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Summaries of Papers in this Issue

The Determination of Chromium and Molybdenum in a Complete Range of Steels by Atomic-absorption Spectrometry with a Nitrous Oxide - Acetylene Flame

Interferences in the absorption of chromium and molybdenum in steels are overcome in the nitrous oxide - acetylene flame by using perchloric acid as the solvent. The method is rapid and suitable for any level of chromium or molybdenum found in steels and other metallic alloys. An alternative method for the determination of molybdenum is necessary when the tungsten concentration exceeds 0·5 per cent.; this involves the use of a perchloric - phosphoric - sulphuric acid mixture to retain the tungsten in solution. A difference read-out technique to obtain good precision for high levels of chromium is described.

D. R. THOMERSON and W. J. PRICE
Pye Unicam Ltd., York Street, Cambridge.

Analyst, 1971, **96**, 321–329.

The Fluorimetric Determination of Zirconium as 8-Hydroxyquinolinate in the Presence of Titanium, Tungsten and Molybdenum

A fluorimetric method is described for the determination of small amounts of zirconium as 8-hydroxyquinolinate. The procedure, which involves extraction of the chelate with chloroform from a hydrofluoric acid - ammonium tartrate solution at pH 9·0, permits the determination of zirconium in the presence of titanium, tungsten and molybdenum.

Excitation is conducted at 398 nm and emission measured at 520 nm. Down to 0·1 μg of zirconium can be determined with a coefficient of variation of 2·3 per cent.

H. O. SCHNEIDER and M. E. ROSELLI
Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

Analyst, 1971, **96**, 330–334.

A Rapid Atomic-absorption Technique for the Determination of Lithium in Silicate Materials

A simple and rapid atomic-absorption procedure is described for the determination of lithium in silicate rocks. Following fusion with sodium borate, the samples are dissolved in dilute nitric acid solution. Determinations of the lithium contents are made directly. The precision of the method, in terms of one standard deviation, was calculated to be 1093 $\pm$ 13 p.p.m. and 34·5 $\pm$ 4·6 p.p.m. Accuracy, as indicated by a comparison with published determinations, is good.

JAMES V. O'GORMAN and NORMAN H. SUHR
Mineral Constitution Laboratories, The Pennsylvania State University, University Park, Pa., U.S.A.

Analyst, 1971, **96**, 335–337.

An Improved Method for the Determination of Bacitracin in Animal Feeds

The determination of small amounts of bacitracin in animal feeds has been studied. It was expected that by using acidified methanol as the only organic solvent in the extraction procedure and phosphate buffer, undesired proteins would not be dissolved and the use of the toxic solvent pyridine might be avoided. Bacitracin could then be determined in the final extract by using microbiological methods, in which methanol interferes less than pyridine. The method reported here has been used for feed mixtures with various zinc bacitracin contents in the range from 5 to 350 p.p.m.

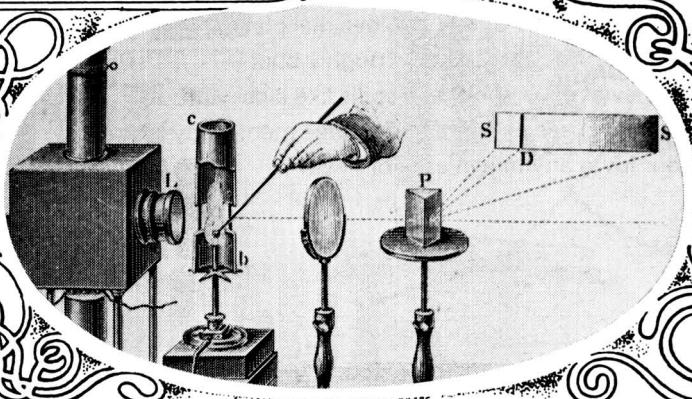
B. GRYNNE
A/S Apothekernes Laboratorium for Specialpræparater, Oslo, Norway.

Analyst, 1971, **96**, 338–342.

Circa **1870**

Early Spectroscopy

The incandescent light source (left) gives continuous spectrum, via condenser lens & prism P, and sodium on rod held in gas flame; this gives sodium absorption line D. (Ronan Picture Library)



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The Determination of Chromium and Molybdenum in a Complete Range of Steels by Atomic-absorption Spectrometry with a Nitrous Oxide-Acetylene Flame

BY D. R. THOMERSON AND W. J. PRICE
(Pye Unicam Ltd., York Street, Cambridge)

Interferences in the absorption of chromium and molybdenum in steels are overcome in the nitrous oxide - acetylene flame by using perchloric acid as the solvent. The method is rapid and suitable for any level of chromium or molybdenum found in steels and other metallic alloys. An alternative method for the determination of molybdenum is necessary when the tungsten concentration exceeds 0·5 per cent.; this involves the use of a perchloric - phosphoric - sulphuric acid mixture to retain the tungsten in solution. A difference read-out technique to obtain good precision for high levels of chromium is described.

EARLIER papers on the determination of chromium and molybdenum in steel with an air - acetylene flame report interferences,[1 to 16] in particular, a serious depression of the chromium and molybdenum absorbance by iron itself. Various methods of overcoming these effects were proposed, but none was completely effective for the whole range of elements found in steels. For this reason atomic-absorption spectrometry with an air - acetylene flame for the determination of chromium and molybdenum in iron and steel never achieved the degree of success associated with it in other fields of analysis.

With the advent of the nitrous oxide - acetylene flame, new interest was kindled, and with it fresh hopes of success. Even so, compared with the amount of published work on the air - acetylene flame, comparatively little work has been reported in which the nitrous oxide flame was used for the determination of chromium and molybdenum in steel.

Kirkbright, Smith and West[17] found that the nitrous oxide - acetylene flame overcame many interferences in the determination of molybdenum in alloy steels; however, their method appears to have some limitations and did not always give consistent results in our hands.

Ramakrishna, West and Robinson[18] made a comprehensive study of the interferences on molybdenum in the nitrous oxide flame. We confirmed their findings concerning the enhancement effect caused by iron in all media; but we could find no evidence under any conditions of the 30 per cent. enhancement due to perchloric acid that they report.

Further studies by McIssac[19,20] showed no interference by iron, but a small enhancement by manganese. Endo, Hata and Nakahara[21] found that the molybdenum response was enhanced by the addition of potassium sulphate, which they report as overcoming all interelemental interferences.

Only two attempts to determine more than 5 per cent. of chromium in steel were found. The first, by Feldman, Blasi and Smith,[22] reported the successful determination of 15 per cent. of chromium by using the 425·4 nm line, but no experimental details were given. The second was by Welz,[23] who reported low results when using synthetic standards with iron and nickel additions; but when calibration was effected with standard steels, good results were obtained at the 17 per cent. level.

A scheme for the determination of chromium and molybdenum in a wide range of steels is therefore considered to be desirable.

Experimental

Apparatus—

All measurements were made with a Unicam SP90A Series 2 atomic-absorption spectrophotometer, which incorporated a SP91 lamp turret accessory and a SP94 nitrous oxide accessory. Air was supplied through a SP93 air compressor, and nitrous oxide and acetylene were obtained from cylinders.

The instrument was also fitted with an inert nebuliser and Unicam high spectral output hollow-cathode lamps. The absorbance peaks were displayed on a SP22 chart recorder.

The only non-standard modification was the fitting of 0·125-mm thick metal shims to the nitrous oxide burner to increase the slot width from the normal 0·46 mm to 0·58 mm. This was necessary to prevent blockage by steel solutions more concentrated than about 0·5 per cent. The fitting of these shims naturally somewhat reduces the previous operating safety margins. Therefore it is important to maintain the gas flow-rates close to the values recommended below. On no account should shims of thickness greater than 0·125 mm be used.

Interferences—

Interference studies of various acids on the absorbance of chromium and molybdenum showed conclusively that perchloric acid was the best solvent. Unlike other acids (sulphuric - phosphoric, nitric - hydrochloric) it did not interfere with the absorption response. Furthermore, experimental evidence was accumulated to suggest that it is essential to obtain both elements in the same oxidation states in both their sample and calibration solutions (otherwise chromium results were on average 10 per cent. low and molybdenum 40 per cent. high), and perchloric acid was also found to ensure complete oxidation.

The interference effects of iron have long been realised, and they exist also in the nitrous oxide - acetylene flame. However, in this flame iron enhances the absorption of both chromium and molybdenum, whereas with air - acetylene there is considerable depression. It was found that the enhancement varies with the iron concentration. In the case of molybdenum this enhancement decreases as the iron concentration increases in the range shown in Fig. 1, whereas for chromium, enhancement increases in the same range of iron concentrations. Therefore, it is still necessary to incorporate iron in the calibration solutions and

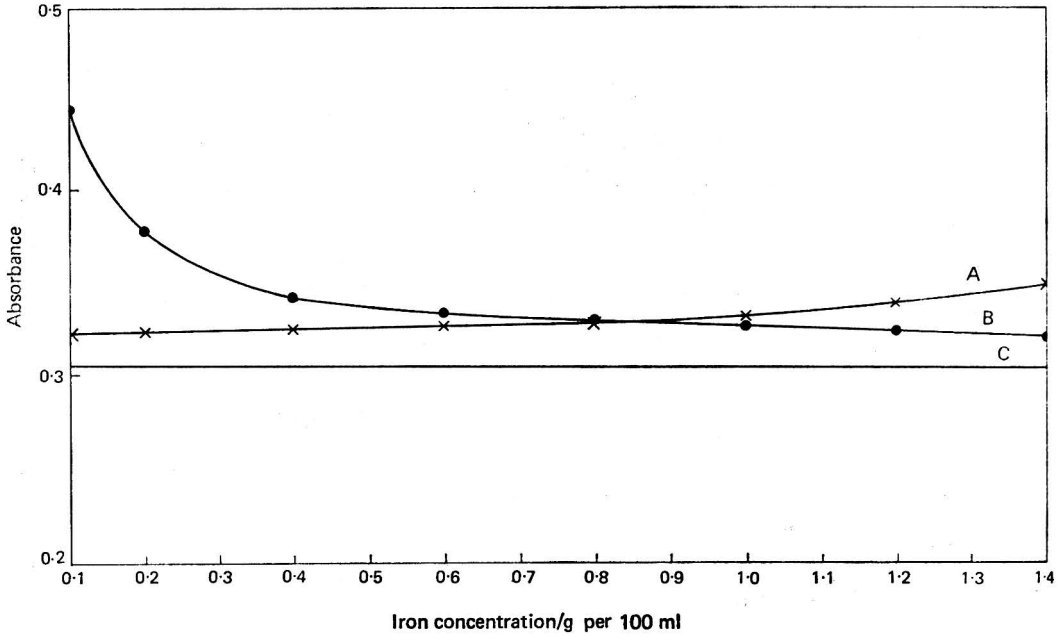

Fig. 1. Effect of iron concentration on the absorbance of 20 mg l^{-1} of chromium and 50 mg l^{-1} of molybdenum: A, chromium; B, molybdenum; and C, aqueous solutions (no iron)

also to add iron to solutions if dilutions are necessary, in order to maintain a constant concentration of iron in all the samples and calibration solutions.

Since most alloyed steels contain only about 60 to 80 per cent. of iron initially in the sample, it is usually necessary to raise their iron content to the equivalent of 100 per cent. in a 1 per cent. sample solution by additions of iron stock solution to the sample solutions before the final dilutions. The improvements in results can be significant.

When chromium standards were prepared by simply adding a stock iron solution in perchloric acid to the chromium calibration solutions, low results were always obtained for concentrations greater than about 5 per cent. This effect probably resulted from a difference in the oxidation states and was overcome only by ensuring that the calibration solutions were prepared and oxidised in an identical manner to the sample solutions (see Proposed Method).

METHOD DEVELOPMENT—

Unfortunately, the presence of tungsten in two of the steels to be analysed posed problems when perchloric acid was used as the solvent. When molybdenum was being determined, the results were only about one-half of their true value. The molybdenum was almost certainly being lost by co-precipitation with the tungsten while the latter was being filtered off as tungstic acid in the preparation of the final solutions.

It was, therefore, thought best to retain the tungsten in solution by dissolving the steel sample in a mixture of sulphuric, phosphoric and perchloric acids. This step is the basis of an alternative procedure. Molybdenum was then successfully determined in the presence of 6·9 per cent. of tungsten while the results obtained in the presence of 19·6 per cent. of tungsten were reasonably acceptable. This method of dissolution cannot be employed in a general scheme for chromium and molybdenum as, when the chromium is determined in this solution, an enhancement effect is observed and results are up to 20 per cent. high. For example, B.C.S. 241/1, which contains 20 per cent. of tungsten and 5·03 per cent. of chromium, gave a result of 6·00 per cent. of chromium; and for B.C.S. 220/1, which contains 6·9 per cent. of tungsten and 5·13 per cent. of chromium, our result was 6·16 per cent. of chromium.

The other disadvantage of this dissolution is the length of time it needs for completion. For a 1-g sample it could take as much as 2 hours, whereas with perchloric acid the average time is about 20 minutes. Hence, it was decided to adopt the perchloric acid solvent for a scheme for determining chromium and molybdenum in all steels except those for which molybdenum is to be determined in the presence of more than 0·5 per cent. of tungsten.

CHOICE OF CALIBRATION RANGE—

The reasonably small range of the molybdenum concentrations posed no calibration problems. In general a range of 5 to 50 mg l^{-1} (corresponding to 0·05 to 0·5 per cent. of molybdenum in a 1-g sample) was used, and sample dilutions were made when necessary. For higher concentrations of molybdenum, a range of 10 to 100 mg l^{-1} was used with a slightly angled burner, and for lower concentrations 2 to 20 mg l^{-1} was used (see Table I) with scale expansion. In all cases the calibration graphs are linear.

Chromium, being present over a much greater range of concentrations, needed a different approach. For chromium concentrations up to 5 per cent., a calibration range of 5 to 50 mg l^{-1}, in conjunction with further dilutions of the sample as necessary, was found to be adequate, but for chromium concentrations between 5 and 25 per cent., large dilutions (of the order of 100 times) were required, and predictably the precision was poor.

This position was greatly improved by using a calibration range of 25 to 300 mg l^{-1} and a fully rotated burner (which in the case of the SP90 is about 50° from the optical axis). This range is equivalent to a concentration of 1·0 to 12·0 per cent. of chromium in a 0·25-g sample made up to 100 ml. The greatest dilution necessary for the range of steels analysed was then five times (Table II). Rotating the burner also reduced the flame noise, which was sometimes a problem with the 5 to 50 mg l^{-1} range and was probably a further reason for the poor precision obtained with results greater than 5 per cent. The calibration graph for the 25 to 300 mg l^{-1} range is slightly curved, while those for the 5 to 50 mg l^{-1} and 10 to 100 mg l^{-1} ranges are linear.

OPERATING PARAMETERS—

A careful study of the observation height and fuel flow-rates showed that for chromium the fuel flow was not critical. The optimum sensitivity was obtained for a "red-feather"

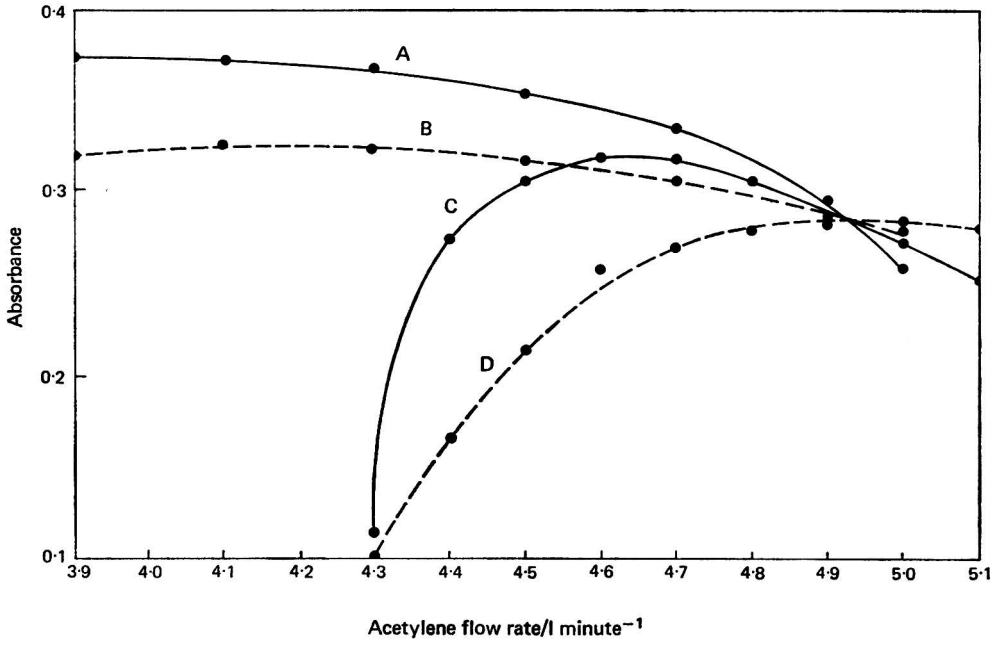

Fig. 2. Effect of fuel flow-rate on absorbance of chromium and molybdenum: A, chromium *plus* 1 per cent. of iron in perchloric acid; B, aqueous chromium solution; C, molybdenum *plus* 1 per cent. of iron in perchloric acid; and D, aqueous molybdenum solution

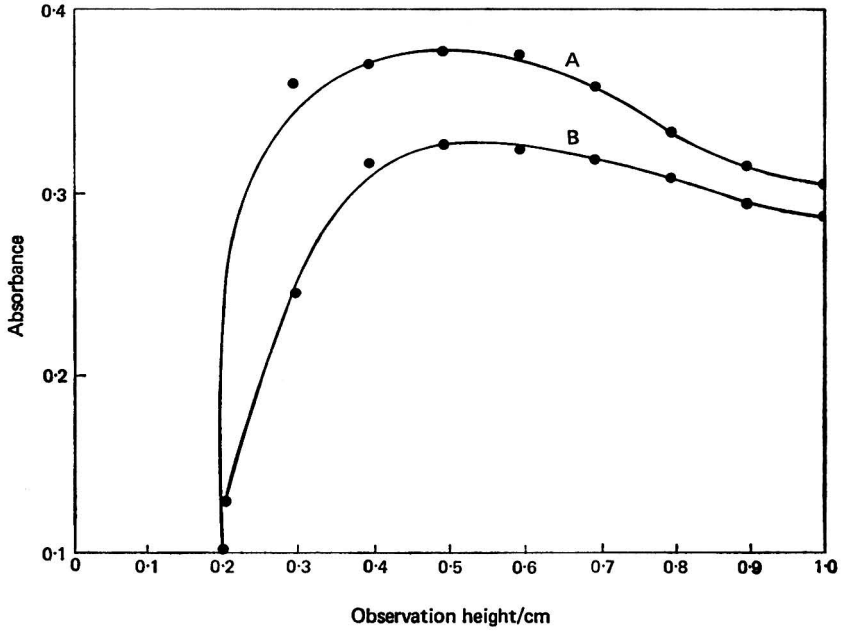

Fig. 3. Effect of observation height on chromium: A, chromium *plus* 1 per cent. of iron in perchloric acid; and B, aqueous chromium solution

height of about 0·5 to 1·0 cm. Conversely, for molybdenum, the fuel flow-rate was found to be very critical so that the flame must be carefully adjusted in order to obtain maximum sensitivity. This corresponds to a fuel-rich flame which has a maximum red-feather height without luminescence (Fig. 2).

The optimum observation height for chromium is 0·60 cm (Fig. 3); but in practice a height of 0·50 cm was preferred, as this somewhat reduced the level of noise contributed by the flame. Similarly, for molybdenum an observation height of 0·5 cm was preferred in practice to the optimum at 0·4 cm because of flame-noise limitations (Fig. 4).

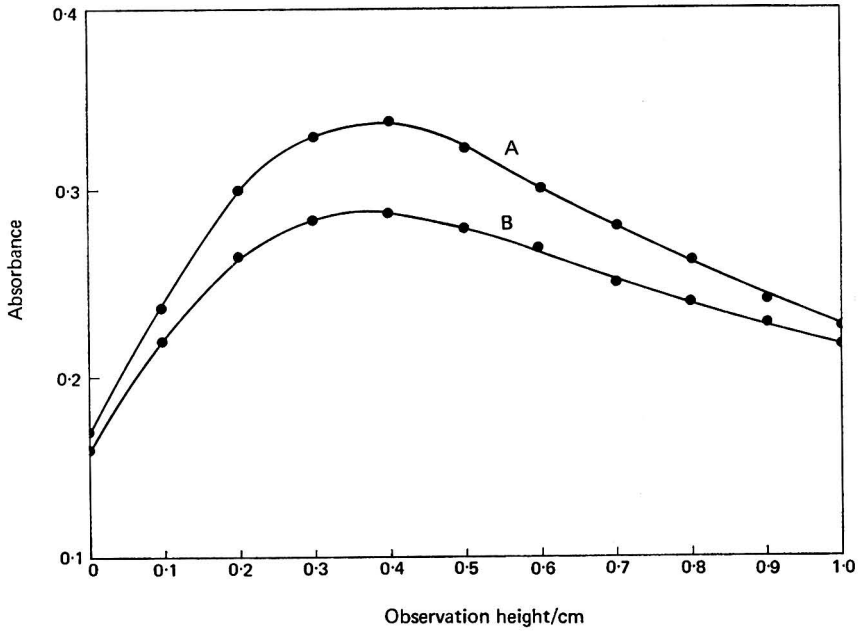

Fig. 4. Effect of observation height on molybdenum: A, molybdenum *plus* 1 per cent. of iron in perchloric acid; and B, aqueous molybdenum solution

Proposed method

Reagents—

Analytical-reagent grade acids were used throughout.

Perchloric acid, sp.gr. 1·54.

Hydrochloric acid, sp.gr. 1·18.

Nitric acid, sp.gr. 1·42.

Perchloric - phosphoric - sulphuric acid mixture—To 300 ml of water add 100 ml of perchloric acid, 100 ml of phosphoric acid (sp.gr. 1·75) and 100ml of sulphuric acid (sp.gr. 1·84).

Stock chromium solution, 1 000 *mg* l^{-1}—Dissolve 1·000 g of 99·99 per cent. chromium metal in 30 ml of hydrochloric acid and dilute to 1 litre.

Stock molybdenum solution, 1 000 *mg* l^{-1}—Dissolve 1·829 g of analytical-reagent grade ammonium molybdate tetrahydrate in water and dilute to 1 litre.

Working stock solutions, 100 *and* 500 *mg* l^{-1}—Prepare these solutions when required by diluting the stock chromium and stock molybdenum solutions with water. These should be prepared at least weekly.

Stock iron solution, 5 *per cent.*—Dissolve 5 g of high-purity iron (B.C.S. 260/2) in 40 ml of hydrochloric acid and 5 ml of nitric acid. When the reaction is complete, add 20 ml of perchloric acid and evaporate until fumes of perchloric acid just appear. Cool and dilute to 100 ml with water.

METHOD 1—

For all chromium determinations and for molybdenum when the tungsten concentration is below 0·5 per cent.

Preparation of sample solutions—Weigh 1·0000 g of sample into a 250-ml beaker and dissolve in 10 ml of hydrochloric acid and 5 ml of nitric acid. After the initial reaction has subsided, add 10 ml of perchloric acid and evaporate until the solution is fully oxidised and fumes of perchloric acid appear. If chromium is present at concentrations greater than 0·3 per cent., this can be seen to be achieved when the solution turns red and perchloric acid refluxes on the sides of the beaker. Fume for about 5 minutes, cool, and dissolve the soluble salts in about 50 ml of water. Filter through a Whatman No. 541 filter-paper, wash well with water, and dilute to 100 ml. For chromium concentrations above 5 per cent., take a 25-ml sample, add 15 ml of stock iron solution, and dilute to 100 ml. This is equivalent to a chromium range of 1·0 to 12·0 per cent. for a calibration range of 25 to 300 mg l^{-1}. For chromium concentrations higher than 12 per cent. it is necessary to dilute the sample further and add 5 per cent. stock iron solution to maintain iron at a constant level of 1 per cent. when making dilutions.

Preparation of calibration solutions—To each of seven 250-ml beakers transfer 1·0 g of high-purity iron and suitable volumes of chromium and molybdenum stock solutions, depending on the required range of the element to be determined (Tables I and II). Use the same dissolution procedure as specified above for the samples. It is imperative that the chromium and molybdenum stock solutions be added before fuming takes place.

TABLE I

MOLYBDENUM CALIBRATION SOLUTIONS

(*i*) For molybdenum up to 0·2 per cent.							
Volume of 100 mg l^{-1} stock solution/ml	0	2·0	5·0	10·0	15·0	20·0	
Concentration/mg l^{-1}	0	2	5	10	15	20	
Molybdenum (1-g sample), per cent. ..	0	0·02	0·05	0·10	0·15	0·20	
(*ii*) For molybdenum up to 0·5 per cent.							
Volume of 500 mg l^{-1} stock solution/ml	0	1·0	2·0	4·0	6·0	8·0	10·0
Concentration/mg l^{-1}	0	5	10	20	30	40	50
Molybdenum (1-g sample), per cent. ..	0	0·05	0·1	0·2	0·3	0·4	0·5
(*iii*) For molybdenum up to 1·0 per cent.							
Volume of 1000 mg l^{-1} stock solution/ml	0	1	2	4	6	8	10
Concentration/mg l^{-1}	0	10	20	40	60	80	100
Molybdenum (1-g sample), per cent. ..	0	0·1	0·2	0·4	0·6	0·8	1·0

NOTE—
A final volume of 100 ml is used throughout.

TABLE II

CHROMIUM CALIBRATION SOLUTIONS

(*i*) For chromium up to 0·5 per cent.							
Volume of 500 mg l^{-1} stock solution/ml	0	1·0	2·0	4·0	6·0	8·0	10·0
Concentration/mg l^{-1}	0	5	10	20	30	40	50
Chromium (1-g sample), per cent. ..	0	0·05	0·1	0·2	0·3	0·4	0·5
(*ii*) For chromium up to 4·0 per cent.							
Volume of 1000 mg l^{-1} stock solution/ml	0	1	2	4	6	8	10
Concentration/mgl^{-1}	0	10	20	40	60	80	100
Chromium (0·25-g sample), per cent. ..	0	0·4	0·8	1·6	2·4	3·2	4·0
(*iii*) For chromium up to 12·0 per cent.							
Volume of 1000 mg l^{-1} stock solution/ml	0	2·5	5	7·5	10	20	30
Concentration/mg l^{-1}	0	25	50	75	100	200	300
Chromium (0·25-g sample), per cent. ..	0	1	2	3	4	8	12

NOTE—
A final volume of 100 ml is used throughout.

METHOD 2—

For molybdenum determinations where the tungsten concentration is above 0·5 per cent.

Preparation of sample solutions—Weigh 1·000 0 g of sample into a 250-ml beaker, add 50 ml of the perchloric - phosphoric - sulphuric acid mixture and heat gently. When the sample has dissolved, oxidise the solution by dropwise additions of nitric acid and evaporate the solutions until the first fumes of perchloric acid appear. Cool, dilute to about 50 ml, filter through a Whatman No. 541 filter-paper and dilute to 100 ml.

Preparation of calibration solutions—To each of seven 250-ml beakers add 1·0 g of high-purity iron and suitable volumes of molybdenum stock solutions, depending on the molybdenum range (Table I). Use the dissolution procedure specified above for the samples.

