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THE ANALYST

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Summaries of Papers in this Issue

Multi-element Atomic-absorption Analyses with a Gas-stabilised Arc as Primary Light Source

The use of a gas and wall-stabilised arc as primary light source for multi-element atomic-absorption measurements was investigated. A solution containing the elements of interest was injected, in aerosol form, into the arc, and the resonance lines excited. The concentration of the solution sprayed into the arc was the only source parameter appreciably influencing the sensitivity (*i.e.*, the degree of atomic absorption by the flame). It was found for fourteen arbitrarily chosen elements that the sensitivity was equal to two thirds of the sensitivity obtained with hollow-cathode lamps as primary sources. The reproducibility of measurement, when non-absorbing reference lines were used, was similar to that obtained with hollow-cathode lamps. The intensity of the background emission of the arc was so low in comparison with the line intensities that it had little effect on the sensitivity. With this source, the atomic-absorption working range can be extended by the variation of the solution concentration in the arc, and this proved useful when lead and zinc were determined simultaneously in a brass sample.

H. G. C. HUMAN, L. R. P. BUTLER and A. STRASHEIM
National Physical Research Laboratory, CSIR, Pretoria, South Africa.

A Comprehensive Scheme for the Analysis of Cement by Atomic-absorption Spectrophotometry

Atomic-absorption spectrophotometry has been applied to the determination of aluminium, calcium, iron, magnesium, manganese, potassium, silicon, sodium, strontium and zinc in cement. Only one sample weighing is necessary, and the results, which agree well with standard values obtained by classical methods of analysis, can be obtained within a few hours.

J. T. H. ROOS and W. J. PRICE
Pye Unicam Ltd., York Street, Cambridge.

Separate and Simultaneous Determination of Zirconium and Hafnium in Nickel-base Alloys with Xylenol Orange

The use of xylenol orange as a spectrophotometric reagent for zirconium and hafnium has been investigated for their determination in the range 0·002 to 0·2 per cent. in complex nickel-base alloys, and the effects of major alloying elements, and likely impurities, have been studied. A simple, direct procedure, based on the formation of the red xylenol orange complexes in 0·8 N hydrochloric acid, has been successfully applied to the determination of either metal in nickel alloys containing chromium, cobalt, iron, molybdenum, titanium and aluminium.

For alloys containing both zirconium and hafnium, a procedure developed for their simultaneous determination is based on the relative effect of acid concentration on the xylenol orange complexes. Preliminary mercury cathode and hydroxide separations are followed by measurement of the total optical densities at three levels of acidity, 0·35, 1·12 and 2·0 N perchloric acid. This "three-point" method has proved satisfactory with synthetic alloy solutions and when applied to complex nickel alloys containing both zirconium and hafnium. Confirmatory evidence of the results was obtained by X-ray fluorescence, emission and mass spectrometry.

The simultaneous procedure provides a simple and sensitive chemical method of differentiating between microgram amounts of zirconium and hafnium, and should be capable of wider application to other alloy systems. The simpler direct method should also prove advantageous when mutual interference does not arise.

H. J. G. CHALLIS
International Nickel Limited, Wiggin Street, Birmingham 16.

An Ultraviolet Spectrophotometric Method for the Characterisation of Some Phenolic Stabilisers in Extracts of Polymer Compositions

A method for extending the usefulness of ultraviolet spectrophotometry in the characterisation of some stabilisers used in polymer compositions is described. As well as the spectra of the stabilisers in ethanol and alkaline ethanolic solutions, the ultraviolet absorptions of the solutions after reaction with nickel peroxide, and after making these solutions alkaline, are also presented. These four spectra together give a more positive identification of the antioxidant. As examples of the method, three sets of spectra are given for phenolic antioxidants, the normal ultraviolet absorptions of which are identical. These spectra show marked differences by which the compounds can now be identified.

L. H. RUDDLE and J. R. WILSON

Imperial Chemical Industries Limited, Plastics Division, Bessemer Road, Welwyn Garden City, Herts.

The Absorptiometric Determination of Silicon in Water
Part VII. Improved Method for Determining the Total Silicon Content of High-purity Water

A method is described for determining the total silicon content of high-purity waters; this method allows more precise results to be obtained than the method given in Part III of this series. Silicon in the water is initially concentrated on a mixture of finely ground cation and anion-exchange resins, which are then ignited and fused with sodium carbonate. The resulting melt is dissolved in water and silicate is determined absorptiometrically as the reduced β-molybdosilicic acid. The standard deviation of analytical results for 1-litre samples containing between 0 and $100\,\mu g$ of silica was about $3\,\mu g$ of silica. Ten analyses and the necessary blank determinations can be carried out in 8 hours.

H. M. WEBBER and A. L. WILSON

Central Electricity Research Laboratories, Cleeve Road, Leatherhead, Surrey

An Improved Technique for Transferring Fractions from a Gas Chromatograph to a Mass Spectrometer

A simple technique is outlined for the collection, storage and mass-spectrometric analysis of small amounts of volatile components separated by a gas - liquid chromatograph. Mass spectra obtained with an A.E.I. MS10c2 mass spectrometer are shown, indicating a collection efficiency of about 95 per cent. A useful modification to the inlet system of the mass spectrometer for the analysis of small samples is described, giving a sensitivity increase of up to thirty times the standard inlet sensitivity. This is demonstrated by a mass spectrum of $0.005\,\mu l$ ($3.5\,\mu g$) of hexane collected from a chromatograph. A maximum working sensitivity for the system is about $0.1\,\mu g$.

W. D. WOOLLEY

Ministry of Technology and Fire Offices' Committee Joint Fire Research Organisation, Fire Research Station, Melrose Avenue, Boreham Wood, Herts.

The Use of Thin-layer Chromatographic Techniques for the Determination of Breakdown Products of Additives to Plating Solutions

The combination of conventional thin-layer chromatographic techniques with a novel form of column chromatography has enabled seventeen derivatives of coumarin in used nickel-plating solutions containing this additive to be isolated.   Ten derivatives were identified as mono-, di- and trihydroxy-coumarins and dihydrocoumarin, melilotic acid, o-coumaric acid and umbellic acid. R_F values and colour reactions of twenty typical coumarin derivatives are also reported.

W.-E. RUPPRECHT

Wilmot Breeden Ltd., Amington Road, Birmingham 25.

Gas-chromatographic Determination of Acetyl and Trimethylsilyl Derivatives of Alkyl Carbamates and their N-Hydroxy Derivatives

Microgram amounts of mixtures of alkyl carbamates, and of urethane and N-hydroxyurethane, as their trimethylsilyl derivatives, and similar mixtures of alkyl N-hydroxycarbamates, as their trimethylsilyl and acetyl derivatives, have been analysed by gas chromatography on SE30 columns. With a programmed temperature rise, the elution temperatures varied linearly with the number of carbon atoms in the alkyl side-chain of a homologous series; the isobutyl analogues were eluted at lower temperatures than the corresponding butyl analogues.   When the corresponding isobutyl derivatives were used as internal standards, the ratios of peak heights (test to standard) varied linearly with the concentration of urethane and N-hydroxyurethane.

R. NERY

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3.

A Method for the Analysis of Cereals and Groundnuts for Three Mycotoxins

A method is proposed for the analysis of samples for three mycotoxins, aflatoxin, ochratoxin and sterigmatocystin, by suitable treatment of a single sample extract.   Based on the subjective evaluation of thin-layer chromatograms of the extract, results can be reproduced with an accuracy of ± 20 per cent.   The method is considered to be satisfactory for the purposes of a field survey when the determination of the approximate level of mycotoxin contamination of cereals and groundnuts in the shortest possible time is of prime importance.   Problems encountered with samples that have high oil contents or that are darkly pigmented are dealt with by appropriate modifications of the method.

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THE ANALYST

Multi-element Atomic-absorption Analyses with a Gas-stabilised Arc as Primary Light Source

BY H. G. C. HUMAN, L. R. P. BUTLER AND A. STRASHEIM

(*National Physical Research Laboratory, CSIR, Pretoria, South Africa*)

The use of a gas and wall-stabilised arc as primary light source for multi-element atomic-absorption measurements was investigated. A solution containing the elements of interest was injected, in aerosol form, into the arc, and the resonance lines excited. The concentration of the solution sprayed into the arc was the only source parameter appreciably influencing the sensitivity (*i.e.*, the degree of atomic absorption by the flame). It was found for fourteen arbitrarily chosen elements that the sensitivity was equal to two thirds of the sensitivity obtained with hollow-cathode lamps as primary sources. The reproducibility of measurement, when non-absorbing reference lines were used, was similar to that obtained with hollow-cathode lamps. The intensity of the background emission of the arc was so low in comparison with the line intensities that it had little effect on the sensitivity. With this source, the atomic-absorption working range can be extended by the variation of the solution concentration in the arc, and this proved useful when lead and zinc were determined simultaneously in a brass sample.

THE ideal primary light source for multi-element atomic-absorption spectroscopy is one capable of emitting the resonance lines of several elements simultaneously with high stability, low background and narrow line widths, together with the ability to select any element or element combination required. Various attempts to produce such a light source have been made,[1,2,3,4] and the gas and wall-stabilised arc developed by Kranz[5,6] was investigated for the following reasons: the high stability of spectral-line emission; the low background continuum, which is probably caused by the failure of the portion of the plasma used as the light source to conduct the current that maintains the arc; and the ability to excite simultaneously the resonance lines of any number of elements introduced simultaneously into the plasma in solution.

EXPERIMENTAL

APPARATUS—

The construction of the arc chamber was similar to that described by Kranz.[5,6] For this investigation thoriated tungsten was used for both electrodes, while nitrogen was used for stabilising the arc and for nebulising the solutions introduced into the arc. The aerosol was introduced into the plasma by two horizontal tubes along the optical axis, as shown in Fig. 1. Because of the high rate of gas consumption of the pneumatic atomiser used, the gas velocity through the sample feed-tubes is high, and the sample is forcibly injected into the plasma.

A current-stabilised, direct current power supply, manufactured by Messrs. R.S.V., Hechendorf, Pils., W. Germany, which gives currents in 5-amp steps up to 35 amps, was used.

