Chem. Soc.*, 1953, 348.
10. Rây, P. C., *J. Chem. Soc.*, 1916, **109**, 607; *"Beilstein's Handbuch Der Organischen Chemie,"* 1938, xxvii, 260.

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Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Methods for the Determination of Hardness, Calcium and Magnesium

Hardness

THE commonly used methods for determining total hardness that depend upon titration with disodium ethylenediaminetetra-acetate (EDTA) cannot be used for all effluents. If, however, the only cations present are calcium, magnesium and the alkali metals, the recommended method for determining calcium and magnesium may be used for the determination of total hardness.

Two methods are described. Method A gives accurate results for total hardness in terms of the definition below, but it involves a lengthy procedure unless a flame photometer is available. Method B may be used when extreme accuracy is not required.

DEFINITIONS—

Total hardness is defined as the sum of all the metallic cations, except those of the alkali metals, calculated in terms of calcium carbonate.

Carbonate (temporary) hardness is defined as that part of the hardness attributable to the bicarbonates of the same cations. Usually, the carbonate hardness is the same as the "methyl orange alkalinity," but this is not so when the effluent also contains the bicarbonates, carbonates or hydroxides of the alkali or alkaline-earth metals.

METHOD A

PRINCIPLE OF METHOD—

The method consists in determining the total cationic strength by ion exchange, followed by determination of the alkali-metal cations. The difference between the results of the two determinations calculated in terms of calcium carbonate is the total hardness.

APPARATUS—

Chromatographic column, consisting of a glass tube 25 cm long with an approximate outside diameter of 1·75 cm, drawn out at its lower end. The upper end should have an open bulb of approximately 100 ml capacity fused to it.

Pack a small quantity of cotton-wool in the constricted end of the tube. Pour in 20 g of Zeo-Karb 225 ion-exchange resin and tap the tube gently to consolidate the resin. Place a small piece of cotton-wool at the top of the resin column.

Activate the resin by passing 1 litre of approximately 2 *N* hydrochloric acid through it, followed by distilled water, until the eluate is free from chloride ion. When the level of the distilled water is about 1 inch above the resin bed, place a stopper in the bulb and allow the column to remain wet until it is required.

REAGENTS—

Sodium hydroxide solution, 0·2 N.
Hydrochloric acid, sp.gr. 1·18.
Barium hydroxide.
Ammonium hydroxide, sp.gr. 0·880.
Ammonium carbonate solution, 5 per cent. w/v.
Indicator solution A—Dissolve 0·125 g of methyl red in 50 ml of 95 per cent. ethanol (or industrial methylated spirit).
Indicator solution B—Dissolve 0·083 g of methylene blue in 50 ml of 95 per cent. ethanol (or industrial methylated spirit).

NOTE—As these indicator solutions, if mixed, deteriorate on storage, they are added separately during the procedure.

Phenolphthalein indicator solution—A 0·1 per cent. solution in 95 per cent. ethanol (or industrial methylated spirit).

PROCEDURE FOR DETERMINING TOTAL CATIONS—

Evaporate 200 ml, or a suitable larger volume if the content of soluble solids is low, in a silica, porcelain or platinum dish and gently ignite the residue at a temperature not exceeding 500° C. Add 1 ml of concentrated hydrochloric acid and 10 ml of distilled water and evaporate again to dryness.

Dissolve the residue in the dish in distilled water and dilute to 100 ml. Place a 250-ml conical flask under the chromatographic tube, remove the stopper and allow 50 ml of the solution to pass through the column. When the water level has sunk nearly to the level of the bed, transfer the remainder of the solution to the tube. When this has nearly passed into the column, wash the bulb with two separate 50-ml portions of distilled water in a similar manner and stopper the bulb as described above.

Add 1 drop of each of indicator solutions A and B to the eluate in the flask and titrate with 0·02 *N* sodium hydroxide solution to a grey colour.

Calculate the total cationic content as calcium carbonate.

$$1 \text{ ml of } 0{\cdot}02\ N \text{ NaOH} \equiv 1{\cdot}0 \text{ mg of } CaCO_3.$$

PROCEDURE FOR DETERMINING ALKALI METALS—

Determine the content of the alkali metals either by flame photometry, or as follows.

Measure 250 ml of the sample into a small beaker. Raise the temperature to the boiling-point and add 1 ml of phenolphthalein indicator solution; then add small quantities of solid barium hydroxide until the colour change indicates a slight excess, care being taken to avoid violent boiling. Remove the precipitate by filtering through a Whatman No. 40 filter-paper, washing it twice, and collect the filtrate and washings in a 250-ml beaker. Add 1 ml of ammonium hydroxide and then ammonium carbonate solution in slight excess. Cover the beaker and boil the mixture. Filter off the precipitate, wash it with distilled water and collect the filtrate and washings in a weighed silica, platinum or porcelain dish. Evaporate the solution to dryness, make the residue slightly acid with sulphuric acid and evaporate again to dryness. Ignite the residue until no more fumes are evolved. The residue consists of alkali-metal sulphates. Cool and weigh the dish. Assuming that 1 mg of residue ≡ 0·7 mg of calcium carbonate, calculate the calcium carbonate equivalent to the alkali-metal sulphates.

CALCULATION OF TOTAL HARDNESS—

Deduct the value obtained for the alkali metals from that obtained for total cations and calculate the total hardness, in terms of calcium carbonate, as milligrams per litre of sample.

METHOD B

PRINCIPLE OF METHOD—

The method consists in determining the amount of standard mixed alkali required to precipitate the "non-alkaline" cations.

REAGENTS—

Hydrochloric acid, 0·1 N.

Hydrochloric acid, 0·5 N.

Mixed alkali solution, 0·5 N—Dissolve 14 g of anhydrous sodium carbonate in about 300 ml of distilled water; also dissolve 11 g of sodium hydroxide in about 300 ml of distilled water. Mix the two solutions and dilute to 1 litre. Determine the normality of the solution by titrating 25 ml with 0·5 *N* hydrochloric acid, using methyl orange indicator solution. Adjust the solution to 0·5 *N* if necessary.

Sodium hydroxide solution, 0·1 N.

Sodium hydroxide solution, 0·5 N.

Methyl orange indicator solution—A 0·04 per cent. aqueous solution.

PROCEDURE—

Measure a suitable volume (usually 100 to 250 ml) of the filtered effluent sample into a conical flask, add 0·2 ml (about 4 drops) of methyl orange indicator solution and neutralise with 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide solution, as appropriate.

Measure into a 100-ml porcelain basin exactly 10 ml of the mixed alkali solution. Add some of the neutralised sample, and evaporate the solution at a temperature just below the boiling-point, replacing the water lost by evaporation by more of the neutralised sample until all has been used. Wash the flask with distilled water and add the washings to the liquid in the basin; continue the evaporation until the volume of the liquid is reduced to about 25 ml.

Filter the solution and collect the filtrate in the original flask. Thoroughly wash the basin with three successive amounts, each of about 5 ml, of hot distilled water, added from a fine jet, and transfer the washings to the filter, allowing each portion to pass through the filter before adding the next. Wash the filter three times with a fine jet of hot distilled water, using about 5 ml each time, and again allow each washing to pass completely through the filter. Cool the filtrate and titrate it with 0·5 *N* hydrochloric acid. Calculate the total hardness (*a*), as milligrams per litre of sample.

$$\text{Total hardness} = \frac{(10 - \text{Volume of } 0{\cdot}5\,N \text{ acid, ml}) \times 25 \times 1000}{\text{Volume of sample taken, ml}}.$$

CALCULATION OF TEMPORARY HARDNESS—

If the original sample is acid, there is no temporary hardness.

If the original sample is alkaline, calculate the alkalinity (*b*) from the formula—

$$\frac{\text{Volume of } 0{\cdot}1\,N \text{ hydrochloric acid required, ml} \times 5 \times 1000}{\text{Volume of sample taken, ml}}.$$

If the total hardness (*a*) exceeds (*b*), the value for (*b*) is the temporary hardness.

If (*b*) exceeds (*a*), there is no permanent hardness and the temporary hardness is given by (*a*).

Calcium and Magnesium

PRINCIPLE OF METHOD—

The method consists in determining calcium separately, and the sum of the calcium and magnesium; magnesium is then calculated by difference. Lead, which would otherwise interfere, is removed as sulphate.

APPLICABILITY—

The method is generally applicable.

REAGENTS—

EDTA solution—Dissolve 3·72 g of crystalline disodium ethylenediamine-tetra-acetate dihydrate (EDTA) in distilled water and dilute to 1 litre.

Hydrochloric acid, sp.gr. 1·18.

Sodium hydroxide solution, 4 M—Dissolve 16 g of sodium hydroxide in 100 ml of distilled water.

Potassium cyanide solution, 10 *per cent. w/v.*

Ammonium hydroxide, dilute—A 10 per cent. aqueous solution of ammonium hydroxide, sp.gr. 0·880.

Buffer solution of pH 10·0—Dissolve 67·5 g of ammonium chloride in 570 ml of ammonium hydroxide, sp.gr. 0·880, and dilute to 950 ml. Dissolve 0·616 g of magnesium sulphate, $MgSO_4.7H_2O$, and 0·93 g of disodium ethylenediaminetetra-acetate dihydrate in 50 ml of distilled water. Mix the two solutions.

The use of 2 ml of buffer solution in 100 ml of the effluent sample is equivalent to 5 mg per litre of magnesium in terms of calcium carbonate together with its equivalent of EDTA.

Sodium sulphate solution—A 10 per cent. w/v solution of the anhydrous salt.