Solutions prepared according to Method 2 can also be used for the determination of tungsten. In this case the appropriate range of tungsten concentrations should be added to the calibration solutions.

TABLE III

INSTRUMENTAL CONDITIONS

	Chromium	Molybdenum
Wavelength/nm	357·9	313·3
Slit width/mm	0·05	0·05
Burner	5-cm N_2O with 0·5-mm slot	
Observation height/cm	0·5	0·5
Nitrous oxide /l minute^{-1}	5·0	5·0
Acetylene/l minute^{-1}	4·2	4·7
Lamp current/mA	7	10

The acetylene flow-rates in the above table should be used only as a general guide. For molybdenum the flow must be carefully adjusted to give a flame with the maximum red-feather height without luminescence. For chromium, a red-feather height of 0·5 to 1·0 cm is sufficient.

ANALYSIS—

Set up the instrument for each element under the conditions specified in Table III. Aspirate the blank and calibration solutions followed by the sample solutions. Plot absorbance *versus* concentration for each element and read the concentration of the element in the sample.

To improve precision of reading at higher concentrations, *e.g.*, above 5 per cent., a difference technique[24] can be used. In the case of chromium, calibration standards are prepared for the range 150 to 250 mg l^{-1}. Zero absorbance is set by using the lower standard and sufficient scale expansion is introduced so that the highest standard gives an absorbance scale reading between 0·8 and 1·0. Samples are then run in the normal way. The low standard should be run between samples so that the operating base-line is maintained and any short-term drifts are immediately detected.

TABLE IV

DETERMINATION OF CHROMIUM IN STEELS

Steel type	B.C.S. No.	Value found, per cent.	Certificate value, per cent.	Certificate range, per cent.	Other major elements present, per cent.
Carbon steel	218/3	0·16, 0·16, 0·16	0·14	0·13 to 0·15	—
Mild steel	321	0·12, 0·11, 0·11	0·11	0·10 to 0·11	—
Low alloy	252/1	0·40, 0·40, 0·40	0·42	0·40 to 0·43	Ni 2·2, Mo 1·1
Nickel - chromium - molybdenum steel	219/3	0·72, 0·72, 0·72	0·76	0·75 to 0·76	Ni 2·5, Mo 0·6
Low alloy	257/1	2·98, 2·88, 2·94	2·97	2·95 to 3·01	—
Austenitic stainless	336	17·3, 17·5, 17·3	17·6	17·6 to 17·7	Ni 9·5, Mo 2·4
Ferritic stainless	339	12·4, 12·4, 12·5	12·4	12·3 to 12·5	—
Austenitic stainless	334	25·4, 25·7, 25·4	25·6	25·5 to 25·7	Ni 20·6
Stainless	341	24·3, 23·9, 23·7	24·0	23·9 to 24·1	—
High-speed steel	241/1	4·70, 4·70, 4·70	5·03	5·00 to 5·05	W 19·6, V 1·6
High-speed steel	220/1	5·04, 5·10, 5·08	5·13	5·12 to 5·16	W 6·9, V 2·1, Mo 5·2

Discussion

The results obtained for a series of British Chemical Standard steels are summarised in Tables IV and V.

The perchloric acid method (Method 1) was chosen for general use because of its speed of operation and accuracy for any steel, except those bearing tungsten, for which a molybdenum determination is required. We feel that this is justified, because one would normally be aware that a particular steel contains tungsten before it is analysed. Even in the presence of large amounts of tungsten chromium is best determined in the perchloric acid solution, though somewhat low results were obtained for B.C.S. 241/1 and B.C.S. 220/1, as will be seen from Table IV. If a range of molybdenum determinations is to be carried out for steels either with or without tungsten, Method 2 can be successfully used throughout for any molybdenum concentration.

Table V

Determination of molybdenum in steels

Steel type	B.C.S. No.	Value found, per cent.	Certificate value, per cent.	Certificate range, per cent.	Other major elements present, per cent.
Carbon steel	218/3	0·03, 0·03, 0·03	0·034	0·032 to 0·036	—
Mild steel	321	0·07, 0·07, 0·07	0·068	0·064 to 0·072	—
Mild steel	324	0·17, 0·17, 0·17	0·17	0·16 to 0·18	—
Low alloy	257/1	0·16, 0·15, 0·15	0·17	0·16 to 0·18	Cr 3
Nickel - chromium - molybdenum steel ..	219/3	0·58, 0·60, 0·60	0·60	0·59 to 0·61	Ni 2·5
Low alloy	252/1	1·08, 1·10, 1·08	1·11	1·09 to 1·13	Ni 2·2
Low alloy	256/1	0·54, 0·54, 0·53	0·53	0·50 to 0·55	Cr 2·4, Mn 1·0
Austenitic stainless ..	336	2·36, 2·40, 2·36	2·43	2·39 to 2·46	Cr 17·6, Ni 9·5
High-speed steel ..	241/1	0·56, 0·56, 0·56	0·52	0·51 to 0·52	W 19·6, Cr 5·0, Co 5·7, V 1·6
High-speed steel ..	220/1	5·30, 5·30, 5·30	5·20	5·15 to 5·27	W 6·9, Cr 5·0, V 2·1

It may be noticed from Fig. 2 that at one particular gas flow-rate both the chromium and the molybdenum absorption responses are independent of the iron concentration. This suggests that if the particular value could be adhered to, there would be no need for iron to be present in the calibration solutions for either chromium or molybdenum. Unfortunately, the observation cannot be utilised in practice as this value would have to be determined for individual instruments (owing to variations in individual flow meters); and in any case the accuracy of the analysis would be critically dependent upon the gas flow-rates. In the case of chromium, for example, if the error in making the flow-meter setting were 0·1 l minute^{-1}, the difference between the absorbance value of chromium with iron present and that without iron present would be 0·01, which on a sample absorbance value of, say, 0·3 would be equivalent to an analytical error of about 3 per cent. The addition of iron to calibration solutions is a far simpler method of ensuring accurate results.

It is important to use pure chromium metal and not potassium dichromate to make up the chromium standards, as potassium reduces ionisation of chromium in the nitrous oxide - acetylene flame in the standards only, thus causing low results to be obtained for the samples.

The method lends itself ideally to a rapid general scheme of analysis suitable for the determination of many elements on one solution. We believe that the problems previously encountered with chromium and molybdenum determinations may now be successfully overcome for a complete range of steels in common use. We have in fact been able to determine high concentrations of chromium in other alloys, specifically Nimonic and Stellite, by the same method, the effects of different compositions always being overcome by maintenance of an iron concentration of 1 per cent. in the prepared solution.

As Welz[23] has already shown, there is no fundamental reason why atomic-absorption spectrophotometry should not give the precision of some other methods, such as volumetric and potentiometric titrations, for high levels of chromium. This precision may be achieved with a difference technique. Coefficients of variation for 6 results (3 different weighings on each of two different occasions) for each of the 2 highest chromium concentrations, samples B.C.S. 334 and B.C.S. 341, were calculated to be 0·46 per cent. and 0·96 per cent., respectively.

High precision in the determination of major components is attainable in atomic-absorption analysis by careful attention to the following—

(1) Reduction of analytical sensitivity so that final readings fall within the most accurate absorbance range. For most instruments this is 20 to 200 times the quoted sensitivity value.[25] Reading accuracy is improved by using a difference technique.

(2) The same care must be taken over sample preparation as in other precision methods.

(3) Frequent checks should be made for sources of instrumental drift.

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The Fluorimetric Determination of Zirconium as 8-Hydroxyquinolinate in the Presence of Titanium, Tungsten and Molybdenum

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A fluorimetric method is described for the determination of small amounts of zirconium as 8-hydroxyquinolinate. The procedure, which involves extraction of the chelate with chloroform from a hydrofluoric acid - ammonium tartrate solution at pH 9·0, permits the determination of zirconium in the presence of titanium, tungsten and molybdenum.

Excitation is conducted at 398 nm and emission measured at 520 nm. Down to 0·1 μg of zirconium can be determined with a coefficient of variation of 2·3 per cent.

Because of the overlap of the absorption bands (within the range 350 to 400 nm) of the complexes formed by 8-hydroxyquinoline with zirconium, titanium, tungsten and molybdenum, the possibility of determining zirconium spectrophotometrically in mixtures of these complexes is excluded unless it is previously separated by extraction, which results in co-extraction of titanium.[1,2]

The present authors[3,4] have studied the luminescence properties of these complexes. At room temperature neither the titanium complex nor the molybdenum complex emits, and emission of the zirconium chelate is much higher than that of tungsten 8-hydroxyquinolinate.

Use has been made of the Van Santen, Schlewitz and Toy[1] extraction method for determining zirconium in attempts to eliminate the effect of competing absorption as well as of collisional quenching when the ratios of titanium, tungsten and molybdenum to zirconium were too high.

The application of this technique to each of these complexes has shown that both titanium and molybdenum are partially extracted and that tungsten is not extracted. Hence, it has proved possible to determine zirconium, within the limits 0·1 to 1·1 μg ml^{-1}, in the presence of the other cations mentioned. Titanium, which causes the most severe interference, can be tolerated up to concentrations ten times higher than that of zirconium.

Experimental

Apparatus—

Absorption spectra were determined with a Perkin-Elmer 400 spectrophotometer. Fluorescence measurements were made with an Aminco spectrofluorimeter (American Instruments Co.), fitted with a 150-W xenon arc lamp and IP28 photomultiplier, and equipped with a X-Y recorder. Silica cells of 10-mm path length were used.

Reagents—

Analytical-reagent grade chemicals and double-distilled water were used throughout.

Metal solutions (1 *mg ml*$^{-1}$)—These were prepared from ammonium molybdate tetrahydrate (Johnson, Matthey Co.), sodium tungstate (Merck) and zirconium oxychloride (Aldrich). The titanium solution was prepared by dissolving an adequate amount of the metal (Johnson, Matthey Co.) in a platinum dish with a few drops of hydrofluoric and nitric acids. After concentrating the solution nearly to dryness, the residue was taken up in 0·5 M tartaric acid.

8-Hydroxyquinoline—This reagent (Merck) was purified by crystallisation from ethanol - water (1 + 1) and by final sublimation in a vacuum.

The chloroform used both for extractions and measurements was purified by washing it successively with 2 per cent. sodium hydroxide and water, drying over anhydrous calcium chloride and distilling with percolation through anhydrous silica gel. The solvent thus obtained shows about a 50 per cent. transmission at 250 nm against water and does not fluoresce.

PREPARATION OF 8-HYDROXYQUINOLINATES—

Chelates were prepared according to the well known techniques of Welcher[5] and Vogel.[6] Trace amounts of 8-hydroxyquinoline present in the chelates were eliminated by sublimation under vacuum. The purified complexes were analysed and their formulae were found to agree to within 5 per cent. with those given by the above-mentioned authors, *i.e.*, Zr(8-hydroxyquinolinate)$_4$, WO_2(8-hydroxyquinolinate)$_2$, TiO(8-hydroxyquinolinate)$_2$ and MoO_2(8-hydroxyquinolinate)$_2$. It is assumed that the composition of the complexes does not change with extraction.

SPECTRAL CHARACTERISTICS OF THE 8-HYDROXYQUINOLINATES—

The absorption and emission spectra of the complexes were determined by directly dissolving the 8-hydroxyquinolinates in chloroform, readings being made immediately after dissolution and de-aeration of the resulting solutions by bubbling nitrogen through them (Note).

Fig. 1 shows the overlap of the absorption spectra bands of the 8-hydroxyquinolinates, together with the higher wavelength absorption band of 8-hydroxyquinoline.

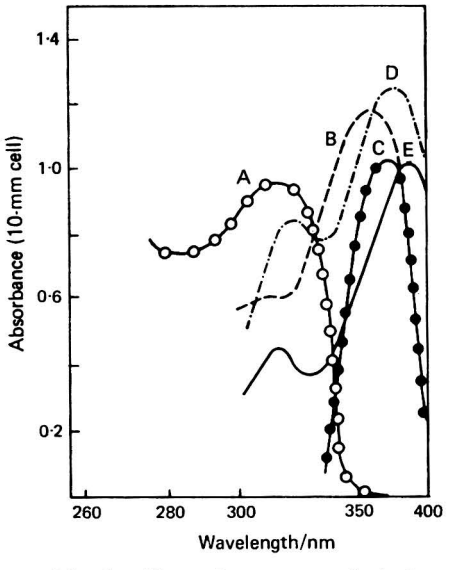

Fig. 1. Absorption spectra of: A, 3×10^{-4} M 8-hydroxyquinoline; B, $2{\cdot}5 \times 10^{-4}$ M tungsten 8-hydroxyquinolinate; C, 3×10^{-4} M molybdenum 8-hydroxyquinolinate; D, 2×10^{-4} M titanium 8-hydroxyquinolinate; and E, 1×10^{-4} M zirconium 8-hydroxyquinolinate. Solvent chloroform

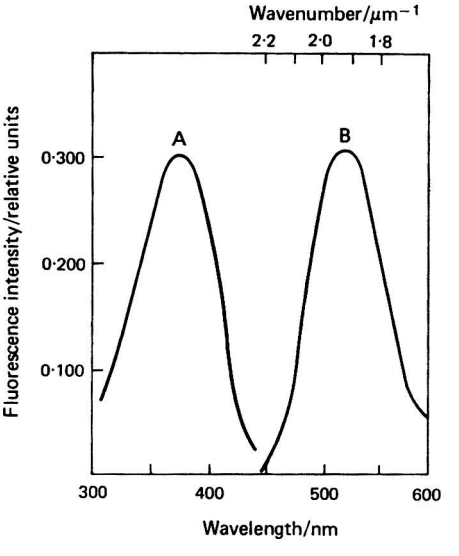

Fig. 2. Excitation spectrum (A) and fluorescence spectrum (B) of zirconium 8-hydroxyquinolinate. Concentration 1×10^{-5} M. Solvent chloroform

The excitation and emission spectra of the zirconium complex can be seen in Fig. 2. Table I includes characteristic values of wavelengths at their maxima, molar extinction coefficients, fluorescence yield and sensitivity for the complexes.

The fluorescence yield was calculated according to the method of Parker and Rees[7] by measuring it against quinine sulphate, the yield of which[8] was taken as equal to 0·55 after correcting the emission spectrum by applying the method of Melhuish.[9]

NOTE—Fluorescence measurements are not reproducible unless the test solutions are aerated to an equal extent, which could be achieved by saturating the system with air, but readings for the fluorescence of zirconium 8-hydroxyquinolinate would then be about 10 per cent. lower than in a de-aerated atmosphere. Therefore, it is advisable to bubble nitrogen through the tightly capped cell (a sleeve-type rubber stopper was used), by using a pair of hypodermic needles.

TABLE I

SPECTRAL CHARACTERISTICS OF 8-HYDROXYQUINOLINATES

8-Hydroxyquinolinate	$\lambda_{abs.}$/ nm	$\epsilon \times 10^{-3}$	$\lambda_{exc.}$/ nm	$\lambda_{em.}$/ nm	$\epsilon \times 10^{-3}$ (398 nm)	Φ	S*
Zirconium	385	10·5	398	520	9·2	0·05	460
Tungsten	358	4·9	370	486	1·2	0·002	2·4
Titanium	380	6·4	—	—	5·8	—	—
Molybdenum	372	3·5	—	—	2·5	—	—
8-Hydroxyquinoline ..	315	3·0	—	—	—	—	—

* S denotes the fluorescence sensitivity calculated by multiplying the fluorescence yield by the molar extinction coefficient at the excitation wavelength of the zirconium complex. Both complexes exhibit analogous widths of emission band; consequently this has not been taken into account in the calculation.

STUDY OF THE INTERFERENCE OF TUNGSTEN, TITANIUM AND MOLYBDENUM—

The sensitivity of emission of the zirconium 8-hydroxyquinolinate is about two hundred times higher than that of the tungsten chelate, when both are excited at 398 nm.

Even although the titanium and molybdenum 8-hydroxyquinolinates do not emit at room temperature, the fraction of light these complexes absorb lowers the emission of zirconium as the molar extinction coefficients at that excitation wavelength are: $5{\cdot}8 \times 10^9$ l mol^{-1} cm^{-1} for titanium and $2{\cdot}5 \times 10^9$ l mol^{-1} cm^{-1} for molybdenum.

The ligand itself does not exhibit fluorescence emission at room temperature; its lower energy band is centred at 315 nm and its absorbance diminishes to zero at about 350 nm. A reference graph of concentration between 1×10^{-6} and 1×10^{-5} M of solutions of zirconium 8-hydroxyquinolinate in chloroform plotted against emission intensity (relative units), as determined by previous calibration at 100 per cent. emission with a 1 μg ml^{-1} solution of quinine sulphate in 0·1 N sulphuric acid, gave a linear relationship for concentrations between 2×10^{-6} and 2×10^{-5} M.

The efficiency and course of the extraction procedure were studied by preparing a spectrophotometric calibration graph for each of the complexes, measurements being made at the wavelength of maximum absorbance. Generally, all of them obey Beer's law at low concentrations.

The extraction technique applied is that of Van Santen, Schlewitz and Toy,[1] which was slightly modified as follows: 10 ml of 2 M hydrofluoric acid in 0·5 M tartaric acid were added to 0·5 ml of a 10 μg ml^{-1} aqueous solution of zirconium, the solution was neutralised to pH 7 with 2 M ammonia solution and the pH finally was adjusted to between 8·9 and 9·0 with 0·1 M ammonia solution. The volume of the aqueous solution was maintained at 45 ml and 1 ml of 1 per cent. solution of 8-hydroxyquinoline in acetone was added. After 30 minutes, the solution was shaken twice, each time for 30 s, with two successive 10-ml portions of chloroform and the extracts were combined and diluted to 25 ml with pure solvent, with the addition of anhydrous sodium sulphate.

The fluorescence intensity of zirconium 8-hydroxyquinolinate was measured at different concentrations and the values obtained were plotted against corresponding zirconium concentrations as determined from the calibration graph from the spectrophotometric readings (Table II).

TABLE II

RECOVERY OF ZIRCONIUM 8-HYDROXYQUINOLINATE IN THE CHLOROFORM EXTRACT

Amount of zirconium (per 45 ml) taken/ μg	Amount of zirconium (per 45 ml) found spectrophotometrically/ μg	Fluorescence intensity
4·95	4·95	7·2
9·90	9·92	18·0
14·85	14·83	29·3
24·75	24·72	39·9
49·50	49·47	50·7

The same extraction procedure was applied to each of the remaining chelates individually and the percentage extraction was calculated on the basis of the respective spectrophotometric calibration graphs. Under these conditions, titanium 8-hydroxyquinolinate follows zirconium in order of extraction and tungsten extraction is nil, most probably because under the conditions used the pH was too far from the optimum for extraction of the tungsten complex.[10]

About 11 per cent. of the molybdenum chelate and about 20 per cent. of the titanium complex are extracted.

The effect of these elements on the emission of zirconium was studied by using mixtures of zirconium with various proportions of the interfering elements and measuring the emission of the chloroform solutions after extraction (Table III).

TABLE III

EXTRACTION OF ZIRCONIUM 8-HYDROXYQUINOLINATE IN THE PRESENCE OF TUNGSTEN, MOLYBDENUM AND TITANIUM

Zirconium concentration 0·33 μg ml^{-1}

Sample No.	Concentration of added interfering element in aqueous phase/μg ml^{-1}			Zirconium found/μg ml^{-1}
	Titanium	Molybdenum	Tungsten	
1	—	—	—	0·31
2	0·33	0·33	0·33	0·30
3	0·66	1·65	1·65	0·31
4	0·99	1·65	1·65	0·30
5	0·99	3·30	3·30	0·31
6	1·65	3·30	3·30	0·32
7	1·98	3·30	3·30	0·30
8	0·33	4·95	4·95	0·31
9	3·30	3·30	3·30	0·27
10	4·95	3·30	3·30	0·20
11	0·33	6·60	6·60	0·25

Samples 1 to 8: standard deviation as coefficient of variation, 2·3 per cent.

Although several other metal 8-hydroxyquinolinates might be capable of being extracted, most of them, mainly the 8-hydroxyquinolinates formed with bivalent cations, could be complexed with cyanide and thus retained in the aqueous phase under the experimental conditions. Other metals, such as aluminium, do not interfere in our simpler system because of the presence of both fluoride and tartrate. However, the proposed technique has been developed with the intention of solving the problem posed by refractory materials and high temperature alloys, such as molybdenum-base alloys, which are of increasing importance in the aerospace industry.

RESULTS

Measurement of the fluorescence emission of zirconium 8-hydroxyquinolinate permits the determination of zirconium at very low concentrations with good reproducibility.

Experimental values obtained in the presence of tungsten, titanium and molybdenum after extraction into chloroform from an aqueous hydrofluoric acid - ammonium tartrate solution at pH 9·0 show that it is possible to determine 0·33 μg ml^{-1} of zirconium in quaternary mixtures in which the contents of molybdenum and tungsten are ten times and the content of titanium is six times as high as the amount of zirconium.

Within these ratios, emission values for the zirconium complex are the same as those for the pure 8-hydroxyquinolinate.

Titanium interference is found to be much more severe than that of molybdenum, which is in agreement with the values for the molar extinction coefficients of their 8-hydroxyquinolinates at the excitation wavelength for zirconium (398 nm), as that of the titanium complex is more than twice that of the molybdenum complex.

A series of determinations conducted at a concentration level of 0·33 μg ml^{-1} gives a coefficient of variation of 2·3 per cent.

Most of the sensitive methods available for the determination of zirconium require the absence of complexing anions such as fluoride, tartrate and citrate, which do not affect the proposed method. Spectrophotometric techniques based on the reaction of zirconium with quercetin are affected by titanium; those based on the reaction with thorin and pyrocatechol violet by both titanium and molybdenum; and interference by titanium, molybdenum and tungsten, among the metals tested, occurs when alizarin is used as the reagent. Also, the well known indirect spectrofluorimetric procedure for determining zirconium with morin, which is greatly dependent on the composition of the system, is affected by fluorides. These limitations therefore preclude the use of these methods without prior application of separation techniques, which outweigh the higher sensitivities claimed for several of them.

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A Rapid Atomic-absorption Technique for the Determination of Lithium in Silicate Materials

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A simple and rapid atomic-absorption procedure is described for the determination of lithium in silicate rocks. Following fusion with sodium borate, the samples are dissolved in dilute nitric acid solution. Determinations of the lithium contents are made directly. The precision of the method, in terms of one standard deviation, was calculated to be 1093 ± 13 p.p.m. and 34·5 ± 4·6 p.p.m. Accuracy, as indicated by a comparison with published determinations, is good.

ATOMIC-ABSORPTION techniques have been described that enable lithium to be determined successfully in silicate rocks and minerals (Stone and Chesher,[1] and Ohrdorf[2]). However, sample preparation is somewhat lengthy and involved, usually entailing dissolution with hydrofluoric and other acids. In our laboratories, a simple scheme of silicate analysis involving fusion with lithium metaborate has been successfully used for some time (Suhr and Ingamells,[3] and Medlin, Suhr and Bodkin[4]). By substituting sodium borate for lithium metaborate, lithium can be determined with adequate precision down to about 5 p.p.m. in the sample. Other elements can be determined with results similar to those already described by Medlin, Suhr and Bodkin.[4,5]

METHOD

INSTRUMENTATION—

A Perkin-Elmer, Model 303, atomic-absorption spectrophotometer with an air - acetylene burner of 4-inch path length and a strip-chart recorder was used. A multi-element hollow-cathode device (Ca–Mg - Al–Li, Westinghouse, Type WL23035) was used and the operating conditions were those recommended in the manufacturer's Analytical Methods Manual. A scale-expansion setting of 3× was used for lithium values of less than 200 p.p.m. For higher values scale expansion was not necessary.

REAGENTS—

Analytical-reagent grade chemicals were used throughout.

Lithium standards—A stock solution of lithium was prepared by dissolving 0·532 4 g of lithium carbonate in 50 ml of dilute nitric acid and diluting to 1 litre with distilled water. This stock solution is equivalent to 5 per cent. of lithium in the sample. Two ranges of standards (equivalent to 0 to 200 p.p.m. and 0 to 2 000 p.p.m. of lithium in the sample) were prepared by making appropriate dilutions of the stock solution with a blank solution (U.S.G.S. Peridotite, PCC-1[6]). This provided lithium standards in a silicate matrix similar to the samples being studied, and eliminated any differences in viscosity between samples and standards.

Nitric acid, 3 per cent.

Anhydrous sodium borate, $Na_2B_4O_7$.

PROCEDURE—

With the exception of substituting sodium borate for lithium metaborate, the procedure has already been outlined in some detail by Suhr and Ingamells[3] and Medlin, Suhr and Bodkin.[4] Briefly, it is as follows: 80·0 mg of 200-mesh sample is mixed with 400 mg of sodium borate by shaking in a plastic vial on a mechanical shaker for about 15 s. The mixture is placed in a graphite fusion crucible (Union Carbide L-4400) and fused for 10 minutes at 1 000 °C in a muffle furnace. The molten bead is poured directly into a beaker containing 40·0 ml of 3 per cent. nitric acid solution. A magnetic stirring bar is added and the mixture placed on a magnetic stirring unit until dissolution is complete (about 10 minutes). This solution, without further treatment, is used for the determination of lithium.

If only a few lithium determinations are to be made, a warm-up period of 20 minutes for the atomic-absorption unit is satisfactory. However, as there is a tendency for the energy output of the hollow cathode to change rapidly in the first 15 to 30 minutes of operation, it is wise to allow a warm-up period of 1 hour or more before proceeding with many determinations. Ten to twenty samples are run between standards to overcome long-term shifts in absorption in the fashion standards A, B, C . . ., samples 1, 2, 3, . . ., . . ., 3, 2, 1, standards . . . C, B, A. An average of the two readings is then taken. We have found this technique to be successful in overcoming any long-term absorption changes.

DISCUSSION

Stone and Chesher[1] found no interferences from the common major elements in silicate rocks and minerals. Consequently we did not systematically investigate the effect of various elements and concentrations thereof on the determination of lithium. In addition, the results given in Table I do not indicate any serious interferences. Good agreement is found between our values and other published values, although there are exceptions. Our values for lithium in iron mica and in magnesium mica are slightly lower than those reported. However, the concentrations in these two samples are not established, as indicated in the footnote to Table I. Also, an independent determination of lithium in the iron mica by a chemical separation - flame photometric technique in our laboratories gave 0·10 per cent. of lithium, which substantiates our lower value.

TABLE I

COMPARISON OF LITHIUM VALUES

Sample	Lithium, p.p.m. Found by atomic-absorption spectrophotometry	Present
Diabase W–1	17	12*
Granite G–1	24	24*
Granite G–2	35	38†
Granodiorite GSP–1	30	33†
Andesite AGV–1	15	13†
Basalt BCR–1	12	15†
Granite GR	65	55‡
Granite GA	88	100‡
Granite GH	40	42‡
Basalt BR	10	12‡
Biotite, iron mica	1090	1400§
Phlogopite, magnesium mica	114	250§
Serpentine UB–N	29	30‖
Diorite, DR–N	42	40‖
Syenite S–1	120	120¶

* Recommended values (Fleischer[7]).
† Average of values reported (Flanagan[6]).
‡ Proposed value (de la Roche and Govindaraju[8]).
§ Preliminary values (de la Roche and Govindaraju[8]).
‖ Proposed value for UB–N and average of four values for DR–N (de la Roche and Govindaraju[9]).
¶ Mean value reported (Sine *et al.*[10]).