In the optical system used (see Fig. 2) a 1-to-1 image of the arc was formed on a diaphragm. Two regions could clearly be distinguished in the plasma flame, *viz.*, a luminous central core and a light blue surround. The diaphragm enabled the three regions indicated in Fig. 1 as h_1, h_2 and h_3, of $\frac{1}{2}$-cm height, to be separated and the most suitable to be used

as the primary light source. A second lens rendered the light parallel before transmitting it through either an air - acetylene or a nitrous oxide - acetylene flame. The light was finally focused on the collimator of the spectrograph, and quartz lenses were used.
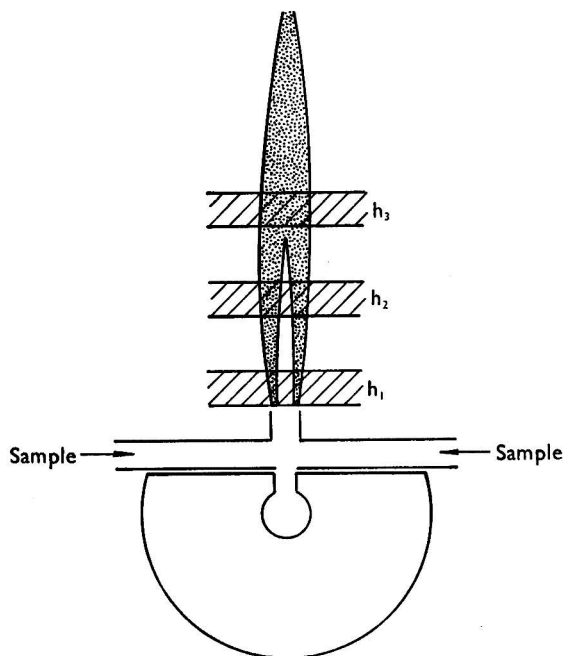
Fig. 1. Diagram showing method of sample introduction into the arc plasma, and the three regions investigated

A medium Hilger spectrograph, equipped with a four-channel direct-reading Strasheim attachment,[2] was used. The entrance slit was set at 30 μm with fixed exit slits of 60 μm.
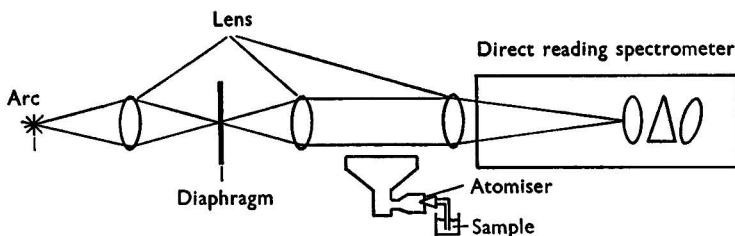
Fig. 2. Optical system

INVESTIGATION OF PARAMETERS INFLUENCING SENSITIVITY—

The influence of certain source parameters on the sensitivity of absorption was investigated. Fig. 3 shows the influence of the solution concentration on the sensitivity at various heights, h, of the plasma flame. These results were obtained for magnesium, with a constant concentration of the solution in the absorbing flame. It is evident that an optimum concentration is obtained for each height, but that the maxima are all equal.
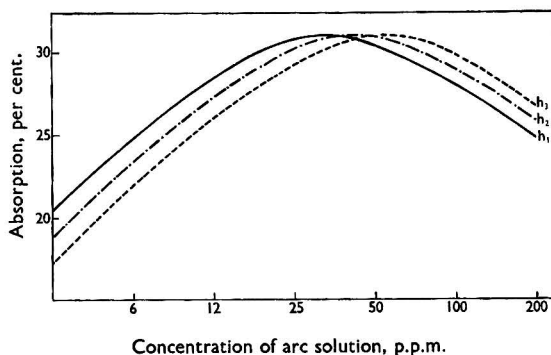
Fig. 3. Variation of absorption with concentration
of the arc solution, with three different regions used as
primary light source (for magnesium)

The arc current was found to have a slight influence on the optimum concentration as well as on the sensitivity. The minimum current at which the arc would burn was 25 amps; by varying the current between this value and a maximum of 35 amps, the results in Table I were obtained. A slight increase in sensitivity was found with increasing current.

TABLE I

OPTIMUM CONCENTRATION OF ARC SOLUTION AT DIFFERENT HEIGHTS
FOR DIFFERENT CURRENTS (FOR MAGNESIUM)

	Optimum concentration of arc solution, p.p.m.		
Current, amps	h_1	h_2	h_3
25	28·2	40·7	52·5
30	31·6	41·7	56·2
35	33·9	43·7	58·9

For all of these tests the tangential gas flow was kept constant at 8 litres per minute. No difference in sensitivity could be detected for gas flows of 6·9 and 9·2 litres per minute at heights of h_1 or h_3.
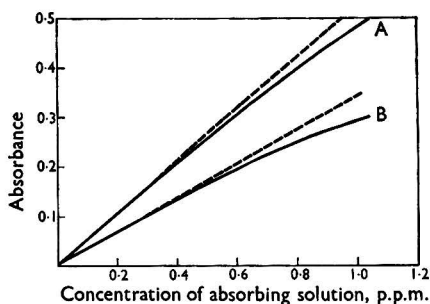
Fig. 4. Working curves: A, with
hollow-cathode lamp as primary source;
B, with Kranz arc as primary source

From these results it was concluded that the only parameter critically affecting the sensitivity was the element concentration in the arc solution. Therefore, for all further tests, the tangential gas flow was kept constant at 8 litres per minute, the current was set at 30 amps, and only the region h_1 of the arc was used as the primary light source. An example of a magnesium working graph, shown in Fig. 4, is obtained by using the Kranz arc as primary

source with these settings and spraying a solution of 30 p.p.m. of magnesium into it. The working graph obtained by using a hollow-cathode lamp at 5 mA is also shown. The ratio of the two sensitivities obtained with the two different sources was 0·65. (The sensitivity ratio is defined as the ratio of the two concentration values giving the same absorption signal, which is taken throughout as 10 per cent. absorption.) It can be seen that the working graph, with the arc as source, deviates from linearity slightly more than the hollow-cathode lamp graph. The line-to-background ratio in the arc was 27:1, while with the hollow-cathode lamp it was better than 100:1.

REPRODUCIBILITY—

Magnesium was used as the test element in an investigation of the reproducibility of measurement, with both the Kranz arc and a hollow-cathode lamp as primary light sources. With the arc, 30 p.p.m. of magnesium were used in the primary solution, together with 30 p.p.m. of calcium added as internal standard. The calcium 422·7 nm line was used as reference line. The standard deviation of the absorption values was determined when various concentrations of magnesium were sprayed into the absorbing flame, and a mean standard deviation of 0·488 per cent. absorption was obtained. When converted into concentration values by means of the working graph, curve A of Fig. 5 was obtained, the shape of which is in accordance with the general shape of curves of this type.[7]
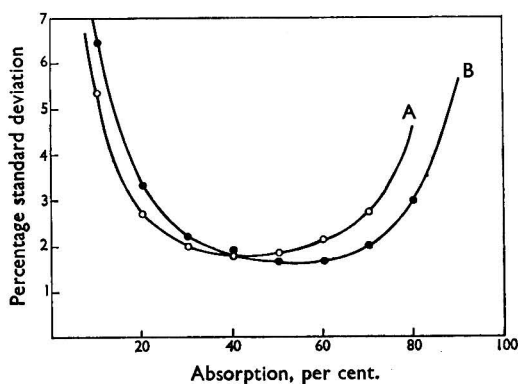
Fig. 5. Relative standard deviation curves:
A, with Kranz arc as source; B, with hollow-cathode lamp as source

With the hollow-cathode lamp as source, the non-absorbing magnesium 383·8 nm line was used as reference line, and a mean standard deviation of 0·584 per cent. absorption was obtained, which resulted in curve B of Fig. 5.

The standard deviation of the absorption values is lower with the Kranz arc as source than with the hollow-cathode lamp. This is probably because the correlation coefficient between the magnesium 285·2 nm and calcium 422·7 nm line intensities in the arc is better than the coefficient obtained between the magnesium 285·2 nm and 383·8 nm lines from the hollow-cathode lamp. The values obtained were 0·988 and 0·972, respectively. From Fig. 5 it is clear that the coefficient of variation of the concentration values with the arc as source is lower for relatively small absorptions, and it reaches its minimum value of 1·77 per cent. at about 40 per cent. absorption. The minimum coefficient of variation of 1·65 per cent. with the hollow-cathode lamp as primary source is obtained at a higher absorption level of 55 per cent. This is as a result of the greater deviation from Beer's law of the working graph obtained with the Kranz arc as primary source.

BACKGROUND—

For insight into the possibilities of universal application of this arc as a primary light source for atomic-absorption spectroscopy, a knowledge of the background emission of the arc is essential. The background emission was photographed with the same spectrograph,
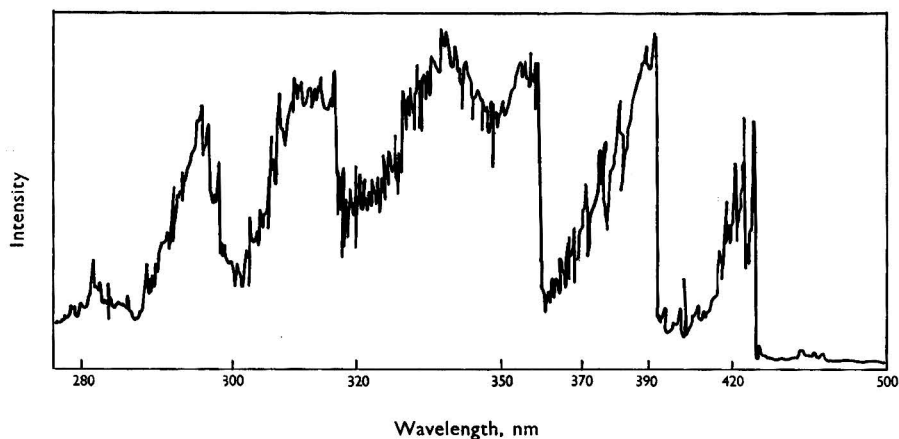
Fig. 6. Densitometer tracing showing background emission from the arc, between 280·0 nm and 500·0 nm. The regions below 280·0 nm and above 500·0 nm are essentially free from background

and the result is shown in Fig. 6. The band systems shown in Table II were identified with the aid of tables by Pearse and Gaydon.[8] The regions of heavy background emission are indicated in Table III, together with resonance lines of the elements in these regions. Of the 65 elements for which atomic-absorption sensitivities have already been measured, only twelve have their most sensitive resonance lines in these high background regions.

TABLE II

BAND SYSTEMS IDENTIFIED IN SPECTRUM OF NITROGEN PLASMA
(MOST INTENSE HEADS OF EACH SYSTEM IN BOLD FIGURES)

System	Wavelengths of band heads detected, nm						
2nd positive system of N_2	405·9	399·8	394·3	**380·4**	375·5	371·0	367·1
	364·1	357·6	353·6*	350·0	337·1*	333·9	330·9
	328·5	326·8	315·9	313·6	311·6	310·4	297·6
	296·2	295·3	281·9	281·4			
1st negative system of N_2^+	427·8	423·6	419·9	416·6	414·0	391·4	388·4
	383·5	358·2	356·3	354·8	353·8		
306·4 nm system of OH	302·1	306·4	**306·7**	307·8	308·9	281·1	
	281·9	282·9					
336·0 nm system of NH	**336·0**	337·0					

* Uncertain because of superposition by N_2^+ and NH heads.