Solochrome black WDFA indicator solution—Dissolve 0·5 g of Solochrome black WDFA (Eriochrome black T) and 4·5 g of hydroxylamine hydrochloride in 100 ml of 95 per cent. ethanol (or industrial methylated spirit). This solution does not deteriorate within 1 month if kept in the dark.

Murexide indicator—Mix thoroughly 0·20 g of murexide and 100 g of sodium chloride. The indicator is used in solid form.

Methyl orange indicator solution—A 0·04 per cent. w/v aqueous solution.

Bromophenol blue indicator solution—A 0·04 per cent. w/v solution in 95 per cent. ethanol (or industrial methylated spirit).

PROCEDURE FOR CALCIUM—

Evaporate 200 ml or a suitable quantity of the effluent sample in a silica, porcelain or platinum dish. Gently ignite the residue at a temperature not exceeding 500° C. Add 1 ml of concentrated hydrochloric acid and 10 ml of distilled water. Add 5 ml of sodium sulphate solution, boil gently for 10 minutes, cool and filter into a 100-ml calibrated flask. Wash the dish and filter-paper and dilute the filtrate to the mark. Measure 50 ml of the solution, neutralise with the sodium hydroxide solution, using methyl orange as indicator, and add 1 ml in excess; then add 5 ml of potassium cyanide solution. Transfer the liquid to a large white dish. Add approximately 0·2 g of murexide indicator, and titrate with EDTA solution until the colour becomes violet. The end-point is indicated when a further addition of 0·1 ml of EDTA solution produces no further colour change. Calculate the calcium as milligrams per litre.

1 ml of EDTA solution ≡ 0·4 mg of calcium.

PROCEDURE FOR MAGNESIUM—

Transfer the remaining 50 ml of the solution to a white dish and neutralise to litmus paper with ammonium hydroxide. Add 5 ml of potassium cyanide solution, 2 ml of the buffer solution and 6 drops of the Solochrome black WDFA indicator solution. Titrate the solution with EDTA solution until the colour becomes blue.

Calculate the sum of the calcium and magnesium so obtained in terms of calcium. Deduct the calcium value obtained from the previous titration and calculate the difference as magnesium. Calculate the magnesium as milligrams per litre.

Calcium × 0·6 = magnesium.

Notes

THE DETERMINATION OF LITHIUM, SODIUM AND POTASSIUM IN MIXTURES WITH THE "EEL" FLAME PHOTOMETER

WHEN the need arose for a fairly rapid method for the determination of sodium, potassium and lithium in aqueous solutions of their chlorides, it was decided to use a flame photometer. Collins and Polkinhorne[1] have shown that the interference due to the presence of acids in the flame-photometric determination of these three alkali metals is negligible below concentrations of 0·001 *N* acid. Domingo and Klyne[2] state that, for sodium at the level of 30 p.p.m., the nitrate, sulphate, dihydrogen phosphate, monohydrogen phosphate, carbonate and acetate give the same readings as for the chloride.

The mutual interference of sodium and lithium was found to be considerable and this was overcome by a method of "bracketing" with synthetic standard solutions of similar composition and concentration to the sample solution. Another source of interference was due to sodium present in glass. For example, distilled water, after standing for 2 days in a glass bottle, gave a reading of 2·5 p.p.m. of sodium. For this reason polythene reagent bottles are used to store solutions.

EXPERIMENTAL

The EEL flame photometer (Evans Electroselenium Ltd.) as described by Collins and Polkinhorne[1] was used in this work.

Stock standard solutions containing 250 p.p.m. of the particular metal chlorides are stored in polythene bottles and dilutions are conveniently made with the aid of an Agla micrometer syringe and a 10-ml burette. The syringe, which will deliver up to 0·5 ml, is calibrated to 0·002 ml and is detachable from the micrometer head; one syringe is used for each element to avoid contamination. The dilutions are made in a single step, in the small beakers supplied with the instrument, and thorough mixing is effected by repeatedly sucking into and discharging from clean dry polythene "spitzas" made by drawing down polythene tubing.

As sodium, potassium and lithium mutually interfere and as each component is variable in the samples for analysis, it is not possible to produce standard curves, so a bracketing technique has been used.

By means of the wide-range graduated sensitivity control of the flame photometer the approximate concentration of each element in the sample can be determined. With use of standard solutions, it was found on our instrument that with 10 p.p.m. of sodium, full-scale deflection was obtained with the sensitivity control reading "10." By obtaining the sensitivity reading that gives full-scale deflection, the approximate concentration of sodium in any solution can be found by direct proportion. Similarly, 10 p.p.m. of lithium and potassium give full-scale deflection with the sensitivity set at 1·8 and 1·3, respectively. Dilutions are always made so that the concentration of each component is less than 10 p.p.m. in order to avoid flame saturation.

PROCEDURE—

The unknown sample is placed in the flame photometer and the sensitivity control adjusted to give full-scale deflection. This procedure is carried out for each element to be determined and from the readings of the sensitivity control a rough idea of the concentration of each component is obtained.

The dilution or dilutions are then made from the sample in order to bring the concentration of each element to below 10 p.p.m. and, after the instrument has been set up to give a full-scale deflection for 10 p.p.m. of the element to be determined, a scale reading is obtained from the diluted sample. This is repeated for each element.

On the basis of these readings, two solutions are made from the standards differing by about 0·2 p.p.m. of each constituent and bracketing the diluted sample. Because of the effect of mutual interferences, it may be necessary to use a trial standard solution before the composition of the bracketing solutions can be predicted accurately, but with some experience this step can usually be eliminated.

The bracketing solutions are then used as standards in the instrument with the diluted sample interposed, and the composition of the sample is obtained by direct proportion.

RESULTS

A number of test solutions of sodium, potassium and lithium chlorides was made up and analysed by the method described above. In Table I a typical set of experimental results is given. The results of analyses of samples of widely varying composition are collected in Tables II, III, IV and V, together with the results of repeated analyses of one solution.

The method has been applied to a number of routine samples with various ratios from 2 per cent. of sodium and 98 per cent. of lithium to 50 per cent. of sodium and 50 per cent. of lithium with good results. Some samples have been checked by an ion-exchange method, which, for a typical sample, gave a total molarity of 0·50. The results by flame photometer were 0·22 *M* for sodium and 0·29 *M* for lithium, giving a total of 0·51 *M*, which agreed reasonably well. It has been found that for lithium solutions alone one reading referred to a 10 p.p.m. standard is sufficient, as the scale is linear for lithium. Sodium, however, is best determined by the bracketing technique, because the scale is not linear. Further, errors caused by pick up of sodium are minimised.

TABLE I

ANALYSIS OF A SOLUTION OF LITHIUM AND SODIUM CHLORIDES

The sample contained 5·43 p.p.m. of sodium and 2·03 p.p.m. of lithium

	Composition of solution		Photometer readings for		Lithium found, p.p.m.	Sodium found, p.p.m.
	Lithium, p.p.m.	Sodium, p.p.m.	Lithium	Sodium		
Test sample ..	—	—	5·50	2·55	2·05	5·45
Bracket 1	5·40	2·00	5·45	2·50		
Bracket 2	5·60	2·20	5·65	2·70		

TABLE II

ANALYSES OF FIVE SOLUTIONS CONTAINING SODIUM AND LITHIUM

Solution No.	Metal present		Metal found			
	Sodium, mg	Lithium, mg	Sodium, mg	Difference, mg	Lithium, mg	Difference, mg
1	255	204	247	− 8	204	—
2	282·5	96·8	274	− 8·5	100	+ 3·2
3	246	164	246	—	172	+ 8
4*	178	237	177	− 1	242	+ 5
5	81·3	217	82	+ 0·7	218	+ 1

* Results of eleven replicate analyses of solution 4—

	Sodium	Lithium
Mean	176·8 mg	239 mg
Standard deviation	1·34	2·47
Coefficient of variation ..	0·76%	1·03%

TABLE III

ANALYSES OF FIVE SOLUTIONS CONTAINING SODIUM AND POTASSIUM

Solution No.	Metal present		Metal found			
	Sodium, mg	Potassium, mg	Sodium, mg	Difference, mg	Potassium, mg	Difference, mg
1	71·2	289·4	76	+ 4·8	284	− 5·4
2	91·9	229·7	94	+ 2·1	240	+ 10·3
3*	157·3	160·4	156	− 1·3	157	− 3·4
4	351·4	57·5	362	+ 0·6	58	+ 0·5
5	315	87	324	+ 9	88	+ 1

* Results of eleven replicate analyses of solution 3—

	Sodium	Potassium
Mean	153·6 mg	156·1 mg
Standard deviation	1·67	1·07
Coefficient of variation ..	1·08%	0·69%

TABLE IV

ANALYSES OF SIX SOLUTIONS CONTAINING POTASSIUM AND LITHIUM

Solution No.	Metal present		Metal found			
	Potassium mg	Lithium mg	Potassium, mg	Difference, mg	Lithium, mg	Difference, mg
1	295	57·6	300	+ 5	62	+ 4·4
2	320	135	324	+ 4	136	+ 1
3	58·78	156	58	− 0·78	160	+ 4
4	113	262	128	+ 15	270	+ 8
5*	223·6	188·2	222·2	− 1·4	187	− 1·2
6	319·6	50·5	316·8	− 2·8	50	− 0·5

* Results of eleven replicates of solution 5—

	Potassium	Lithium
Mean	220·6 mg	186 mg
Standard deviation	1·83	2·5
Coefficient of variation ..	0·83%	1·34%

DISCUSSION OF RESULTS

In the experimental work described above, the ratios of the metals for each series of determinations were such that a single appropriate dilution of the "unknown" solution would bring all constituents into the 0 to 10 p.p.m. range. When the atomic ratios were high, the accuracy of determination of the lesser constituent or constituents was, of course, less. The errors can be reduced by making two or three separate dilutions of the unknown, so that scale readings on the instrument are as high as possible.