Precision, as indicated in Table II, and the observed detection limit of 5 p.p.m. in the sample are more than adequate for most geochemical studies. The detection limit could easily be improved by using greater scale expansion, but we did not find it necessary.

Table II

Precision results for lithium

Sample	Concentration, p.p.m.	Standard deviation, p.p.m.	Number of determinations*
Granite G–2	34·5	4·6	26
Biotite, iron mica	1093	13	16

* For G–2 determinations were made on ten sub-samples on four different days. For iron mica determinations were on three sub-samples on six different days. No results were rejected; the odd number of determinations is caused by not running all of the sub-samples each day.

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An Improved Method for the Determination of Bacitracin in Animal Feeds

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The determination of small amounts of bacitracin in animal feeds has been studied. It was expected that by using acidified methanol as the only organic solvent in the extraction procedure and phosphate buffer, undesired proteins would not be dissolved and the use of the toxic solvent pyridine might be avoided. Bacitracin could then be determined in the final extract by using microbiological methods, in which methanol interferes less than pyridine. The method reported here has been used for feed mixtures with various zinc bacitracin contents in the range from 5 to 350 p.p.m.

ZINC bacitracin is frequently used as an additive in animal feeds and has a growth-promoting effect. In the absence of chemical methods for the determination of bacitracin in feedingstuffs, the antibiotic has to be assayed microbiologically[1] after solvent extraction. Although zinc bacitracin is known to be soluble in water,[2] experiments show that an acid as well as an organic solvent is required to dissolve all the zinc bacitracin present in a feedingstuff sample.[3]

The method most widely used for the determination of bacitracin involves the acid - pyridine extraction of zinc bacitracin as described by Craig.[4] Following this extraction, methanol is added to the extract in order to precipitate proteins and, after removal of the pyridine, the amount of bacitracin present is determined microbiologically.[1] This method is referred to in this paper as the pyridine method.

In this work an attempt has been made to simplify the extraction of zinc bacitracin by using an organic solvent that is less toxic than pyridine and would not necessarily have to be removed before the microbiological stage of the assay for bacitracin.

METHOD

REAGENTS—

Phosphate buffer solution, pH 6·5—Dissolve 22 g of potassium monohydrogen orthophosphate and 28 g of potassium dihydrogen orthophosphate in 1 litre of distilled water.

Acidified methanol—A 2 per cent. v/v solution of concentrated hydrochloric acid (sp.gr. 1·19) in methanol.

Bromocresol purple solution—Dissolve 0·1 g of the indicator in 18·5 ml of 0·01 N sodium hydroxide and dilute the solution to 250 ml with water.

It is recommended that reagents of analytical-reagent grade are used in the preparation of these solutions.

Zinc bacitracin stock solution—Dissolve 34 mg of zinc bacitracin of known potency, *i.e.*, 2 500 i.u. of bacitracin (Note 1), in 5 ml of 0·1 N hydrochloric acid. Add 10 ml of water and adjust the pH to 5 with N sodium hydroxide solution. Dilute the resulting solution to 50 ml to give a concentration of bacitracin equivalent to 50 i.u. ml^{-1}. When stored at a temperature between 5 and 8 °C, this solution is stable for 1 week.

NOTE 1—The zinc bacitracin was standardised against the Second International Standard for Bacitracin. This substance is available from Division of Biological Standards, National Institute of Medical Research, Mill Hill, London, N.W.7. It contains 74 i.u. mg^{-1} of bacitracin.

PREPARATION OF STANDARD SOLUTION—

Dilute the stock solution with sufficient phosphate buffer to give a solution containing 0·05 to 0·1 i.u. ml^{-1} of bacitracin. This solution should be prepared immediately before use.

PREPARATION OF STANDARD SOLUTION MODIFIED WITH EXTRACT OF UNSUPPLEMENTED FEED—

Carry out the extraction and dilution of an unsupplemented sample (Note 2) of the feedingstuff under test as described below (under Procedure A or B). Add a suitable amount of stock solution to the diluted extract to give a solution containing 0·05 to 0·1 i.u. ml^{-1}.

NOTE 2—If no unsupplemented samples are available for blanks, the following procedure is recommended. Add a known increment of zinc bacitracin to the unknown sample and determine its recovery by assay. Use the recovery value for calculation of the potency of the unknown sample. An alternative method is the use of autoclaved samples although with this technique it was not always possible to obtain complete inactivation of zinc bacitracin. Also, some feed components decomposed giving dubious blanks.

PREPARATION OF ASSAY SOLUTIONS OF FEEDINGSTUFFS—

Procedure A for feedingstuffs containing 20 *to* 350 *p.p.m. of zinc bacitracin* (*Note* 3)—Weigh a 10-g sample of feedingstuff into a mortar. Triturate the sample for 2 minutes with 25 ml of acidified methanol in order to obtain a solution of pH 2 (if necessary, add concentrated hydrochloric acid dropwise until the required pH is obtained). Then add 25 ml of phosphate buffer and transfer the resulting mixture to a 250-ml Erlenmeyer flask, shaking it for 20 minutes following the transfer. Centrifuge the mixture for 5 minutes at 4 000 r.p.m. and dilute the supernatant liquid thus obtained with the phosphate buffer to give a final solution containing 0·05 to 0·1 i.u. ml^{-1} of bacitracin (Note 4).

Procedure B for feedingstuffs containing 5 *to* 20 *p.p.m. of zinc bacitracin*—Triturate a 10-g sample of feedingstuff in a mortar with 25 ml of acetone in order to remove the fat (if any). Decant the acetone, let the feed sample dry in air at a temperature below 35 °C and carry out the zinc bacitracin extraction as in the above procedure.

Transfer 20 ml of the supernatant liquid into a 250-ml round-bottomed flask, add 1 ml of bromocresol purple solution and then add N sodium hydroxide solution dropwise until the indicator has changed colour (at pH 6·5). Immerse the round-bottomed flask in water at 30 °C, connect it to a rotary evaporator and evaporate the extract to dryness at reduced pressure. Next, dissolve the residue in phosphate buffer solution and transfer this mixture quantitatively into a calibrated flask, diluting to the mark. A solution containing 0·05 to 0·1 i.u. ml^{-1} of bacitracin (Note 4) results, *e.g.*, if the residue of a feedingstuff sample containing 5 p.p.m. of bacitracin is dissolved to 10 ml, the solution will contain 0·084 i.u. ml^{-1} while the residue from a sample containing 20 p.p.m. yields 0·067 i.u. ml^{-1} when dissolved to 50 ml.

NOTE 3—It has recently been shown (Grynne, unpublished work) that this method, with slight modifications, is satisfactory for high-potency feed supplements containing mainly calcium carbonate or kaolin. The potencies of the supplements tested were 10^4 or 4×10^4 p.p.m. of zinc bacitracin.

NOTE 4—For transforming international units into milligrams of bacitracin most of the feed industry has accepted the definition 42 i.u. = 1 mg, which has been used in this work, but it should be mentioned that the Second International Standard for Bacitracin contains 74 i.u. mg^{-1}. (The potency of the U.S.A. Food and Drugs Administration Working Standard is 62 i.u. mg^{-1}.)

MICROBIOLOGICAL DETERMINATION OF THE BACITRACIN CONTENT IN THE ASSAY SOLUTION—

Single-point assay—Test the assay solution against the standard or the modified standard solution by means of the agar diffusion method described in detail by Grove and Randall,[1] with the slight modification that test plates with a single layer of about 10 ml of agar should be used instead of two-layer plates. Holes in the agar can be used instead of cylinders, but paper discs should be avoided since these increase the interference by non-antibiotic components. Calculate the amount of zinc bacitracin in the feedingstuff from the following equation—

$$\frac{c \times v \times e \times 1\,000}{g \times d \times 42} = \text{p.p.m. of zinc bacitracin in the feed,}$$

where c is the concentration of bacitracin found (by assay) in international units per millilitre; v is the volume of the assay solution in millilitres; e is the volume of extraction liquids used (acidified methanol, hydrochloric acid and phosphate buffer) in millilitres; g is the weight of the feed sample in grams; d is the volume of extract diluted to v ml in millilitres; and 42 is the amount of bacitracin in international units per milligram of zinc bacitracin.

Two-point assay (*samples with more than* 100 *p.p.m. of zinc bacitracin*)—Dilute the extract (in the work described in this paper a two-point assay was carried out on samples 13 to 16, see Table II) to two levels (approximately 0·1 and 0·2 i.u. ml^{-1} of bacitracin) and test against two zinc bacitracin standard solutions with 0·1 and 0·2 i.u. ml^{-1}, by using a satisfactory assay design. For example, twelve agar plates, each with four cylinders filled with the four different solutions, are used for one sample. Carry out the calculation as suggested by Pitton,[5] check the validity of the assay and then, if this is satisfactory, calculate the potency of the test solution.

Results and discussion

The solubility of zinc bacitracin present in feedingstuffs was studied. Several organic extraction liquids (methanol, ethanol, propanol, ethylene glycol monoethyl ether and dimethylformamide) in combination with various amounts of hydrochloric acid were tried and it was shown that methanol had the best properties, *i.e.*, lower toxicity and boiling-point than pyridine, the property of not dissolving undesired proteins, and the ability to dissolve zinc bacitracin.

Experiments were initiated to study the possibility of avoiding the time consuming evaporation of the extraction liquids prior to the microbiological test. Preliminary attempts to investigate the influence of the extraction liquids on the size of the inhibition zones were therefore made and by means of zinc bacitracin standard solutions it was demonstrated that the inhibition zones were considerably increased by the presence of more than 1 per cent. v/v of pyridine. Similar experiments with methanol showed that the microbiological test was not seriously affected by the presence of up to 15 per cent. v/v of methanol in the assay solution (Table I).

Table I

Influence of extraction liquids on the zone sizes

Solution* number	Pyridine content of assay solution, per cent. v/v	Methanol content of assay solution, per cent. v/v	Zone diameter/mm	Apparent change of bacitracin potency† caused by solvent, per cent.
1	0	—	17·10	—
2	0·3	—	16·94	−5
3	0·5	—	17·16	+4
4	1·0	—	17·24	+8
5	2·0	—	20·10	+320
6	—	2	16·94	−5
7	—	4	16·96	−4
8	—	8	17·02	−2
9	—	10	16·94	−5
10	—	15	16·94	−5
11	—	20	16·80	−10
12	—	25	16·22	−28

* Each of solutions 2 to 12 was tested in thirty-six cylinders against an equal number of cylinders filled with solution 1.

† Bacitracin potency of all solutions = 0·1 i.u. ml^{-1}. Diluent, phosphate buffer solution (pH 6·5).

A more detailed examination of the influence of 15 per cent. v/v of methanol was carried out. In this examination, six zinc bacitracin solutions (0·05, 0·1, 0·2 i.u. ml^{-1} of bacitracin, with and without 15 per cent. v/v of methanol) were tested microbiologically in a (3 + 3) design. On the basis of the observed zone diameters (fourteen zones from each solution) statistical calculations were carried out as suggested by Pitton.[5] It could be shown that the apparent change of antibiotic potency, due to the methanol, was 2 per cent. Further, the two regression lines were found to be parallel and linear.

It was now calculated that when Procedure A was followed, convenient concentrations of methanol (15 per cent. v/v or less) and of bacitracin (0·05 to 0·1 i.u. ml^{-1}) were obtained only for diluted extracts originating from feedingstuffs containing at least 20 p.p.m. of zinc bacitracin. In order to show that the proposed method was suitable for zinc bacitracin in feedingstuffs, assays were carried out on samples with 20 to 350 p.p.m. of zinc bacitracin. The good agreement between the results by Procedure A and the pyridine method is shown

in Table II. Similarly, the results for feedingstuffs containing 5 to 20 p.p.m. of zinc bacitracin (obtained by use of Procedure B, Table II) were in good agreement with those obtained with the pyridine method.

It was thought that soluble ingredients from the basic feed might be able to influence the extraction of bacitracin as well as the formation of inhibition zones by inactivating the bacitracin during its extraction, by reducing the diffusion rate or by affecting the micro-organism. Modified standard solutions were therefore produced by adding blank extracts to the standard solutions. It seemed, however, to make no difference whether a standard or a modified standard was used (Table II).

Unfortunately, there are numerous recipes for the compression of feedingstuffs, most of which are unknown to the analyst, and it is still not fully known whether or not any feed components could interfere in the bacitracin extraction and the microbiological assay. The precise rôle of the zinc atom in the antibacterial activity of bacitracin has also not yet been defined, and attention should be drawn not only to the concentration of zinc, but also to possible metal binding agents[6] or other metals[7] present in the extracts, assay solutions, agar media, etc.

Therefore, to minimise inaccuracy in the assay, it is recommended that either modified standards are used, as described in this work or, alternatively, that the quantitative zinc bacitracin recovery of the feed is checked.

TABLE II

COMPARISON OF METHANOL AND PYRIDINE FOR THE EXTRACTION OF BACITRACIN FROM FEEDINGSTUFFS*

	Sample tested against standard	Sample tested against modified standard	
	Procedure A‡		Pyridine method
Sample number†	Bacitracin, p.p.m.	Bacitracin, p.p.m.	Bacitracin, p.p.m.
1	19	22	21
2	20	21	21
3	27	31	22
4	27	26	28
5	25	25	23
6	48	42	44
7	49	49	45
8	51	51	48
9	105	113	95
10	148	151	153
11	233	235	221
12	327	347	333
13§	94	—	90
14§	132	—	131
15§	191	—	206
16§	260	—	262
	Procedure B‡		
17	3·6	3·5	3·9
18	5·0	4·6	4·8
19	5·3	5·2	4·5
20	4·5	5·1	5·2
21	10·1	9·5	10·6
22	10·0	10·0	9·8
23	9·0	9·4	9·6
24	9·3	9·0	10·0
25	9·1	9·2	11·0
26	20	21	21
27	21	20	18
28	20	22	24

* Each result is the mean of three determinations, *i.e.*, extraction and biological test.

† For assays 1 to 16, 20 and 25 the same basic feed was used. Remaining samples were of various compositions.

‡ Procedure A required no evaporation of methanol prior to the microbiological test, whereas procedure B did require an evaporation.

§ Assays 13 to 16 were two-point assays, while the remainder were single-point assays.

Conclusions

An improved method for the determination of bacitracin in different feedingstuffs containing 5 to 350 p.p.m. of zinc bacitracin has been developed. The most suitable organic extraction liquid tried out, in combination with hydrochloric acid, appeared to be methanol. The maximum amount of methanol in feedingstuff extracts that did not interfere in the assay was found to be 15 per cent. v/v. For feedingstuffs containing at least 20 p.p.m. of zinc bacitracin the evaporation of the solvent prior to the microbiological test was unnecessary. The results obtained with this method compared favourably with those obtained with the pyridine method.

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Chromatographic Separation and Titrimetric Determination of the Aphicide Menazon in Paste Formulations

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Paste formulations of menazon were chromatographed on a column of acidic alumina. The emulsifying agents and other active ingredients were eluted with hexane, diethyl ether and chloroform. Menazon was finally eluted with dioxan and was then titrated potentiometrically with 0·1 N perchloric acid in dioxan - acetic acid (1 + 1).

MENAZON (I.S.O. common name for *S*-(4,6-diamino-1,3,5-triazin-2-ylmethyl)dimethyl phosphorothiolothionate), which combines high systemic toxicity to aphid species with very low toxicity to humans,[1] is considered one of the best aphicides currently available.

Excluding powders for seed dressing, the formulations containing this active ingredient, because of its very low solubility in any solvent, come in the form of thick pastes or emulsifiable suspensions. At the present time, the methods of analysis for menazon, both in formulations and residues, are exclusively based on the colorimetric determination of phosphorus after ashing and oxidation of the sample. Paper[2,3,4] or thin-layer[5] chromatographic methods have been used for the separation and detection of menazon residues; but, in any case, the quantitative determination has been carried out by colorimetry, even though different colorimetric procedures were used. For example, Camoni, D'Antonio, Gandolfo, Leoni, Ramelli and Sampaolo[3] used Calderbank and Turner's technique,[2] slightly modified following Beremblum and Chain,[6] for determining menazon in olive oil, while Plant Protection Ltd.,[4] the European manufacturer of menazon, reports the use of Calderbank and Turner's method[2] based upon the technique of Chen, Toribara and Warner[7] for determining menazon residues in various crops. Previously, Calderbank and Turner's method has been used for formulations also. It provides for a separation of the active ingredient on an ion-exchange resin before the colorimetric determination of phosphorus. This method is laborious because of the need for ashing of the sample, its dissolution and its passage through the resin, the last step needing rather critical conditions.

Our laboratories have conducted investigations into an analytical method for a formulation containing menazon, other active ingredients and a very high percentage of emulsifying and dispersing agents. It was found that menazon recoveries were not quantitative when Calderbank and Turner's method was used. In fact, with both pure and technical menazon in paste formulations we have obtained large errors with deviations of −14 to −16 per cent. We were not able to explain such large errors, so to obviate these difficulties we tried other analytical techniques. In view of the weak basicity of the menazon molecule (pK_a 3·8),[2] a titrimetric method in non-aqueous medium was tested. Pure menazon gave good results; however, direct determinations on the formulation were not possible because of some interferences caused by the surfactants and by active ingredients other than menazon. Therefore, we thought of exploiting both the very low solubility of menazon in most organic solvents and its polarity characteristics to separate it from the co-formulants by column chromatography. Many chromatographic adsorbents were tested, but only acidic alumina was found to be efficient. The co-formulants were eliminated by elution with hexane, diethyl ether and chloroform. The menazon was finally eluted with dioxan. The active ingredient thus separated was potentiometrically titrated in the non-aqueous solvent dioxan - acetic acid (1 + 1) with 0·1 N perchloric acid by using glass - calomel electrodes.[8] The recoveries were quantitative. This method has been successfully applied to the industrial control of only one type of paste formulation, but it seems reasonable to assume that it can be applied to other kinds of liquid, paste or powder formulations based on menazon.

Method

Apparatus—

Chromatographic column—300 × 25 mm i.d., fitted with a sintered-glass medium-porosity septum and with a Teflon stopcock.

Potentiometer—This is equipped with glass - calomel electrodes. A Beckmann Research pH meter was used.

Magnetic stirrer.

Reagents—

Aluminium oxide, acidic—Brockmann grade I.

Solvents—Diethyl ether, chloroform, glacial acetic acid, dioxan and hexane, all analytical-reagent grade.

Perchloric acid—A 0·1 N solution in glacial acetic acid.

Procedure—

Fill the chromatographic column with sufficient alumina to make a layer 4 cm deep after settling. Wet the column with hexane and allow a 3 to 4-cm layer of this solvent to remain over the alumina. Accurately weigh into a 100-ml beaker sufficient of the sample to contain approximately 0·5 g of menazon. Add to the beaker 5 g of acidic alumina and mix well with a glass rod to a homogenous and dry powder (help the homogenisation if necessary with 8 to 10 drops of diethyl ether). Transfer the powder to the column by using a powder funnel. Add an additional 3 g of alumina to the beaker. Mix well, stirring the mixture and scraping the beaker walls with a glass rod. Transfer this powder to the column. Rinse the beaker, the glass rod, the funnel and the column walls with two 5-ml portions of hexane. Cover the alumina column with a glass-wool plug. Allow the solvent used for the washings to be completely absorbed, then elute sequentially with 150 ml of hexane, 350 ml of diethyl ether and 400 ml of chloroform. The elution rate for all the solvents is not important. Discard the three eluates, unless they contain other active ingredients to be determined. (In our case, the hexane fraction contained pyrethrins, while the diethyl ether fraction contained piperonyl butoxide). Finally, elute the menazon with dioxan. Collect 300 ml of eluate in a tall 500-ml beaker, completely evaporate the dioxan on a water-bath with the aid of a current of air, and take up the residue in 100 ml of dioxan - acetic acid (1 + 1). Titrate this solution with 0·1 N perchloric acid in glacial acetic acid solution, by using a potentiometer equipped with a glass - calomel electrode and with magnetic stirring.

Calculation—1 ml of 0·1 N perchloric acid is equivalent to 28·1 mg of menazon (molecular weight 281).

$$\text{Menazon content of the sample, per cent.} = \frac{a \times 2{\cdot}81}{w}$$

where a is the volume in millilitres of 0·1 N perchloric acid used, and w is the sample weight in grams.

Results and discussion

The formulations analysed were thick suspensions containing 40 per cent. of active ingredient, together with a high percentage of emulsifying and dispersing agents and with other active ingredients. The accuracy of the method was established by recovery tests on mixtures containing all the ingredients *plus* pure menazon, and with tests on some industrial formulations. The results are shown in Tables I and II.

Table I

Determinations on formulations containing pure menazon

Menazon added/g	Menazon found/g	Recovery, per cent.	Mean recovery, per cent.
0·50	0·501	100·2	99·25
0·50	0·492	98·4	
0·50	0·495	99·0	
0·50	0·497	99·4	

Three blanks each gave a menazon determination of 0·000.

TABLE II

DETERMINATION ON FORMULATIONS CONTAINING TECHNICAL QUALITY MENAZON

Menazon present, per cent.	Menazon found, per cent.	Recovery, per cent.	Mean recovery, per cent.
40	39·2	98·00	98·56
40	39·7	99·25	
40	39·2	98·00	
40	39·6	99·00	

Blank tests were carried out, and no interferences were observed from reagents or co-formulants.

The method is faster and simpler than the commonly accepted colorimetric method. Furthermore, it does not need the preparation of specific reagents. It has been found to give satisfactory results for production control purposes, but impurities arising from decomposition of a formulated sample on long storage may be eluted with the menazon fraction and be titrated with the perchloric acid. The method cannot therefore be recommended on the basis of the work so far carried out for the determination of menazon in stored samples. The method has been shown to eliminate the incorporated co-formulants under examination; other co-formulants will have to be examined in detail to ensure that there is no interference in the menazon titration.

Trace amounts of water present in the sample could interfere in the titration, but they would be adsorbed first by the alumina added to the sample before the transfer to the column.

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A Rapid and Accurate Method for the Determination of Molybdenum in Plant Materials with Toluene-3,4-dithiol

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A rapid and accurate method is described for determining molybdenum in plant materials with toluene-3,4-dithiol. The molybdenum is allowed to react with the reagent in a solution with a sulphuric acid concentration of about 6 N. Prior separation of the element from the acid digest of the plant material is unnecessary, there being no loss of sensitivity or accuracy if this process is omitted.

Several workers have demonstrated the rôle of molybdenum in the metabolic processes of plants[1,2,3,4] and higher animals.[5] With the latter much attention has recently been focused on the interaction of this element with copper and inorganic sulphate ions that occur in the ruminant.[6,7] In all such organisms molybdenum is needed in small amounts and therefore very sensitive methods must be used for its determination. In addition, speed and accuracy of determination are often necessary in the study of the rôle of this element in these biological systems. In this paper a rapid and accurate, but yet very sensitive, method for determining molybdenum in plants with toluene-3,4-dithiol is described, which does not involve prior separation of the element.

Method

Apparatus—

A Unicam SP500 spectrophotometer was used for absorbance measurements in 1-cm glass cells.

Reagents—

Toluene-3,4-dithiol, 0·2 per cent. solution—Warm a 1-g ampoule of dithiol on a steam-bath to melt the yellowish solid. Then pour the molten compound into 0·5 N sodium hydroxide, with stirring to ensure its complete dissolution, and make the volume up to 500 ml with 0·5 N sodium hydroxide; add 10 ml of thioglycollic acid (anti-oxidant). This reagent keeps for at least 3 months in completely filled glass-stoppered bottles stored in a refrigerator.

Standard molybdenum solution—Prepare a 1000 μg ml^{-1} stock stolution of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$ in 0·5 N sulphuric acid; prepare from this solution the required concentrations by appropriate dilution.

The other reagents used were of analytical-reagent grade and de-ionised water was used for making the solutions.

Procedure—

Wet oxidise a suitable weight of oven-dry plant material (1 to 5 g) with nitric acid (sp.gr. 1·42), sulphuric acid (sp.gr. 1·84) and 60 per cent. perchloric acid. For each sample weight taken, use an amount of sulphuric acid that will give a concentration of this acid of about 6 N in the final aqueous solution in which the molybdenum - toluene-3,4-dithiol complex is to be precipitated. If the sample weight is between 1 and 2 g, simply pour the cooled sulphuric acid digest directly into a 50-ml Nessler tube in which the complex is to be formed, rinsing the flask with water and adding the rinsings. If the sample weight is more than 2 g, filter the digest through a sintered-glass funnel to remove the silica, if a comparatively large amount of the latter is present, and then transfer the filtrate and rinsings to the Nessler tube. Mix the aqueous solution and make the volume up to about 30 ml. Add 3 ml of 0·2 per cent.

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toluene-3,4-dithiol and mix the solution with a glass rod. Allow the tube to stand in a water-bath at a temperature of 75 °C for 20 minutes. Remove the tube, cool it and quantitatively transfer the contents to a 100-ml separating funnel. Add 5·0 ml of carbon tetrachloride and shake the funnel vigorously for 1 minute to extract the green complex into the organic layer. Filter the green solution through cotton-wool and then read the absorbance of the solution at 682 nm.[8] Construct a standard graph for the molybdenum concentration range of 0 to 2·5 p.p.m.

Results

Precision test—

This work was carried out with replicate determinations of molybdenum on the pasture plants *Brachiaria ruziziensis* (1-g samples were used), *Chloris gayana*, *Panicum maximum*, *Pennisetum purpureum* and *Paspalum commersonii* (2-g samples were used for the last four). The results obtained are shown in Table I.

Table I

Replicate determination of molybdenum in leaves of five pasture plants

Plant	Number of replicates	Molybdenum content, p.p.m.	Coefficient of variation
Brachiaria ruziziensis	20	4·25	1·4
Chloris gayana	20	0·25	10·0
Panicum maximum	20	0·39	10·2
Pennisetum purpureum.. ..	20	0·34	5·8
Paspalum commersonii ..	20	0·39	7·7

Accuracy test—

The accuracy of the method was tested by comparing the molybdenum contents of plant tissues determined by the present method and by Piper and Beckwith's method.[9] The results are shown in Table II.

Table II

Molybdenum contents of pasture plants by the present method and by Piper and Beckwith's method

Plant	Plant part	Molybdenum content, p.p.m.	
		Present method	Piper and Beckwith's method
Brachiaria ruziziensis	Leaf	4·25	4·21
Chloris gayana	Leaf	0·25	0·23
Panicum maximum	Leaf	0·39	0·38
	Stem	0·25	0·27
Brachiaria congo	Stem	0·52	0·50
Pennisetum purpureum	Leaf	0·34	0·38
Paspalum commersonii	Leaf	0·39	0·41

Possible interference by copper—

Piper and Beckwith[9] noted that a standard copper solution containing about 20 μg, when complexed with dithiol, gave an absorption comparable with that of 2 μg of molybdenum when pentyl acetate was used as the solvent, but in the present work the same copper concentration gave an absorbance equivalent to the blank reading at 682 nm. Hence, no appreciable interference from copper is experienced in this method.