TABLE III

REGIONS OF HEAVY BACKGROUND

Region, nm	Resonance lines involved			
291·0 to 297·8				
305·1 to 316·0	Aluminium 309·2	Molybdenum 313·3		
328·0 to 358·3	Silver 328·0	Chromium 357·9	Lanthanum 357·4	Lutetium 335·9
	Rhenium 346·0	Rhodium 343·5	Ruthenium 349·9	
371·0 to 391·4	Scandium 391·1			
415·0 to 427·8	Calcium 427·7	Dysprosium 421·1		

Although the line-to-background ratio of the silver resonance line at 328·0 nm under optimum sensitivity conditions (200 p.p.m. in primary solution) was only 13 : 1, it still gave 67 per cent. of the sensitivity obtained with a hollow-cathode lamp as primary source. Up to an absorbance of 0·30, the working graph shows no larger deviation from linearity than was observed for magnesium (see Fig. 4). For the elements chromium, molybdenum, aluminium and calcium the sensitivity ratios found were 0·65, 0·71, 0·70 and 0·63, respectively.

The corresponding line-to-background ratios, with optimum concentrations of the arc solutions, were 50:1, 40:1, 19:1 and 60:1, respectively. The slight influence of the background is shown by the different sensitivity ratios of the three chromium and two aluminium lines (see next section and Table IV). For aluminium a slightly higher sensitivity was found for the 396·1 nm line than for the 309·2 nm line with the arc as source. With the hollow-cathode lamp as source, the reverse was found, hence the high sensitivity ratio for the 396·1 nm line. Because of the high sensitivity ratios for those elements with resonance lines in the high background regions, it can be accepted that the background emission is so low as to have practically no effect on absorption measurements, and that the Kranz arc can be applied successfully as a primary light source for most elements.

TABLE IV

RESULTS OF SENSITIVITY COMPARISON TESTS

Element	Line	Optimum concentration of arc solution, p.p.m.	Sensitivity ratio	Detection limit, p.p.m.
Aluminium	309·2	1000	0·70	—
	396·1	500	0·87	—
Silver	328·0	200	0·67	—
Barium	553·5	500	0·71	—
Calcium	422·7	30	0·63	—
Cadmium	228·8	300	0·45	—
Cobalt	240·7	500	0·68	—
Chromium	357·9	200	0·65	—
	359·4	200	0·68	—
	360·5	200	0·70	—
Copper	324·8	150	0·72	—
Dysprosium	421·1	1000	—	3·4
Erbium	400·8	1000	—	4·0
Iron	248·3	200	0·72	—
Magnesium	285·2	30	0·65	—
Molybdenum	313·3	1000	0·71	—
Nickel	232·0	300	0·53	—
Lead	283·3	750	0·80	—
Scandium	391·1	1000	—	2·4
	402·0	1000	—	3·3
Strontium	460·7	30	0·68	—
Yttrium	410·2	1000	—	28
Zinc	213·8	200	0·60	—

SENSITIVITY COMPARISON WITH HOLLOW-CATHODE LAMPS—

The maximum sensitivities obtained with the Kranz arc as primary source were compared with the sensitivities obtainable with hollow-cathode lamps. The hollow-cathode lamps were always run with currents giving maximum absorption sensitivity coupled with sufficient light intensity, these values ranging from 5 to 15 mA. Table IV shows the results for several arbitrarily chosen elements. For the elements dysprosium, erbium, aluminium, molybdenum, scandium and yttrium, a 5-cm nitrous oxide - acetylene flame was used as the absorbing medium, while the remaining elements were investigated with a 10-cm air - acetylene flame. For dysprosium, erbium, scandium and yttrium no hollow-cathode lamps were available and, instead of the sensitivity ratios, the detection limits obtained are given (defined as the concentration giving an absorption signal equal to twice the standard deviation of the background value). Attempts to obtain absorption signals for samarium and gadolinium were unsuccessful. The samarium resonance line at 429·6 nm was not sufficiently excited to be detected, even with 5000 p.p.m. of samarium in the arc. The gadolinium resonance line at 368·4 nm could easily be detected, but even 1000 p.p.m. of gadolinium in the nitrous oxide - acetylene flame gave no absorption signal.

DETECTION LIMITS—

As shown under Reproducibility it is clear that, if a proper reference line is used, the reproducibility of measurement with the Kranz arc as source is just as good as that with a hollow-cathode lamp. Thus, the same ratios listed in Table IV for the relative sensitivities are applicable when considering the relative detection limits.

EXTENSION OF WORKING RANGE—

A disadvantage of the atomic-absorption technique is the relatively narrow working range. With a Kranz arc as source, the sensitivity can easily be decreased by using solution concentrations other than the optimum in the arc. Fig. 7 shows that the working range for magnesium can be extended to the lower sensitivity side by a factor of 8, by increasing the concentration of the arc solution from 30 to 1000 p.p.m. This procedure, however, inevitably results in a working graph with greater curvature because of self-absorption of the emission line.
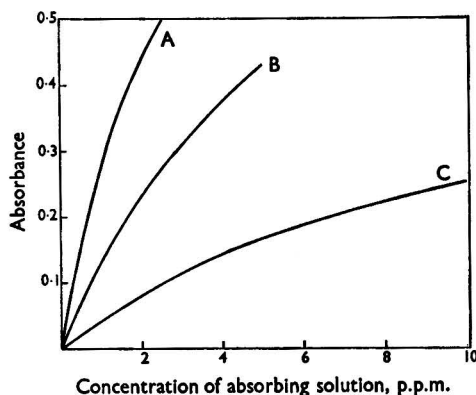
Fig. 7.   Working graphs: A, 30 p.p.m. of magnesium in arc; B, 300 p.p.m. of magnesium in arc; C, 1000 p.p.m. of magnesium in arc

APPLICATION TO METAL ANALYSIS

Lead, nickel and iron were determined simultaneously in the brass sample NBS 124b, with the Kranz arc as a primary source. With the optimum concentrations of the three elements in the arc, a 0·030 per cent. w/v solution of the sample gave absorption signals on the linear part of the working graph, and the concentration values in Table V were obtained.

TABLE V

ANALYSIS OF BRASS SAMPLES

Sample			Element	Results obtained by using present technique, per cent.	Chemical values, per cent.
NBS 124b	..	..	Lead	4·53	4·64
			Nickel	0·74	0·75
			Iron	0·28	0·26
BCS 183	..	..	Lead	1·80	1·83
			Zinc	1·82	1·86

In the simultaneous determination of lead and zinc in sample BCS 183 (brass), it was found that a 0·015 per cent. w/v solution of the sample absorbed 6 per cent. of the lead resonance line and 53 per cent. of the zinc resonance line, with optimum concentration values in the primary source. The latter absorption signal was considered to be too high for good accuracy. The zinc concentration in the primary source was, therefore, increased from 200 p.p.m. to 2400 p.p.m., resulting in an absorption signal of only 12·2 per cent. In this way the values in Table V were obtained for this sample.

For both of these determinations the lead 405·8 nm line was used as reference, and a reproducibility test was carried out on the sample NBS 124b. The standard deviations (obtained from twenty-six measurements) of the percentage absorption values were 0·54 per cent. absorption for iron, 0·83 per cent. absorption for nickel and 0·27 per cent. for lead. These values indicate that the lead 405·8 nm line is excellent for the lead resonance line, but is less suitable for the iron resonance line and is even worse for the nickel resonance line. This low standard deviation of the lead percentage absorption values transforms to a relative

standard deviation (of the concentration values) of 1·35 per cent. at an absorption level of 20 per cent., which was the actual absorption signal that the sample gave. A minimum relative standard deviation of 1·22 per cent. was obtained at about 40 per cent. absorption.

DISCUSSION

Of the fourteen elements for which the Kranz arc sensitivities were compared with sensitivities obtained with hollow-cathode lamps as primary sources, the mean sensitivity ratio is 0·66 (the secondary lines of aluminium and chromium are not considered). Rann[9] showed that, when a plasma at atmospheric pressure is used as primary light source, the sensitivity depends on the value of the damping constants of the resonance lines in the primary source and in the absorbing medium, as well as on the degree of self-absorption in the primary source. Thus, to be able to predict the relative sensitivity expected for a particular element, these parameters need to be known.

The reason for the optimum value of concentration of the solution in the primary source (see Fig. 3) is that a compromise is achieved between decreasing line-to-background ratios for lower concentrations, and increasing self-absorption at higher concentrations. Although the emissivity of the arc in the higher regions (h_2 and h_3) is less, the same absorption signal can be obtained by increasing the concentration of the solution sprayed into the arc. This indicates that the temperature and arc region are not critical for obtaining maximum sensitivity, but that the concentration of the element is the only critical parameter.

CONCLUSION

The Kranz arc can be used most successfully as a primary light source for atomic-absorption spectroscopy. The advantages of using such a source are the possibility of determining several elements simultaneously; only solutions of the desired element combinations are required; and the working range can be extended to lower sensitivities.

In comparison with hollow-cathode lamps, the arc source has the disadvantage of having poorer sensitivities and more curved working graphs. However, the reproducibility of measurement, when using a suitable reference line, is just as good as with hollow-cathode lamps as primary sources.

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A Comprehensive Scheme for the Analysis of Cement by Atomic-absorption Spectrophotometry*

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Atomic-absorption spectrophotometry has been applied to the determination of aluminium, calcium, iron, magnesium, manganese, potassium, silicon, sodium, strontium and zinc in cement. Only one sample weighing is necessary, and the results, which agree well with standard values obtained by classical methods of analysis, can be obtained within a few hours.

METHODS currently used for the analysis of cement samples[1] frequently involve lengthy procedures and tedious separations. Sample preparation normally involves hydrochloric acid attack followed by dehydration of the precipitated silica, which is then separated by filtration. The determination of individual elements is usually performed gravimetrically or titrimetrically, although emission-flame photometry has been used for several elements.[2,3]

Takeuchi and Suzuki[4] have determined sodium, potassium, magnesium, manganese and calcium in cement by atomic-absorption spectrophotometry, reporting good precision for all but calcium. Capacho-Delgado and Manning[5] have described the determination of several elements, including aluminium, silicon and titanium. The determination of aluminium, calcium, iron, magnesium, manganese, potassium and sodium has also been reported by Crow, Hime and Connolly.[6]

To compensate for possible matrix effects these authors used standard cement samples for the preparation of calibration standards.

Price and Roos[7] have described the determination of silicon in cement by atomic-absorption spectrophotometry after dissolution of the sample in a mixture of hydrochloric and hydrofluoric acids. They reported enhancement of the silicon absorption by calcium, aluminium and iron in the samples. Addition of vanadium to both standard solutions and samples compensated for this effect.

EXPERIMENTAL

APPARATUS—

The present work was carried out with a Unicam SP90 atomic-absorption spectrophotometer equipped with a recorder, lamp turret and nitrous oxide flame accessories, and a standard set of interchangeable stainless-steel burner heads. Hollow-cathode lamps were obtained from Pye Unicam Limited, Cambridge. High spectral output lamps were used for aluminium, silicon and zinc. Polythene or PTFE apparatus was used whenever solutions contained uncomplexed hydrofluoric acid.

REAGENTS—

Aluminium solution, 2 per cent. Al^{3+}—A 20·0-g sample of pure aluminium metal was dissolved in the minimum amount of hydrochloric acid $(1 + 1)$, with heating, and diluted to 1 litre.

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* Paper presented at the Second SAC Conference 1968, Nottingham.