TABLE V

ANALYSES OF SEVEN SOLUTIONS CONTAINING SODIUM, LITHIUM AND POTASSIUM

Metal present			Metal found					
Sodium, mg	Lithium, mg	Potassium, mg	Sodium, mg	Difference, mg	Lithium, mg	Difference, mg	Potassium, mg	Difference, mg
26·05	29·8	378	24	− 2·05	30	+ 0·2	380	+ 2
118·8	145·5	184·6	116	− 2·8	142	− 3·5	182	− 2·6
115·8	195·8	61·15	154	− 1·8	194	− 1·8	60	− 1·15
395	24·53	18·78	392	− 3	24	− 0·53	18	− 0·78
23·8	349	22·5	24	+ 0·2	348	− 1	22	− 0·5
229	155·1	96·93	228	− 1	158	+ 2·9	96	− 0·93
118·2	306·3	96·93	114	− 4·2	304	− 2·3	96	− 0·93

When conditions are chosen such that the instrument readings are reasonably high, the errors are small, and precision of the order of a few parts in a thousand can be obtained with replication, as shown by the results given above.

Another factor influencing the accuracy of the method when absolute values rather than ratios are required is the accuracy of dilution of the unknown sample. When accurate readings are obtained for all constituents in a single dilution, the ratio can then be determined quickly with high accuracy by several replications. The total alkali-metal concentration can be determined by hydrogen-ion exchange followed by titration, and hence the absolute concentrations of the individual metals can be determined.

REFERENCES

1. Collins, G. C., and Polkinhorne, H., *Analyst*, 1952, **77**, 430.
2. Domingo, W. R., and Klyne, W., *Biochem. J.*, 1949, **45**, 400.

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USE OF ALUMINA COLUMNS IN THE DETERMINATION OF MERCURY AS DITHIZONATE

FOR the colorimetric determination of small amounts of mercury, dithizone, HDz, is clearly the reagent of choice. The methods in use for trace-metal determination with dithizone fall into three distinct groups: the mixed colour method, a mono-colour method after removal of excess of dithizone by an alkaline stripping solution and the Irving reversion technique. A mono-colour method, the dithizone being removed by an alkaline stripping solution, has been recommended by a joint committee of the Society for Analytical Chemistry and the Association of British Chemical Manufacturers.[1]

Studies of the behaviour of metal dithizonates on alumina columns have been made by Bach,[2] Tanaka, Ashizawa and Shibata[3] and Erämetsä.[4] The work of Bach and of Erämetsä suggested that mercury dithizonate could be easily separated from excess of dithizone, since dithizone, and many other metal dithizonates, are much more strongly adsorbed on alumina than mercury dithizonate. It would appear from a study of the literature that little use has been made of this separation for quantitative analysis. It has been found, however, that this affords a convenient basis for an improved mono-colour method for the determination of mercury.

EXPERIMENTAL

STABILITY OF MERCURY DITHIZONATE—

Bach has pointed out that some decomposition of metal dithizonates may occur on adsorption and elution from alumina columns. We observe that this is most marked for mercury dithizonate when the dithizonate is very strongly adsorbed. This is dependent on the solvent used, as well as the alumina. Hence mercury dithizonate is weakly adsorbed from chloroform solutions, but much more strongly adsorbed from solutions in carbon tetrachloride and toluene. It is not eluted by pure carbon tetrachloride or toluene, but can be eluted by the addition of 1 per cent. of ethanol to the solvent.

When mercury dithizonate is adsorbed from carbon tetrachloride or toluene solutions, subsequent elution with the solvent containing 1 per cent. of ethanol gives an eluate containing mercury, but a coloured zone extends over the whole column, and cannot be removed by further elution. This does not occur with chloroform, and for this reason chloroform has been used as a solvent for all subsequent work.

RECOVERY OF MERCURY DITHIZONATE FROM ALUMINA COLUMNS—

In order to check that elution is quantitative with chloroform, a solution of mercury dithizonate free from excess of dithizone was prepared by shaking a purified solution of dithizone with excess of an acid solution of mercuric sulphate. An aliquot was diluted to a volume convenient for spectrophotometric measurement and a similar aliquot was placed on an alumina column. After the column had been washed with chloroform, the eluate was diluted to the same volume. The extinctions of both solutions were measured in a 1-cm cell at 490 mμ. Table I shows the results of several such experiments, and demonstrates that the elution is quantitative.

TABLE I

RECOVERY OF MERCURY DITHIZONATE FROM ALUMINA COLUMNS

Volume of aliquot, ml	Final volume, ml	Extinction at 490 mμ after direct dilution	Extinction after passage through alumina column and dilution to volume
1	10	0·790	0·770
3	45	0·532	0·527
5	62·5	0·657	0·657
7	62·5	0·910	0·890

METHOD

REAGENTS AND APPARATUS—

Sulphuric acid, 5 N—AnalaR.

Ammonia solution—Dilute 10 ml of ammonium hydroxide, sp.gr. 0·880, to 100 ml.

Chloroform—AnalaR.

Alumina for chromatography—The alumina used in these experiments was "Alumina for Chromatography" (obtainable from The British Drug Houses Ltd.). The material from a freshly opened container was frequently found to be too absorbent and gave low recoveries even with chloroform. The activity could be reduced to a satisfactory level by exposing the alumina to the laboratory atmosphere overnight. An alumina of satisfactory activity should allow mercury dithizonate to pass through almost as quickly as the solvent (*i.e.*, a small retention volume), but should still strongly adsorb unreacted dithizone. The activity is further checked by the method described above for recovery of mercury dithizonate from alumina columns.

Sodium sulphate, anhydrous—AnalaR.

Hydroxylamine hydrochloride—AnalaR.

Dithizone solution, 0·05 per cent. in chloroform—This is purified by the usual method to remove its yellow oxidation product and any metal dithizonates present as impurities. The oxidation product is not strongly held by the alumina and would be eluted with the mercury dithizonate if it were present. Hence hydroxylamine hydrochloride should be present in the extraction to prevent oxidation, as in other methods involving use of dithizone. Extract 25 ml of the chloroform solution with 25 ml of ammonia solution. Separate and reject the chloroform layer. Acidify the aqueous layer with 5 *N* sulphuric acid and extract with 25 ml of chloroform.

Alumina column—A glass tube 20 to 30 cm long and 8 mm in diameter, with the lower end drawn out to a taper 1 to 2 mm in diameter. A small pledget of cotton is placed in the taper, alumina is packed in to a depth of 5 to 7 cm and is surmounted by a 1 to 2-cm layer of anhydrous sodium sulphate. After being washed with chloroform, the column is ready for use.

PROCEDURE—

The following procedure is used after the usual preliminary extractions, see, *e.g.*, Laug and Nelson.[5] If necessary, organic matter should be removed by oxidation, precautions being taken to avoid loss of mercury by volatilisation.[1]

Transfer to a 100-ml separating funnel an aliquot containing from 10 to 75 μg of mercury; adjust the acidity to 1 to 2 *N* with sulphuric acid. Add 0·5 g of hydroxylamine hydrochloride and then 2 ml of the dithizone solution. Shake well and allow to separate. The chloroform layer should remain dark green. Run the chloroform layer on to the top of the alumina column.

Shake the aqueous layer in the separator with 2 ml of chloroform and add the chloroform to the column. Repeat until the chloroform extract is colourless, and continue to elute with chloroform.

A brown band due to excess of dithizone appears at the top of the alumina and a rapidly moving orange band of mercury dithizonate below it. As the orange band approaches the bottom of the column, collect the eluate in a 25-ml calibrated flask protected from light. When the eluate becomes colourless, remove the flask and dilute to the mark with chloroform. Measure the extinction at 490 mμ in a 1-cm cell, against a reagent blank. Determine the quantity of mercury by reference to a calibration curve constructed from readings obtained for known quantities of mercury.

Influence of other metals

The order of adsorbability of dithizonates given by Bach[2] and Erämetsä[4] has been confirmed; with the exception of copper and silver, most metal dithizonates are much more strongly adsorbed by alumina than is mercury dithizonate.

The acidity of the solution to be extracted by dithizone minimises the interference by copper, and, in practice, a separation on the column is achieved without difficulty. Silver, however, interferes, and must be removed, *e.g.*, by the methods of Laug and Nelson[5] or of the A.B.C.M. - S.A.C.[1] Zinc does not interfere, but a bright red band due to this metal is often observed just below the dithizone band.

Results

Recoveries of mercury in presence of 100 μg of added copper by the method described above are shown by the following results—

Mercury added, μg	10	20	40	60
Mercury found, μg	9	21	41	62
Recovery, %	90	105	103	103

Some typical calibration results were as follows—

Mercury taken, μg	5	10	20	40	60
E_{1cm} at 490 mμ with a final volume of 25 ml ..	0·082	0·117	0·247	0·487	0·742

"Keto - enol" tautomerism

According to Fischer,[6] many metals, mercury amongst them, form two dithizonates, which he termed keto and enol forms. The keto form contains twice as much dithizone per atom of metal as the enol form. Recently considerable doubt has been thrown on Fischer's formulae for these compounds by Irving and Bell.[7]

In the work described only the orange so called "keto" form was encountered, but it was felt that some information on the influence of alumina on this tautomerism was needed. The reddish purple mercury enol-dithizonate was prepared by treating dithizone with a large excess of mercury in alkaline solution. It is unstable and slowly reverts to the orange keto form. When an aliquot was passed down an alumina column, the eluate consisted of the orange keto form. The addition of more dithizone solution to the column and further elution with chloroform produced a further and equivalent amount of the keto form. A similar aliquot of the enol form was completely converted to the keto form, with acid and excess of dithizone. The mercury dithizonate was determined in the usual way, and the results were as follows, the final volume of each solution being 25 ml—

Solution tested		1st eluate	2nd eluate	Keto form
Extinction at 490 mμ ..	(1)	0·545	0·422	0·920
	(2)	0·130	0·120	0·265

These would indicate that the enol form decomposes on the column, half of the mercury originally added as the enol form being retained on the column. This residual mercury is eluted by more dithizone.