Completeness of extraction of the complex—

Extractions were carried out in two sets of ten replicate determinations of molybdenum in *Brachiaria ruziziensis*. In one set 10·0 and 5·0-ml volumes of carbon tetrachloride were used in two consecutive extractions of the complex. In the second set 5·0 and 5·0-ml volumes of the solvent were used for the extractions. With both sets, the molybdenum concentration

in the second extraction amounted to no more than that found in the blanks, which indicates that the coefficient of extraction of the complex into carbon tetrachloride on vigorously shaking the mixture is very good, especially as the aqueous volume was several times larger than the organic phase. Thus a single extraction of the complex with 5·0 ml of carbon tetrachloride was sufficient.

Discussion and conclusion

The results of both the precision and accuracy tests show that molybdenum can be determined accurately with toluene-3,4-dithiol in plant tissues without prior separation of the molybdenum. The high level of acidity at which the complex is formed prevents interference by tungsten,[9] which reacts with the reagent at a much lower acidity level[10] (pH 0·5 to 2·0). The small amount of silica that may be present in the acidic solution was found to have no effect on the formation and extraction of the complex. Because of the high sulphuric acid concentration small amounts of sulphate tend to precipitate before the addition of the reagent.

The good coefficient of extraction of the complex into carbon tetrachloride with vigorous shaking for 1 minute permits a small volume of the organic layer to be used in conjunction with the large aqueous volume in which the complex is formed. Thus there is no loss of sensitivity and a considerable saving in time, which would otherwise have been spent needlessly on the prior separation of the molybdenum.

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Determination of Sub-microgram Amounts of Cobalt in Plants and Animal Tissues by Extraction and Atomic-absorption Spectroscopy

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Atomic-absorption spectroscopy is used to determine cobalt in plants and animal tissues after solvent extraction of the 1-nitroso-2-naphthol complex from an acidic solution of the sample. The solvent is evaporated and the complex is dissolved in ethyl methyl ketone for aspiration. The method is more sensitive than, and as accurate and precise as, a colorimetric procedure involving nitroso-R salt, and is much less tedious.

Cobalt can be determined in the range 0·05 to 1 μg in the final 1-ml sample solution (corresponding to 0·05 to 1 p.p.m.). With a 5-g sample from 0·01 to 0·2 p.p.m. of cobalt in samples (dry matter) can be determined. The method can, therefore, be applied readily to pasture samples containing 0·1 p.p.m. of cobalt and less, levels that are considered likely to cause deficiency in ruminants. At 0·07 p.p.m. of cobalt the coefficient of variation is 14 per cent. At higher concentrations (0·14 p.p.m.) of cobalt in pasture and liver samples the coefficients of variation are 11 and 6 per cent., respectively.

COBALT concentrations of less than 0·1 p.p.m. (dry-matter basis) are common in pasture and liver samples from the South West of Western Australia, where marginal cobalt deficiency in sheep and cattle is widespread.[1] Established procedures for analysis of agricultural materials[2,3,4,5] for cobalt generally involve a preliminary separation followed by a spectrophotometric or spectrographic determination. For most methods working in the range from 1 to 5 μg of cobalt is recommended and therefore at least 10 g of dry matter per sample of normal pastures (0·1 to 0·5 p.p.m. in dry matter) are required. Such amounts of sample are often not available from field trials, especially when pasture samples are sorted for the analysis of individual species. Consequently, when only 5-g samples are available and concentrations of cobalt are less than 0·2 p.p.m., it may be necessary to determine amounts of cobalt of less than 1 μg. For samples from areas where cobalt deficiency occurs, amounts of cobalt of only 0·05 μg per 5-g sample (0·01 p.p.m. in dry matter) can be determined with this method.

Marston and Dewey's method,[3] with modification of the conditions for colour development with nitroso-R salt, has given satisfactory results in these laboratories, but the method is tedious. Forster and Zeitlin[6] have increased the sensitivity of the nitroso-R salt method, but their investigations also show that the conditions for colour development must be strictly controlled.

Holland and Bozic[7] list a number of reagents and their applications to the determination of cobalt in a variety of samples, and Ure and Mitchell[8] have described an atomic-absorption method for the determination of cobalt in soil extracts, but little attempt appears to have been made to lower the limit of detection in agricultural samples.

The method described depends on the selectivity of 1-nitroso-2-naphthol for separation of cobalt and replaces the final colorimetric determination by atomic-absorption spectroscopy, which is rapid and specific. Increased sensitivity is achieved by aspirating the metal in a volatile solvent. Copper interferes, so is removed by extraction with dithizone.

METHOD

REAGENTS—

1-Nitroso-2-naphthol solution—Dissolve 0·5 g of 1-nitroso-2-naphthol in 100 ml of ethanol. Use reagent that has been recrystallised from light petroleum after extraction in a Soxhlet apparatus.

Ammonium citrate solution—Prepare a 1 M solution of ammonium citrate in water and purify by extraction with a solution of dithizone in chloroform.

Dithizone solution—Dissolve 1 g of dithizone in 500 ml of chloroform.

Bromophenol blue indicator, 0·4 per cent. solution.

Ethyl methyl ketone—Distil and store over anhydrous sodium sulphate.

Nitric, perchloric and sulphuric acids—Analytical-reagent grade concentrated acids are satisfactory.

Ammonia solution, concentrated, sp.gr. 0·89.

Standard cobalt solution—Prepare a 1 p.p.m. solution of cobalt as follows. Add 2 ml of 1-nitroso-2-naphthol solution to 0·5 ml of cobalt stock solution (100 μg ml^{-1}). Stand this solution for 1 hour at room temperature and make the volume up to 50 ml with ethyl methyl ketone. This solution will remain stable for several weeks and is used to optimise instrumental conditions and to provide a check on the recovery of cobalt from standards carried through the procedure.

APPARATUS—

A Techtron, model AA-3, atomic-absorption spectrophotometer with a 100-mm laminar flow burner AB41 and an adjustable nebuliser was used. A recorder would be an advantage but was not used in this work. The cobalt hollow-cathode lamp was manufactured by Atomic Spectral Lamps Pty. Ltd., Melbourne, Australia. The conditions used were: lamp current, 10 mA; wavelength, 240·7 nm; slit width, 50 μm; and flame, air - acetylene.

PROCEDURE—

Digest 5 g of sample with a mixture of nitric, perchloric and sulphuric acids,[9] and dissolve the residue in 50 ml of water (Note 1). Filter through sintered glass if necessary and transfer the solution into a 100-ml separating funnel. Add 10 ml of ammonium citrate solution and one drop of bromophenol blue indicator.

Plant material—Adjust the pH of the solution to 3·5 (green - grey) with concentrated ammonia solution; add 2 ml of 1-nitroso-2-naphthol solution (Note 2), mix, and stand the solution for one hour at room temperature. Add 1 ml of 18 N sulphuric acid and mix. Extract the cobalt complex by using successive portions of 10, 5 and 5 ml of chloroform and shaking the mixture for 60, 30 and 30 seconds respectively. Combine the extracts in a 30-ml beaker. Place the beaker on a steam-bath and evaporate to dryness (Note 3). Dissolve the residue in exactly 1·0 ml of ethyl methyl ketone (Notes 2 and 4), taking care to wash the sides of the beaker with the solvent. To minimise evaporation of solvent and for ease of handling during aspiration, transfer the ethyl methyl ketone solution to small stoppered test-tubes (Quickfit MF24/0). Treat a blank and standards (0·1, 0·4, 0·7 and 1·0 μg of cobalt) similarly.

Animal tissues—Adjust the pH of the solution to 3 (yellow - green) with concentrated ammonia solution. Add 10 ml of dithizone solution and extract the copper by shaking the mixture vigorously for 30 s. Discard the chloroform layer. Repeat with 5-ml portions of dithizone solution until the green colour of the reagent persists in the chloroform layer and finally with 5 ml of chloroform. Add one drop of bromophenol blue indicator to the solution in the separating funnel, adjust the pH to 3·5, and proceed as described above for plant material.

ASPIRATION—

Adjust the acetylene flow while aspirating ethyl methyl ketone to give a slightly rich flame and set the nebuliser to give an aspiration rate of 3 to 4 ml minute^{-1} of ethyl methyl ketone. Aspirate the 1 p.p.m. standard solution and optimise instrumental conditions. This solution should give about 30 per cent. absorption. Aspirate solvent between sample determinations to prevent the flame from lifting off the burner.

Aspirate each sample twice and average the two readings.

NOTES—

1. Contrary to the findings of Hiscox,[10] dry ashing overnight at 550 °C and wet ashing with a mixture of nitric, perchloric and sulphuric acids were equally effective in extracting cobalt from plant material. A sample of lucerne tops gave 0·44 p.p.m. of cobalt after dry ashing (four determinations) and 0·41 p.p.m. after wet ashing (six determinations).
2. Blockage of the nebuliser may occur when more than 2 ml of 1-nitroso-2-naphthol solution are used or if moisture is present in the ethyl methyl ketone.
3. The standards taken through the procedure with each batch of samples have shown extremely efficient and consistent recovery of cobalt. Continued heating of the extract on the steam-bath after evaporation of the chloroform produced a dark residue, which was slow to dissolve in ethyl methyl ketone. Tests showed that heating for periods up to 30 minutes had no effect on the recovery of cobalt.
4. Ethyl methyl ketone was selected from eleven solvents (acetone, pentyl alcohol, pentyl acetate, butanol, ethanol, hexyl methyl ketone, methanol, methyl pentyl ketone, ethyl methyl ketone, isobutyl methyl ketone, and ethyl methyl ketone - pentyl acetate (1 + 1)). Ethyl methyl ketone and acetone gave the greatest (3-fold) enhancement of absorption compared with cobalt in aqueous solution. Ethyl methyl ketone was selected because it has a lower vapour pressure than acetone.

INTERFERENCES—

Table I shows that more than about 500 μg of copper interfered seriously with the recovery of cobalt from standard solutions.

TABLE I

EFFECT OF COPPER ON RECOVERY OF 0·5 μg OF COBALT

Copper present/μg	0	100	200	500	1500	2000	2500
Cobalt recovered/μg	0·50	0·46	0·50	0·35	0·30	0·19	0·12

Copper interference has been reported by Saltzmann.[4] Beck[11] also first extracted copper when separating cobalt with 1-nitroso-2-naphthol from biological ash. Samples of dried animal liver have been found to contain up to 0·1 per cent. of copper (up to 5 mg of copper in a 5-g sample). It is therefore necessary to extract this type of sample with dithizone. This step is not necessary for plant samples.

There was no interference from iron or nickel. Other ions that form 1-nitroso-2-naphtholates,[12] *viz.*, neptunium(V), palladium(II), plutonium(IV), thorium(IV) and uranium(VI), are not present in biological samples in appreciable amounts.

RESULTS AND DISCUSSION

Table II compares results obtained by the atomic-absorption procedure and the colorimetric method of Marston and Dewey.[3] The comparable accuracy of the two methods is indicated by the good agreement of the results.

Table III gives the precision of the method. The liver samples were extracted with a solution of dithizone in chloroform to remove copper.

The precision obtained for pasture sample E is acceptable considering the low concentration of cobalt in the sample. The coefficient of variation of 11 per cent. for pasture sample F was calculated from analyses performed independently by four analysts on different days over a period of several months, and is comparable with that obtained by Ure and Mitchell[8] for the determination of cobalt in water - ethanol solutions of soil extracts. These workers achieved coefficients of variation of 3·25 per cent. and 10 per cent. for solutions containing 0·8 p.p.m. and 0·12 p.p.m. of cobalt, respectively. The precision of the method is as good as or better than that of the colorimetric (nitroso-R salt) method of Marston and Dewey, which has given a coefficient of variation of 14 per cent. for plant samples containing 0·16 p.p.m. of cobalt.[13]

Table IV shows the recovery of cobalt added to samples carried through the procedure. The recoveries of cobalt fall within the range to be expected for a method with a coefficient of variation of 10 per cent.

In some cases the recoveries were low, especially when 5-g samples with high ash content were involved. This probably accounts for the 9 per cent. coefficient of variation for the lucerne sample in Table III. Sample weights ranged from 1 to 5 g in this set of analyses and there was a tendency for the larger samples to give a lower result. This increased the range of the values obtained, and hence the coefficient of variation is greater than is to be expected from a sample containing cobalt at the relatively high concentration of 0·42 p.p.m.

TABLE II

COMPARISON OF THE ATOMIC-ABSORPTION METHOD WITH A COLORIMETRIC METHOD

Sample		Cobalt, p.p.m. on dry basis: Atomic-absorption	Cobalt, p.p.m. on dry basis: Colorimetric
Mixed pasture	19400/67	0·84	0·79
	19402/67	0·40	0·37
	13294/68	0·11	0·13
	13295/68	0·08	0·08
	13296/68	0·11	0·14
Clover	7844/68	0·07	0·07
	7873/68	0·04	0·04
Lucerne	8813/67	0·05	0·06
	8814/67	0·03	0·04
Liver	8123/68	0·70	0·71
	8136/68	0·13	0·15
	8146/68	0·09	0·09
Rock phosphate	10439/68	1·2	1·6
	10441/68	5·9	5·7

TABLE III

PRECISION OF THE METHOD

Sample	Number of analyses	Mean cobalt content, p.p.m.	Coefficient of variation, per cent.
Pasture E	6	0·07	14
Pasture F	36	0·12	11
Liver	6	0·14	6
Liver	6	0·25	6
Lucerne	11	0·42	9

TABLE IV

RECOVERY OF COBALT ADDED TO SAMPLES

Sample	Weight/g	Cobalt/μg: In sample	Cobalt/μg: Added	Cobalt/μg: Found	Recovery, per cent.
Pasture F	2·0	0·22	0·10	0·31	90
	5·0	0·55	0·60	1·15	100
	5·0	0·55	0·60	1·24	115
Rose clover.. ..	4·0	0·26	0·20	0·46	100
	4·0	0·26	0·20	0·40	70
Grass	3·0	0·045	0·10	0·133	88
Weeds	3·0	0·21	0·20	0·42	105
Lucerne	1·0	0·42	1·0	1·44	102
	4·0	1·68	1·0	2·61	93
Lucerne	2·0	0·22	0·50	0·69	94
	5·0	0·55	0·50	0·89	70
Lupin	4·0	0·51	0·50	1·02	102
	4·0	0·51	0·50	0·94	86
Liver	2·0	0·28	0·20	0·49	105
	5·0	0·70	0·40	1·09	98
Liver	0·5	0·10	0·20	0·30	100
Rock phosphate ..	0·4	0·24	0·50	0·74	100
	1·0	0·61	1·0	1·52	91

Conclusion

The method described is suitable for the determination of cobalt in samples of plants and animal tissue and has the advantage that not more than 5 g of sample are required. Precision of the method is acceptable for amounts of cobalt corresponding to critical concentrations in the feed of ruminants. The procedure has been applied successfully to the determination of cobalt in phosphate rock, and it should be suitable for analysis of soil extracts or fusion digests when the direct method of Ure and Mitchell is not sufficiently sensitive.

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Study of Slightly Soluble Metal Chelates by Anodic Stripping Voltammetry

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Anodic stripping voltammetry has been used to study the chelates of ammonium 1-pyrrolidinecarbodithioate with metal ions. A technique is described in which polarographic waves are obtained when the chelates are highly insoluble. The metal is deposited at a hanging mercury drop electrode in the absence of chelating agent. The chelating agent is then added to the solution and the anodic stripping peak is recorded. Metal-to-ligand ratios and dissociation constants are calculated from the shift in the anodic stripping peak compared with the stripping peak obtained in the absence of chelating agent. Advantages include the ability to use polarographic techniques when the chelates are insoluble (and non-reducible), increased sensitivity, and the possibility of using concentrations rather than activities for calculations involving dilute solutions.

POLAROGRAPHY offers a rapid and simple means for studying metal-ion complexes. The change in the potential at which a metal ion is reduced at a dropping-mercury electrode when it becomes complexed is related to the nature and stability of the complex. By using appropriate equations, the metal-to-ligand ratio and the instability constant of the complex can be calculated:

$$E_{\frac{1}{2}(c)} = E_{\frac{1}{2}(s)} + \frac{RT}{n\mathrm{F}} \ln K_d - \frac{RT}{n\mathrm{F}} \ln \frac{f_s k_c}{f_c k_s} - \frac{RT}{n\mathrm{F}} p \ln C_x f_x \ldots \quad \ldots \quad (1)$$

where $E_{\frac{1}{2}(c)}$ is the half-wave potential of the complexed metal ion; $E_{\frac{1}{2}(s)}$ is the half-wave potential of the simple metal ion; K_d is the overall dissociation constant of the complex; f_s and f_c are the activity coefficients of the simple and complexed metal ion, respectively; k_c/k_s, *i.e.*, $(D_c/D_s)^{\frac{1}{2}}$ is the ratio of square root of diffusion coefficients of complexed and simple metal ion; p is the ligand-to-metal ratio in the complex; C_x is the concentration of ligand; and f_x is the activitity coefficient of ligand.

The ratio $\frac{f_s k_c}{f_c k_s}$ can be taken as nearly unity.

Certain difficulties limit the usefulness of conventional polarography for these determinations. First, the concentration of the metal ion must be at least 10^{-5} M and usually greater to obtain a well-shaped polarographic wave. As the complexing agent should be in concentrations about 100-fold or more greater than the metal ion,[1] it follows that measurements cannot be made when a small amount of complexing agent is available. Also, several metal-ion chelates, as well as the chelating agents themselves, have low solubilities, so that polarographic waves will not be observed. It becomes apparent that a more sensitive technique is required.

Anodic stripping voltammetry[2,3] is up to 1 000 times more sensitive than conventional polarography for measuring metal ions in solution. Popova and Stromberg[4] have determined the composition of hydroxyzinc complexes taking part in the electrode process at a stationary mercury-film electrode by anodic stripping voltammetry. The effects of hydroxide-ion concentration on the potentials of anodic peaks of zinc were determined and results were identical with those obtained with a dropping-mercury electrode and agreed also with polarographic results obtained by earlier investigators. Rozhdestvenskaya, Songina and Muldagelieva[5] have determined the stability constants for thiourea and ammine complexes of copper by measuring the shift in the potential of the beginning of anodic dissolution of a copper anode in the

presence of the complexing agents. They used conventional polarographic equations[1] to calculate the constants.

The present paper reports an investigation of anodic stripping voltammetry at a hanging mercury drop electrode for the study of the stoicheiometry and stability of complexes, in particular those which have slight solubility. A technique is described in which anodic stripping peaks are obtained in the presence of a ligand that forms an insoluble chelate with the metal ion.

Chelates of ammonium 1-pyrrolidinecarbodithioate (APDC) with metal ions were chosen for this study. APDC was described by Malissa and co-workers[6,7] as a chelating agent for over thirty metals in acidic solutions. Stetter and Exler[8] at the same time described the use of sodium 1-pyrrolidinecarbodithioate for the solvent extraction of metals in soils. Because it can be used in acidic media, APDC has found wide use in atomic-absorption spectroscopy for the solvent extraction of metals into isobutyl methyl ketone prior to analysis.[9] Despite the importance of APDC and its chelates, little work has been reported on the measurement of stabilities of metal - APDC chelates, mainly because of the generally low solubility of these chelates in aqueous solution. Low solubility is characteristic of other dithiocarbamate chelates, and hence several authors have established stability series based on solubility or solvent extraction.[10] Jansen[11,12,13] has determined the stabilities of copper chelates of di-alkyldithiocarbamic acids and of APDC in water and in water - ethanol mixtures. The chelates were made soluble in various water - ethanol mixtures and the stability constant values determined were extrapolated to the corresponding value in wholly aqueous solution.

Experimental

Reagents—

Analytical-reagent grade chemicals were used without further purification. The purity of the ammonium 1-pyrrolidinecarbodithioate was determined by amperometric titration with copper.[14] Mass spectral analysis indicated that this compound had the correct structure (Matkovich, C. E., and Christian, G. D., unpublished work); there was some concern that it may have had a different structure.[15] A stock 0·10 M solution of APDC was prepared.

Apparatus—

Polarographic measurements were made with a Chemtrix single-sweep Polarographic Analyzer System, Model SSP-3. A Lingane H-cell[16] equipped with a saturated calomel electrode was used for measurements. The hanging mercury drop electrode was prepared and used as previously described,[17] and solutions were stirred with a magnetic stirring bar and a magnetic stirrer.

Procedure—

Twenty millilitres of a 10^{-5} M solution of the metal ion in 0·2 M potassium nitrate were placed in the polarographic cell and were de-aerated with nitrogen. The solution was then pre-electrolysed for 120 s while stirring it at a constant rate. The following pre-electrolysis potentials were used (*versus* S.C.E.): zinc, −1·7 V; cadmium, −1·0 V; and lead, −0·8 V. Following pre-electrolysis, stirring of the solution was stopped and an anodic stripping peak was recorded in the usual manner, which established the stripping peak potential of the unchelated metal. A fresh mercury drop was then suspended from the electrode and the pre-electrolysis step repeated. However, after pre-electrolysis, the circuit was opened and an aliquot of the stock APDC solution was added to the cell to give the desired concentration of APDC (about 10^{-3} to 10^{-2} M). After stirring the solution briefly, the circuit was closed and the anodic stripping peak was recorded; the peak occurred at a more negative potential than that of the free metal ion. Successive peak shifts were obtained over a range of APDC concentrations in increments up to 10^{-2} M by repeating the procedure, starting with a fresh metal-ion solution.

A graph of log (APDC) *versus* E_p (peak potential) was prepared, and the ligand-to-metal ratio was calculated from the slope of the graph while the dissociation constant was calculated from the intercept. The calculations were based on equation (1) except that $E_{\frac{1}{2}}$ values were replaced by stripping peak potentials.

Results and discussion

APDC cathodic and anodic peaks—

Halls, Townshend and Zuman[18] have studied the anodic polarographic waves of mono-alkyldithiocarbamates at the dropping-mercury electrode. They observed a single two-electron wave in very alkaline medium, which split into two one-electron waves as the pH was decreased. These waves were all accompanied by adsorption pre-waves at suitably high concentrations. No cathodic waves were observed. When the metal ions cadmium, mercury(II) and lead were added to the solutions, precipitates formed, which caused the anodic waves to disappear. No cathodic waves corresponding to soluble complexes were found.

In the present study, a single-sweep cathodic scan of 10^{-4} M APDC in 0·2 M potassium nitrate produced a cathodic peak at −0·82 V *versus* S.C.E., the height of which increased with increasing APDC concentration. A cadmium solution gave a peak at −0·61 V. When successively increasing amounts of APDC were added to a 10^{-5} M cadmium solution (in 0·2 M potassium nitrate), a white precipitate occurred while the cadmium peak at −0·61 V decreased; the peak disappeared when the APDC-to-cadmium ratio was equal to 2. Excess of APDC resulted in appearance of the peak at −0·82 V, but no cathodic peaks were found corresponding to a cadmium - APDC chelate, which further illustrates the difficulty in applying conventional cathodic polarographic techniques to the study of slightly soluble complexes.

When anodic stripping analysis was performed on 10^{-3} M APDC in 0·2 M potassium nitrate, two anodic stripping peaks were recorded, at −0·61 and −0·40 V, the second wave being about ten times larger than the more negative wave. These waves were not present, however, when anodic stripping peaks of metals were recorded in the presence of APDC.

A stripping peak for uncomplexed cadmium at 10^{-5} M concentration occurred at −0·54 V, which shifted negatively by about 300 mV when 10^{-3} to 10^{-2} M APDC was added after pre-electrolysis. Two new APDC anodic stripping peaks appeared at −0·45 and −0·1 V, but only when the APDC concentration exceeded a certain level, the more positive peak being much larger than the other peak. With 10^{-6} M cadmium and 10^{-4} to 10^{-3} M APDC, these two peaks occurred at all the concentrations of APDC with potentials of −0·5 and −0·3 V, respectively; therefore, their magnitude and potential depended on the concentrations of cadmium and APDC. It is unlikely that one or both of the APDC waves, in the presence or absence of cadmium, are associated with adsorption processes similar to those reported by Halls *et al.*[18] The same two APDC anodic stripping peaks that were recorded in the presence of cadmium were observed in the presence of lead. The potential region of these waves was not scanned in studies with zinc.

It is difficult to explain the stripping peaks of APDC. The results of Halls *et al.*,[18] in which adsorption pre-waves existed, would suggest that at negative potentials the APDC is adsorbed and then it (or the electrode) is oxidised as the potential is scanned to more positive values.

Attempts were made to take measurements in a buffered solution at pH 3 by using an orthophosphate buffer at 0·2 M ionic strength,[19] but a white precipitate was formed when APDC was added to the buffer.

Metal - APDC stability measurements—

APDC-to-metal ratios and dissociation constants were determined from the magnitude of the negative shift of the metal stripping peak in the presence of various amounts of APDC, by using conventional equations.[1] A precipitate always occurred when APDC was added to the metal-ion solution after pre-electrolysis, but as the APDC was in 100 to 1000-fold excess, the amount of APDC precipitated was assumed to be negligible.

The peak potential was dependent on the peak heights, and hence on the concentration of the metal ion, the stirring rate and the plating time. These variables must therefore be strictly controlled in order to maintain the activity of the metal-amalgam constant for each measurement. Reversibility criteria are the same as for conventional techniques. If the metal amalgam is irreversibly stripped, the peak, even if recorded, may occur at a more positive potential than the reduction peak of the free metal ion.

The magnitude of the initial potential shift depended on the stability of the chelate, the concentration of the chelating agent and the values of n and p. As here we can deal with

more dilute solutions of chelating agent than in conventional methods, the magnitude of the shift may be significantly smaller, which imposes a limitation on the method. While quantitative results are not available, it appears that for reproducibility, a shift of 100 mV is desirable. Based on this assumption, limiting values of dissociation constants with various values of n, p and ligand concentration (C_x) to achieve this shift are listed in Table I, and it can be concluded from these results that the chelate must be more stable as n and p become larger and as C_x becomes smaller in order to achieve this minimal shift of the peak.

TABLE I

MAXIMUM DISSOCIATION CONSTANTS FOR A 100-mV INITIAL POTENTIAL SHIFT

C_x/M	n	p	K_d
10^{-3}	1	1	$10^{-4.7}$
10^{-4}	1	1	$10^{-5.7}$
10^{-3}	1	2	$10^{-7.7}$
10^{-4}	1	2	$10^{-9.7}$
10^{-3}	2	1	$10^{-6.3}$
10^{-4}	2	1	$10^{-7.3}$
10^{-3}	2	2	$10^{-9.3}$
10^{-4}	2	2	$10^{-11.3}$
10^{-3}	2	3	$10^{-12.3}$
10^{-4}	2	3	$10^{-15.3}$

TABLE II

LIGAND-TO-METAL RATIOS AND STABILITIES OF METAL - APDC CHELATES IN 0·2 M POTASSIUM NITRATE

Metal	p*	K_d*	p†	K_d†
Pb^{2+}	2·2	$2·0 \times 10^{-21}$	2	$2·0 \times 10^{-21}$
Cd^{2+}	2·3	$2·6 \times 10^{-16}$	2·3	$3·4 \times 10^{-16}$
Zn^{2+}	1·7	$5·2 \times 10^{-11}$	2·5	$4·9 \times 10^{-10}$

* Based on peak potential.
† Based on half-peak potential.

Results of this study are summarised in Table II. The pH of the unbuffered solutions varied from 6·3 for 10^{-3} M APDC to 6·7 for 10^{-2} M APDC; before adding APDC it was 5·6. The results are the average of two determinations each (three for lead). Calculations were made by using both the peak potential and the half-peak potential. Except for zinc, for which the potential shift was smallest, results by the two methods agreed closely. Reproducibility was best by using peak potentials and use of these is recommended for calculations.