Lanthanum chloride solution, 5 per cent. La^{3+}—A 58·6-g sample of lanthanum oxide was dissolved in 800 ml of 20 per cent. hydrochloric acid, with heating, and diluted to 1 litre.

Vanadium chloride solution, 2·5 per cent.—A 25·0-g sample of vanadium trichloride was dissolved in 500 ml of water containing 20 ml of analytical-reagent grade hydrochloric acid (sp.gr. 1·16); the solution was filtered and diluted to 1 litre.

Boric acid solution, 4 per cent.—A 40-g sample of analytical-reagent grade boric acid was dissolved in 800 ml of water, with heating, and the solution made up to 1 litre.

Stock solutions, 1000 p.p.m.—Stock solutions of aluminium, iron, manganese, magnesium and zinc were prepared by dissolving 1·000 g of the pure metal in the minimum volume of hydrochloric acid (1 + 1) and making up to 1 litre. Calcium, potassium, sodium and strontium stock solutions were prepared from analytical-reagent grade salts, while the stock silicon solution was prepared from sodium silicate, and standardised gravimetrically for silica. For the preparation of calibration graphs, the stock solutions were further diluted as required.

All solutions were made up with de-ionised water and stored in polythene bottles, and all acids used were of analytical-reagent grade.

INTERFERENCES—

The effects of the other constituents of cement on the determination of a particular element were investigated over those concentration ranges likely to occur in cement samples. As expected, silicon was found to suppress the absorption by aluminium, iron, calcium, magnesium and zinc. Addition of vanadium (to give an over-all concentration of 0·5 per cent. of vanadium chloride) was found to overcome the effect of silicon on aluminium, iron and zinc; addition of lanthanum (to give a final concentration of 0·5 per cent. of La^{3+}) instead of vanadium overcame the silicon interference on all the above elements. Similarly, interference of aluminium on calcium and magnesium was eliminated by the addition of lanthanum.

With the nitrous oxide - acetylene flame the only interference observed in the determination of strontium was from calcium, which caused considerable enhancement of the strontium absorption. The addition of lanthanum to both standards and samples fully compensated for this effect, and also for the enhancement, in an air - acetylene flame, of potassium absorption by calcium. It was also found that the presence of lanthanum compensated for the enhancement of silicon absorption by calcium, aluminium and iron in the samples.

Hydrofluoric acid in the amounts used in this investigation were not found to interfere in any of the determinations.

DEVELOPMENT OF THE METHOD—

Addition of lanthanum chloride solution to a solution containing even a small concentration (0·5 per cent. v/v) of hydrofluoric acid causes precipitation of insoluble lanthanum fluoride. This precipitate is not re-dissolved, even in the presence of relatively high concentrations of nitric or hydrochloric acids. It was, therefore, necessary to complex the excess of hydrofluoric acid (after dissolution of the sample) prior to addition of lanthanum. Aluminium and beryllium are reported[8] to be the most effective complexing agents for higher concentrations of hydrofluoric acid. As the use of beryllium in routine analysis is precluded by its toxicity and, as aluminium is normally determined in cement, the possible use of boric acid was investigated. Addition of 2 g of boric acid to 1 ml of hydrofluoric acid (40 per cent.) in 50 ml of water was found to prevent precipitation of lanthanum fluoride when 20 ml of lanthanum solution were added. The presence of boric acid effectively prevented hydrofluoric acid attack of glassware by such solutions.

The presence of boric acid was not found to influence the absorption of light by any of the elements determined in cement; nevertheless, it is recommended that boric acid also be added to the standard solutions to compensate for any impurities in the boric acid solution.

With a 10-cm air - acetylene flame, the most suitable concentration ranges for calcium and magnesium are 3 to 30 p.p.m. and 0·2 to 2·5 p.p.m., respectively. These concentration ranges require a 50 or 100-fold dilution of the original sample solution as described under Procedure. With an emission burner head giving an absorption path of 1 cm, however, the method becomes less sensitive for both calcium and magnesium by a factor of 5 to 10, thus making it possible to work with much higher concentrations of these elements. A 10-fold dilution only of the original sample solution is then required, leading to both an increase in precision and a saving in time.

PROCEDURE

STANDARDS—

Prepare the following series of calibration standards by appropriate dilution of the respective stock solutions.

Aluminium solutions—Equivalent to 0 to 125 p.p.m. of alumina in 0·5 per cent. lanthanum (La^{3+}) solution.

Iron solutions—Equivalent to 0 to 125 p.p.m. of iron(III) oxide in 0·5 per cent. lanthanum solution.

Manganese solutions—Equivalent to 0 to 2·5 p.p.m. of manganese(III) oxide in 0·5 per cent. lanthanum solution.

Silicon solutions—Equivalent to 0 to 750 p.p.m. of silica in 0·5 per cent. lanthanum solution.

Sodium solutions—Equivalent to 0 to 12·5 p.p.m. of sodium oxide in 0·5 per cent. lanthanum solution.

Strontium solutions—Equivalent to 0 to 12·5 p.p.m. of strontium oxide in 0·5 per cent. lanthanum solution.

Zinc solutions—Equivalent to 0 to 0·5 p.p.m. of zinc oxide in 0·5 per cent. lanthanum solution.

Each member of the above series should also contain 25 ml of boric acid and 5 ml of hydrochloric acid (sp.gr. 1·16) per 100 ml of solution.

Calcium solutions—Equivalent to 0 to 200 p.p.m. of calcium oxide in 0·5 per cent. lanthanum solution.

Magnesium solutions—Equivalent to 0 to 12·5 p.p.m. of magnesium oxide in 0·5 per cent. lanthanum solution.

Potassium solutions—Equivalent to 0 to 2·5 p.p.m. of potassium oxide in 0·5 per cent. lanthanum solution.

These solutions should also contain 2·5 ml of boric acid solution and 5 ml of hydrochloric acid (sp.gr. 1·16) per 100 ml of solution.

SAMPLE PREPARATION—

Weigh 0·500 g of the powdered sample into a 100-ml polythene or PTFE beaker. Wash down the sides of the beaker with about 20 ml of water. Add, with stirring, 10 ml of hydrochloric acid (sp. gr. 1·18), breaking up any gritty particles with the end of the stirring rod. When the sample has dissolved (except for any precipitated silica) rinse down the glass rod, remove it from the beaker and add 1·0 ml of hydrofluoric acid (40 per cent.). Carefully swirl the mixture until all precipitated silica is dissolved, then add 50 ml of boric acid solution and mix thoroughly. Transfer the solution quantitatively to a 200-ml calibrated flask, add 20 ml of stock lanthanum chloride solution and dilute to the mark with water (solution A). Transfer 10·0 ml of solution A to a 100-ml calibrated flask, add 5 ml of hydrochloric acid and 9·0 ml of stock lanthanum chloride solution, and dilute to the mark with water (solution B).

Solution A is used for the determination of aluminium, iron, manganese, silicon, sodium, strontium and zinc, while solution B is used for the determination of calcium, magnesium and potassium. The instrumental conditions for these determinations are given in Table I.

TABLE I

INSTRUMENTAL CONDITIONS FOR TEN ELEMENTS

Element	Wave-length, nm	Slit width, mm	Burner	Acetylene flow-rate, litres per minute	Oxidant, litres per minute	Burner height, cm	Lamp current, mA
Aluminium	309·3	0·10	5-cm N_2O - C_2H_2	4·0	Nitrous oxide, 5·0	1·0	10
Calcium	422·7	0·08	Air - C_2H_2 emission	1·4	Air, 5·0	2·0	12
Iron	248·3	0·10	10-cm air - C_2H_2	1·4	Air, 5·0	1·0	15
Magnesium	285·2	0·08	Air - C_2H_2 emission	1·4	Air, 5·0	2·0	4
Manganese	279·5	0·15	10-cm air - C_2H_2	1·4	Air, 5·0	1·0	12
Potassium	766·5	0·15	10-cm air - C_2H_2	1·0	Air, 5·0	1·0	12
Silicon	251·6	0·10	5-cm N_2O - C_2H_2	4·3	Nitrous oxide, 5·0	1·0	15
Sodium	589·0	0·10	10-cm air - C_2H_2	1·0	Air, 5·0	1·0	12
Strontium	460·7	0·10	5-cm N_2O - C_2H_2	4·0	Nitrous oxide, 5·0	1·0	12
Zinc	213·9	0·10	10-cm air - C_2H_2	1·0	Air, 5·0	1·0	12

RESULTS

The results obtained for the analysis of standard cement samples by the proposed method are given in Table II. Also included in this table are figures for the precision (expressed as the coefficient of variation) for the determination of seven of the elements. These were calculated from the results obtained for six replicate analyses of the same standard cement sample (N.B.S. 1015).

TABLE II

ANALYSIS OF STANDARD SAMPLES

| Constituent | N.B.S. 1013 | | N.B.S. 1015 | | Coefficient of variation, per cent. |
	Certificate	Atomic absorption	Certificate	Atomic absorption	
Alumina, Al_2O_3	3·30	3·18 3·21	5·04	4·99 5·06	1·0
Calcium oxide, CaO.. ..	64·26	64·6 63·9	61·37	60·4 59·5	0·7
Iron(III) oxide, Fe_2O_3	3·07*	3·21 3·17	3·27*	3·36 3·38	0·7
Magnesium oxide, MgO ..	1·39	1·37 1·37	4·25	4·35 4·24	0·7
Manganese oxide, Mn_2O_3 ..	0·05	0·052 0·052	0·06	0·059 0·059	1·3
Potassium oxide, K_2O ..	0·32	0·38 0·38	0·87	0·85 0·85	—
Silica, SiO_2	24·2	24·7 23·7	20·6	21·0 20·3	1·2
Sodium oxide, Na_2O ..	0·20	0·19 0·19	0·16	0·17 0·16	—
Strontium oxide, SrO ..	0·08	0·080 0·085	0·11	0·095 0·098	0·9
Zinc oxide, ZnO	—	0·008 0·008	—	0·011 0·011	—

* Capacho-Delgado and Manning[5] found 3·17 and 3·37 per cent. of Fe_2O_3 for N.B.S. 1013 and N.B.S. 1015, respectively.

The complete analysis of five samples was performed in about 1 normal working day made up as follows. Dissolution of five samples required 1¼ hours; the preparation of dilutions for atomic-absorption analysis ¼ hour; the setting up of the instrument took 5 minutes; the measurement of five samples for one element 5 minutes and the measurement of six standards for one element 6 minutes, thus the time required for ten elements was about 3 hours; and the preparation of calibration graphs and the calculation of results required 3½ hours. The total time, therefore, required was 8 hours.

No significant increase in the time required for analysis would be caused by an increase in the number of samples analysed.

DISCUSSION

Suppression of the absorption of several elements in the flame by silicon (as silicate ions) is a well established phenomenon, having been noted by several authors.[9,10,11] However, the suppression of aluminium absorption appears not to have been noted previously. This effect operates only if, in addition to aluminium and silicon, a third element such as calcium is present. The interference is, therefore, dependent on the formation of a complex silicate, such as calcium aluminium silicate. This does not occur in the presence of an excess of vanadium or lanthanum.