The following scheme indicates the sequence of reactions—

$$2HgDz \xrightarrow[\text{column}]{\text{Alumina}} HgDz_2 + Hg/Al_2O_3$$

$2HgDz$ —(Excess of dithizone and acid)→ $2HgDz_2$

Hg/Al_2O_3 —(Excess of dithizone)→ $HgDz_2$

We thank the Directors of The British Drug Houses Ltd., Graham Street, London, N.1 for permission to publish these results.

REFERENCES

1. Joint A.B.C.M. - S.A.C. Committee, "Recommended Methods for the Analysis of Trade Effluents," *Analyst*, 1956, **81**, 176.
2. Bach, R. O., *Ind. y Quím.*, 1950, **12**, 283.
3. Tanaka, M., Ashizawa, T., and Shibata, M., *Chem. Researches (Japan), Inorg. & Anal. Chem.*, 1949, **5**, 35.
4. Erämetsä, O., *Acta. Chem. Fenn.*, 1943, **16**, 13.
5. Laug, E. P., and Nelson, K. W., *J. Ass. Off. Agric. Chem.*, 1942, **25**, 399.
6. Fischer, H., *Angew. Chem.*, 1937, **50**, 919.
7. Irving, H., and Bell, C. F., *Nature*, 1952, **169**, 756.

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November 29th, 1956

THE DETERMINATION OF TRACES OF MERCURY IN APPLES

THE method of Klein[1] for the determination of mercury residues in foods has been successful in our hands when applied to tomatoes. The use of this method on apples, however, gave very low recoveries of mercury. Losses occurred during the combustion step and it was thought that these might be due to volatilisation of the mercury in the large volume of carbon dioxide evolved at this stage. This was borne out by the fact that by passing a stream of carbon dioxide from a cylinder through a solution of 5 μg of mercury, added as mercuric chloride, in 50 per cent. nitric acid boiling under reflux, the mercury content of the solution was reduced to less than 1 μg after ½ hour.

It therefore seemed likely that a method avoiding complete combustion might prove more satisfactory, and the combustion method of Laug and Nelson,[2] as modified by Kunze[3] with the addition of selenium, was tried; the determination of mercury in the resulting solution was still carried out by the method of Klein.[1] These modifications resulted in recoveries of between 90 and 95 per cent. of mercury added as phenylmercuric chloride at the microgram level. Determinations were then carried out on samples of apples that had been sprayed with mercurial formulations and mercury was found to be present in the range 0·01 to 0·10 p.p.m. Determinations on unsprayed fruits gave results equivalent to less than 0·01 p.p.m. of mercury, which is regarded as the limit of reliable application of the method. The method as finally used is described in detail below, the apparatus used being originally based on that of Klein,[1] but modified in the light of experience with the method.

METHOD

APPARATUS—

A 500-ml two-necked round-bottomed flask carrying a 50-ml dropping funnel by a B19 joint in one neck and a 10-inch air condenser of 1-inch diameter by a B29 joint in the other. To the top of the air condenser is fitted a double-surface water condenser, also by a B29 joint.

REAGENTS—

Sulphuric acid, concentrated.
Nitric acid, concentrated.
Hydrogen peroxide, 30 per cent. w/v.
Ammonia solution, sp.gr. 0·880.
Acetic acid, 30 per cent. v/v.
Hydrochloric acid, 0·1 N.

Sodium hypochlorite solution—A solution having 5·0 per cent. w/v of available chlorine. It should be free from mercury.

Hydroxylamine hydrochloride solution—A 20 per cent. w/v aqueous solution. Extract the solution with dilute dithizone solution until the chloroform layer remains green, remove excess of dithizone with chloroform and filter.

Sodium thiosulphate solution—A 1·5 per cent. w/v aqueous solution. Prepare daily as required.

Chloroform—Purify by the method of the Lead Panel Report[4] and then distil in all-glass apparatus; add absolute ethanol at the rate of 10 ml per litre.

Dithizone, stock solution—Dissolve 100 mg of dithizone per litre in purified chloroform.

Dithizone, working solution—This solution contains 4 mg per litre; prepare it from the stock solution as required.

Mercuric chloride solution—This should contain 1 mg of mercury per ml. Dilute it as required for standard amounts of mercury.

Selenium powder.

For amounts of mercury greater than 5 μg use stronger working solutions of dithizone and 2-cm or 1-cm cells, according to the Table given by Klein.[1] Keep solutions of sodium hypochlorite and dithizone in a refrigerator when not in use.

PROCEDURE—

Dice several apples and place a representative sample of about 50 g of the fruit in the flask. Add 0·1 g of selenium powder and mix well. Add a few glass beads, assemble the apparatus, maintaining a rapid flow of water through the condenser, and add, through the tap funnel during 10 minutes, 20 ml of a cold (3 + 1) mixture of sulphuric and nitric acids. Heat gently until the frothing abates, adding more nitric acid dropwise as necessary to prevent charring, and then heat under reflux at full heat for 3 hours. Cool, add 10 ml of hydrogen peroxide and heat under reflux for ½ hour. Wash down the condensers with 50 ml of water, cool and filter through Whatman No. 541 filter-paper or spin in a centrifuge (½ hour at 2000 r.p.m. and 30 cm radius) and decant. Dilute to 250 ml, titrate 1 ml with 0·1 *N* sodium hydroxide and adjust the remainder to an acidity of 1 *N* with ammonia solution.

Transfer to a 500-ml separator, and add 5 ml of hydroxylamine hydrochloride solution and 10 ml of dithizone working solution. Shake for 1 minute, allow to separate and transfer the chloroform layer to a small separator containing 25 ml of 0·1 *N* hydrochloric acid and 5 ml of hydroxylamine hydrochloride solution. Re-extract twice with 5 ml of dithizone reagent and transfer each extract to the second separator. Shake the second separator well and, after allowing to settle, transfer the chloroform layer to a third separator containing 50 ml of 0·1 *N* hydrochloric acid, washing the aqueous layer with 2 to 3 ml of chloroform and adding the washings to the bulk. To the third separator add 2 ml of sodium thiosulphate solution, shake for 1 minute and run off the chloroform. Wash with 2 to 3 ml of chloroform, rejecting the washings, add 3 to 5 ml of sodium hypochlorite solution and shake for 1 minute. Add 5 ml of hydroxylamine hydrochloride solution (wetting the stopper and neck of separator with this reagent) and shake for 1 minute. Blow off the chlorine, shake again and then extract twice with 2 to 3 ml of chloroform, rejecting the extracts. Add 3 ml of acetic acid and 10·0 ml of dithizone solution, shake well and allow to separate. Dry the stem of the funnel and insert a loose plug of cotton-wool. Run off a little of the solution and then read the optical density of the rest in a 4-cm cell against chloroform at 490 mμ.

Prepare a standard curve by adding suitable dilutions of the mercuric chloride solution (0 to 5 μg of mercury) to 50 ml of 0·1 *N* hydrochloric acid, 5 ml of hydroxylamine hydrochloride solution and 3 ml of acetic acid. Saturate with chloroform, run off all excess and extract the mercury with 10·0 ml of dithizone solution as described above. Read the optical densities at 490 mμ. Carry out a blank determination on the reagents.

RESULTS

Table I shows the results of several recovery experiments carried out on tomatoes and apples by the Klein and the proposed methods. Mercury was added as a dilute solution of phenylmercuric chloride (5 μg of mercury per ml).

TABLE I

DETERMINATION OF KNOWN AMOUNTS OF MERCURY IN FRUITS

Sample	Method	Mercury added, μg	Mercury found, μg	Recovery, %
Tomatoes	Klein	5	4·5	90
Apples	Klein	5	0·5	10
Apples	Klein	5	1·0	20
Apples	Proposed	5	4·5	90
Apples	Proposed	5	4·7	94

We thank the Government Chemist for permission to publish this Note.

REFERENCES

1. Klein, A. K., *J. Ass. Off. Agric. Chem.*, 1952, **35**, 537; *see also* "Official Methods of Analysis," Eighth Edition, The Association of Official Agricultural Chemists, Washington, D.C., 1955, p. 438.
2. Laug, E. P., and Nelson, K. W., *Ibid.*, 1942, **25**, 400.
3. Kunze, F., *Ibid.*, 1948, **31**, 439.
4. Analytical Methods Committee, "The Determination of Lead in Foodstuffs," *Analyst*, 1954, **79**, 397.

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THE TITRIMETRIC DETERMINATION OF SULPHATE WITH LEAD NITRATE AS TITRANT AND DITHIZONE AS INDICATOR

LEAD sulphate is appreciably soluble in aqueous solutions, but almost completely insoluble when organic solvents are present. The classical method of determining lead as the sulphate depends on the almost complete insolubility of lead sulphate in water - ethanol mixtures. In the present work it has been found that lead sulphate is insoluble in water - acetone mixtures. If lead nitrate solution is added to a weakly acid water - acetone solution containing sulphate ions, and to which a little dithizone has been added, the dithizone will not change from its green colour to the red colour of lead dithizonate until an excess of lead over sulphate ions is present. This reaction forms the basis of the method described.

METHOD

REAGENTS—

Lead nitrate, 0·02 N—Prepared from reagent grade solid.