Stability studies of these metals with other dithiocarbamates have been reported. Malatesta[20] determined the relative stabilities of diethyldithiocarbamates, based on solubility, and showed that they decreased in the order copper(II) > lead > zinc > thallium(I). Šedivec and Vašák[21] obtained the same order for copper(II), lead, cadmium and zinc from potentiometric titrations, and this order for these metals was also obtained by replacement extraction procedures.[22] The same order has been obtained in the present work for lead, cadmium and zinc with APDC. In addition, no shift of the stripping peak for thallium (thallium(I) solutions were used) was obtained, which indicated that no stable chelate was formed with APDC. This follows the same trend as indicated above for diethyldithiocarbamates. The duplication of the order of stability is to be expected. It has been demonstrated that there are no significant differences in the chelating properties of several derivatives of dithiocarbamate.[10] The p-values obtained by us agree with those reported by Malissa and Kolbe-Rohde[23] for the APDC chelates of cadmium and zinc. It is unlikely that stepwise formation of the complexes occurs to a significant extent; it is negligible in most instances of formation of dithiocarbamates because of a rather drastic shift of the equilibrium towards precipitation or extraction of the metal chelate.[10]

It is difficult to determine whether a soluble metal chelate is formed at the electrode surface or whether a precipitate is formed. The numbers in Table II would be reasonable solubility product (K_{sp}) values for these chelates. Approximate calculations based on the equations of Reinmuth[24] and Shain and Lewinson[25] indicate that for 10^{-3} M APDC and the

conditions of electrolysis used by us the concentration of metal ion in the diffusion layer at the electrode surface would be more than sufficient to exceed a reasonable solubility product and hence cause precipitation of the chelate in the diffusion layer. It is quite probable, then, that the figures in Table II represent K_{sp} values.

Janssen,[11] by extrapolation of results obtained with non-aqueous solutions, calculated the dissociation constant for the copper(II) - APDC chelate in water to be $1{\cdot}6 \times 10^{-21}$. The relative stability of the copper and lead chelates of diethyldithiocarbamate were shown to be close,[20,21] and the value obtained by Janssen is close to that given for lead in Table II. This would suggest that solubility products and dissociation constants for metal dithiocarbamates may not be very different, and that solubility studies are a good indication of relative stabilities of slightly soluble chelates.

Other techniques of stripping analysis should be useful for studying complexes. For example, anodic stripping pulse voltammetry[26] extends the sensitivity limit 10-fold over d.c. stripping analysis. In addition, millimolar or lower concentrations of supporting electrolyte can be used, so that little or negligible error results in neglecting activity coefficients in calculations, *i.e.*, activity is approximately equal to concentration.

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The Stability of Dilute Solutions of Mercury(I) Perchlorate

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Centinormal solutions of mercury(I) perchlorate undergo changes in reducing normality during storage. These solutions should be standardised frequently or prepared daily from the stable decinormal solution.

MERCURY(I) perchlorate is a useful titrant for submillinormal concentrations of iron(III),[1] copper(II),[2] iodine,[3] hexacyanoferrate(III),[4] and cerium(IV).[5] Some of these titrations can conveniently be carried out with 0·01 N titrant. Although 0·1 N mercury(I) perchlorate has been shown to be stable when stored in the dark,[6,7] no information is available concerning the stability of more dilute solutions.

EXPERIMENTAL

REAGENTS—

Prepare the reagents in the manner described by Stock and Merrer.[4] Store solutions of mercury(I) perchlorate (*a*), approximately 0·1 N in N perchloric acid, and (*b*), solution (*a*) accurately diluted 10-fold in N perchloric acid, and of approximately 0·1 N potassium hexacyanoferrate(III), in the dark. Add a few drops of mercury to one half of each batch of mercury(I) perchlorate solutions. Leave the other halves untreated.

APPARATUS—

Use conventional apparatus for amperometric titration at zero potential with respect to a saturated calomel electrode. Shield the titration cell and microburette from the light and clean and condition for use the rotating platinum electrode before each set of titrations.[2,4]

RESULTS AND DISCUSSION

Iodimetric standardisation[8] of the potassium hexacyanoferrate(III) solution at intervals of approximately 4 weeks gave a normality of 0·1001 ± 0·0002 that remained unchanged up to the termination of the stability trials. Table I gives the apparent normalities of the mercury(I) perchlorate solutions, as determined by triplicate amperometric titrations of hexacyanoferrate(III) in 0·5 N potassium thiocyanate - 0·01 N perchloric acid medium.[4] The fifteen determinations on each of the two portions of 0·1 N mercury(I) perchlorate have standard deviations of 0·0011 (stored over mercury) and 0·0024 (no mercury), respectively.

TABLE I

EFFECT OF AGEING ON THE REDUCING NORMALITY OF MERCURY(I) PERCHLORATE

Storage condition	Age/days 7	39	60	124	181
Over mercury. . . . (nominally 0·1 N)	$0{\cdot}095_1 \pm 0{\cdot}001_3$	$0{\cdot}095_6 \pm 0{\cdot}001_6$	$0{\cdot}096_0 \pm 0{\cdot}000_6$	$0{\cdot}095_8 \pm 0{\cdot}000_4$	$0{\cdot}096_9 \pm 0{\cdot}001_2$
Over mercury. . . . (nominally 0·01 N)	$0{\cdot}010_0 \pm 0{\cdot}000_2$	$0{\cdot}010_2 \pm 0{\cdot}000_1$	$0{\cdot}010_6 \pm 0{\cdot}000_1$	$0{\cdot}010_5 \pm 0{\cdot}000_1$	$0{\cdot}010_6 \pm 0{\cdot}000_1$
No mercury . . . . (nominally 0·1 N)	$0{\cdot}096_5 \pm 0{\cdot}002_8$	$0{\cdot}095_3 \pm 0{\cdot}000_7$	$0{\cdot}094_6 \pm 0{\cdot}001_8$	$0{\cdot}095_8 \pm 0{\cdot}003_0$	$0{\cdot}096_4 \pm 0{\cdot}002_9$
No mercury . . . . (nominally 0·01 N)	$0{\cdot}008_6 \pm 0{\cdot}000_1$	$0{\cdot}006_8 \pm 0{\cdot}000_1$	$0{\cdot}006_7 \pm 0{\cdot}000_1$	$0{\cdot}006_6 \pm 0{\cdot}000_1$	$0{\cdot}006_7 \pm 0{\cdot}000_2$

These deviations are comparable with the ranges of mean results for each portion (0·0018 and 0·0019, respectively). Any variation in concentration with time is probably no greater than the experimental error. To this extent, the present work confirms the reported stability of 0·1 N mercury(I) perchlorate when stored over mercury[7,9] and when mercury is omitted.[3,9] Although the more dilute (initially 0·0096 N) solutions eventually stabilised, this took several weeks.

Pugh[6] reported initially that 0·1 N mercury(I) perchlorate slowly lost its reducing power. This loss was later found to be apparent, and due to a trace of chlorine in the standardising system (Andrews's method).[7] The present sets of results for the two storage methods, obtained almost simultaneously and without change of reagents or technique, appear to be real. Limited observation of a second batch of 0·01 N mercury(I) perchlorate showed that the effects are not reproducible. In this case, a loss in reducing power occurred in the portion of the solution that was stored over mercury as well as in the portion from which mercury was absent.

Possible causes of increase in normality during the storage of mercury(I) perchlorate over mercury are carry-over of a trace of mercury(II) oxide or slight incompleteness of reduction of mercury(II) during the preparation of the solution. Traces of oxidising agents in the perchloric acid could account for the fall in reducing normality during storage, but hardly when storage is carried out over mercury. Another possibility is the presence of sufficient chloride to bring about slow nucleation and eventual separation of mercury(I) chloride. However, if such an effect occurs in 0·01 N mercury(I) perchlorate, it should be detectable in the companion 0·1 N solutions, yet no precipitates were visible at the termination of the storage trials.

Reagents, preparative methods and storage conditions that give satisfactory 0·1 N mercury(I) perchlorate solutions do not necessarily give 0·01 N solutions that are immediately stable. The latter solutions should be made up daily by dilution of 0·1 N mercury(I) perchlorate solutions, or at least should be standardised daily.

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The Amperometric Titration of Submillinormal Concentrations of Cerium(IV) with Mercury(I) Perchlorate

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The amperometric titration with mercury(I) perchlorate of 1×10^{-4} N cerium(IV) in 0·01 N potassium iodide - 0·2 N perchloric acid solution is precise and accurate to about 2 per cent.

ALTHOUGH the Ce^{4+}/Ce^{3+} couple is more strongly oxidising than the Hg^{2+}/Hg_2^{2+} couple, mercury(I) and cerium(IV) salts in acidic solution react only slowly. The complete oxidation of mercury(I) with an excess of cerium(IV) requires at least 30 minutes with the solution at near-boiling temperature,[1] although this heating time can be greatly reduced by silver(I) - manganese(II) catalysis.[2] Gold(III) chloride catalysis is reported to permit the direct titration with mercury(I) of cerium(IV) in dilute sulphuric acid.[3] The present work concerns the amperometric mercury(I) titration of cerium(IV) by methods similar to those used for iron(III),[4] copper(II),[5] iodine[6] and hexacyanoferrate(III).[7]

EXPERIMENTAL

REAGENTS—

Mercury(I) perchlorate, 0·1 N or 0·01 N in N perchloric acid—Prepare this solution in the manner described by Berka, Vulterin and Zyka,[8] diluting it as necessary with N perchloric acid. Centinormal mercury(I) perchlorate solution should either be prepared daily or standardised daily by titration of iodine[6] or hexacyanoferrate(III).[7,9]

Cerium(IV) sulphate, 0·1 N in N sulphuric acid—Standardise this solution against arsenic(III) oxide.[10] Dilute it as required with N sulphuric acid.

Perchloric acid, approximately 0·4 N.

Potassium iodide, approximately 0·02 N *in boiled-out water.*

APPARATUS—

Use conventional apparatus for amperometric titration at a rotating platinum electrode of zero potential. (All potentials are recorded with respect to the saturated calomel electrode.) Clean and recondition the rotating platinum electrode,[5] but use cerium(IV) in the preliminary titration.

The sensitivity of the rotating platinum electrode used in the present work was 0·0393 μA per micromole of hexacyanoferrate(III) per litre, measured at zero potential in de-oxygenated 0·5 N potassium thiocyanate - 0·01 N perchloric acid at 25 °C.

PROCEDURE—

Transfer 50 ml of 0·4 N perchloric acid and 50 ml of 0·02 N potassium iodide to the titration cell. Then use method (A) or method (B) as described by Stock[5] for the titration of copper(II), with 0·1 N or 0·01 N cerium(IV) sulphate in place of copper(II) sulphate.

RESULTS AND DISCUSSION

All observations were made at room temperature (in the range 24 to 28 °C).

VOLTAMMETRY—

The current - potential graphs were obtained manually. The current was noted 1 minute after each potential change.

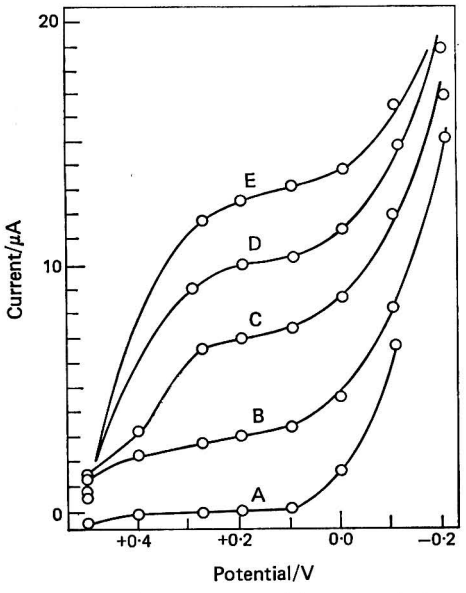

Fig. 1. Current - potential graphs at stages in the titration of 100 μN cerium(IV) in 2 μN gold(III) chloride - N sulphuric acid. Percentage equivalent of mercury(I) perchlorate added: curve B, 0; curve C, 50; curve D, 100; curve E, 150. Curve A shows the residual current of the medium

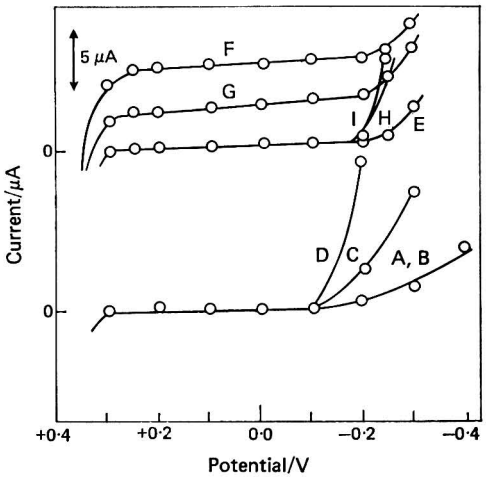

Fig. 2. Current - potential curves at stages in the titration of 100 μN cerium(IV) in N potassium thiocyanate (lower set of curves; residual current, curve A) and in 0·01 N potassium iodide - 0·20 N perchloric acid (upper set of curves; residual current, curve E). Percentage equivalent of mercury(I) perchlorate added: curves B and F, 0; curve G, 50; curves C and H, 100; curve D, 200; curve I, 150

Fig. 1 shows the effect of adding mercury(I) to a 100 μN cerium(IV) solution in N sulphuric acid that is 2 μN in gold(III) chloride. Similar graphs resulted when gold(III) was omitted, and when perchloric acid (both with and without gold(III)) replaced sulphuric acid. Qualitatively, mercury(I) merely augments the current from the drawn-out wave of cerium(IV).[11]

Mercury(I) becomes a strong reducing agent in a thiocyanate or iodide medium. However, thiocyanate suppresses the current caused by cerium(IV), so it cannot be used. Within the region where the current is essentially independent of potential, the current remains virtually zero as mercury(I) is added up to the point where it is in a large excess (Fig. 2, graphs B, C and D). The addition of cerium(IV) to an acidic iodide medium gives a well-defined and

TABLE I

TITRATION OF CERIUM(IV) IN ACIDIC IODIDE MEDIA

Cerium(IV) concentration/μN	Potassium iodide concentration/N	Perchloric acid concentration/N	Apparent mercury(I) normality		
			Method A[5]	L-curve method[5]	Method B[5]
1000	0·01	0·20	0·1024 ± 0·0017	0·1018 ± 0·0007	0·1018 ± 0·0007
100	0·01	0·20	0·1022 ± 0·0011*	0·1001 ± 0·0017*	0·1001 ± 0·0012*
100	0·01	0·50	0·1032 ± 0·0006	0·1012 ± 0·0000	0·1012 ± 0·0001
100	0·01	0·02	0·1053 ± 0·0001	0·1032 ± 0·0007	0·1041 ± 0·0000
100	0·01	0·10	0·1032 ± 0·0007*	0·1000 ± 0·0007*	0·1001 ± 0·0007*
100	0·005	0·10	0·1054 ± 0·0030	0·1032 ± 0·0011	0·1032 ± 0·0011
100	0·10	Zero	0·111†	0·109†	0·110†
50	0·01	0·20	0·0102 ± 0·0007‡	0·0100 ± 0·007‡	0·0099 ± 0·0006‡
10	0·01	0·20	0·0100 ± 0·0001‡	0·0097 ± 0·0002‡	0·0094 ± 0·0003‡
5	0·01	0·20	0·0114 ± 0·0012‡	0·0109 ± 0·0020‡	0·0093 ± 0·0009‡

* Five runs.
† One run.
‡ Titrant diluted 10-fold.

stable limiting current. Measured at zero potential, this current is proportional to the concentration of cerium(IV). Progressive addition of mercury(I) causes the current to fall to or near the residual value and then to remain substantially unchanged by an excess of mercury(I) (Fig. 2, curves F, G, H and I).

AMPEROMETRIC TITRATIONS—

Table I summarises the results of triplicate amperometric titrations with 0·1013 N mercury(I) perchlorate at zero potential of cerium(IV) in acidic iodide media. A medium that is 0·01 N in potassium iodide and 0·2 N in perchloric acid is satisfactory (these concentrations are not critical). However, underconsumption of titrant tends to occur when the concentration of acid or of iodide is too low; this may be caused by incomplete reaction of cerium(IV) with iodide. Preliminary experiments in a potassium iodide - 0·2 N perchloric acid medium indicate an overconsumption of titrant when the iodide concentration is greater than approximately 0·03 N. A probable cause is slight oxidation of iodide by traces of dissolved oxygen.

In the recommended medium cerium(IV) at a concentration of 100 μN can be titrated with a precision of approximately ± 2 per cent. The titration is still reasonably precise at a cerium(IV) concentration of 10 μN, but only method (A)[5] gives acceptable accuracy at such a low concentration. None of the methods yields acceptable results at cerium(IV) concentrations lower than 10 μN.

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Polarographic Behaviour of Zinc, Nickel, Copper, Cobalt and Cadmium in Monoethanolamine Solution

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Polarographic characteristics of copper, cobalt, nickel, zinc and cadmium in a solution 0·5 M in monoethanolamine and 0·1 M in potassium chloride, at pH 11·0, have been observed, well defined waves being obtained in all instances. The method described is suitable for the quantitative determination of these metals individually and for the differentiation of nickel from cobalt and from zinc.

Nitrogen-containing organic compounds, especially bases, have been found to be the most suitable complex-forming agents for zinc, nickel, copper, cobalt and cadmium.[1] The reduction of the complexes of these metals at the dropping-mercury electrode has been studied by various workers to enable their stability constants to be calculated and procedures to be developed for their determination in the presence of other metals.[2,3] These complexing agents are important mainly because they are specific and selective, which the ordinary inorganic supporting electrolytes are not. Among the most important organic supporting electrolytes are pyridinium chloride,[4] EDTA with sodium hydroxide,[5] triethanolamine with sodium hydroxide[6] and glycine with potassium chloride.[7] The use of monoethanolamine in the polarographic determination of tellurium(IV) has previously been reported by the present authors.[8] In the present paper the polarographic characteristics of copper, cobalt, nickel, zinc and cadmium, and also the quantitative determination of these metals individually and in the presence of each other, are described.

Experimental

Apparatus—

Polarograms in all instances were recorded at 25° ± 0·1° C with a manual Adept polarograph and pH values of the solutions were measured with a Marconi pH meter. A saturated calomel electrode was used as the reference and was connected to the polarographic cell by a potassium chloride - agar bridge. Oxygen was expelled by passing a stream of hydrogen through the test solution. Double-distilled mercury was used and the values of the constants m and t were 2·80 mg s^{-1} and 3·0 s, respectively, in distilled water and on open circuit.

Solutions—

All of the metal chloride or sulphate solutions were prepared from B.D.H. analytical-reagent grade materials and the metal contents were determined by classical methods. A 4 M solution of monoethanolamine was prepared from the freshly distilled reagent.

Effect of monoethanolamine concentration—

The graph of $\log i/(i_d - i)$ *versus* E indicated that reduction of cadmium, zinc and copper from the monoethanolamine complexes occurs reversibly whereas that of cobalt and nickel is irreversible. The slope of the logarithmic analysis graph for the former three metals indicates a two-electron process. The shape of the waves does not change when the ethanolamine concentration is increased from 0·3 to 3 M, although the half-wave potentials are shifted to more negative values. By using the equation

$$\frac{d\,E_{1/2}}{d\log[NH_2C_2H_4OH]} = \frac{0{\cdot}059\,p}{n},$$

where p is the number of ligands and n is the number of electrons involved in the reduction, the value of p was found to be 2, thus indicating the following type of process—

$$[M(NH_2C_2H_4OH)_2]^{2+} + 2e \rightleftharpoons M^0(Hg) + 2NH_2C_2H_4OH$$

For the irreversible reductions of nickel and cobalt, which were studied in the presence of 0·1 M potassium chloride, a shift of half-wave potentials with increasing ethanolamine concentration was also observed, but because of the irreversibility no deductions can be made regarding the composition of the complex. Comparison of wave heights (I in Table I) indicates that nickel - ethanolamine and cobalt - ethanolamine complexes also are reduced in a two-electron step.

TABLE I

POLAROGRAPHIC CHARACTERISTICS IN MONOETHANOLAMINE
Monoethanolamine, 0·5 M; potassium chloride, 0·1 M; pH, 11·0

Metal	$E_{1/2}$/V	I	Range of concentrations/mM	$E_{3/4} - E_{1/4}$ and from log plot/mV	Remarks
Nickel	−0·97	3·398	0·3 to 4·5	45	Irreversible
Cobalt	−1·33	2·665	0·290 to 3·0	50	,,
Cadmium	−0·75	2·434	0·56 to 5·6	29	Reversible
Zinc	−1·27	2·695	0·325 to 5·0	30	,,
Copper	−0·42	2·847	0·317 to 3·17	30	,,

EFFECT OF METAL-ION CONCENTRATION—

The values of the diffusion-current constant (I in Table I) in 0·5 M ethanolamine - 0·1 M potassium chloride solution at pH 11·0 were found to be constant over the metal-ion concentration range given in Table I. Therefore, the polarographic results can be used for the quantitative determination of these metals and also for their differentiation when the half-wave potentials differ by 0·2 V. A procedure for the determination of nickel in the presence of zinc or cobalt has therefore been developed.

GENERAL PROCEDURE FOR THE DETERMINATION OF COPPER, ZINC, COBALT, NICKEL AND CADMIUM—

A calibration graph for each of the metals was prepared by recording the polarograms of the metal at various concentrations in 0·5 M monoethanolamine - 0·1 M potassium chloride solution at pH 11·0. The i_d values of the polarograms were plotted against the concentration

TABLE II

POLAROGRAPHIC DETERMINATION OF NICKEL AND ZINC IN ADMIXTURE
Monoethanolamine, 0·5 M; potassium chloride, 0·1 M; pH, 11·0; sensitivity, 1/20

Sample No.	Concentration added/mM		Concentration found/mM	
	Nickel	Zinc	Nickel	Zinc
1	0·879	1·306	0·880	1·306
2	0·293	1·634	0·293	1·634
3	1·174	3·268	1·181	3·245
4	2·050	2·615	2·047	2·615
5	2·940	1·962	2·935	1·960

TABLE III

POLAROGRAPHIC DETERMINATION OF NICKEL AND COBALT IN ADMIXTURE, WITH CONDITIONS AS IN TABLE II

Sample No.	Concentration added/mM		Concentration found/mM	
	Nickel	Cobalt	Nickel	Cobalt
1	0·587	0·589	0·587	0·586
2	0·440	0·058	0·437	2·065
3	1·029	1·617	1·032	1·614
4	2·051	1·176	2·048	1·179
5	2·637	0·441	2·640	0·438

of the metal ion, and then the polarogram of the solution containing the metal at unknown concentration was recorded under identical conditions. The i_d values of this wave were referred to the calibration graph (which was linear) and the concentration of the metal in the solution thus determined.

MIXED POLAROGRAMS OF NICKEL - ZINC AND NICKEL - COBALT—

From the individual polarograms of nickel, zinc and cobalt it is obvious that it is possible to differentiate nickel from zinc and from cobalt in monoethanolamine solution. Consequently, a series of polarograms was recorded with synthetically mixed solutions of the cations and the i_d values measured for each metal were referred to its calibration graph. Tables II and III show the concentrations of nickel and zinc and nickel and cobalt present, respectively, in the mixed solution and the concentrations found from the polarograms. The results obtained in both instances are accurate and reproducible.

EFFECT OF FOREIGN IONS—

The effect of the anions acetate, oxalate, nitrate, fluoride, salicylate, citrate, sulphate and formate on the polarograms of these metals was studied; in all instances the $E_{\frac{1}{4}}$ value remained almost constant. The advantages of this base electrolyte over those previously reported[4,5,6,7] are that it can be used without the need of any maximum suppressor. Moreover, only a limited number of metals undergo reduction; most metals either do not undergo reduction (*e.g.*, tungsten, chromium, cerium, etc.) or are precipitated (*e.g.*, thorium, iron and manganese).

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The Determination of Methanol in Hair Spray

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Interference by ethanol and other hair spray constituents in the colorimetric determination of methanol is obviated by distillation of the spray, extraction with light petroleum of the sodium chloride saturated distillate, and use of a standard graph of methanol concentration *versus* optical density.

THE well-known method of determining methanol by oxidation to formaldehyde with potassium permanganate and sulphuric acid[1] followed by colour development with chromotropic acid cannot be applied directly to hair sprays because of the interfering effects of ethanol and of other hair spray constituents. The interference of ethanol in the colorimetric determination of formaldehyde has been shown recently by Still, Wilson and Lynch.[2] Although the presence of ethanol reduces the intensity of the colour, it does not affect the rectilinearity of the relationship between the methanol concentration and the colour intensity, as may be seen in Fig. 1.

To obviate these interferences, the following method is recommended, in which the other interfering constituents of the hair spray are removed by distillation followed by extraction with light petroleum of the sodium chloride saturated distillate, and the interference by ethanol is eliminated by a procedure of standard additions of methanol.

METHOD

REAGENTS—

Reagents used were of analytical-reagent quality except where otherwise stated.

Potassium permanganate solution—Prepare a 5 per cent. w/v aqueous solution.

Chromotropic acid solution—Prepare a 0·5 per cent. w/v aqueous solution freshly.

Light petroleum, 40 to 60 °C boiling range.

Sulphuric acid, concentrated, sp.gr. 1·84.

Sodium chloride, commercial grade.

Sodium metabisulphite.

Standard methanol solution—Transfer 1 ml of methanol by pipette into a 1-litre standard flask and make up to the mark with distilled water.

Antifoam S—Available from Thompson & Capper Ltd.

APPARATUS—

A Hilger and Watts Uvispek spectrophotometer was used to determine the optical densities of the various solutions.

PROCEDURE—

Spray the sample into a 25-ml calibrated flask until the liquid level is well above the mark. Leave it to stand for about 20 minutes to allow air bubbles to disperse, then withdraw sample down to the mark. Transfer the 25 ml to a 1-litre distillation flask containing about 100 ml of water and add a few porcelain chips and a pinch of antifoam S. Distil and collect about 80 ml of distillate. Transfer the distillate to a 250-ml separating funnel and saturate with commercial sodium chloride. Extract with 100 ml of light petroleum, shaking the funnel vigorously for about 2 minutes. Allow the layers to separate and run the lower aqueous layer through a No. 31 filter-paper into a 250-ml calibrated flask. Wash the light petroleum layer with 25 ml of saturated sodium chloride solution, allow the layers to separate and filter the washings through the same filter-paper into the flask. Rinse the filter-paper with distilled water and dilute to the mark (solution A).