Although either vanadium or lanthanum chlorides can be used as releasing agents for aluminium, iron and zinc in the presence of silicate ions, lanthanum is recommended because vanadium does not adequately release calcium, magnesium and manganese from refractory silicates, whereas lanthanum does; lanthanum fully compensates for the enhancement of the strontium absorption, by calcium in the samples, while vanadium, because of its higher ionisation energy, compensates only partially; and vanadium salts are not readily available in a sufficiently pure form.

The use of lanthanum salts as releasing agents for calcium and magnesium is frequently criticised on the ground that laboratory-reagent grade lanthanum compounds may contain significant amounts of magnesium and calcium impurities, resulting in large values for the reagent blanks. However, by using the shorter path-length emission burner with consequent reduction in sensitivity, the calcium and magnesium concentrations can be increased by a factor of five or ten over those normally used in the long-path burner. However, the same amount of lanthanum is found to give complete releasing action, and the readings for the blank solutions are proportionately reduced to acceptable limits.

Atomic absorption has proved to be an extremely rapid and versatile method for the analysis of cement.   Not only is it possible to determine ten elements after one sample weighing, and without any chemical separations, but the reproducibility is comparable with that expected for the accepted methods of analysis.   The only possible exceptions are the determinations of the major constituents, silicon and calcium.   For example, a standard deviation of 0·7 per cent. was obtained for calcium, for which standard gravimetric procedures are usually expected to give nearer 0·2 per cent.

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Separate and Simultaneous Determination of Zirconium and Hafnium in Nickel-base Alloys with Xylenol Orange*

By H. J. G. CHALLIS

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The use of xylenol orange as a spectrophotometric reagent for zirconium and hafnium has been investigated for their determination in the range 0·002 to 0·2 per cent. in complex nickel-base alloys, and the effects of major alloying elements, and likely impurities, have been studied. A simple, direct procedure, based on the formation of the red xylenol orange complexes in 0·8 N hydrochloric acid, has been successfully applied to the determination of either metal in nickel alloys containing chromium, cobalt, iron, molybdenum, titanium and aluminium.

For alloys containing both zirconium and hafnium, a procedure developed for their simultaneous determination is based on the relative effect of acid concentration on the xylenol orange complexes. Preliminary mercury cathode and hydroxide separations are followed by measurement of the total optical densities at three levels of acidity, 0·35, 1·12 and 2·0 N perchloric acid. This "three-point" method has proved satisfactory with synthetic alloy solutions and when applied to complex nickel alloys containing both zirconium and hafnium. Confirmatory evidence of the results was obtained by X-ray fluorescence, emission and mass spectrometry.

The simultaneous procedure provides a simple and sensitive chemical method of differentiating between microgram amounts of zirconium and hafnium, and should be capable of wider application to other alloy systems. The simpler direct method should also prove advantageous when mutual interference does not arise.

ADDITION of minor amounts of zirconium or hafnium, or both, beneficially affects the mechanical properties, weldability and mechanical working of various nickel-base alloys. However, because of their close chemical similarity, the separate determination of these two elements in alloys containing aluminium, chromium, cobalt, iron, manganese, molybdenum and titanium presented a difficult analytical problem.

Consideration of the numerous spectrophotometric reagents available for zirconium and hafnium[1,2] indicated that xylenol orange and arsenazo III offered distinct advantages in high sensitivity and selectivity. As arsenazo III was not available for the initial tests, xylenol orange was chosen for detailed examination, particularly as exploratory tests indicated that this reagent not only offered the possibility of a direct method for both elements, but also their simultaneous determination appeared feasible. Following lengthy separations, Cheng[3,4,5] used xylenol orange to determine zirconium or hafnium in high-temperature alloys, but his attempt to determine hafnium in the presence of zirconium by control of acidity was unsuccessful.

In the investigation reported below, initial experiments were concerned with the development of simple, rapid and direct methods for the separate determination of each of the elements and later, after studies of the effect of acidity and alloying metals on colour reaction, methods were established covering the range 0·002 to 0·2 per cent. of zirconium or hafnium in complex nickel alloys. However, the problem of mutual interference remained. On a practical basis, alloys in which zirconium alone is used could safely be assumed to be hafnium free, but the converse was not true, because commercial grades of hafnium contain 2 to 3 per cent. of zirconium. These initial experiments, together with the information available in the literature, formed a useful background to the development of a procedure for the simultaneous determination of each element in the presence of one another.

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* Paper presented at the Second SAC Conference 1968, Nottingham.

EXPERIMENTAL

APPARATUS—

A Unicam SP600 spectrophotometer was used for all optical-density measurements made at 535 nm, with 1-cm cells.

SEPARATE DETERMINATION OF ZIRCONIUM AND HAFNIUM—

Effect of acidity—The effect of acidity on 50 μg of zirconium or hafnium was studied by preparing solutions containing varying amounts of hydrochloric or perchloric acids (0·3 to 2 N) per 25 ml and 2 ml of xylenol orange solution (0·05 per cent.). The results (Fig. 1) confirmed the optimum acidity for zirconium to be 0·8 N and that for hafnium 0·35 N. Careful control of acidity was obviously essential, for although the zirconium and hafnium complexes were only slightly affected between 0·7 and 0·9 N, and 0·3 and 0·4 N, respectively, the reagent blank values were high, with optical-density readings of 0·10 at 0·35 N and 0·14 at 0·8 N. These values were, however, stable and reproducible. In subsequent tests, a preliminary acid determination was carried out on an aliquot of each solution by titration, in order that another aliquot could be exactly adjusted to the desired acidity before addition of the xylenol orange reagent.
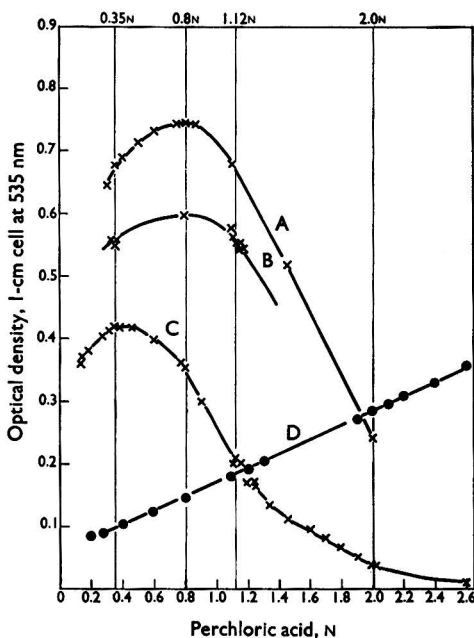
Fig. 1. Effect of perchloric acid on zirconium - xylenol orange and hafnium - xylenol orange complexes. Curve A, 50 μg of zirconium; curve B, 40 μg of zirconium; curve C, 50 μg of hafnium; and curve D, reagent blank

Calibration graphs—Based on Cheng's recommendations,[3,5] satisfactory calibration graphs were constructed for zirconium up to 50 μg per 25 ml in 0·8 N hydrochloric or perchloric acids, and for hafnium up to 100 μg per 25 ml in the same acids at 0·35 N concentration.

The graphs for zirconium were almost identical in the two acids and complied with the Beer - Lambert law; similarly, graphs for hafnium at 0·35 N concentration were equally satisfactory. The similarity of graphs obtained in hydrochloric and in perchloric acids was convenient, as the use of mixed acids was made practical.

Effect of other metals—The effect of nickel and of other metals likely to occur in nickel - chromium high-temperature alloys was studied by analysis of synthetic solutions of Specpure (Johnson, Matthey Limited) metals in 0·8 N hydrochloric acid. After correcting for any background colour, metals in the amounts shown in Table I were found to have no apparent

TABLE I

EFFECT OF NICKEL AND OTHER METALS ON THE DIRECT DETERMINATION OF ZIRCONIUM

Metals	Weight, g	Per cent. (calculated on 0·05-g sample)	Zirconium added, µg	Zirconium found (apparent), µg
Nickel	0·05	100	—	N.D. (<0·5)
			10	10·2
			20	20·3
			50	51
Aluminium	0·05	100	—	N.D. (<0·5)
			10	10, 10·2
Cerium	0·003	6	—	N.D. (<0·5)
			10	9·8, 10·1
			10*	9·8
Cobalt	0·02	40	—	N.D.
Chromium	0·02	40	—	N.D.
Copper	0·05	100	—	N.D. (<0·5)
			10	10·0, 9·8
Nickel - chromium	0·05	80 nickel - 20 chromium	—	N.D.
			10	10·2
Nickel - chromium - cobalt ..	0·05	60 nickel - 20 chromium - 20 cobalt	10	9·8, 10·2
Manganese	0·05	100	—	N.D.
			10	10·0, 10·1
Magnesium	0·05	100	—	N.D.
			10	9·9, 9·8, 10·2
Thorium	0·004	8	—	N.D.
			10	9·9, 10·3
Yttrium..	0·02	40	—	N.D.
			10	10·0, 10·5
Titanium	0·05	100	—	N.D. (<0·5)
			10	9·8, 9·9
			50	49·0

* After fuming with perchloric acid and reduction with hydrochloric acid.
N.D.—Not determined.

effect on xylenol orange or on the determination of added zirconium (10 µg). Iron and molybdenum, however, produced slight positive errors, equal to an apparent zirconium content of 0·001 per cent. for each 1 per cent. of iron, and 0·001 per cent. for 10 per cent. of molybdenum (Table II). Reduction of iron by ascorbic acid was not entirely satisfactory, but with tin(II)

TABLE II

INTERFERENCE OF IRON AND MOLYBDENUM IN DIRECT METHOD

Weight, g	Per cent. (calculated on 0·05-g sample)	Apparent zirconium, per cent.
Iron—		
0·0005	1	0·001
0·001	2	0·002
0·0025	5	0·007
0·005	10	0·015
0·05	100	0·13
Molybdenum—		
0·005	10	0·001
0·01	20	0·003
0·025	50	0·006
0·05	100	0·010

chloride the error was almost eliminated, and it was thus possible to make determinations in the presence of at least 10 per cent. of iron (calculated on a 0·05-g sample). Unfortunately, tin(II) chloride could not be used in the presence of molybdenum because a dark brown colour was produced; this colour faded rapidly. However, in the samples to be analysed, iron and molybdenum did not exceed about 5 per cent.; it was, therefore, considered preferable to leave any iron in the iron(III) state and apply slight corrections for both of the interfering elements. Although the alloys concerned did not contain tungsten, niobium or tantalum, it should be noted that alloying amounts of these elements can interfere because of precipitation.

In attempting to apply the direct method to hafnium two complications arose; first, the mutual interference of zirconium and hafnium; secondly, the considerably increased interference of iron at the optimum acidity of 0·35 N, amounting to an apparent 0·2 per cent. of hafnium for each 1 per cent. of iron present. Obviously, direct determination at 0·35 N acidity would be unsatisfactory for alloys containing minor or alloying amounts of iron. Provided that zirconium was present only as an impurity arising from the hafnium present (i.e., about one fiftieth of the hafnium content), a more satisfactory alternative was to sacrifice some sensitivity and determine hafnium in 0·8 N hydrochloric acid.