Acetone—This should be free from traces of heavy metals. If it gives a red solution when a trace of dithizone is added, it should be distilled.

Dithizone solution—An approximately 0·1 per cent. solution in acetone; the solution should be prepared freshly just before use.

Ammonia solution, dilute, approximately 0·02 N.

Nitric acid, dilute, approximately 0·02 N—This should be prepared from dilute (2 *N*) bench reagent acid that has stood for some time. If concentrated nitric acid is diluted directly, there may be traces of nitrous acid present, which will rapidly oxidise dithizone.

Bromophenol blue indicator solution—A 0·1 per cent. w/v solution in ethanol.

Acetic acid, 20 per cent. v/v—This should be free from traces of heavy metals.

PROCEDURE—

The procedure as described is suitable for direct titration of sulphate ion in solutions of ammonium and alkali-metal sulphates, and forms the basis of the practical applications described.

By pipette put into a 250-ml conical flask an aliquot portion of the sample solution containing about 0·2 to 0·4 milli-equivalents of sulphate ion. Add water to give a total volume of about 10 ml and then 2 drops of bromophenol blue indicator solution and either dilute ammonia solution or dilute nitric acid until the solution has a pale green colour. Add 1 ml of 20 per cent. v/v acetic acid, 50 ml of acetone and 1 ml of 0·1 per cent. dithizone solution. Titrate with 0·02 *N* lead nitrate until the solution changes from green to a permanent purple colour. Near the end-point add the lead nitrate drop by drop, shaking for a few seconds between additions.

Under the conditions described the end-point is sensitive to 0·03 ml of 0·02 *N* lead nitrate.

Results—In the course of work in this laboratory it was necessary to determine the concentrations of a number of supersaturated solutions of sodium sulphate. After suitable dilution of the solutions, the method as outlined above was used, and for four solutions was checked by the normal barium sulphate gravimetric method. The results were as follows—

Sodium sulphate by gravimetric method, % w/v ..	24·10	23·00	22·85	21·40
Sodium sulphate by proposed method, % w/v ..	24·00	22·95	22·90	21·40

PROCEDURE WHEN METALS THAT REACT WITH DITHIZONE ARE PRESENT—

When copper, zinc or other metals that react with dithizone are present, addition of dithizone immediately gives rise to a red dithizonate, making it impossible to carry out the titration as described. If only traces of the metals are present, the difficulty can be overcome by adding sufficient dithizone to combine with all the metals present, plus an excess so that the solution

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BULLETIN

ANNUAL GENERAL MEETING, MARCH 1st, 1957

THE eighty-third Annual General Meeting of the Society was held at 2.45 p.m. on Friday, March 1st, 1957, in the meeting room of the Royal Society, Burlington House, London, W.1. The Chair was occupied by the President, Dr. K. A. Williams, A.Inst.P., M.Inst.Pet., F.R.I.C. The financial statement for the year ending October 31st, 1956 was presented by the Honorary Treasurer and approved, and the Auditors for 1957 were appointed. The Report of the Council for the year ending March, 1957, was presented by the Honorary Secretary and adopted.

The President announced that the following had been elected Officers for the coming year:—

President—J. H. Hamence, M.Sc., Ph.D., F.R.I.C.

Past Presidents serving on the Council—D. W. Kent-Jones, J. R. Nicholls, George Taylor and K. A. Williams.

Vice-Presidents—N. L. Allport, J. Haslam and A. A. Smales.

Honorary Treasurer—A. J. Amos.

Honorary Secretary—R. E. Stuckey.

Honorary Assistant Secretary—S. A. Price.

Ordinary Members of Council (for the ensuing two years)—D. C. M. Adamson, W. Cule Davies, D. C. Garratt, H. M. N. H. Irving, E Q. Laws and J. G. Sherratt.

S. G. Burgess, R. C. Chirnside, D. D. Moir, F. C. J. Poulton and A. F. Williams, having been elected members of the Council in 1956, will, by the Society's Articles of Association, remain members of the Council for 1957.

A. N. Leather (Chairman of the North of England Section), Magnus A. Pyke (Chairman of the Scottish Section), P. J. C. Haywood (Chairman of the Western Section), R. Belcher (Chairman of the Midlands Section), D. F. Phillips (Chairman of the Microchemistry Group), J. E. Page (Chairman of the Physical Methods Group) and S. K. Kon (Chairman of the Biological Methods Group) will be *ex-officio* members of the Council for 1957.

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, April 3rd, 1957

AN Ordinary Meeting of the Society, organised by the Physical Methods Group, will be held at 7 p.m. on Wednesday, April 3rd, 1957, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1.

The subject of the meeting will be "Fluorimetry," and the following papers will be presented and discussed:—

"The Spectrometry of Fluorescence," by E. J. Bowen, M.A., D.Sc., F.R.S.

"Some Experiments with Spectrofluorimeters and Filter Fluorimeters," by C. A. Parker, B.Sc., Ph.D., F.R.I.C.

"Spectrofluorimetry," by Professor R. T. Williams, D.Sc., Ph.D.

"A Direct Reading Fluorimeter," by L. Brealey, B.Sc., and R. E. Ross, A.M.Brit.I.R.E.

Ordinary Meeting of the Midlands Section, March 28th, 1957

AN Ordinary Meeting of the Section will be held at 7 p.m. on Thursday, March 28th, 1957, in the Gas Showrooms, **Nottingham.**

A discussion on "The Analysis of Complex Sulphur Compounds" will be opened by C. E. Kendall, B.Sc., A.R.I.C.

Ordinary Meeting of the Midlands Section, April 10th, 1957

AN Ordinary Meeting of the Section will be held at 7 p.m. on Wednesday, April 10th, 1957, at the University, Edmund Street, **Birmingham, 3.**

The following paper will be presented and discussed:—

"The Analytical Chemistry of Beryllium," by E. Booth.

Ordinary Meeting of the Biological Methods Group, April 4th, 1957

AN Ordinary Meeting of the Group will be held at 7 p.m. on Thursday, April 4th, 1957, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1.

The following papers will be presented and discussed:—

"Experience in the Microbiological Assay of Vitamins and Amino Acids by Large-plate Methods," by D. F. Harris and J. S. Simpson, F.I.M.L.T.

"Quantitative Analysis of Immunologically Specific Substances in Agar-gel Plates," by J. G. Feinberg, B.Sc., D.V.M., M.Sc.

BRITISH STANDARDS INSTITUTION

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to members of the Society, and can be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

Draft Specifications prepared by Sub-Committee SFE/45/8—Analysis and Testing of Coal and Coke.

CX(SFE)2296—Draft B.S. Methods for the Analysis and Testing of Coal and Coke (Revision of B.S. 1016) Part 6—Ultimate Analysis of Coal.

CX(SFE)2147—Draft B.S. Methods for the Analysis and Testing of Coal and Coke (Revision of B.S. 1016) Part 7—Ultimate Analysis of Coke.

Draft Specifications prepared by Technical Committee FHC/4—Solvents and Allied Products.

CX(FHC)2046—Draft B.S. for (A) Carbon Tetrachloride (Revision of B.S. 575).

CX(FHC)2047—Draft B.S. for (B) Tritolyl Phosphate (Revision of B.S. 1999).

Draft Specification prepared by Sub-Committee RUC/10/5—Chemical Methods for Rubber.

CW(RUC)9214—Draft B.S. Methods for the Determination of Sulphur (Revision of Part 3 of B.S. 903).

Draft Specification prepared by Technical Committee LBC/5—Hydrometers.

CX(LBC)1856—Draft B.S. for Density - Composition Tables for Aqueous Solutions of Nitric Acid (Revision of B.S.975).

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is a blue-green colour. Under these conditions the end-point is less sharp, but the results are of moderate accuracy. If, however, considerable amounts of reactive metals are present, it is necessary to remove them with an ion-exchange column.

A specific problem to which this procedure was applied was the determination of sulphate in potassium carbonate solutions containing considerable amounts of copper. The potassium carbonate solutions were approximately 2 *N* and contained 0·3 to 1·0 per cent. w/v sulphate ion. The procedure was as follows.

Take 20 g of Amberlite IR-120(H) resin in a 250-ml beaker, and add by pipette 10 ml of the carbonate solution. Effervescence takes place as carbon dioxide is evolved. Set aside with occasional swirling for 5 minutes, and then pour the supernatant liquid down an ion-exchange column of the same resin. (The column used had an internal diameter of about ½ inch and was packed to a length of about 6 inches.) Collect the runnings from the column in a 200-ml calibrated flask. Wash the resin in the beaker with small portions of water, and pour the washings on the column. Continue to run the column at about 10 ml per minute until nearly 200 ml of liquid have been collected, and then dilute to the mark.

Add to a 250-ml flask an aliquot of the solution containing 0·2 to 0·4 milli-equivalents of sulphate ion. Evaporate to about 10 ml, cool, and determine the sulphate ion by the procedure described above.

Results—Results by the method described and by the gravimetric method, with and without prior ion-exchange treatment, were as follows—

Sulphate by direct gravimetric method, % w/v	0·272	0·294	0·286	0·315	0·294	0·976
Sulphate by gravimetric method after ion-exchange treatment, % w/v	0·296	0·304	0·317	0·328	0·297	1·055
Sulphate by proposed method, % w/v ..	0·290	0·319	0·304	0·340	0·306	1·090

It will be seen from the figures for the gravimetric method without ion-exchange treatment that the presence of potassium salts leads to low results.

Application to the determination of sulphur in organic compounds

Oxidise 10 to 20 mg of the sample in a sealed tube at 280° C with 0·2 ml of fuming nitric acid for 3 hours. Transfer the contents of the tube to a 250-ml beaker, and add 0·6 ml of *N* ammonia solution. Place the beaker on a water bath and evaporate the contents to dryness. Add about 1 ml of water and evaporate to dryness again. Dissolve the residue in 10 ml of water and determine the sulphate as outlined under "Procedure."