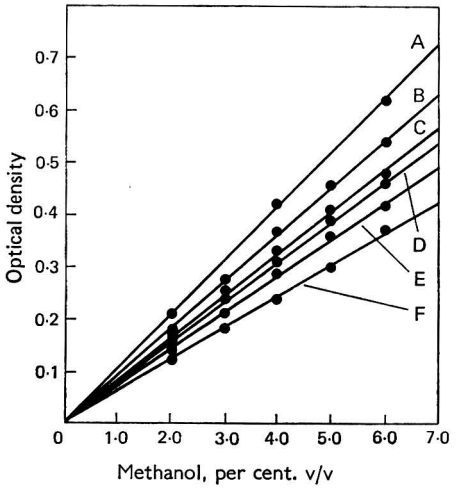

Fig. 1. Variation of calibration graphs with increasing concentrations of ethanol: A, water; B, water - ethanol (75 + 25); C, water - ethanol (50 + 50); D, water - ethanol (40 + 60); E, water - ethanol (30 + 70); and F, water - ethanol (20 + 80)

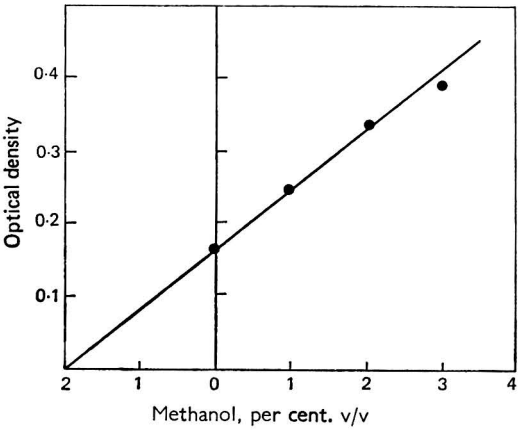

Fig. 2. Determination of methanol by extrapolation. The intercept on the x axis gives the percentage of methanol originally present in the hair spray

Transfer by pipette 10 ml of the solution A to a 1-litre calibrated flask and dilute to the mark with water. Transfer 1 ml of this solution by pipette into a 10-ml stoppered graduated cylinder. Add 0·1 ml of sulphuric acid and 1·0 ml of potassium permanganate solution, mix well and allow to stand, with occasional shaking, for exactly 5 minutes. Immediately decolorise the mixture with a pinch of sodium metabisulphite and add 0·2 ml of chromotropic acid solution followed by 6 ml of sulphuric acid down the side of the cylinder. Mix the contents and place the cylinder in a water-bath at 100 °C for exactly 5 minutes. Remove and cool it in water to room temperature. Measure the optical density of the solution in a 1-cm cell at 570 nm against a blank prepared with 1 ml of distilled water. The optical density should read not more than 0·2. If the reading is high, dilute solution A accordingly.

To determine the optical density with 1 per cent. of methanol added, transfer 10 ml of standard methanol solution and 10 ml of solution A by pipette into a 1-litre flask and dilute to the mark. Then take 1 ml for colorimetric determination. Similarly, for 2 per cent. of added methanol, take 20 ml of standard methanol solution and add 10 ml of solution A and dilute to 1 litre. Plot the optical density against the percentage of methanol added. Extrapolate the graph to cut the x axis and read off the concentration (v/v) of methanol in the sample hair spray directly from the x axis.

The recovery of methanol by this method was determined by the addition of methanol to hair sprays known to be free from methanol. The results of one of these determinations is shown in Table I. A typical graph obtained in the determination of methanol by the recommended method is shown in Fig. 2.

TABLE I

RECOVERIES OF METHANOL FROM HAIR SPRAY

Added, per cent. v/v	Found, per cent. v/v	Recovery, per cent.
1·0	1·03	103·0
2·0	2·03	101·5
3·0	3·01	100·3
5·0	4·90	98·0
10·0	9·90	99·0

For the brands of hair spray containing methanol that we have examined, the content of methanol was found to be less than 5 per cent.

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Proton Chemical Shifts for Solvents and other Simple Substances

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The chemical-shift values for the principal nuclear magnetic resonance peaks for fifty solvents and other low molecular-weight substances dissolved in deuterochloroform, deuterodimethyl sulphoxide, benzene (or deuterobenzene), pyridine (or deuteropyridine), trifluoroacetic acid and heavy water are recorded. The values are useful for the rapid identification and determination of these substances when present as impurities in other organic compounds.

Nuclear magnetic resonance spectroscopy is a valuable technique for the rapid identification and determination of solvent and other simple impurities in organic compounds. If a proton resonance peak in the nuclear magnetic resonance spectrum of a solution of an organic compound can, from its chemical shift, be assigned to a known solvent or other impurity, the amount of impurity relative to the organic compound can be determined by comparing the height of the integrator step for the impurity peak with that for a peak associated with a known proton-containing group in the compound.

The technique is well established and during the last eight years has been used routinely in our laboratory for the determination of simple impurities in research samples of pharmaceutical products.[1] The sensitivity of the method depends on the nature of the impurity and of the major component. Thus about 0·2 per cent. of acetone in a steroid or in a cephalosporin is readily detected; for chloroform the lower limit is about 0·5 per cent. However, although tables giving the nuclear magnetic resonance absorption spread for many solvents as undiluted liquids have appeared,[2] no tables listing the chemical shifts of the characteristic peaks for solvent and other impurities at low concentration in a variety of important nuclear magnetic resonance solvents have been published. The chemical-shift values depend on the nature of the solvent used.

We have, therefore, assembled in Table I (see page 372) the chemical-shift values for prominent nuclear magnetic resonance peaks for 50 common solvents and other substances we have encountered as impurities in research samples of organic compounds. The values are for solutions at low concentration (0·01 to 0·05 M) in solvents that we use for routine nuclear magnetic resonance measurements, namely, deuterochloroform, deuterodimethyl sulphoxide, pyridine (or deuteropyridine), benzene (or deuterobenzene), heavy water and trifluoroacetic acid. The peaks are normally sharp and are readily identified in the spectra of larger organic molecules. Although the chemical-shift values listed were measured on 60-MHz spectrometers, the values can be, and are, used for the examination of 100-MHz spectra.

The peaks listed in Table I are singlets unless described as doublets (d), triplets (t), quadruplets (q) or multiplets (m). Splittings arising from couplings with hydroxyl and imino protons are often not observed during normal measurements. Nevertheless, alcohols sometimes show couplings of this type and give rise to more complicated patterns, particularly when examined in dimethyl sulphoxide. Peaks for water and for hydroxyl and imino protons, which are strongly temperature and concentration dependent, have been omitted from the table. The chemical-shift values are probably accurate to within $\pm 0{\cdot}02$ p.p.m., but may be less reliable for heavy water solutions and for solutions in which solute - solvent interaction can occur. Many alcohols esterify rapidly in trifluoroacetic acid solution and yield confused

spectra containing peaks for both the free alcohol and its ester. The ratio of the two components may change appreciably during the short time interval between the recording of the spectrum and of the integral trace. In Table I only peaks associated with free alcohols have been listed. Anisyl alcohol is attacked by trifluoroacetic acid, forming a mixture of products that give a series of broad nuclear magnetic resonance bands. The peaks for compounds in heavy water solution are often dependent on the pD of the solution; addition of either acid or alkali might cause significant shifts from the values listed in the table.

The chemical-shift values for compounds at low concentration in complexing solvents are often concentration dependent. The effect, which is shown by acetonitrile and chloroform in benzene and dimethyl sulphoxide solution respectively is, however, not large enough to prevent identification of the substance concerned.

Liquid paraffin and petroleum jelly give peaks in the same positions as those shown by petroleum spirit, but the relative intensities of the peaks differ. Silicone oil in deuterochloroform solution forms a sharp peak at τ 9·92.

Experimental

The spectra were recorded at 38 °C and at a sweep rate of 1 Hz s^{-1} on either a Varian A60 or a Varian A60D nuclear magnetic resonance spectrometer, and chemical shifts are expressed in τ units relative to either tetramethylsilane (for deuterochloroform, pyridine, benzene and trifluoroacetic acid solutions) or sodium 3-(trimethylsilyl)propane-1-sulphonate (for dimethyl sulphoxide and heavy water solutions), used as internal standards.

The substances and solvents used were, when possible, of AnalaR or spectroscopic quality. Deuterochloroform and deuterodimethyl sulphoxide were dried and stored over fresh molecular sieves ($\frac{1}{16}$-inch pellets, Union Carbide, Type 4A).

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TABLE I

CHEMICAL-SHIFT VALUES (τ-UNITS) FOR SOLVENTS AND OTHER SIMPLE SUBSTANCES IN SIX NUCLEAR MAGNETIC RESONANCE SOLVENTS

Impurities	Solvents					
	$CDCl_3$	$(CD_3)_2SO$	C_5D_5N or C_5H_5N	C_6D_6 or C_6H_6	D_2O	CF_3COOH
Acetic acid	7·87	8·05	7·87	8·37	7·84	7·72
Acetone	7·83	7·88	8·00	8·38	7·78	7·59
Acetonitrile	8·02	7·91	8·15	9·33	7·95	7·92
Anisyl alcohol	5·39, 6·21	5·56, 6·26	5·08, 6·32	5·64, 6·68	5·43, 6·19	reacts
Benzene	2·63	2·60	2·67	2·70	2·56	2·67
Bromoform	3·15	2·25	2·14	4·11	insoluble	3·13
n-Butanol	6·33 (t, 6), 9·06 (t, 7)	6·59 (t, 6), 9·11 (t, 7)	6·20 (t, 6), 9·11 (t, 7)	6·62 (t, 6), 9·17 (t, 7)	6·40 (t, 6), 9·11 (t, 7)	6·09 (t, 6), 8·99 (t, 7)
t-Butyl alcohol	8·72	8·86	8·63	8·94	8·77	8·34
Chloroacetic acid	5·86	5·72	5·56	6·69	5·75	5·76
Chloroform	2·73	1·65	1·59	3·59	insoluble	2·75
Cyclohexane	8·57	8·58	8·62	8·60	insoluble	8·53
1,2-Dibromoethane	6·37	6·16	6·30	7·12	6·21	6·38
Dichloroacetic acid	4·02	3·32	3·28	4·57	3·79	8·94
1,2-Dichloroethane	6·27	6·07	6·22	7·01	6·08	6·28
Dichloromethane	4·70	4·21	4·38	5·54	insoluble	4·74
Diethyleneglycol dimethyl ether	6·40 (m), 6·62	6·51 (m), 6·72	6·43 (m), 6·73	6·60 (m), 6·87	6·37 (m), 6·63	6·03 (m), 6·37
Diethyl ether	6·52 (q, 7), 8·80 (t, 7)	6·58 (q, 7), 8·87 (t, 7)	6·62 (q, 7), 8·88 (t, 7)	6·73 (q, 7), 8·90 (t, 7)	6·44 (q, 7), 8·83 (t, 7)	6·17 (q, 7), 8·66 (t, 7)
Diisopropyl ether	8·88 (d, 6)	8·96 (d, 6)	8·91 (d, 6)	9·50 (d, 6)	8·88 (d, 6)	8·66 (d, 6)
Dimethylacetamide	6·99, 7·06, 7·92	7·01, 7·18, 8·01	7·18, 7·30, 8·04	7·41, 7·89, 8·36	6·95, 7·11, 7·92	6·61, 7·40
Dimethylformamide	1·99, 7·05, 7·12	2·02, 7·08, 7·24	7·28, 7·34	7·60, 8·02	2·09, 7·00, 7·14	1·60, 6·63, 6·74
Dimethyl sulphoxide	7·38	7·48	7·51	8·09	7·30	7·02
Dioxan	6·30	6·39	6·39	6·62	6·25	5·91
Ethanediol	6·24	6·58	5·99	5·79	6·34	5·92
Ethanol	6·28 (q, 7), 8·76 (t, 7)	6·51 (q, 7), 8·91 (t, 7)	6·14 (q, 7), 8·71 (t, 7)	6·61 (q, 7), 9·03 (t, 7)	6·36 (q, 7), 8·84 (t, 7)	5·99 (q, 7), 8·61 (t, 7)
Ethyl acetate	5·88 (q, 7), 7·96, 8·75 (t, 7)	5·92 (q, 7), 7·98, 8·79 (t, 7)	5·94 (q, 7), 8·06, 8·90 (t, 7)	6·09 (q, 7), 8·32, 9·06 (t, 7)	5·86 (q, 7), 7·92, 8·77 (t, 7)	5·69 (q, 7), 7·78, 8·63 (t, 7)
Ethyl formate	1·96, 5·78 (q, 7), 8·71 (t, 7)	1·77, 5·83 (q, 7), 8·76 (t, 7)	1·78, 5·86 (q, 7), 8·90 (t, 7)	2·40, 6·17 (q, 7), 9·15 (t, 7)	1·84, 5·72 (q, 7), 8·71 (t, 7)	1·75, 5·55 (q, 7), 8·59 (t, 7)
Formic acid	1·98	1·82	1·46	2·76	1·78	1·81

Furfuraldehyde	0·32, 2·29 (m), 2·75 (m), 3·40 (m)	0·31, 1·86 (m), 2·41 (m), 3·18 (m)	0·22, 2·13 (m), 2·69 (m), 3·44 (m)	0·64, 3·11 (m), 3·43 (m), 4·18 (m)	0·48, 2·06 (m), 2·41 (m), 3·22 (m)	0·49, 2·06 (m), 2·28 (m), 3·19 (m)
Isobutyl methyl ketone	7·88, 9·08 (d, 6)	7·92, 9·12 (d, 6)	8·00, 9·17 (d, 6)	8·39, 9·26 (d, 6)	7·81, 9·12 (d, 6)	7·63, 8·99 (d, 6)
Isopropyl acetate	7·98, 8·78 (d, 6)	8·00, 8·79 (d, 6)	8·05, 8·87 (d, 6)	8·31, 8·98 (d, 6)	insoluble	7·81, 8·66 (d, 6)
Isopropyl alcohol	5·97 (m), 8·80 (d, 6)	8·94 (d, 6)	5·84 (m), 8·71 (d, 6)	6·24 (m), 8·99 (d, 6)	8·82 (d, 6)	8·63 (d, 6)
Methanol	6·52	6·80	6·43	6·91	6·65	6·32
Methyl acetate	6·33, 7·95	6·39, 7·98	6·45, 8·08	6·72, 8·37	6·32, 7·91	6·14, 7·79
Methyl iodide	7·84	7·79	reacts	8·53	insoluble	7·89
Morpholine	6·31 (m), 7·15 (m)	6·48 (m), 7·32 (m)	6·33 (m), 7·19 (m)	6·50 (m), 7·51 (m)	6·30 (m), 7·21 (m)	5·76 (m), 6·42 (m)
Nitromethane	5·68	5·56	5·61	6·91	5·59	5·60
Petroleum spirit (boiling range 60 to 80 °C)	8·72, 9·10 (t, 4)	8·72, 9·11 (t, 4)	8·80, 9·14 (t, 4)	8·78, 9·11 (t, 4)	insoluble	8·68, 9·09 (t, 4)
Potassium acetate*	insoluble	8·40	7·89	insoluble	8·09	7·72
Propane-1,2-diol	8·83 (d, 6)	8·96 (d, 6)	8·64 (d, 6)	8·90 (d, 6)	8·87 (d, 6)	not examined
Propanol	6·40 (t, 7), 8·40 (m), 9·07 (t, 7)	8·55 (m), 9·13 (t, 7)	6·25 (t, 7), 8·30 (m), 9·03 (t, 7)	6·24 (t, 7), 8·60 (m), 9·20 (t, 7)	6·39 (t, 7), 8·43 (m), 9·11 (t, 7)	6·12 (t, 7), 8·24 (m), 8·98 (t, 7)
Propionic acid	7·58 (q, 7), 8·82 (t, 7)	7·74 (q, 7), 8·97 (t, 7)	7·54 (q, 7), 8·80(t, 7)	7·98 (q, 7), 9·11 (t, 7)	7·53 (q, 7), 8·90 (t, 7)	7·43 (q, 7), 8·72 (t, 7)
Pyridine	~1·40 (m), ~2·31 (m), ~2·72 (m)	~1·39 (m), ~2·17 (m), ~2·60 (m)	~1·29 (m), ~2·42 (m), ~2·79 (m)	~1·50 (m), ~2·95 (m), ~3·30 (m)	~1·50 (m), ~2·10 (m), ~2·54 (m)	~1·08 (m), ~1·21 (m), ~1·79 (m)
Succinimide	7·25	7·37	7·36	8·50	7·22	6·96
1,1,2,2-Tetrachloroethane	4·04	3·08	3·10	5·04	insoluble	4·09
Tetrahydrofuran	6·26 (m), 8·15 (m)	6·37 (m), 8·22 (m)	6·33 (m), 8·36 (m)	6·99 (m), 9·13 (m)	6·25 (m), 8·12 (m)	5·96 (m), 7·94 (m)
Toluene	2·81, 7·66	2·78, 7·68	2·78, 7·78	2·90, 7·87	insoluble	2·78, 7·67
1,1,1-Trichloroethane	7·28	7·20	7·39	8·42	insoluble	7·30
Triethylamine	7·44 (q, 7), 8·97 (t, 7)	7·53 (q, 7), 9·01 (t, 7)	7·57 (q, 7), 9·04 (t, 7)	7·60 (q, 7), 9·05 (t, 7)	7·41 (q, 7), 8·98 (t, 7)	6·61 (q, 7), 8·56 (t, 7)
Trimethyl borate	6·52	6·54	6·42	6·87	reacts	5·91
Trimethyl phosphate	6·20 (d, 11)	6·28 (d, 11)	6·29 (d, 11)	6·63 (d, 11)	6·18 (d, 11)	6·05 (d, 11)

* Similar peaks are given by other salts of acetic acid.

NOTE—The peaks listed are singlets, unless described as doublets (d), triplets (t), quadruplets (q), multiplets (m). Coupling constants (in Hz) are given in parentheses.

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Fluidic Devices: Design and Applications for Analytical Sampling Processes

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A range of commercially available fluidic devices has been evaluated for application to analytical sampling processes.

As a result of this work a modified bistable fluidic element incorporating a control assembly has been constructed. Details of its design and performance are given.

Fluidic devices, which are widely used in the electronics field,[1] consist of a solid block of glass, plastic or steel in which shallow channels have been cut (Fig. 1). They operate with streaming gas or liquid on the basis of the Coanda or wall attachment effect. It would seem that the stream-switching properties of these devices have considerable application to sampling processes in analysis; they have already found application in industrial process control.[2]

The purpose of the present work was to evaluate the analytical usefulness of the commercially available fluidic elements. On the basis of this preliminary investigation the most suitable type of fluidic device for analytical sampling was selected and subsequently modified to incorporate the control assembly.

A liquid or gas stream (the power stream) is pumped through the nozzle C_1 (Fig. 1) across a widened chamber and reaches one outlet, either 01 or 02. The jet strikes the sharp angle of the splitter and is divided into two parts only if the chamber is large enough to avoid wall attachment of the jet. This effect can be explained qualitatively in the sense that a laminar stream on entering the widened chamber will change its previously parabolic velocity profile, *i.e.*, the centre velocity decreases, the jet broadens and turbulence conditions are set up. Fluid from both sides of the stream is entrained and causes a pressure drop between the stream and the walls of the chamber. Fluid in a counterflow streams into the area of lower pressure to equalise the pressure difference established but, as soon as disturbances occur within the flow, this counterflow is not equal on both sides of the stream and the jet is forced towards one wall. As the zone between the stream and that wall becomes narrower, the counterflow diminishes and the pressure difference cannot be compensated for. The jet locks on to one wall (Fig. 2).[3] Obviously the jet attached to one side can move over to the other if the pressure difference between the control nozzles C_2 and C_3 is varied. The deflection angle θ will be

$$\tan \theta = \frac{w_{1,2}\,\Delta p}{w_0 p_0}$$

where p_0 is the inlet pressure, $w_{1,2}$ and w_0 are the widths of control nozzles and power nozzle, respectively, and Δp is the pressure difference between C_2 and C_3.

If the jet is attached to one side, the pressure will be reduced on the other so that a low pressure results in the passive outlet. Therefore, vents are introduced to provide sufficient liquid or air to compensate for the low pressure in the passive outlet.

The splitter distance from the power nozzle has a large effect on the performance of unvented elements but less effect on vented elements.[4] As a general rule this distance should not be less than six times the width of the power nozzle.

Experimental

Fluidic devices were obtained from the Plessey Company (fluidic experimental kit). Sampling experiments were carried out mainly with water. However, several experiments were conducted with the modified fluidic device (described on p. 376) to test its performance

with organic solvents, *e.g.*, methanol, chloroform and benzene. An investigation of the splitting efficiency of the modified fluidic device for aqueous solutions containing particulate matter was carried out. In this instance a suspension of 1 to 2 per cent. of barium carbonate was used.

Results and discussion

Three types of fluidic devices may be applied to sampling processes—

Bistable element—This is the most commonly used element; it is shown schematically in Fig. 3.

Automatic re-setting bistable element—With suitable geometry of the unit it is possible to arrange for automatic re-setting of the switching process, which can be achieved by varying the geometry of the chamber such that the angle between the input and normal output is different from the corresponding angle with the sample line (Fig. 4).

A similar effect is obtained if the diameter of the control nozzle on the side of the sample outlet is increased with respect to the opposite control nozzle.

"AND"-gate—In this type of fluidic device the direction of the outflow of the vents is vertical to the previous momentum of the jet, creating a vortex in the vents (Fig. 5). If the jet (1) acts upon a second jet (2) of the same momentum and pressure, an outflow will occur in (3).

To fulfil the requirements as sampling elements in automatic analysis, these three devices needed some modification. The nature of the controlling jet was the first consideration. The switching of liquid streams can be controlled either by a by-pass line from the sample stream itself or by a liquid stream of density similar to that of the sample stream. As switching by a second liquid stream, *e.g.*, water, causes problems arising from dilution of the sample stream, systems were investigated in which the sample stream itself operated the switching process.

Modified bistable element—

The scheme used to modify the bistable fluidic device is shown in Fig. 6. The sample line has two by-pass lines connected to it; the first is connected to the control beam on the side of the sample outlet and contains a flow restrictor, R, in series, while the second line passes through a water-pump constrictor, P, and through the valve, E, to waste. In normal flow conditions with E open, the liquid will flow through the waste outlet because of the reduced pressure in C_3 caused by the pump P. If E is closed, there is an immediate build-up of pressure in C_3, which exceeds the pressure in C_2, and the stream is switched to the sample outlet line.

The incorporation of small vents prevents the occurrence of low pressure in the passive outlet, but causes entrainment of gas in the liquid stream. Therefore, a small proportion of the flow from the sample and waste outlet lines, or from the main supply, is fed back to cover the vents. In this case it is necessary that this liquid is held at atmospheric pressure.

Modified automatic re-setting bistable element—

It is possible to apply this type of fluidic in a similar manner to the normal bistable element. One of the control ports is covered with liquid under atmospheric pressure. The operation is thus controlled by varying the pressure on the opposite control port, *i.e.*, by the water-pump effect used to operate the fluidic element (Fig. 7).

"AND"-gate element—

The "AND"-gate furnishes one of the simplest arrangements for sampling processes because the vents serve as outlets and the problem of covering the vents does not arise. On the other hand, the vortex that is established in one of the vents causes an appreciable amount of back-pressure giving rise to a liquid column in the sampling line (Fig. 8).

From the results of this preliminary survey it was concluded that there was little difference in the performance of these three types, *i.e.*, as regards ease of operation, response time, etc., and that any selection would have to be made after consideration of ease of production and reliability.

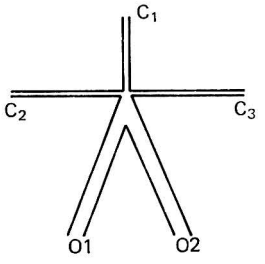


Fig. 1. Arrangement of channels in a typical fluidic device

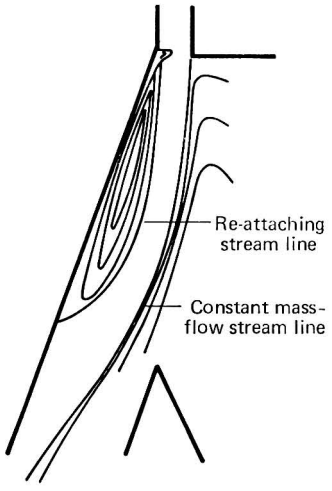


Fig. 2. Flow profile illustrating the wall attachment effect

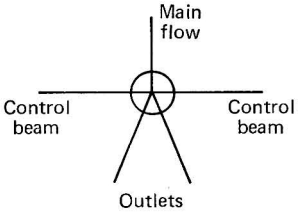


Fig. 3. Schematic diagram of a bistable fluidic element

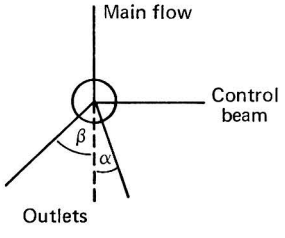


Fig. 4. Schematic diagram of automatic re-setting bistable element

As the commercial fluidic elements used in the preliminary study were primarily intended for use with gas streams, they were not ideally suited to use with liquids. It was particularly apparent that the switching process was slightly dependent on the surface adhesion properties of the material from which the fluidic was constructed. A modified fluidic was therefore designed, based on the principle of the automatic re-setting bistable element. This was constructed from stainless-steel sheet in sandwich fashion, partly for ease of assembly but also because of the low surface adhesion properties of this material towards water.

For the outer layers 3-mm thick polished steel sheet was used, while the three inner layers of the sandwich were of 1·5-mm thick sheet. The geometry of the arrangement was achieved by cutting the plates A and B into suitably shaped sections (Fig. 9). The whole assembly consisted of a five-layer sandwich with two flat plates making up the outer layers and an intermediate plate separating plates A and B. No adhesive was used because of the difficulty of avoiding blockage of the channels. The various sections of plates A and B were attached to the intermediate plate by countersunk screws and the outer plates bolted through the whole assembly. All channels in the assembly were made 1·5 mm wide and connection was made with 3 mm i.d. glass tubing (the connection points to the fluidic were 4 mm diameter holes with the glass connections fitted by means of a thin PVC sleeve).