The direct method for zirconium or hafnium was checked by analysis of synthetic nickel-base solutions with a basic composition similar to that of the commercial high-temperature alloy "Nimonic"* alloy 105. Satisfactory recoveries were obtained of 0·02 to 0·10 per cent. of added zirconium or hafnium (Table III).

TABLE III

DIRECT DETERMINATION OF ZIRCONIUM OR HAFNIUM IN SYNTHETIC NICKEL-BASE ALLOY*

Test No.	Zirconium, per cent. Added	Found	Test No.	Hafnium, per cent. Added	Found
1	Nil	<0·001	5	Nil	<0·001
2	0·020	0·020	6	0·020	0·020
3	0·040	0·040	7	0·040	0·039
4	0·100	0·102	8	0·100	0·098

* Cr 15, Co 20, Mo 5, Ti 2, Al 5, Fe 2, Mn 1 per cent.; balance Ni.

SIMULTANEOUS DETERMINATION OF ZIRCONIUM AND HAFNIUM—

The major problem of the separate determination of the two elements remained, and the final solution was based on a study of the relative effect of acid concentration on their relevant xylenol orange complexes.

In an attempt to determine hafnium in the presence of zirconium, Cheng[5] used the difference in optical densities in 0·6 and 1·0 N perchloric acid but obtained results 20 to 30 per cent. high, which he attributed to difficulty in control of acidity. In the present investigation, control of acidity by the preliminary titration technique has presented no difficulty. Further study of his acidity curves, and of those shown in Fig. 1, suggested that measurement of optical densities at other levels of acidity would be more advantageous.

Cheng[5,6] also suggested an alternative procedure involving the masking of zirconium with hydrogen peroxide, but tests have shown that such a procedure would be inapplicable in the presence of titanium. Experiments were, therefore, concentrated on the effect of acidity based on the following observations.

The optical density of the pure zirconium complex is the same at 0·35 N (optimum for hafnium) and 1·1 N. Consequently, in solutions containing both elements, any difference between the measurements at these two levels of acidity will be a function of the hafnium present. A value for hafnium in such solutions should, therefore, be obtainable from a calibration graph constructed from the difference between optical-density readings at 0·35 and 1·1 N. This value, converted into a corresponding optical-density reading for hafnium at 0·35 N, and subtracted from the total reading at this acid concentration, should give the optical density attributable to zirconium at 0·35 N, thus enabling the amount present to be determined.

* "Nimonic" is a trade mark.

TABLE IV

SIMULTANEOUS DETERMINATION OF HAFNIUM AND ZIRCONIUM IN SYNTHETIC
SOLUTIONS BY "TWO-POINT" METHOD

Added, μg		Recovered, μg	
Hafnium	Zirconium	Hafnium	Zirconium
50	20	48·5, 52, 45	22, 20, 23
20	20	24, 18	19, 20
20	40	26, 22	37, 40
30	40	33·5	37·5
10	40	14	38

Appropriate calibration graphs were constructed for hafnium (in micrograms) plotted against optical density at 0·35 N *minus* optical density at 1·1 N also for zirconium at 0·35 N. The proposed method was checked by determinations of the total optical density at 0·35 and 1·1 N for synthetic solutions containing 10 to 50 μg of hafnium and 20 to 40 μg of zirconium. Results obtained by this "two-point" method (Table IV) were promising, but only approximately correct, probably because of the steep slope of the hafnium acidity curve at 1·1 N concentration.

From a further consideration of the curves in Fig. 1, measurement of optical density at a third point, 2·0 N, was introduced. This full procedure was designated the "three-point" method. At that level of acidity, the optical density due to hafnium is considerably reduced, to about one tenth of that at 0·35 N, whereas readings for zirconium are about one third. Correction for hafnium at 2·0 N can, therefore, be made on the basis of the approximate content calculated from the readings at 0·35 and 1·1 N described in the "two-point" method, to provide a more accurate zirconium value. In turn, the latter value can be converted into an equivalent reading at 0·35 N which, subtracted from the total optical-density readings at this point, enables the hafnium content to be calculated at the optimum acidity.

Briefly, the "three-point" method involves prior construction of calibration graphs for zirconium and hafnium at 0·35 and 2·0 N, also for hafnium readings corresponding to the optical density at 0·35 N *minus* optical density at 1·1 N. Zirconium and hafnium contents can then be calculated from the total optical-density readings obtained in perchloric acid solutions at 0·35, 1·1 and 2·0 N. This procedure was checked by analysis of synthetic solutions containing 20 to 50 μg of zirconium and 10 to 50 μg of hafnium. The recoveries obtained (Table V) showed a definite improvement over corresponding values calculated from "two-point" readings, and were sufficiently promising to justify application of the method to actual samples.

TABLE V

SIMULTANEOUS DETERMINATION OF HAFNIUM AND ZIRCONIUM IN SYNTHETIC
SOLUTIONS: COMPARISON OF "TWO-POINT" AND "THREE-POINT" METHODS

Added, μg		Recovered, μg			
		"Two-point" method		"Three-point" method	
Hafnium	Zirconinm	Hafnium	Zirconium	Hafnium	Zirconium
50	20	48	22·5	50·5	20·5
50	20	46	20·5	51·5	20·5
50	20	49	20·3	51	19
20	20	23·5	18·5	21	19·5
20	50	25	46	21	49
10	50	14·5	46	10	49

The experimental work on the direct method showed clearly that removal of interfering elements, particularly iron and molybdenum, would be essential. It was, therefore, decided to include mercury cathode and hydroxide separations.[4,5] The full, proposed procedure was tested by addition of varying amounts of zirconium and hafnium to synthetic solutions containing the equivalent of 1 g of a nickel-base alloy, and satisfactory recoveries were obtained at the equivalent of 0·01 to 0·05 per cent. of zirconium and 0·02 to 0·20 per cent. of

hafnium (Table VI). Attempts were made to electrolyse in perchloric acid solution to avoid the hydroxide separation, which had been included to eliminate the interference by sulphates and sulphuric acid, but results were then less satisfactory. The procedures described below were, therefore, finally adopted.

TABLE VI

DETERMINATION OF HAFNIUM AND ZIRCONIUM IN SYNTHETIC SOLUTIONS OF A NICKEL-BASE ALLOY* BY "THREE-POINT" METHOD

Test No.	Added, per cent.		Found, per cent.	
	Hafnium	Zirconium	Hafnium	Zirconium
1	—	—	N.D. <0·002	0·003
2	0·02	—	0·021, 0·022	0·002, 0·003
3	0·03	—	0·032	0·002
4	0·05	—	0·052	0·002
5†	0·10	0·01	0·098, 0·102, 0·100	0·010, 0·011, 0·009
6†	0·20	0·02	0·201	0·020
7†	0·02	0·02	0·022	0·019
8†	0·05	0·03	0·052	0·030
9†	0·10	0·05	0·096	0·050

* Alloy composition, per cent.: Cr 20, Co 16, Ti 2·5, Al 1·5; balance Ni.
† Tests 5 to 9 corrected for Zr in alloy blank (0·003 per cent.).

DETERMINATION OF ZIRCONIUM AND HAFNIUM IN NICKEL-BASE ALLOYS

METHOD I: DIRECT DETERMINATION OF ZIRCONIUM OR HAFNIUM

This method is suitable for either zirconium or hafnium in amounts up to about 0·2 per cent. when present alone in nickel-base alloys, substantially free from tungsten, niobium and tantalum.

REAGENTS—

Standard zirconium solution—Transfer 0·1000 g of high-purity zirconium to a platinum dish (3 inches in diameter is suitable). Add 20 ml of water, then hydrofluoric acid (40 per cent.), dropwise, until the metal is dissolved (about 0·5 ml will be required). Add 5 ml of sulphuric acid (sp.gr. 1·84), evaporate to dense fumes of sulphuric acid and fume for 10 minutes. Cool, wash the sides of the dish with water and fume again for 5 minutes. Cool, wash the sides of the dish with water and then fume almost to dryness. Cool, dissolve the salts in 20 ml of hydrochloric acid (1 + 1) and heat to boiling for 5 minutes, cool, transfer to a 100-ml calibrated flask and dilute to the mark. Dilute 5 ml of this solution to 500 ml with 0·8 N hydrochloric acid.

1 ml of final solution ≡ 10 μg of zirconium.

Standard hafnium solution—Transfer 0·1000 g of high purity hafnium to a platinum dish and proceed as described for the preparation of 100 ml of standard zirconium solution. Dilute 10 ml of this solution to 500 ml with 0·8 N hydrochloric acid.

1 ml of final solution ≡ 20 μg of hafnium.

Xylenol orange solution, 0·05 per cent.—Dissolve 0·0500 g of xylenol orange in 50 ml of water and dilute to 100 ml (Note 1).

PREPARATION OF CALIBRATION GRAPHS FOR ZIRCONIUM AND HAFNIUM—

Add 6·0 ml of standard zirconium (*or* hafnium) solution to a 25-ml graduated flask and, to another 25-ml flask, add 1·75 ml of hydrochloric acid (sp.gr. 1·18). Dilute each solution to the mark and check the normalities of the respective solutions by titrating 1 ml with 0·1 N sodium hydroxide (Note 2).

Prepare a series of calibration solutions by the separate addition of 0·0, 1·0, 2·0, 3·0, 4·0, 5·0 and 6·0 ml of the standard zirconium (*or* hafnium) solution to each of seven 25-ml calibrated flasks; add the amount of hydrochloric acid (sp.gr. 1·18) calculated to produce a final acidity of 0·80 N (Note 2). Dilute the solutions to 20 ml, add 2 ml of 0·05 per cent. xylenol orange solution and dilute to the mark.

Measure the optical densities of the solutions at 535 nm, with a 1-cm cell, against water in the compensating cell. Correct for the reagent blank and construct a calibration graph for optical densities against micrograms of zirconium (*or* hafnium).

Dilute 1 ml of each solution to 10 ml and check the normalities against 0·10 N sodium hydroxide. If the normality of a solution is not within 0·80 ± 0·01 N, the determination should be repeated, with appropriate correction for acid addition.

PROCEDURE—

Determine a reagent blank value with each batch of samples. For zirconium or hafnium contents up to 0·2 per cent., transfer a 0·5-g sample to a 150-ml beaker and dissolve in 15 ml of hydrochloric acid (sp.gr. 1·18) *plus* 5 ml of nitric acid (sp.gr. 1·42). Add 5 ml of perchloric acid (sp.gr. 1·54) and evaporate to fumes, with a glass cover on the beaker, until carbides are destroyed and chromium salts oxidised. Cool to room temperature, add 15 ml of hydrochloric acid (sp.gr. 1·18), warm to dissolve salts and boil for 5 minutes, with the cover on, to reduce chromium salts. Add 10 ml of water, boil and filter through a 9-cm No. 42 Whatman filter-paper into a 50-ml calibrated flask. Wash any precipitate on to the filter-paper with water, continue washing and dilute the filtered solution to 50 ml. Examine the filter-paper for zirconium or hafnium (Note 3).