Results—In a trial of the method the results were as follows—

Compound	Sulphur found, %	Theoretical sulphur content, %
Sulphanilic acid	18·38, 18·47, 18·56, 18·44, 18·54, 18·30, 18·49, 18·54, 18·49	18·51
1-Cystine	26·50 26·38, 26·71, 26·58, 26·65	26·68

Modification when chloride ions are present

As lead chloride is not very soluble, the method gives erroneous results for sulphate if more than traces of chloride are present. Chloride, however, can be readily removed by evaporating the solution to dryness with an excess of nitric acid. Sufficient ammonium or alkali-metal ions must be present to hold back the sulphate ions.

This procedure has not been worked out as an application to any definite practical problem, but it has been demonstrated that sulphate ions can be determined in the presence of a considerable excess of hydrochloric acid. In an experiment 15 ml of 0·02 *N* sulphuric acid were taken, and 10 ml of *N* hydrochloric acid, 0·6 ml of *N* ammonia solution and 0·2 ml of concentrated nitric acid were added. The solution was evaporated to dryness on the water bath, the residue was dissolved in about 1 ml of water, and the solution was again evaporated to dryness.

Sulphate was then determined as described under "Procedure," a titre of 15·0 ml of 0·02 *N* lead nitrate being obtained.

The Distillers Company Limited
Research and Development Department
Great Burgh, Epsom, Surrey

E. E. Archer
October 16th, 1956

EXPERIENCE OF THE MICROBIOLOGICAL ASSAY OF VITAMINS AND AMINO ACIDS BY LARGE-PLATE METHODS

MOST recent papers[1,2,3,4,5,6] on plate assay of vitamins and amino acids have recommended methods involving use of Petri dishes with the usual (2 + 2) design. Although these methods are an obvious improvement on tube and flask methods, the use of Petri dishes has many disadvantages. Large plates lend themselves to the more complete reduction of errors and to the conduct of multiple comparisons. Lees and Tootill[7] have summarised their work in this field and deal adequately with the errors inherent in Petri-dish methods. Our experience of both methods confirms the undoubted superiority of large-plate methods.

In response to demands for improved precision and for an increased throughput of samples, the large-plate method of assay, as used in this laboratory for antibiotics, vitamin B_{12}, etc., was successfully adapted for use in the assay of other water-soluble vitamins and of amino acids.

The culture media employed are those recommended by Barton-Wright,[8] Davis "New Zealand" agar being used to produce a 1·2 per cent. gel.

A (2 + 2) design has been employed and this has usually been of the 8 × 8 quasi Latin-square type, accommodating six samples and two standards. All samples have been assayed by two operators using different dilutions, designs and plates. Zones of exhibition are well defined (see Fig. 1), except those produced by pyridoxine hydrochloride, and readings are made, again by different operators, with needle-point calipers to the nearest 0·1 mm, measured directly on the surface of the medium. As pointed out by Harrison, Lees and Wood,[9] this method of reading avoids errors due to parallax and gives the maximum information from the assay. Smaller plates of the 6 × 6 and 4 × 4 Latin-square type are used for smaller numbers of samples. A (3 + 3) design is used for standard checks and higher precision assays and to determine or check the nature of the dose - response line.

Strict bacteriological control of all stock cultures and suspensions and care at all stages in preparing culture media are of vital importance for successful assays. Glass-distilled water is used throughout and all glassware is cleaned in potassium hydroxide solution and then with hydrochloric acid, followed by sterilisation. Incubation of the washed seeding inoculum at 37° C for 2 hours before inoculation of the melted assay medium eliminates endogenously stored vitamins and amino acids.

The method has been employed since October 1953, and by its means assays of riboflavin, nicotinic acid, calcium D-pantothenate, folic acid, pyridoxine hydrochloride, thiamine hydrochloride, tryptophan, inositol and biotin have been successfully carried out; preliminary assays of choline, *p*-aminobenzoic acid and seven different amino acids indicate that for these also the method could eventually become a routine procedure. Use of the method has shown a 50 per cent. increase in throughput of samples, a decrease in assay staff and materials required and an improved precision compared with the earlier tube and flask methods. Results are available within 24 hours. If urgently required, they can be made available within 5 hours by using the pre-incubation technique described originally by Goyan, Dufrenoy, Strait and Pratt.[10] The silver impregnation of developed zones, also described by these workers, has not been used.

Results of the determination of riboflavin in five samples by the tube and large-plate methods on two successive days are shown in Table I. Each individual result is the mean of two determinations by different operators.

TABLE I

DETERMINATION OF RIBOFLAVIN IN VARIOUS SAMPLES

	Riboflavin found by tube assay			Riboflavin found by large-plate assay		
	Day 1, μg per ml	Day 2, μg per ml	Mean, μg per ml	Day 1, μg per ml	Day 2, μg per ml	Mean, μg per ml
Pure riboflavin solution ..	18·7	21·9	20·3	19·6	21·0	20·3
Infant cereal food extracts ..	2·00	2·23	2·12	2·04	2·03	2·04
Riboflavin concentrates ..	54·9	47·8	51·4	54·6	51·0	52·8
Multivitamin solution ..	24·3	27·7	26·0	27·2	24·6	25·9
Multivitamin solution ..	13·7	15·3	14·5	14·7	15·6	15·2

It will be noted that the maximum deviation of any single result, expressed as a percentage of the mean, is ± 16 per cent. for the tube assay and ± 7·0 per cent. for the large-plate method.

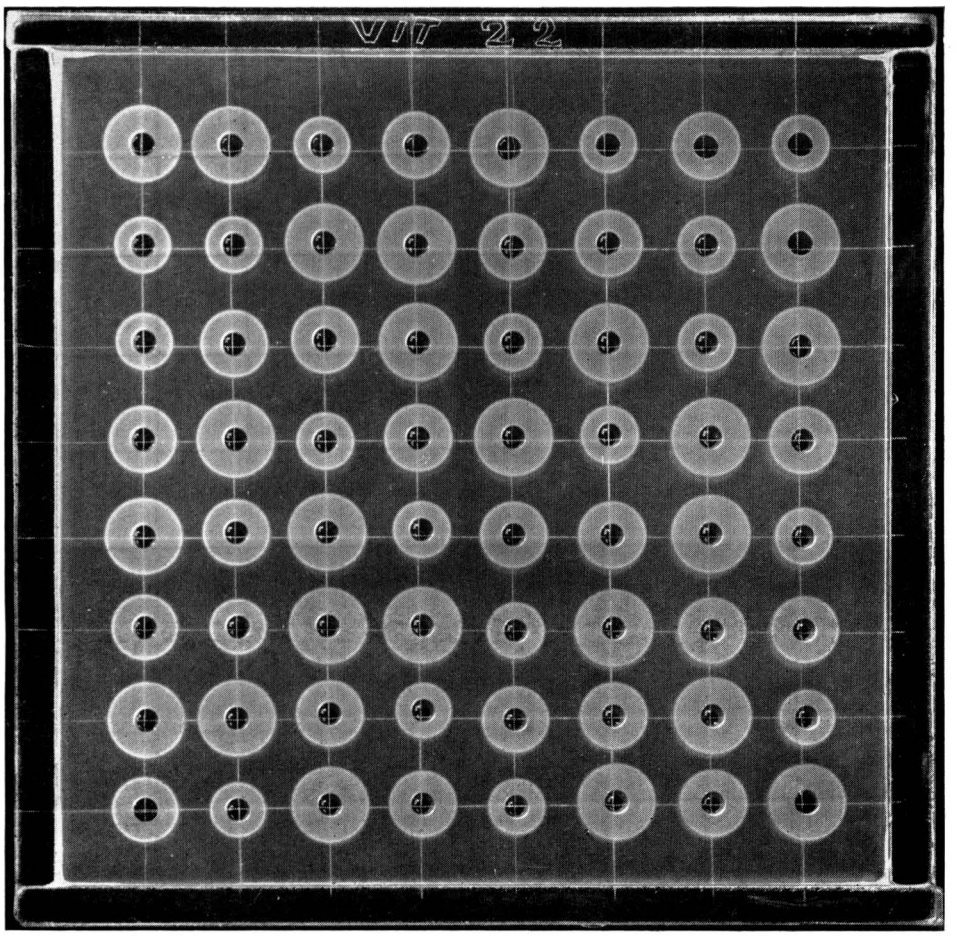

Fig. 1. An 8 × 8 quasi Latin-square design for the large-plate assay of vitamins and amino acids

[To face p. 210

REFERENCES

1. Jones, A., and Morris, S., *Analyst*, 1949, **74**, 333.
2. ——, ——, *Ibid.*, 1950, **75**, 613.
3. ——, ——, *Ibid.*, 1951, **76**, 588.
4. Morris, S., and Jones, A., *Ibid.*, 1953, **78**, 15.
5. Jones, A., and Morris, S., *Ibid.*, 1954, **79**, 586.
6. Bacharach, A. L., and Cuthbertson, W. F. J., *Ibid.*, 1948, **73**, 334.
7. Less, K. A., and Tootill, J. P. R., *Ibid.*, 1955, **80**, 123.
8. Barton-Wright, E. C., "The Microbiological Assay of the Vitamin B Complex and Amino-Acids," Sir Isaac Pitman & Sons Ltd., London, 1952.
9. Harrison, E., Lees, K. A., and Wood, F., *Analyst*, 1951, **76**, 696.
10. Goyan, F. M., Dufrenoy, J., Strait, L. A., and Pratt, R., *J. Amer. Pharm. Ass.*, 1947, **36**, 65.