The water-pump used to control the pressure in one of the control lines was fed from the main inlet line while the reservoir (plate B) was connected to the waste outlet (W — W'). When the device is operating, the flow of solution through either outlet induces a slight vacuum on the other outlet, which would result in entrainment of air through the open outlet

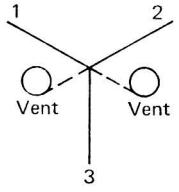


Fig. 5. Schematic diagram of an "AND"-gate fluidic element

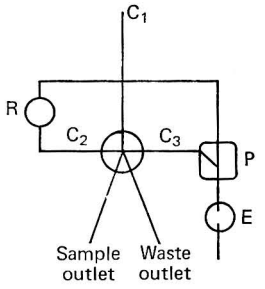


Fig. 6. Schematic diagram of the modified bistable fluidic element

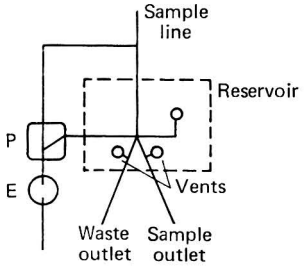


Fig. 7. Schematic diagram of the modified automatic resetting bistable element

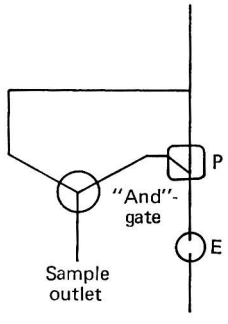


Fig. 8. Schematic diagram of the modified "AND"-gate fluidic element

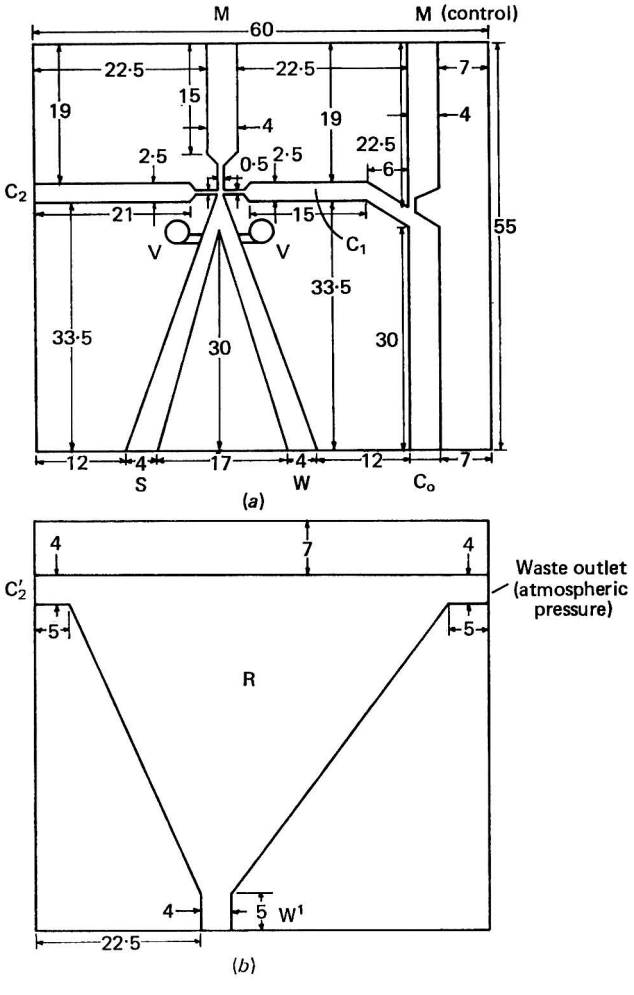
line. This effect was eliminated by incorporating two 4 mm diameter vent holes drilled into plate A (Fig. 9) and connected to the outlet lines by capillary channels. These vents are connected to the reservoir via holes of similar size in the intermediate plate. Thus by connecting the vents and the control port ($C_2 - C_2'$) to the reservoir, which is maintained at atmospheric pressure, any back-pressure in the passive line is immediately compensated for by solution flowing from the reservoir. The high rates of solution flow involved ensure that the reservoir is rapidly flushed out; hence there is little risk of contamination between successive samples. Control switching is carried out by placing an electromagnetic valve downstream from C_0; when this valve is closed, the pressure build-up in C_1 causes the stream to switch over to the sample outlet, S.

The response of the device is instantaneous and only a small delay results from the necessity to flush out the small amount of solution remaining from the previous sample. With this device it is possible to sample with extreme accuracy from a flowing stream, and further it can readily be adapted for continuous automatic analysis. It is also possible to connect several of these devices to different sample points and to obtain automatic sequential sampling, with the impulse required for sample change-over being derived from a common control line.

Although these devices were originally intended to work at fast flow-rates, the lowest permissible flow-rate with the present dimensions of the fluidic was found to be 80 ml minute^{-1} to maintain effective switching. It would be possible, however, to lower the flow-rates by reducing the dimensions of the fluidic.

The suitability of this device for stream sampling with organic solvents was examined. Effective switching was achieved with methanol, benzene and chloroform. The minimum flow-rates required for effective switching were, in all cases, about 10 to 20 per cent. lower than for water.

Sampling of process streams containing particulate matter is a particular problem with conventional valve systems, in which sedimentation around the valve can occur. With a fluidic device, however, the sample stream is continuously in motion so that this problem does not arise. The performance of the modified fluidic device was therefore tested by using a fine suspension of barium carbonate (1 to 2 per cent.) in water. At this concentration efficient switching was observed.



M = Main inlet line

C_1 = Active control port connected via channel C_0 to electromagnetic valve

C_2 = Passive control port connected to C'_2 channel to reservoir R

W = Waste outlet connected to W' channel to reservoir

S = Sample outlet

V = Vents connected via channels to sample and waste lines, and to reservoir by identical holes in intermediate plate

Fig. 9. Construction of the modified automatic re-setting element (all dimensions are given in millimetres): (*a*), plate A; and (*b*), plate B

Conclusions

Fluidic devices for sampling systems have the great advantage that they do not have any moving parts, they are resistant to heat and vibration and, further, are easily constructed. They should have particular advantage for sampling of highly corrosive liquids and gases.

One major limitation is that as the output from a fluidic device is a momentum of liquid rather than a static pressure it is undesirable to have any restrictions in the output sample line. Generally, high flow-rates are required and no large particles or coagulated material within the flow can be tolerated.

As well as the obvious application for automatic sampling of process streams this technique would also appear to have an application in improving the precision of the sampling process in gas chromatography (Deans, D., personal communication) and also as a sample-modulation device for atomic spectroscopy.

This work was carried out with the aid of a grant from the Technicon Corporation. One of us (H.v.S.) thanks the German Academic Exchange Service for providing financial support.

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An Attachment to a Simple Trace Reader for Use in Reaction Rate Analysis

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An attachment to a commercial chart reader that facilitates the measurement of the gradients of sloping traces is described. The performance of the attachment was investigated by using traces generated by the L.K.B. Reaction Rate Analyzer. A measurement rate of ten traces per minute with a coefficient of variation of 1 per cent. was achieved.

Some analytical instruments, *e.g.*, the L.K.B. Reaction Rate Analyzer, produce an output in the form of a sloping trace on a recorder chart. The output of the instrument is fast and

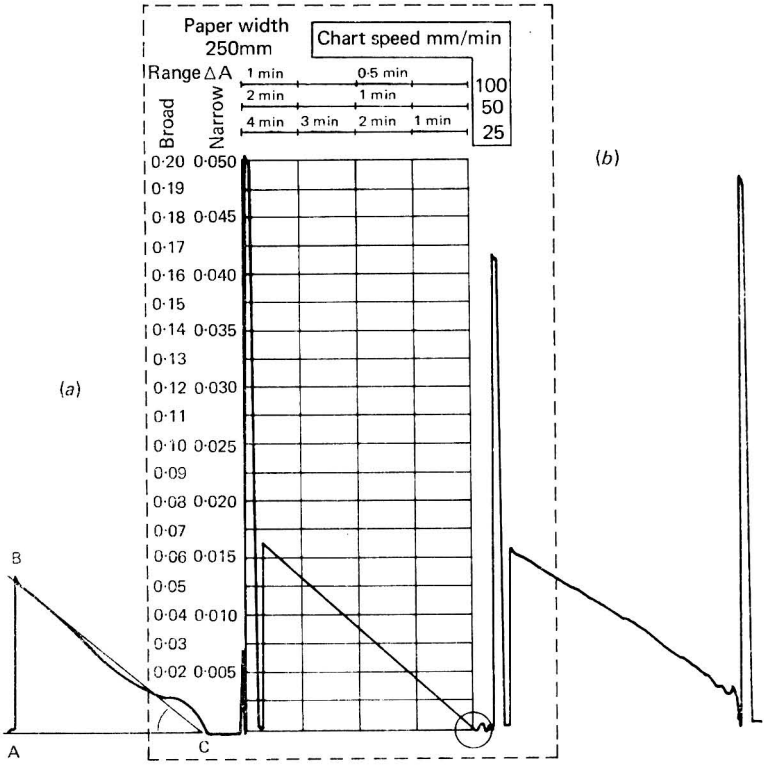

Fig. 1. Typical tracing from reaction rate analysis: (*a*) geometric determination of the gradient; and (*b*) L.K.B. scale for the direct measurement of tracings in enzyme units

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it is particularly useful for enzyme analysis but it is slow and tedious to read the trace and calculate results from it. The use of enzyme analysis in clinical chemistry is rapidly expanding so that there is a need to facilitate the measurement of the recorder trace.

To interpret the recorded trace from such an instrument it is necessary to measure the gradient of the trace, AB/AC (Fig. 1 (*a*). This gradient can be calculated in one of three ways: by measurement of distances AB and AC and calculation of the ratio; by measurement of the angle ACB and reading the corresponding value of the tangent or value in enzyme units from tables; or by use of a scale (Fig. 1 (*b*) with a standard base length and graduated in terms of the units required. All of these methods are adequate when the number of analyses is small (about 20), but are less satisfactory when large numbers of analyses are involved (about 50). We have therefore developed an attachment for a simple trace reader[1]* whereby gradients can be measured and the analytical result printed out directly in enzyme units.

Instrumentation

The instrument is based on the trace reader developed by Dawson, Milner and Mawston.[1] The operating principle of the attachment is the third method of gradient determination outlined above, whereby a fixed base-line is provided by the perpendicular distance between the pivot point of a rotatable ruler (Fig. 2 (*a*) and the cursor slide-bar of the trace reader. The ruler and the cursor are linked (Fig. 2 (*b*) so that when the ruler is rotated to align it with the recorder trace, the cursor of the trace reader moves a distance that is proportional to the tangent of the angle between the ruler and its zero position. The cursor is linked to a potentiometer so that a voltage is generated that is proportional to the gradient of the recorder trace. This voltage can be used to supply linearising and digitising circuits

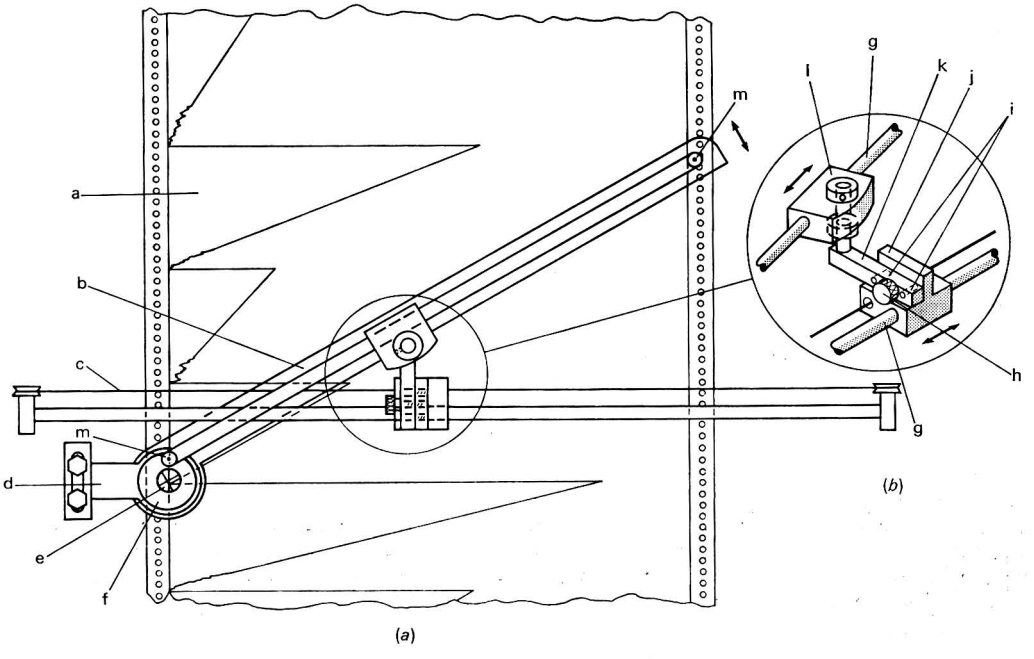

Fig. 2: (*a*) Plan of attachment to trace reader. (a) Chart. (b) Perspex ruler. (c) Drive cord. (d) Bracket supporting rotating arm. (e) Perspex graticule. (f) Ball race. (g) Slide bars. (h) Locking screw. (i) Locating dowels. (j) Trace reader carriage. (k) Link bar and pivot. (l) Attachment carriage. (m) Slide bar supporting pillars; (*b*) Details of linkage between rotating rule and slide wire of trace reader

* Available from Chemical Electronics, Birtley, Co. Durham.

whose output, which can be in terms of concentration units, can, in turn, be used to drive a printer or tape punch.

When it is necessary to use the instrument in its peak reading mode[1] the attachment can be easily separated from the cursor by releasing screw h (Fig. 2 (*b*). A rest is provided at the side of the instrument for the rotating arm when it is not in use.

Observations

To assess the usefulness of the device, a number of recorder tracings from the L.K.B. Reaction Rate Analyzer were used and results were calculated by using a ruler and set-square method and then the trace reader. Three aspects were examined: the reproducibility of the two methods of measuring gradients; the time taken to read a batch of analytical traces; and the comparability of the results obtained by different operators. Measurements of recorder traces were made by two operators in two ways: firstly, a group of ten traces was measured sequentially ten times, and secondly, a group of forty traces was measured once. This enabled assessments to be made of the reproducibility of the two methods of measurement and the influence on each of different operators.

Table I

Mean and standard deviation of the measured slopes of ten traces, each measured ten times by two operators each using manual and trace-reader methods

Method	Operator	Reading rate/traces minute^{-1}		Trace number 1	2	3	4	5
Manual	A	4	Mean*	60·2	27·0	39·2	172·3	94·8
			S.D.*	0·44	0	0·42	1·89	0·79
	B	4	Mean	60·2	27·0	39·1	171·6	94·9
			S.D.	0·42	0	0·32	0·84	0·99
Trace reader ..	A	10	Mean	60·1	26·7	39·3	173·8	95·7
			S.D.	0·36	0·44	0·36	2·13	0·63
	B	10	Mean	59·9	26·4	38·9	171·1	95·4
			S.D.	0·49	0·28	0·19	1·28	0·85

Method	Operator	Reading rate/traces minute^{-1}		Trace number 6	7	8	9	10
Manual	A	4	Mean*	76·1	38·6	44·6	21·0	37·5
			S.D.*	0·74	0·52	0·52	0	0·53
	B	4	Mean	76·5	38·7	44·6	20·9	37·8
			S.D.	0·85	0·48	0·52	0·32	0·42
Trace reader ..	A	10	Mean	76·9	39·1	44·7	20·6	37·7
			S.D.	0·66	0·37	0·47	0·34	0·33
	B	10	Mean	76·7	38·7	44·4	20·3	37·4
			S.D.	0·12	0·12	0·30	0·22	0·27

Results calculated from 10 measurements of 10 analytical traces.

* Arbitrary units.

The results presented in Table I show no consistent differences between mean values or standard deviations obtained by using different techniques or with different operators, except that the trace-reader method is 2·5 times faster than the manual method. The mean coefficient of variation of a reading is plotted against the mean value of the reading (Fig. 3). The theoretical curve is calculated on the assumption that the predominant source of error is constant and occurs in the setting of the angular position of the rotatable ruler. This leads to an expression of the form—

$$\text{Coefficient of variation} = K/\sin\theta\cos\theta$$

If θ is the angular position of the ruler and K is an arbitrary constant, the minimum of the curve corresponds to an angle of 45°. The distribution of points derived from the trace-reader measurement shows a shallow minimum somewhat displaced towards a larger angle

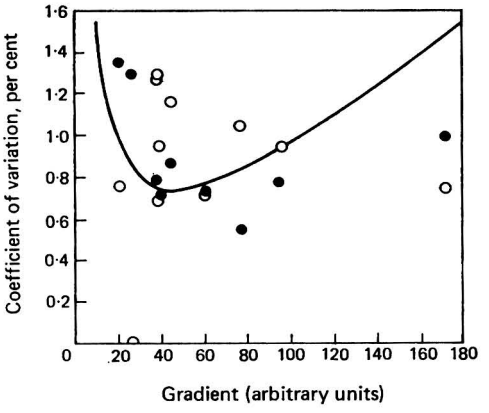

Fig. 3. Relationship between the coefficient of variation of a measurement and the gradient of the recorder trace: ○ Determined manually; ● determined by trace reader; —— theoretical curve

(50°), whereas the distribution of points from the manual method is less systematic, with a general trend to a lower coefficient of variation with increased gradient. The results presented in Table II show close agreement between operators and methods, and suggest that any difference between them is less than 2 per cent.

Table II

MEAN AND STANDARD DEVIATION OF THE MEASURED SLOPES OF FORTY TRACES, EACH MEASURED ONCE BY TWO OPERATORS EACH USING MANUAL AND TRACE-READER METHODS

	Manual		Trace reader	
Operator	Mean	S.D.	Mean	S.D.
A	54·4	15·59	54·9	15·80
B	55·4	15·81	54·7	15·95

Results calculated from single measurements of forty analytical traces from different specimens.

Conclusion

The most important benefits from using this instrumental method for the measurement of chart records produced in reaction rate analysis are the 2·5-fold increase in measurement rate and the reduction in operator fatigue. There was no significant difference in precision between the methods. The precision of measurement is dependent upon the gradient of the trace, and for traces having an angle between 20 and 75° it varies between 0·6 and 1·4 per cent. (coefficient of variation). The usefulness of the trace reader can be extended to the measurement of other angular functions (*e.g.*, sine, cosine) by appropriate programming of its biased diode network.

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Accepted *January 20th*, 1971

Analyst, May, 1971, Vol. 96, *pp.* 384–392

Analytical Methods Committee

REPORT PREPARED BY THE FLUORINE SUB-COMMITTEE

The Determination of Small Amounts of Fluoride in Solution

THE Analytical Methods Committee has received the following Report from its Fluorine Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Sub-Committee was: Dr. E. J. Newman* (Chairman since October, 1969), Mr. R. W. Fennell* (Chairman to October, 1969), Dr. E. J. Dixon* (resigned November, 1968), Mr. J. K. Foreman*, Mr. G. S. Goff, Mr. R. J. Hall,* Mr. W. C. Hanson* (resigned May, 1970), Dr. R. F. Milton, Mr. J. W. Ogleby, Mr. B. T. Saunderson*, Dr. J. M. Skinner* and Mr. C. A. Watson,* with Mr. P. W. Shallis as Secretary. The members marked with an asterisk took part in the work of the Microgram Group of the Sub-Committee, which was responsible for the preparation of this Report. In addition, the following were corresponding members of the Sub-Committee: Professor R. Belcher, Mr. H. A. Foner, Mr. H. Green, Mr. R. Waspe and Dr. J. E. Whitley.

INTRODUCTION

In 1965 the Analytical Methods Committee set up the Fluorine Sub-Committee "To investigate methods for determining fluorine and to recommend standardised methods for its determination at all levels in both organic and inorganic materials."

At the outset, it was apparent to the Sub-Committee that a wide variety of methods had been described for the determination of fluorine, and that the problems of separation could be formidable. The Sub-Committee therefore decided to approach the problem in two stages: first, to establish reliable procedures for the end determination of fluorine, and secondly, to use these procedures in studies of the separation of fluorine.

It was decided also to divide the Sub-Committee into two working groups, concentrating their efforts on the determinations of microgram and milligram amounts of fluorine in the final solution. This Report arises from the work of the Microgram Group of the Sub-Committee.

The methods available to the Microgram Group included colorimetric, radiochemical and enzymatic methods. The colorimetric methods depended either on the bleaching effect of fluoride ions on certain coloured metal complexes, or on the formation of blue ternary complexes between alizarin fluorine blue (alizarin complexan; 1,2-dihydroxyanthraquinon-3-ylmethylamine-*NN*-diacetic acid), a rare-earth cation and fluoride.

Since the formation of the Sub-Committee, the ion-selective fluoride electrode has been introduced and has been the subject of many reported investigations. Preliminary studies of the use of this electrode have been made by the Sub-Committee and further work is planned.

The Microgram Group decided that it would first examine colorimetric procedures, as few laboratories have facilities for radiochemical methods, and enzymatic methods have not so far received widespread attention. Many colorimetric methods were available that depended on the bleaching effect of the fluoride ion, but it was the opinion of the Group that the majority of these were becoming less widely used since the introduction of the

quantitative alizarin fluorine blue methods by Belcher, Leonard and West.[1] Nevertheless, the Group decided to investigate the method based on the bleaching of the zirconium - Solochrome cyanine R complex[2] because of its apparent simplicity and speed. The alizarin fluorine blue method was also selected for early consideration.

The zirconium - solochrome cyanine R method

The method depends on the bleaching effect of fluoride ions on the red complex that is formed from the reaction of zirconyl ions and Solochrome cyanine R (Colour Index 43820). The method was proposed originally by Megregian,[3] but the conditions used in the method studied by the Group were derived in the laboratory of one of its members who had established the optimum conditions required consistent with a high sensitivity to fluoride.[2] The reaction is carried out in very dilute hydrochloric acid solution and the concentration of fluoride is measured by the decrease in the absorbance of light of wavelength 540 nm by the zirconium - Solochrome cyanine R complex.

This method is very sensitive and can be used to determine amounts of fluoride ranging from 0 to 2·5 μg with a claimed accuracy of better than 0·1 μg. No doubt this method would be useful for the rapid determination of small quantities of fluoride in reasonably pure solutions, but the Group found that it did not satisfy certain of the desirable features of a recommended method.

Most members of the Group found that the colour of the zirconium complex faded; in some laboratories the fading was slight and ceased after a short period of time, whereas in other laboratories the fading was serious and continued for several hours. It was thought that the degree and rate of fading were associated with the previous history of the glassware in which the reaction was carried out or of the spectrophotometer cells in which the absorbance measurements were made, but the Group was unable to establish the reason for the fading phenomenon. It was therefore necessary to specify short periods of time for both the bleaching reaction with fluoride and for the solution to remain in the cell before completing the absorbance measurement. This is inconvenient for practical use; it means that each standard and each test solution must be treated with the colour reagent and measured individually—a batch of standards or samples cannot be treated simultaneously.

After a series of preliminary experiments had been carried out by members of the Group, a collaborative trial was conducted. This was designed to provide information on the reproducibility of calibration and on the performance of the method in the hands of different operators. The results obtained were subjected to statistical analysis.

The accuracy of the measurements was constant over the range 0 to 2·5 μg of fluoride. It was found that if an operator calibrated and performed an analysis on the same day, the standard deviation for the estimated value was 0·07 μg of fluoride. However, because of day-to-day variations in calibration, when calibrations and analyses were made on different days, the standard deviation of the estimated value rose to 0·10 μg of fluoride.

There was evidence of operator differences large enough to be important. In some laboratories, differences in mean levels of the order of 0·08 μg of fluoride were obtained between operators calibrating on the same day and using the same spectrophotometer. It therefore appears that an analyst should use only his own calibration values. These findings were not in themselves too serious, but, when they were considered together with the other weaknesses of the method referred to previously, the Group concluded that it would not be able to recommend this method as a standard procedure.

Recommended method for the determination of amounts of fluoride up to 40 μg

The method involves the formation of the blue ternary compound between alizarin fluorine blue, lanthanum ions and fluoride ions, and the concentration of fluoride is determined by measurement of the light absorbance of the blue colour at a wavelength of 625 nm.

In aqueous solution at about pH 5 alizarin fluorine blue is orange and it reacts with several metal cations to form red to mauve complexes. The red alizarin fluorine blue - metal complexes of lanthanum, cerium and praseodymium combine with fluoride ions to form blue ternary compounds. The ternary compounds contain equimolar amounts of alizarin fluorine blue, lanthanon and fluoride.[4]

The original alizarin fluorine blue method for fluoride made use of the reaction involving cerium, but sensitivities were later found to be in the order lanthanum > cerium > praseodymium,[5,6,7] so that methods described more recently have usually employed the reaction with lanthanum. It has also been shown that sensitivity is further enhanced by conducting the reaction in an acetone - water medium, and that the sensitivity increases with acetone content up to a concentration of 20 per cent. by volume.[5,7,8]

Most workers have followed the original procedure in the use of an acetate-buffered medium for the reaction. However, Hall showed that the use of a succinate buffer increased both the sensitivity and the speed of the reaction with fluoride.[9] Other members of the Group also reported that, in their experience, variations of acetate concentration altered the

TABLE I

COMPARISON OF SUCCINATE AND ACETATE BUFFERS

(For simplification, absorbance readings have been multiplied by 1000)

Laboratory	Amount of fluoride/μg	Mean absorbance	Deviations from mean absorbance: Day 1		Day 2		Day 3	
Succinate procedure—								
A	10	128	0		0		+1	
	20	257	−17		+8		+10	
	30	390	−12		+8		+3	
	40	526	−9		+6		+2	
	50	644	−12		+14		−2	
B	10	132	−3	−3	−1	−2	+5	+3
	20	269	+7	−4	−1	−7	+3	0
	30	400	+16	−1	−5	−9	0	+1
	40	530	+1	0	−1	−7	+7	0
	50	649	−4	+1	−4	−9	+10	+8
C	10	132	0	+3	−2	−1	−1	−1
	20	263	+3	+4	−3	+1	−1	−4
	30	392	+6	+5	−3	0	−5	−4
	40	512	+6	+9	0	−2	−9	−5
	50	638	+6	+10	−1	0	−6	−8
D	10	131	+12	−6	+4	−1	−2	−7
	20	285	−8	+3	−1	0	+4	0
	30	401	+1	−1	+10	0	−3	−6
	40	544	−8	−6	+7	+4	+6	−4
	50	674	+1	−4	+13	+6	−5	−9
Acetate procedure—								
A	10	118	+1		−1		−1	
	20	241	−5		+3		+1	
	30	356	−5		+4		+2	
	40	478	−3		+7		−4	
	50	585	−8		+3		+4	
B	10	125	+1	0	−1	−2	+4	0
	20	255	0	−1	−5	−3	+8	−2
	30	381	+4	−1	−5	−2	+4	−2
	40	510	−5	+2	−2	−7	+10	+3
	50	628	+4	−2	−3	−6	+9	−2
C	10	110	0	−7	−5	+17	+3	−10
	20	238	−1	−8	−10	+26	+1	−11
	30	369	+4	+1	−7	+22	+1	−19
	40	488	+14	+5	−11	+20	−27	+1
	50	615	+15	+3	+13	−7	+3	−26
D	10	123	−5	+3	+10	−7	+9	−8
	20	272	−10	+4	−4	−14	−5	+38
	30	392	−16	+2	+40	−12	−2	−12
	40	533	−10	−25	+43	−25	+41	−27
	50	650	−24	+8	+16	0	+1	−2

Laboratories B, C and D carried out the tests in duplicate.

sensitivity and the rate of the reaction with fluoride, and caused shifts in the wavelength of maximum light absorption of the blue fluoride complex.

The Group therefore decided that the use of a succinate buffer was worthy of further examination, and a study was undertaken by one of the members. Various features of the resulting method were confirmed by other members of the Group and, after a series of collaborative tests, this method was finally adopted as the basis of the recommended procedure contained in this Report. It was the experience of most members of the Group that the succinate buffer solution was stable for at least 5 days. Succinate solution is, however, a good medium for supporting organic growth, and it is possible that in some circumstances the buffer solution would be stable for only a few hours. For this reason an instruction is given in the recommended method (see Appendix) to the effect that the succinate buffer solution should be prepared freshly immediately before use.

Experimental work

Choice of method—

A complete literature survey of the alizarin fluorine blue method was made and certain basic conditions for the reaction were thereby established. Thus, it was decided to use a concentration of 2×10^{-4} M of the alizarin fluorine blue - lanthanum chelate and to carry out the reaction in an acetone - water medium containing 20 per cent. by volume of acetone.

Variation of the sensitivity of the reaction was only slightly influenced by changes in succinate concentration. A final succinate concentration of 0·01 M was chosen, but there is no significance in the fact that this is half the concentration used by Hall.[9] Changes in the succinate concentration also had no noticeable effect on the wavelength of maximum light absorption, which occurred at 625 nm. Maximum sensitivity occurred at pH 4·6 and the molar absorptivity of the fluoride complex was approximately 13 100. The calibration graph obeyed Beer's law for amounts of fluoride up to 40 μg. The colour was fully developed within 30 minutes and remained stable for at least 24 hours.