Place three separate 5-ml aliquots (Note 4) of the sample solution into 25-ml calibrated flasks (A, B and C). Dilute the solution in flask A to 25 ml and check for normality as described under Preparation of calibration graphs for zirconium and hafnium. To flasks B and C add the amount of hydrochloric acid (sp.gr. 1·18) calculated to produce final acidities of 0·80 N.

To solution C add 10 ml of water and 2 ml of xylenol orange solution; dilute solutions B and C to 25 ml. Measure the optical densities of solutions B and C at 535 nm against water, with 1-cm cells, correct the optical density of solution C for reagent blank and background (B), and then calculate the zirconium or hafnium content from the calibration graph. Make corrections for iron and molybdenum, if necessary (Note 5).

NOTES—

1. Prepare a calibration graph with each new batch of xylenol orange.

2. The 1·75 ml of hydrochloric acid (sp.gr. 1·18) added should, after dilution with water to 25 ml, produce a 0·80 N solution. Subsequent acidity corrections of the calibration and sample solutions are made on the basis of these titrations.

3. Ignite the filter-paper, fuse the residue with sodium carbonate (0·5 g), dissolve the cooled melt in 10 ml of hydrochloric acid (1 + 1), boil the solution for 3 minutes and make up to 25 ml. Check the acidity of a 5-ml aliquot and examine for zirconium *or* hafnium as described under Procedure.

4. For 0·1 to 0·2 per cent. of zirconium take a 2-ml aliquot.

5. Iron and molybdenum cause slight positive errors, which can be corrected by subtracting 0·001 per cent. of zirconium (*or* 0·002 per cent. of hafnium) for each 1 per cent. of iron present and 0·001 per cent. (*or* 0·002 per cent. of hafnium) for each 10 per cent. of molybdenum.

METHOD II: SIMULTANEOUS DETERMINATION OF ZIRCONIUM AND HAFNIUM
IN NICKEL-BASE ALLOYS

This method is suitable for the determination of zirconium and hafnium in amounts up to about 0·2 per cent. when both metals are present in nickel-base alloys substantially free from tungsten, niobium and tantalum.

REAGENTS—

As described in Method I, except that the final standard solutions are diluted to 500 ml with 0·8 N perchloric acid.

PREPARATION OF CALIBRATION GRAPHS—

Add 6 ml of standard zirconium (*or* hafnium) solution to a 25-ml graduated flask and, to another similar flask, add 3·0 ml of perchloric acid (sp.gr. 1·54). Dilute each solution to the mark and determine normalities by titrating 1 ml with 0·1 N sodium hydroxide (Note 6).

Based on Method I, determine the optical densities of calibration solutions containing—

(*a*) 0 to 60 μg of zirconium in 0·35 N perchloric acid;
(*b*) 0 to 60 μg of zirconium in 2·0 N perchloric acid;
(*c*) 0 to 100 μg of hafnium in 0·35 N perchloric acid;
(*d*) 0 to 100 μg of hafnium in 1·12 N perchloric acid; and
(*e*) 0 to 100 μg of hafnium in 2·0 N perchloric acid.

Check the acidities of the solutions and correct for reagent blanks in 0·35, 1·12 and 2·0 N perchloric acid. Construct calibration graphs for solutions (a), (b), (c) and (e) to give graphs 1, 2, 3 and 4, respectively. Construct a calibration graph for differences between optical densities at 0·35 N *minus* optical densities at 1·1 N against micrograms of hafnium (graph 5).

PROCEDURE—

Determine a reagent blank value with each batch of samples.

Transfer a 1·0-g sample to a 250-ml beaker and dissolve it in 30 ml of hydrochloric acid (sp.gr. 1·18) and 10 ml of nitric acid (sp.gr. 1·42). When dissolved, cool, add 20 ml of sulphuric acid (1 + 1) and evaporate slowly to fumes of sulphuric acid. Cool, dissolve in 40 ml of water by boiling for about 15 minutes, with a cover on the beaker. Filter the solution through a 9-cm No. 42 Whatman filter-paper and wash the beaker with water, transferring any precipitate to the paper. Ignite the filter-paper in a platinum crucible, fuse the residue with sodium carbonate (0·5 g), dissolve the melt in 10 ml of sulphuric acid (1 + 3), boil and, if clear, add the solution to the original filtrate (Note 7).

Dilute the solution with water to 150 ml; transfer it to a mercury-cathode cell and electrolyse at 4 amps until spot tests confirm the absence of iron and chromium. Boil the electrolyte until any yellow colour caused by titanium is discharged, then evaporate to fumes of sulphuric acid. Cool, dissolve in 40 ml of hydrochloric acid (1 + 1), boil for 5 minutes, cool and adjust the pH to between 8 and 9 by addition of ammonia solution (1 + 1). Boil the solution for 5 minutes to precipitate zirconium and hafnium together with the aluminium and titanium present in the alloys (Note 8). Allow the precipitate to settle, filter it on to a 11-cm No. 42 Whatman filter-paper and wash it free from sulphates with ammonium nitrate (1 per cent. w/v).

Dissolve the precipitate off the paper with four 25-ml portions of hot 0·8 N perchloric acid, collecting the solution in a 250-ml beaker, and wash the paper with water until free from perchloric acid. Reserve the filter-paper to test for zirconium and hafnium (Note 9). Evaporate the solution just to fumes of perchloric acid, cool, add 20 ml of hydrochloric acid (1 + 1) and boil for 5 minutes to ensure complete breakdown of zirconium and hafnium complexes. Add 20 ml of water and filter through a 9-cm Whatman No. 42 filter-paper into a 100-ml graduated flask. Wash the beaker and paper with water and dilute to 100 ml (Note 9).

DETERMINATION OF ZIRCONIUM AND HAFNIUM BY "THREE-POINT" METHOD—

Transfer 5-ml aliquots of blank and sample solutions to a 25-ml graduated flask, dilute to the mark and determine acidity by titration of 1 ml with 0·1 N sodium hydroxide. Add the calculated amount of perchloric acid (sp.gr. 1·54) to a further 5 ml of sample solution to produce a final acidity of 0·8 N. Add about 10 ml of water and 2 ml of xylenol orange solution (0·05 per cent.); dilute to 25 ml. Measure the optical density of the sample solution at 535 nm, with a 1-cm cell, against water in the corresponding cell to determine whether the total absorbance exceeds about 0·8 (Note 10).

Transfer three separate 5-ml (or appropriate) aliquots of the sample and reagent blank solutions to 25-ml graduated flasks and add the amount of perchloric acid (sp.gr. 1·54) calculated to produce final acidities of 0·35, 1·12 and 2·0 N in the sample and blank solutions (Note 6). Prepare three acidic solutions for determination of the xylenol orange reagent blank at 0·35, 1·12 and 2·0 N. Dilute the solutions to about 20 ml, add 2 ml of xylenol orange solution (0·05 per cent.) and dilute to 25 ml. Measure the optical density of each solution at 535 nm, with a 1-cm cell. Finally, check the normality of the solutions by titrating 1 ml with 0·1 N sodium hydroxide solution.

CALCULATION OF ZIRCONIUM AND HAFNIUM CONTENTS—

Correct the optical density values at 0·35, 1·12 and 2·0 N for the respective acid and reagent blanks (Note 11).

From the optical-density reading of the sample at 1·12 N, subtract that for 0·35 N, and from this difference value read off from graph 5 the approximate hafnium content in micrograms (Note 12).

By using graph 4, convert hafnium content into the corresponding optical-density reading at 2·0 N, subtract this reading from total absorbance value at 2·0 N, and convert the difference into micrograms of zirconium by using graph 2. With graph 1, convert micrograms of zirconium into an equivalent optical-density reading at 0·35 N, subtract that value from total absorbance at 0·35 N and, from the difference, obtain the hafnium content in micrograms by using graph 3.

Calculate the percentages of zirconium and hafnium.

NOTES—

6. The 3·0 ml of perchloric acid (sp.gr. 1·54) added should, after dilution with water to 25 ml, produce a 1·12 N solution. Subsequent acidity corrections of the calibration, blank and sample solutions are made on the basis of these titrations.

7. If the solution is not clear (because of traces of silica), filter the precipitate on to a 7-cm No. 42 Whatman filter-paper and wash with water. Ignite the paper in a platinum crucible, cool, add one drop of sulphuric acid (sp.gr. 1·84) and 1 ml of hydrofluoric acid (40 per cent.). Evaporate to dryness; heat to 700° C to expel all of the acid. Fuse any residue with sodium carbonate, etc., as before, and add the extract and earlier filtrate to the main solution.

8. If insufficient titanium or aluminium is present to act as co-precipitant, add a solution containing 40 mg of pure titanium or aluminium to the samples and blanks.

9. Ignite the filter-papers in platinum crucibles, fuse with sodium carbonate (0·5 g), dissolve the melt in 10 ml of hydrochloric acid (1 + 1), boil for 3 minutes and make up to 50 ml. Examine the solution for zirconium *plus* hafnium at an acidity of 0·8 N. Only traces are normally detected (<0·001 per cent.); larger amounts of zirconium and hafnium should be determined by the "three-point" method.

10. If the total absorbance exceeds 0·8 use smaller aliquots for the determinations.

11. The acid and reagent blanks are practically identical at the three levels of acidity.

12. An approximate zirconium content can be calculated by subtracting the equivalent optical-density reading for hafnium at 0·35 N (obtained from graph 3) from total absorbance reading at 0·35 N. The difference is caused by zirconium, and the content can be obtained from graph 1.

TABLE VII

DETERMINATION OF ZIRCONIUM IN NICKEL-BASE ALLOYS*

Alloy	Sample No.	Zirconium (nominal), per cent.	Zirconium, per cent.	
			Direct method	Separation method (Cheng)
A	1	0·02	0·019, 0·020, 0·020	0·019
	2	0·025	0·029, 0·029, 0·029	0·030
	3	0·05	0·051, 0·048, 0·051	0·048
	4	0·10	0·096, 0·097, 0·100	0·103
	5	0·20	0·189, 0·200, 0·202	0·185
B	1	—	0·003, 0·004, 0·003, 0·003	0·0034, 0·0032, 0·0027, 0·0032
	2	0·02	A. 0·029, 0·0295† B. 0·0285, 0·028	—
	3	0·06	A. 0·064, 0·0635 B. 0·0655, 0·0645	—
	4	0·10	A. 0·1035, 0·1035 B. 0·1015, 0·1015	—
C	1	0·06	0·067, 0·069	0·064
	2	0·10	0·104, 0·105, 0·108	0·105, 0·100, 0·096
D	1	0·08	0·090, 0·090	—
	2	0·06	0·066, 0·067	—
	3	0·10	0·105, 0·106	—
	4	0·12	0·126, 0·127	—
	5	0·14	0·143, 0·142	—
E	1	—	0·001	0·0015

* Compositions of alloys tested, per cent.—

Alloy	Cr	Co	Mo	Ti	Al	Fe	Mn	Si	Cu	Ni
A	11	20	5	2	5	1 max.	0·5 max.	1 max.	0·2 max.	Balance
B	20	16	—	2·5	1·5			As for A		
C	20	—	—	2·3	1·3			As for A		
D	15	20	5	1·2	4·5			As for A		
E	20	—	—	—	—	—	—	—	—	Balance

† *A* and *B* separate samples tested in duplicate on aliquots from same solution.