J. S. SIMPSON
D. F. HARRIS

GLAXO LABORATORIES LTD.
GREENFORD, MIDDX.

September 16th, 1955

THE ELECTROPHORETIC ISOLATION OF DYE-STUFFS IN FOODS

SINCE the recent amended legislation[1] that considerably limited the number of permitted dyes available for use in foods, the need has been felt for a method that would permit the extraction of these substances in a pure state, and in sufficient quantity for identification procedures to be carried out.

The isolation of dye from foods has always been found difficult owing to the adsorption of dye on protein, and the subsequent loss of the dye-stuff when removing the protein from extracts.

Enzyme hydrolysis of foods by amylases and proteases followed by resolution of the dyes on cellulose columns is a useful but limited procedure, since the eluates contain large amounts of amino acids, and carbohydrates that interfere with the dye absorption spectra. This means that less sensitive methods of identification have to be used.

After the studies of Anderson, Lock and Martin[2] on the electrophoresis of food dyes, it became apparent that the use of electrophoresis to isolate the pure dyes from foods would overcome objectionable features of the above technique.

The method is based on work carried out by Anderson, Lock and Martin,[2] and Anderson and Martin,[3] and was satisfactorily applied to biscuits, jams and cream confectionery.

METHOD

Extract 100 g of material for 6 hours at 45° C by slowly stirring it with 250 ml of *n*-butanol saturated with 2 *N* hydrochloric acid. Filter the mixture, using a Buchner funnel, and concentrate the filtrate by evaporation under vacuum, the distillation temperature not being allowed to exceed 45° C. Spread the residue, which should be a dark, thick and resinous material, zone fashion on a 24-inch × 5-inch strip of Whatman 3 MM paper, and carry out electrophoresis at 600 V with the apparatus described by Foster,[4] with a current of 10 mA and using a buffer solution of pH 12 as the electrolyte, prepared by mixing 1000 ml of 0·05 *M* sodium tetraborate and 1600 ml of 0·1 *N* sodium hydroxide.

From 4 to 6 hours electrophoresis is usually sufficient to isolate the component dyes into discrete zones. Dry the electropherogram,[5] cut out the colour zones, elute the dyes with distilled water and record their absorption spectra. Perform a blank test with each extraction.

With products containing large quantities of dye-stuff, the eluates from the electropherograms contain sufficient material for a paper-chromatographic procedure to be used for identification. The phenol - water - formic acid solvent used by Anderson and Martin[3] was found to give excellent results in this procedure.

I thank the Directors of William Arnott Pty. Ltd., for permission to publish this Note.

REFERENCES

1. New South Wales (Australia) Government Gazette No. 6, 21/1/55.
2. Anderson, J. R. A., Lock, L. C., and Martin, E. C., unpublished work.
3. Anderson, J. R. A., and Martin, E. C., *Anal. Chim. Acta*, 1953, **8**, 530.
4. Foster, A. B., *Chem. & Ind.*, 1952, 1050.
5. Lederer, M., "Introduction to Paper Electrophoresis and Related Methods," Elsevier Publishing Co., Amsterdam, 1955, p. 23.

WILLIAM ARNOTT PTY. LTD.
GEORGE STREET
HOMEBUSH, N.S.W., AUSTRALIA

JOHN F. WILLIAMS

October 8th, 1956

EFFECT OF LIGHT ON QUININE IN TONIC WATER

In the course of checking the quinine content of a number of tonic waters, considerable variation was found between bottles that had been filled at the same time and with the same batch of liquid. The bottles, which were of the customary colourless glass, had been in stock for a short time and, other causes of the variation having been investigated with negative results, the possibility of exposure to light as the source of the trouble was examined. Four bottles showing the faint blue fluorescence characteristic of solutions of quinine sulphate were selected from the batch. Two were kept in the dark, and two were kept exposed to daylight on a window sill. After 25 days, fluorescence was to be seen only in the bottles that had been stored in the dark, and all four bottles were opened and their contents analysed. The method of analysis used was that given by the Association of Official Agricultural Chemists,[1] except that the titration was performed by adding an excess of 0·02 *N* acid to the alcoholic solution of the alkaloid and titrating with 0·02 *N* alkali, since it was found that a sharper end-point was obtainable in this way when determining such small amounts of quinine. The results for the samples kept in the dark were 38 and 27 p.p.m. of quinine sulphate, whereas for the two samples exposed to daylight the results were lower, being 2 p.p.m. of quinine sulphate for both samples.

To confirm these findings, ten bottles of freshly bottled tonic water from the same maker were obtained. Four were treated in my laboratory as before (two kept in the dark, two exposed to daylight) and another four were treated similarly, with the kind co-operation of Mr. J. G. Sherratt, at his laboratory in Warrington. After 50 days the samples were analysed, in this laboratory by the A.O.A.C. method and in Mr. Sherratt's laboratory by a method based upon the fluorescence of quinine sulphate under conditions of controlled acidity. The results are shown in Table I.

TABLE I

QUININE FOUND IN TONIC WATER AFTER BEING KEPT FOR 50 DAYS

	Quinine sulphate found in tonic water kept in the dark		Quinine sulphate found in tonic water exposed to daylight	
Analyst	1st bottle, p.p.m.	2nd bottle, p.p.m.	1st bottle, p.p.m.	2nd bottle, p.p.m.
A. A. D. C. ..	92	92	7*	Nil
J. G. S. ..	~ 100	~ 100	< 25	< 25

* This sample developed an unpleasant odour.

As before, fluorescence had disappeared from the exposed samples, but was visible in the others. The samples kept in the dark also gave a positive reaction in the thalleioquin test, whereas the exposed samples gave no reaction. No differences in pH (approximately 3) or flavour could be detected among them, but one sample (marked with an asterisk in Table I) developed an unpleasant odour. All samples were clear and colourless, but those exposed to daylight showed a minute amount of a light brown flocculent deposit, while those kept in the dark were free from deposit. The deposit was thoroughly dispersed in the liquid before an analysis was made.

The deposit from another exposed bottle was separated and examined microscopically. It consisted mainly of amorphous particles with no evidence of any micro-organisms, and gave a positive reaction for iron but no reaction in the thalleioquin test. It should, perhaps, be noted that, although the loss of quinine in these experiments was about 4 to 8 mg per 100 ml, the deposit from 100 ml weighed considerably less than 1 mg.

REFERENCE

1. "Official Methods of Analysis," Eighth Edition, The Association of Official Agricultural Chemists, Washington, D.C., 1955, p. 617, para. 3284 (*b*).

TRAFFORD LABORATORY
ASHBURTON ROAD
MANCHESTER, 17

A. A. D. COMRIE
October 10th, 1956

Apparatus

A SIMPLE AUTOMATIC APPARATUS FOR SPOTTING LARGE VOLUMES OF LIQUID ON CHROMATOGRAM PAPERS

MIXTURES of substances to be separated by paper chromatography are frequently obtained as dilute solutions. To get an adequate quantity of solute in a small spot on the paper necessitates

either the repeated application and drying of very small volumes, a lengthy and tedious process, or the concentration of the dilute solution, possibly under reduced pressure, which again may need considerable attention.

By using the apparatus described here, which is easily constructed in the laboratory, a volume of up to 0·5 ml can be applied to the paper as a single spot. The apparatus needs no attention once it is started, and it is switched off automatically when the predetermined volume has been delivered, resulting in a considerable saving of worker's time.

Essentially it consists of an Agla micrometer syringe (Burroughs Wellcome & Co. Ltd.) driven by a slow speed synchronous electric motor. A Perspex disc is fixed to the moving part of the micrometer in such a way as to make contact with a micro switch when the zero mark is reached; the motor is thereby switched off.

Details of the apparatus are shown in Fig. 1; A is a synchronous electric motor (1 revolution per minute; Sangamo & Weston Ltd., Enfield, Middlesex; Type S7), to the shaft of which is attached the bent rod, B ($\frac{1}{8}$-inch brass). The bent rod engages with a pin, C, which is fixed near the circumference of the Perspex disc, D ($2\frac{1}{2}$ inches in diameter; $\frac{1}{8}$-inch thick). This disc is attached to the moving part of the micrometer by a single screw through the centre, the ratchet mechanism being removed. The micro switch, E (Burgess Products Co. Ltd., Sapcote, Leics.; type CRS) is fixed to the body of the micrometer by the brass bracket, F, adjustment of height being made by the screw and slot G, so that the switch is just actuated by the Perspex disc when the micrometer reaches the zero mark. It is wired up in series with the synchronous motor and an a.c. mains supply, the terminals marked "normally closed" being used. A very small movement of the switch contact then breaks the circuit and the motor stops.