The Group then conducted a collaborative exercise to compare the succinate-buffered method with the acetate-buffered method. The succinate-buffered procedure was essentially that described in the recommended method and, for the acetate-buffered tests, the equivalent strength of a pH 4·6 acetate buffer was substituted for succinate. Tests were carried out in

Table II

Calibration figures obtained with the recommended method

(For simplification, absorbance readings have been multiplied by 1 000)

Laboratory	Amount of fluoride/μg	Mean absorbance	Deviations from mean absorbance: Day 1		Day 2		Day 3	
A	10	128	0	0	0	−2	−2	+2
	20	264	−1	+1	+3	−4	−1	+3
	30	405	−1	+8	+5	−5	−3	−2
	40	540	+1	+5	0	−6	−2	0
	50	671	+1	+6	+5	−3	−7	−5
B	10	133	+8	+3	+4	−6	−10	0
	20	261	+11	−2	+6	−12	−3	+1
	30	392	+9	+4	+7	−5	−9	−9
	40	526	+15	−2	+3	−2	−5	−8
	50	639	−1	−2	+4	0	0	−4
C	10	125	+5	0	−7	−2	+2	−1
	20	261	+4	+1	−5	−2	−3	+2
	30	391	+6	−3	−9	+3	0	+1
	40	522	+5	+6	−3	−2	−2	−3
	50	643	+6	+6	−6	−12	+2	+2
D	10	138	+2	−1	−1	−3	0	+1
	20	275	0	−5	+2	+4	−2	+1
	30	406	−13	−11	+1	+2	+4	+4
	40	553	−2	0	−3	0	+3	+2
	50	685	−15	−5	+8	0	+7	+7

each laboratory on three different days in order to assess the reproducibilities of the methods. The results are shown in Table I.

The results show that the succinate procedure is slightly more sensitive than the acetate procedure, and that the use of the succinate buffer led to better agreement both between and within laboratories. Moreover, day-to-day variations were less serious when the succinate buffer was used. Although two laboratories were able to obtain quite good reproducibility by the acetate method, the Group concluded that, on balance, the succinate buffer was preferable to the acetate buffer. Some operators reported difficulties in the preparation of the alizarin fluorine blue reagent solution, and modifications were made to the instructions for its preparation.

A second collaborative exercise was carried out along the same lines as the first, but with succinate buffer only. For this exercise, the method used was identical to the recommended method. The results of this experiment are shown in Table II. Statistical examination of these results showed that each laboratory obtained a linear relationship between absorbance and the amount of fluoride present. The best straight line fitting the results from each laboratory was calculated and, by using the deviations of each measurement from the values calculated for this line, the precision of the method was derived. It was found that, within 95 per cent. confidence limits, the precision of the method was ± 4 per cent., which the Sub-Committee considered reasonable for a colorimetric procedure. There were no indications of significant day-to-day variations within laboratories. There were statistically significant calibration differences between the laboratories, which were not necessarily of practical significance.

INTERFERENCES—

One of the collaborating laboratories carried out interference studies, using the recommended method. Each substance used in these studies was dissolved in water and a solution in water containing 30 μg of fluoride was also prepared. The solutions were treated by the recommended procedure given in the Appendix, except that the absorbances at 625 nm were measured against water in the reference cell instead of a reagent blank. The reason for adopting this approach was that interferences can arise in two ways. The interfering species could have an adverse effect on the complex formation between alizarin fluorine blue and lanthanum or it could influence the formation of the ternary complex between the alizarin fluorine blue - lanthanum chelate and fluoride ions.

Interferences of the first kind were studied by measuring the effects of foreign species on reagent blanks, that is, in the absence of fluoride. Such interferences were readily apparent in the recommended procedure because blanks in this method measured against water as reference have optical densities of 0·3 to 0·4 absorbance units.

Interferences of the second kind were observed when the absorbance of 30 μg of fluoride in the presence of a foreign substance was significantly different from the calibration value when measured against a blank containing the same concentration of the foreign substance.

It is important to realise that an interference of the first kind could cause a serious error by altering the background absorbance value; an example of this effect can be seen in the interference from zinc in Table III.

Salt effects—Up to 1·0 g of potassium chloride had no effect on the blank value or on the recovery of fluoride. Larger amounts enhanced the blank value, and in the presence of 2·0 g of potassium chloride, the absorbance of the fluoride-containing solution increased to the same extent. At even higher concentrations of potassium chloride the fluoride recovery, measured by the absorbance difference between the two solutions, was low. At least 5·0 g of sodium perchlorate monohydrate had no significant effect on either the blank or the recovery of 30 μg of fluoride.

It was concluded that the effects of changes in ionic strengths of solutions likely to be encountered in analysis are negligible.

Anions—The anions tested were all added in the form of neutralised solutions of their potassium salts, except for bromate and sulphide, which were added as sodium salts. The study of salt effects demonstrated that chloride and perchlorate did not interfere, even when present in appreciable amounts. The following anions caused no significant error when present in 100-mg amounts: bromate, bromide, iodide, nitrate, nitrite, selenate and tetraborate.

Sulphate had no effect when 1 and 10-mg amounts were added; 100 mg reduced the absorbance of the blank by about 9 per cent., but had no effect on the increase in absorbance expected from 30 μg of fluoride.

Silicate had no effect at the 1-mg level, and in the presence of 10 mg the absorbance of the blank was reduced by about 3 per cent.; the net absorbance of 30 μg of fluoride was unaffected.

Tartrate could be tolerated at the 1-mg level but in the presence of 10 mg the absorbance of the blank was reduced by about 18 per cent., and in this case the absorbance increase caused by 30 μg of fluoride was 9 per cent. high.

Citrate had no effect in a 1-mg amount, but 10 mg caused a reduction of the blank absorbance of 27 per cent.; the net absorbance of 30 μg of fluoride was also low by about the same amount. Moreover, it was observed that the colours of both the blank and the solution containing fluoride were fading in the presence of 10 mg of citrate. When the absorbances were measured again 90 minutes later, both had faded by a further 12 per cent.

Carbonate and sulphide caused serious interferences. When 1 mg of either was present the reagent blank absorbances were extremely high, and the net absorbances (*i.e.*, the recoveries) of 30-μg amounts of fluoride were very low. At the 0·1-mg level, absorbance

TABLE III

STUDY OF METAL INTERFERENCES

Ion added	Amount	Absorbance value of blank	Absorbance value of solution containing 30 μg of F^-	Absorbance value of solution containing 30 μg of F^-, read *versus* blank
None	—	0·327	0·738	0·411
NH_4^+	1 mg	0·325	0·735	0·410
Ba^{2+}	1 mg	0·330	0·735	0·405
Be^{2+}	1 mg	0·292	0·318	0·026
	100 μg	0·315	0·680	0·365
Li^+	1 mg	0·340	0·745	0·405
Mg^{2+}	1 mg	0·335	0·745	0·410
Ca^{2+}	1 mg	0·345	0·755	0·410
Al(III)	1 mg		Precipitated	
	100 μg	0·140	0·390	0·250
CrO_4^{2-}	1 mg	0·350	0·720	0·370
Cr(III)	1 mg		Precipitated	
	100 μg	0·350	0·755	0·405
Fe(II)	1 mg	0·715	0·745	0·030
	100 μg	0·655	1·100	0·445
Fe(III)	1 mg		Precipitated	
	100 μg	0·580	0·995	0·415
Mn^{2+}	1 mg	0·500	0·845	0·345
	100 μg	0·330	0·740	0·410
Cu(I)	1 mg		Turbid yellow solution formed	
Cu(II)	1 mg	0·350	0·370	0·020
	100 μg	0·335	0·760	0·425
Ni^{2+}	1 mg	0·680	0·705	0·025
	100 μg	0·415	0·660	0·245
Co^{2+}	1 mg	0·835	0·835	0·000
	100 μg	0·615	0·990	0·375
Zn^{2+}	1 mg	0·250	0·320	0·070
	100 μg	0·460	0·850	0·390
Ag^+	1 mg	0·410	0·820	0·410
Hg(II)	1 mg	0·470	0·865	0·395
Ce(IV)	1 mg	0·450	0·880	0·425
Ti(IV)	1 mg		Precipitated	
V(V)	1 mg	0·210	0·225	0·015
Mo(VI)	1 mg	0·250	0·660	0·410

values of blanks containing carbonate and sulphide were also rather high, but the net absorbances of 30 μg of fluoride were satisfactory.

These tests were repeated, except that the solutions containing carbonate or sulphide were treated, before adding the other reagents, with 1 ml of M perchloric acid, set aside for 5 minutes and then neutralised with 1 ml of M sodium hydroxide. This treatment somewhat

reduced the interferences in the reagent blanks, although the readings were still higher than normal, and the net absorbances obtained from solutions of 30 μg of fluoride that originally contained 1 mg of sulphide or 5 mg of carbonate were satisfactory.

The presence of 1 mg of orthophosphate reduced the absorbance of the blank by about 20 per cent., but the blue fluoride complex was formed quantitatively. Acetate at the 100-mg level enhanced the blank value and reduced the absorbance due to fluoride. Oxalate at the 1-mg level reduced both the blank value and the sensitivity to fluoride. Determinations of fluoride in the presence of acetate, orthophosphate and oxalate, at the levels quoted, could be made by suitable modifications to the test procedure. The easiest approach would probably be to use the method of "standard additions."

Metals—Interferences from cations were numerous. Studies were made of metal interferences at 1-mg and 100-μg levels, and only the cations of Groups IA and IIA of the periodic system and the ammonium ion could be tolerated in 1-mg amounts. The results of the study of metal interferences are given in Table III.

The opinion of the Group concerning this interferences study was that it did not markedly conflict with earlier reports of interference in the alizarin fluorine blue method for fluoride. It was therefore considered unnecessary for interference effects to be the subject of collaborative studies and the work described above was accepted.

Conclusions

The Group has established conditions for the alizarin fluorine blue method for the determination of fluoride in aqueous solution when the medium is buffered with succinate. This has two principal advantages over the more common practice of buffering with acetate, namely, that the method is much less influenced by alterations in buffer strength and it is more sensitive. The limitations caused by interferences are no more restricting than when acetate buffer is used.

Appendix

Recommended method for the determination in aqueous solution of amounts of fluoride not greater than 40 μg

Principle of method—

The sample is treated with a solution of the lanthanum - alizarin fluoride blue chelate in a succinate-buffered medium at pH 4·6, diluted to a known volume with acetone and water, and set aside for 30 minutes to allow the blue ternary lanthanum - alizarin fluorine blue - fluoride compound to form.

The fluoride is determined spectrophotometrically by measuring the absorbance of the ternary compound at a wavelength of 625 nm.

Range—

For fluoride contents in the range 2 to 40 μg in the sample taken.

Applicability—

The method is applicable to aqueous sample solutions. The following impurities can be tolerated in amounts up to those indicated.

Salts—

5·0 g: sodium perchlorate monohydrate.
1·0 g: potassium chloride.

Non-metals—

100 mg: bromate, bromide, iodide, nitrate, nitrite, selenate, tetraborate.
10 mg: sulphate.
1 mg: acetate, citrate, silicate, tartrate.
100 μg: oxalate, phosphate.
10 μg: carbonate, sulphide.

Metal and ammonium ions—

1 mg: ammonium, barium, calcium, lithium, magnesium.
200 μg: chromate.
100 μg: copper(II), manganese(II), molybdenum(VI).
50 μg: chromium(III)
20 μg: beryllium.
10 μg: cerium(IV), silver, titanium(IV), zinc.
5 μg: aluminium, cobalt(II), mercury(II), nickel.
3 μg: iron(II), iron(III).
2 μg: vanadium(V).

REAGENTS—

Reagents of the highest available purity should be used.

Water—Further purify glass-distilled water by passing it through a mixture of strongly acidic cation-exchange resin and strongly basic anion-exchange resin.

Succinate buffer, 0·1 M, pH 4·6—Dissolve 5·9 g of succinic acid in 300 ml of water and neutralise the solution to pH 4·6 with 0·5 M sodium hydroxide solution by using a pH meter; dilute to 500 ml with water. This solution must be prepared freshly before use.

Alizarin fluorine blue solution, approximately 0·002 2 M—Triturate 0·44 g of alizarin fluorine blue with 5 ml of 0·5 M sodium hydroxide solution, and dilute to 200 ml with water. Add 50 ml of 0·1 M succinate buffer, pH 4·6, check the pH of the solution with a pH meter and adjust it if necessary to between 4·5 and 4·8. Dilute to 500 ml with water and, if necessary, filter. Store this solution in a refrigerator.

Lanthanum nitrate solution, approximately 0·002 M—Dissolve 0·43 g of lanthanum nitrate hexahydrate in 500 ml of water.

Lanthanum - alizarin fluorine blue reagent solution—Mix equal volumes of alizarin fluorine blue solution, approximately 0·002 2 M, and lanthanum nitrate solution, approximately 0·00 2 M. This mixture must be made up freshly each day.

Acetone.

Fluoride stock solution—Dissolve 2·210 3 g of sodium fluoride, previously dried at 140 °C for 1 hour, in water to produce 1 litre. One millilitre of this solution contains 1·00 mg of fluoride.

Fluoride dilute standard solution—Dilute 10·0 ml of fluoride stock solution with water to produce 1 litre immediately before use. One millilitre of this solution contains 10·0 μg of fluoride.

CALIBRATION—

Transfer suitable volumes of dilute standard fluoride solution, accurately measured to cover the range 0 to 40 μg of fluoride ion, to a series of standard 50-ml calibrated flasks, and dilute each to 10 ml with water. Treat the contents of each flask as described below.

Add 5·0 ml of 0·1 M succinate buffer (pH 4·6), 10·0 ml of lanthanum - alizarin fluorine blue reagent solution and 10·0 ml of acetone, mixing thoroughly after each addition (see Note 1). Dilute to the mark with water, mix thoroughly and set aside for 30 minutes. Measure the absorbance of each solution containing fluoride in a 10-mm cuvette at a wavelength of 625 nm, using in the reference cuvette the solution prepared as described but containing no added fluoride.

By graphical construction or otherwise, establish the relationship between the amount of fluoride and the absorbance (see Note 2).

NOTES—

1. The order of addition of reagents may influence the rate of colour development and the stated order should be strictly observed.

2. For routine purposes, it is necessary to establish the calibration once only for each set of reagents. Fresh calibration is required when reagents are changed or in order to obtain results of the highest possible accuracy.

PROCEDURE—

Transfer the sample solution previously neutralised to between pH 4 and 5 containing not more than 40 μg of fluoride in a volume of not greater than 20 ml to a standard 50-ml calibrated flask. Add 5·0 ml of 0·1 M succinate buffer, pH 4·6, and complete the colour development as described under Calibration. At the same time, prepare a blank on the reagents in the same way, using water in place of the sample. Measure the absorbance of the sample solution against the reagent blank solution as reference, as described under Calibration. Calculate the amount of fluoride present in the sample by reference to the calibration relationship.

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Book Review

IDENTIFICATION TECHNIQUES IN GAS CHROMATOGRAPHY. By D. A. LEATHARD and B. C. SHURLOCK. Pp. x + 282. London, New York, Sydney and Toronto: Wiley-Interscience, a division of John Wiley & Sons Ltd. 1970. Price £5.

The subject matter is very well presented, and results in a valuable and easily readable book. Ancillary techniques for identification are well covered with the exception of thin-layer chromatography, which is merely given a mention in Chapter 3. By contrast, rather a lot of space is given over to a discussion on Rohrschneider plots, which is followed by evidence to show their rather limited use in practice. The chapter on selective abstraction is particularly useful. The precautions to be exercised when using columns of boric acid or FFAP are worthy of note.

In Chapter 6, the opening paragraph of section 1 (page 111), while true, is expressed in such a way that the reader may erroneously assume that variation in results is a function only of the apparatus. Apart from this, the difficulties associated with PGC are well presented. The reviewer questions the formation of aromatics from cycloalkanes (page 103): certainly under normal hydrogenating conditions the reverse is true.

It is good to see some considerable discussion on little used but potentially valuable detectors such as the piezoelectric detector, the mass detector, and the often neglected gas-density balance. The gas-density balance diagrams are somewhat clearer than one normally finds in publications. While it is correct to state that the mass detector has been used to weigh 10-μg samples, it is also true that the limit of detection is 0·5 μg. Development of the piezoelectric detector as a quantitative device is feasible, but changes in frequency resulting from stationary phase bleed from the column on to the detector, and from the detector itself, may make the long-term stability questionable.

The statement at the beginning of Chapter 8 that, in general, detector response is normally a linear function of the amount of eluate is just not true.

Most of the book is devoted to the identification of gross amounts of materials. It would have been desirable to give more explicit information on the somewhat more difficult task of identifying trace constituents.

The book is forward looking and is not merely a collection of the works of other people. As such, it is to be thoroughly recommended both to gas chromatographers and to those who make use of gas chromatography.

T. A. GOUGH

Chromatographic Separation and Titrimetric Determination of the Aphicide Menazon in Paste Formulations

Paste formulations of menazon were chromatographed on a column of acidic alumina. The emulsifying agents and other active ingredients were eluted with hexane, diethyl ether and chloroform. Menazon was finally eluted with dioxan and was then titrated potentiometrically with 0·1 N perchloric acid in dioxan - acetic acid (1 + 1).

PAOLO MAINI

Società Italo Americana Prodotti Antiparassitari, Centro Esperienze e Ricerche, 40015 Galliera (Bologna), Italy.

Analyst, 1971, **96**, 343–345.

A Rapid and Accurate Method for the Determination of Molybdenum in Plant Materials with Toluene-3,4-dithiol

A rapid and accurate method is described for determining molybdenum in plant materials with toluene-3,4-dithiol. The molybdenum is allowed to react with the reagent in a solution with a sulphuric acid concentration of about 6 N. Prior separation of the element from the acid digest of the plant material is unnecessary, there being no loss of sensitivity or accuracy if this process is omitted.

H. SSEKAALO

Animal Health Research Centre, P.O. Box 24, Entebbe, Uganda.

Analyst, 1971, **96**, 346–348.

Determination of Sub-microgram Amounts of Cobalt in Plants and Animal Tissues by Extraction and Atomic-absorption Spectroscopy

Atomic-absorption spectroscopy is used to determine cobalt in plants and animal tissues after solvent extraction of the 1-nitroso-2-naphthol complex from an acidic solution of the sample. The solvent is evaporated and the complex is dissolved in ethyl methyl ketone for aspiration. The method is more sensitive than, and as accurate and precise as, a colorimetric procedure involving nitroso-R salt, and is much less tedious.

Cobalt can be determined in the range 0·05 to 1 μg in the final 1-ml sample solution (corresponding to 0·05 to 1 p.p.m.). With a 5-g sample from 0·01 to 0·2 p.p.m. of cobalt in samples (dry matter) can be determined. The method can, therefore, be applied readily to pasture samples containing 0·1 p.p.m. of cobalt and less, levels that are considered likely to cause deficiency in ruminants. At 0·07 p.p.m. of cobalt the coefficient of variation is 14 per cent. At higher concentrations (0·14 p.p.m.) of cobalt in pasture and liver samples the coefficients of variation are 11 and 6 per cent., respectively.

J. JAGO, P. E. WILSON and B. M. LEE

Government Chemical Laboratories, 30 Plain Street, Perth, Western Australia, 6000.

Analyst, 1971, **96**, 349–353.

Study of Slightly Soluble Metal Chelates by Anodic Stripping Voltammetry

Anodic stripping voltammetry has been used to study chelates of ammonium 1-pyrrolidinecarbodithioate with metal ions. A technique is described in which polarographic waves are obtained when the chelates are highly insoluble. The metal is deposited at a hanging mercury drop electrode in the absence of chelating agent. The chelating agent is then added to the solution and the anodic stripping peak is recorded. Metal-to-ligand ratios and dissociation constants are calculated from the shift in the anodic stripping peak compared with the stripping peak obtained in the absence of chelating agent. Advantages include ability to use polarographic techniques when the chelates are insoluble (and non-reducible), increased sensitivity, and the possibility of using concentrations rather than activities for calculations involving dilute solutions.

PHILLIP H. DAVIS and GARY D. CHRISTIAN

Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506, U.S.A.

Analyst, 1971, **96**, 354–358.

The Stability of Dilute Solutions of Mercury(I) Perchlorate

Centinormal solutions of mercury(I) perchlorate undergo changes in reducing normality during storage. These solutions should be standardised frequently or prepared daily from the stable decinormal solution.

R. J. MERRER and J. T. STOCK

Department of Chemistry, The University of Connecticut, Storrs, Connecticut 06268, U.S.A.

Analyst, 1971, **96**, 359–360.

The Amperometric Titration of Submillinormal Concentrations of Cerium(IV) with Mercury(I) Perchlorate

The amperometric titration with mercury(I) perchlorate of 1×10^{-4} N cerium(IV) in 0·01 N potassium iodide - 0·2 N perchloric acid solution is precise and accurate to about 2 per cent.

R. J. MERRER and J. T. STOCK

Department of Chemistry, The University of Connecticut, Storrs, Connecticut 06268, U.S.A.

Analyst, 1971, **96**, 361–363.

Polarographic Behaviour of Zinc, Nickel, Copper, Cobalt and Cadmium in Monoethanolamine Solution

Polarographic characteristics of copper, cobalt, nickel, zinc and cadmium in a solution 0·5 M in monoethanolamine and 0·1 M in potassium chloride, at pH 11·0, have been observed, well defined waves being obtained in all instances. The method described is suitable for the quantitative determination of these metals individually and for the differentiation of nickel from cobalt and from zinc.

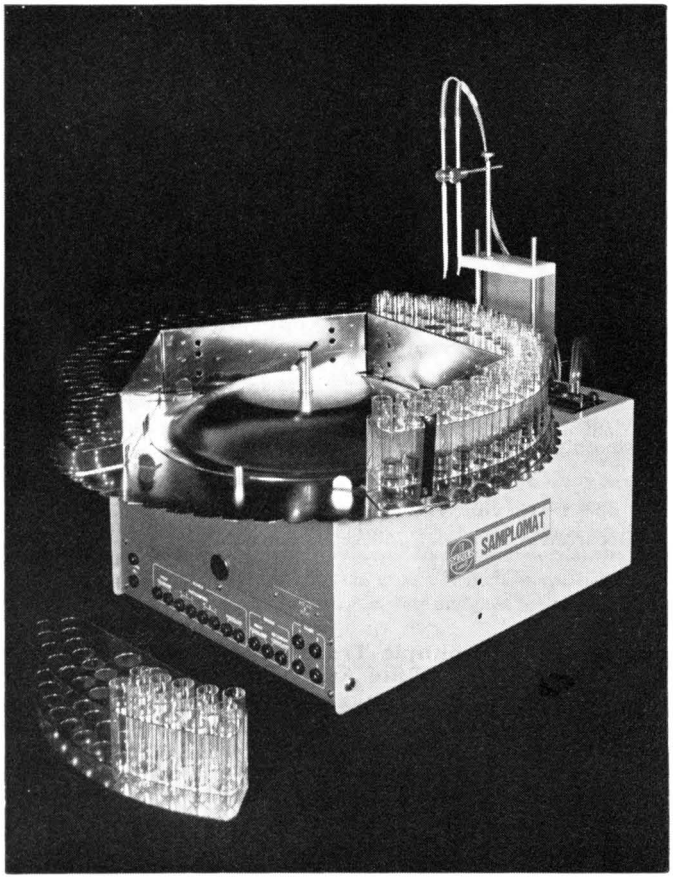
A. L. J. RAO and B. K. PURI

Department of Chemistry, Punjabi University, Patiala, India.

Analyst, 1971, **96**, 364–366.

SAMPLOMAT*

The Adaptable System for Automating Colorimetric and other Analytical Methods



This equipment can be used to automate the introduction of samples into an optical instrument and the subsequent reading and recording of results. It is suitable for most analyses which finish with a liquid sample requiring photometric or similar measurement. Additionally its design, and the many facilities which are built-in as standard equipment, make it eminently suitable for other uses.*

Manufactured by STRUERS of Copenhagen.
Available ex-stock from
CAMLAB Limited
Nuffield Road,
Cambridge CB4 1TH
Telephone 0223-62222

*See: An Apparatus for Rapid Automatic Determination of Mercury in Digested Samples (Analyst, March 1971, Vol 96 pp 223-229).

The Determination of Methanol in Hair Spray

Interference by ethanol and other hair spray constituents in the colorimetric determination of methanol is obviated by distillation of the spray, extraction with light petroleum of the sodium chloride saturated distillate, and use of a standard graph of methanol concentration *versus* optical density.

CHIA CHWEE LEONG and THENG CHYE YAM

Department of Chemistry, Ministry of Science and Technology, Outram Road, Singapore, 3.

Analyst, 1971, **96**, 367–369.

Proton Chemical Shifts for Solvents and other Simple Substances

The chemical-shift values for the principal nuclear magnetic resonance peaks for fifty solvents and other low molecular-weight substances dissolved in deuterochloroform, deuterodimethyl sulphoxide, benzene (or deuterobenzene), pyridine (or deuteropyridine), trifluoroacetic acid and heavy water are recorded. The values are useful for the rapid identification and determination of these substances when present as impurities in other organic compounds.

R. A. FLETTON and J. E. PAGE

Glaxo Research Ltd., Greenford, Middlesex.

Analyst, 1971, **96**, 370–373.

Fluidic Devices: Design and Applications for Analytical Sampling Processes

A range of commercially available fluidic devices has been evaluated for application to analytical sampling processes.

As a result of this work a modified bistable fluidic element incorporating a control assembly has been constructed. Details of its design and performance are given.

B. FLEET and L. H. von STORP

Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1971, **96**, 374–379.

An Attachment to a Simple Trace Reader for Use in Reaction Rate Analysis

An attachment to a commercial chart reader that facilitates the measurement of the gradients of sloping traces is described. The performance of the attachment was investigated by using traces generated by the L.K.B. Reaction Rate Analyzer. A measurement rate of ten traces per minute with a coefficient of variation of 1 per cent. was achieved.

J. B. DAWSON, G. W. FISHER and W. ANNAN

Departments of Medical Physics and of Chemical Pathology, University of Leeds, The General Infirmary, Leeds, 1.

Analyst, 1971, **96**, 380–383.

The Determination of Small Amounts of Fluoride in Solution

Report prepared by the Fluorine Sub-Committee.

ANALYTICAL METHODS COMMITTEE

9/10 Savile Row, London, W1X 1AF.

Analyst, 1971, **96**, 384–392.

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As examples of specific utilisation, we mention for HAP, which is endowed with notable ion exchange properties, in particular with respect to sodium, the removal of radioactive sodium from irradiated biological samples.
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COX Erba (Cerous Oxalate)
CUC Erba (Cuprous Chloride)
CUS Erba (Cupric Sulphide)
HAP Erba (Hydrated Antimony Pentoxide)
HMD Erba (Hydrated Manganese Dioxide)
TDO Erba (Tin Dioxide)
ZPH Erba (Zirconium Phosphate)

They are supplied in packs of 100 and 1000 g. A complete kit is also available which includes: 100 g. of each of the selective ion retention media and of the anionic and cationic resins; 60 plastic columns; 1 bottle of quartz wool for column packing; 113 cards for the optimal use of the retention media.
With suitable combinations of these cards it is possible to program every possible separation of the radioions present in the sample under examination. Further information may be found in the publication « Carlo Erba selective ion retention media » available on request.

CARLO ERBA Divisione Chimica Industriale / Via C. Imbonati 24 / 20159 Milano

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