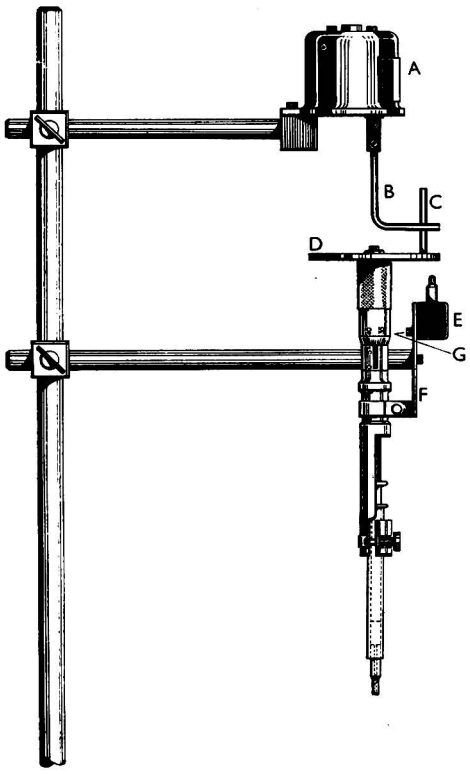

Fig. 1. Apparatus for spotting large volumes of liquid on chromatogram papers

To use, the micrometer is turned back slightly further than the volume to be delivered and the syringe, containing the sample, is clamped with its piston touching the moving part of the

micrometer. Final adjustment of volume is made by moving the micrometer by hand, the chromatogram paper is placed in position, touching the nozzle of the syringe, and the motor and driving rod B are swung into position and clamped, coaxial with the micrometer. The sample is now discharged on to the paper at the rate of 10 μl ($\equiv$ 1 revolution) per minute until the zero mark is reached and the motor is switched off. The total volume delivered is reproducible within a few microlitres. The chromatogram paper should be supported clear of the bench at the point of application, *e.g.*, by resting on the lower half of a Petri dish. An electric hair drier is used to hasten evaporation of the solvent, and by adjusting its position the diameter of the applied spot can be kept to 5 mm or less. It is convenient to have a socket for the hair drier wired in parallel with the synchronous motor. Thus the drier also is switched off at the end of the application and overheating of the spot is avoided.

Thanks are due to Mr. G. W. Coote for the drawing.

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
UNIVERSITY OF READING

W. G. DUNCOMBE*
B. W. E. PEAPLE

August 30th, 1956

(* PRESENT ADDRESS: THE WELLCOME RESEARCH LABORATORIES, BECKENHAM, KENT)

A SEPARATOR FOR REMOVING HEAVY MINERALS FROM FLOUR AND OTHER FOODSTUFFS

SEPARATING funnels of conventional design have long been used for the separation of minerals and extraneous matter from flour and other finely divided foodstuffs, carbon tetrachloride or some other inert liquid of suitable specific gravity being used as a flotation medium for the flour.

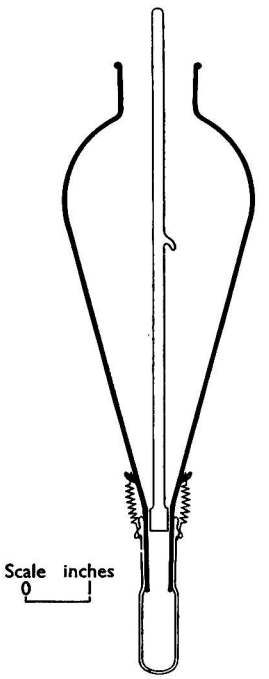

Fig. 1. Separator

Such a funnel is not entirely satisfactory when the amount of sediment to be separated is very small or when it is desired to separate successive fractions of sediment for microscopic examination. The small bore of the stopcock and the constriction of the funnel near the stopcock make it difficult to remove the sediment expeditiously or quantitatively for further examination. A

cylindrical settling vessel with a removable cap has been described by Wynter Blyth[1] for the removal of sediment from samples of water, but it is not of a suitable shape or size for the present purpose. Also a large funnel of smoothly tapering section closed by a screw-clip instead of a tap has been described by Kent-Jones, Amos, Elias, Bradshaw and Thackeray[2] for the flotation of extraneous matter from cereal digests.

The funnel shown in Fig. 1 is also of smoothly tapering section, but it is fitted with a receiver for the sediment. It consists of a wide-mouthed conical funnel with an A10 ground joint at the bottom end, over which fits a short tube having an A10 neck with lugs provided for attaching it to the funnel by means of springs. Inside the narrow throat of the funnel a ground seating is made to take a glass rod, which acts as a stopper, so that the small tube may be removed at any stage of the sedimentation.

In use, the tube is first attached and the funnel is filled three-quarters full with the solvent selected. About 20 g of flour or other material are then introduced on the surface of the liquid and the suspension is stirred slowly and intermittently. Too vigorous agitation of the suspension prevents the heavy material from settling. When it is desired to remove the sediment, the glass-rod stopper is placed in its seating and the tube may then be removed. The liquid is decanted from the sediment, which can be spun in a centrifuge and washed if necessary, and dried before examination.

Quantitative recovery of a mineral sediment from flour is very difficult to achieve, even when the difference in density is very great. Reduced iron, with a density of 7·4 g per ml, cannot be separated quantitatively from flour, density 1·45 g per ml, by means of carbon tetrachloride sp.gr. 1·54. This is due to the very fine state of division of a fraction of the reduced iron. With the apparatus described it is, however, possible to achieve up to 90 per cent. recovery of inorganic materials such as reduced iron, aerating agents, Chalk B.P., some powder improvers and metallic fragments.

These funnels are obtainable from A. D. Wood (Cambridge) Ltd., 10 Walnut Tree Avenue, Cambridge.

I thank the Directors of Spillers Limited for permission to publish this work.

REFERENCES

1. Wynter Blyth, A. H., "Foods: Their Composition and Analysis," Seventh Edition, Charles Griffin & Co. Ltd., London, 1927, p. 544.
2. Kent-Jones, D. W., Amos, A. J., Elias, P. S., Bradshaw, R. C. A., and Thackray, G. B., *Analyst*, 1948, **73**, 128.

SPILLERS CENTRAL LABORATORY
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A. W. HARTLEY
November 8th, 1956

British Standards Institution

NEW SPECIFICATION*

B.S. 998 : 1957. Vacuum Salt for Butter and Cheese Making. Price 3s. 6d.

AMENDMENT SLIP*

A PRINTED slip bearing an amendment to a British Standard has been issued by the Institution, as follows—

PD 2677—Amendment No. 1 (December, 1956) to B.S. 1428 : Part B2: 1953. Ammonia Distillation Apparatus (Markham).

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

Publications Received

TECHNIQUE OF ORGANIC CHEMISTRY. Volume III. Part I: SEPARATION AND PURIFICATION. Edited by ARNOLD WEISSBERGER. Second Edition. Pp. x + 873. New York and London: Interscience Publishers Inc. 1956. Price $17.50; 120s.

A TEXTBOOK OF DAIRY CHEMISTRY. By EDGAR R. LING, Ph.D., M.Sc., F.R.I.C., A.R.C.S. Third Edition. Volume II: PRACTICAL. Pp. xii + 140. London: Chapman & Hall Ltd. 1956. Price 16s.

BIOLOGICAL TREATMENT OF SEWAGE AND INDUSTRIAL WASTES. Volume I: AEROBIC OXIDATION. Edited by BRO. JOSEPH MCCABE, F.S.C., and W. W. ECKENFELDER, jun. Pp. viii + 393. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1956. Price $10.0; 80s.

STUDIES IN URBAN HOUSEHOLD DIETS 1944–49. Second Report of the National Food Survey Committee, Ministry of Agriculture, Fisheries and Food. Pp. 141. London: Her Majesty's Stationery Office. 1956. Price 5s. 6d.

DETERMINATION OF MOISTURE IN SOLIDS. By P. J. GEARY, B.Sc. (Eng.). Pp. iv + 51. Chislehurst: British Scientific Instrument Research Association. 1956. Price* (in Great Britain) 7s. 6d.; (overseas) 9s. 6d.

Brief notes and selected literature references.

PHYSICAL METHODS IN CHEMICAL ANALYSIS. Volume III. Edited by WALTER G. BERL. Pp. xii + 652. New York and London: Academic Press Inc. 1956. Price $15.00; 107s.

ORGANIC ANALYSIS. Volume III. Edited by J. MITCHELL, jun., I. M. KOLTHOFF, E. S. PROSKAUER and A. WEISSBERGER. Pp. viii + 546. New York and London: Interscience Publishers Inc. 1956. Price $11.50; 92s.

ORGANIC SYNTHESIS. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 36. Editor-in-Chief: N. J. LEONARD. Pp. vi + 120. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1956. Price $3.75; 30s.

PAINT TRADE MANUAL OF RAW MATERIALS AND PLANT. Compiled by H. W. CHATFIELD, Ph.D., B.Sc., F.R.I.C., M.I.Chem.E. Second Edition. Pp. 282. London: Scott Greenwood & Son Ltd. 1956. Price 24s.

MOLECULES AND CRYSTALS IN INORGANIC CHEMISTRY. By A. E. VAN ARKEL. Second English Edition. Pp. x + 270. London: Butterworths Scientific Publications; New York: Interscience Publishers Inc. 1956. Price 30s.

ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Volume XV. WAXES TO ZYMOSTEROL *and* INDEX TO VOLUMES I–XV. Edited by RAYMOND E. KIRK and DONALD F. OTHMER. Pp. xii + 936. New York and London: Interscience Publishers Inc. 1956. Price $30.00; 210s. (single volume): $25.00; 175s. (subscription).

ASPECTS OF RIVER POLLUTION. By LOUIS KLEIN, M.Sc., Ph.D., F.R.I.C., M.Inst.S.P., with J. R. ERICHSEN JONES, Ph.D., D.Sc., and H. A. HAWKES, M.Sc., A.M.Inst.S.P. Pp. xii + 621. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1957. Price 84s.; $14.50.

SEMIMICRO QUALITATIVE ORGANIC ANALYSIS: THE SYSTEMATIC IDENTIFICATION OF ORGANIC COMPOUNDS. By NICHOLAS D. CHERONIS and JOHN B. ENTRIKIN. Second Edition. Pp. xiv + 774. New York and London: Interscience Publishers Inc. 1957. Price $9.00; 72s.

* An additional charge of 1s. is made when cash does not accompany order.

Errata

DECEMBER (1956) ISSUE, p. 691, 13th line. *For* "150°C" *read* "105°C."

FEBRUARY (1957) ISSUE, p. 74, 8th line. After title of paper *add* "by R. C. Chirnside, F.R.I.C."