APPLICATION OF METHODS

The proposed direct Method I was applied to the analysis of complex nickel-base alloys containing chromium, cobalt, manganese, molybdenum, aluminium, titanium, copper, silicon and iron, with zirconium up to 0·2 per cent. Typical results are given in Table VII. The replicate values showed satisfactory reproducibility and close agreement with results obtained by lengthy separation methods.[4,5] It was interesting to note that with sample B1, to which no zirconium had been added, a mass-spectrographic test confirmed the presence of about 0·002 per cent. of zirconium compared with the average chemical value of 0·003 per cent. The accuracy of the direct method was confirmed by the determination of zirconium in a British Chemical Standard Magnesium alloy B.C.S. No. 307 containing 0·56 per cent. of zirconium, for which duplicate values of 0·55 per cent. were obtained.

TABLE VIII

DETERMINATION OF HAFNIUM AND ZIRCONIUM IN HAFNIUM STANDARDS (ALLOY B)

| | | Simultaneous "three-point" method | | | | | | |
| | | Hafnium | | | Zirconium | | | Direct method |
Mark	Nominal hafnium, per cent.	Number of tests	Range, per cent.	Average, per cent.	Number of tests	Range, per cent.	Average, per cent.	hafnium,* per cent.
B5	Nil	5	N.D.<0·002	N.D.<0·002	5	0·003 to 0·004	0·003	N.D.<0·005
B6	0·02	4	0·022 to 0·026	0·025	4	0·002 to 0·005	<0·005 (0·003)	0·022
B7	0·05	3	0·050 to 0·055	0·054	2	0·002 to 0·005	<0·005 (0·003)	0·050
B8	0·10	3	0·099 to 0·102	0·100	2	0·003 to 0·005	<0·005 (0·004)	0·11
B9	0·20	5	0·161 to 0·173	0·167	4	0·004 to 0·007	0·005	0·16

* Direct results after applying correction for zirconium present.

For the determination of hafnium in a series of nickel-base alloy samples, the full "three-point" procedure was used to ascertain the impurity levels of zirconium (Table VIII). Considering the complexity of the problems involved, replicate tests showed reasonable reproducibility up to 0·2 per cent., at which level the zirconium content was about 0·005 per cent. Confirmation of the hafnium values was obtained by mass-spectrographic tests, which also confirmed the presence of zirconium, but indicated a sensitivity factor greater than 1 (Table IX). The direct Method I was successfully applied also to this series of alloys and, after making slight corrections for the zirconium present, the results were in close agreement with those produced by the more lengthy Method II (Table VIII).

TABLE IX

COMPARISON OF CHEMICAL AND MASS-SPECTROGRAPHIC RESULTS ON HAFNIUM STANDARDS

| | | Hafnium, per cent. | | Zirconium, per cent. | |
Mark	Nominal hafnium, per cent.	Chemical	Mass-spectrographic	Chemical	Mass-spectrographic
B5	Nil	N.D. <0·002	<0·001	<0·005 (0·003)	(0·002)
B6	0·02	0·025	0·02	<0·005 (0·003)	(0·01)
B7	0·05	0·054	0·04	<0·005 (0·003)	(0·01)
B8	0·10	0·100	0·1	<0·005 (0·004)	(0·02)
B9	0·20	0·167	0·2	0·005	(0·02)

Finally, several complex nickel-base alloy samples containing added zirconium and hafnium were examined by the full Method II, with the results given in Table X. In the absence of any alternative chemical method, critical evaluation of these results is difficult, but the values are within working limits of the nominal additions. Confirmation of the results was, however, obtained by emission and X-ray fluorescence spectrometry, by using chemically analysed standards containing either hafnium or zirconium (Table X).

TABLE X

DETERMINATION OF HAFNIUM AND ZIRCONIUM IN NICKEL-BASE ALLOY D

Mark	Nominal hafnium, per cent.	Nominal zirconium, per cent.	Simultaneous "three-point" method		Spectrographic method Hafnium, per cent.	X-Ray method Zirconium, per cent.
			Hafnium, per cent.	Zirconium, per cent.		
D6	—	0·05	<0·002	0·056	<0·01	0·06
D7	0·01	—	0·012 (5)	0·001	0·011	<0·005
D8	0·01	—	0·010, 0·011, 0·011	0·002, 0·002, 0·002	0·011	<0·005
D9	0·10	0·06	0·094, 0·098, 0·105	0·065, 0·062, 0·060	0·11	0·07, 0·05
D10	0·03	0·08	0·042, 0·040	0·081, 0·085	0·037	0·09, 0·09
D11	0·03	0·12	0·040, 0·041, 0·040, 0·046	0·12, 0·125, 0·122, 0·115	0·037	0·13, 0·14

CONCLUSIONS

It was concluded that the direct xylenol orange procedure (Method I) offered a simple and reproducible means of determining zirconium (0·002 to 0·2 per cent.) in complex nickel alloys, with the further advantage of considerable saving in time over separation methods. Direct determination of hafnium is, however, complicated by the presence of alloying amounts of iron and of zirconium present in commercial grades of hafnium. By sacrificing sensitivity and determining hafnium at an acid concentration of 0·8 N, instead of the optimum of 0·35 N, results have been obtained on alloys low in zirconium that were in good agreement with those obtained by the more lengthy simultaneous Method II.

Information obtained during development of the direct method has provided the background necessary for the successful evolution of a simultaneous procedure for determination of zirconium and hafnium. Careful control of acidity is essential, but the check procedure described makes possible easy adjustment to the required normality. From analysis of synthetic solutions, the accuracy has been shown to be within 5 per cent. of the amounts present. In the absence of alternative chemical methods, the accuracy obtained on actual samples could not be ascertained, but replicate tests on alloys were reproducible to within about 5 per cent. which, in view of the difficulties involved and the levels of contents present, is considered acceptable. Confirmatory evidence of blanks and of the magnitude of the zirconium-to-hafnium ratios in the alloys was provided by X-ray fluorescence, emission and spark-source mass spectrometry. Although the methods have been developed primarily for analysis of complex nickel-base alloys, they are capable of wider application, particularly if interfering metals are absent or can be removed by mercury-cathode electrolysis. As shown in the preliminary tests, the direct method can be applied in the presence of many other base materials (not iron) when mutual interference does not arise. It has been used for the analysis of copper, titanium-base and magnesium-base samples containing up to 0·5 per cent. of zirconium, and higher values (up to 5 per cent.) have also been determined in nickel alloys, showing fairly good agreement with gravimetric results. Alloys rich in tungsten, niobium and tantalum, however, present special problems, and further experimental work would be necessary before the method could be applied to such materials.

The simultaneous procedure provides a simple chemical means of differentiating between microgram amounts of zirconium and hafnium and, by inclusion of mercury cathode and hydroxide separations, the method has been successfully applied to a series of complex nickel-base alloys. It also offers the possibility of wider application to other alloy systems.

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An Ultraviolet Spectrophotometric Method for the Characterisation of Some Phenolic Stabilisers in Extracts of Polymer Compositions

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A method for extending the usefulness of ultraviolet spectrophotometry in the characterisation of some stabilisers used in polymer compositions is described. As well as the spectra of the stabilisers in ethanol and alkaline ethanolic solutions, the ultraviolet absorptions of the solutions after reaction with nickel peroxide, and after making these solutions alkaline, are also presented. These four spectra together give a more positive identification of the antioxidant. As examples of the method, three sets of spectra are given for phenolic antioxidants, the normal ultraviolet absorptions of which are identical. These spectra show marked differences by which the compounds can now be identified.

ULTRAVIOLET-ABSORBING stabilisers normally added to plastic materials are divided into two classes, antioxidants and ultraviolet absorbers.

Antioxidants, as the name implies, are added to polymers to hinder oxidation of the polymer chain during the heating processes in the manufacture and subsequent fabrication of a polymer composition. The antioxidants most often used are alkyl-substituted phenols, but substituted aromatic amines and other compounds, such as phosphites and thio-esters, are also used. Each polymer type usually has its own series of antioxidants, with properties suitable only for that polymer, although some antioxidants are in common use for many polymers. Antioxidants also have applications as additives in petroleum and lubricating oil, and some are permitted for use in foodstuffs. The scheme described in this paper has been used only for examination of extracts of plastic materials, especially polyolefins, and application of the procedure to oil or food may not be possible because of interference from the raw materials.

Ultraviolet absorbers are used to protect the polymer from degradation by both sunlight and artificial light. They are also substituted phenols, the most common types being hydroxy-benzophenones, hydroxybenzotriazoles and salicylate esters. These compounds have wavelengths of maximum absorption in the ultraviolet region of the spectrum between 300 and 350 nm, whereas the antioxidants absorb at shorter wavelengths, with λ_{max}. between 260 and 290 nm. The additives may be present at any concentration from 100 p.p.m. to 0·5 per cent., although 0·1 to 0·2 per cent. is the usual range.

The determination of the stabiliser content of polymer compositions must be carried out to correlate moulding or weathering properties with additive content and, with competitive materials, the stabilisers must also be identified.

The determination is carried out, after extraction of the additive with a suitable solvent, either by measurement of the ultraviolet absorption of the extract or, for antioxidants, by the Metcalfe and Tomlinson[1] iron(III) chloride reduction procedure. The stabiliser content can also be determined by direct ultraviolet absorption measurement of a thin film of the polymer.[2]

The identification of unknown stabilisers is more complicated and may involve the use of most of the physical methods of analysis. The stabiliser must first be obtained as a pure compound, usually by thin-layer chromatography.[3] After elemental analysis and molecular weight determination, the fraction can be examined by colour tests[4] and by measurement of the ultraviolet, infrared, nuclear magnetic resonance and mass spectra of the compound. This full treatment is required only for new stabilisers; for a characterisation of well known compounds the simplest method is by direct comparison of the ultraviolet absorption spectra

with those of a series of known stabilisers. For some compounds this will probably be suffi-
cient, but many substituted phenols have similar spectra, and for three of the most frequently
used antioxidants the ultraviolet spectra are identical. Topanol OC, Ionox 330 and Binox M
(see Table I for their chemical constitution) in ethanolic solution all have $\lambda_{max.} = 277$ nm,
with a shoulder at 282 nm.[2] To extend this procedure the spectra of alkaline solutions of
the phenols have then been measured either directly against a solvent blank or as "difference
spectra" measured against the neutral solution.[5] This still gives almost identical spectra
for the three compounds mentioned above.

TABLE I

WAVELENGTHS OF MAXIMUM ABSORPTION IN ETHANOL, ETHANOL - POTASSIUM HYDROXIDE,
ETHANOL - NICKEL PEROXIDE AND ETHANOL - NICKEL PEROXIDE - POTASSIUM HYDROXIDE

Trade name	Chemical constitution	Ethanolic solution, $\lambda_{max.}$ (nm)	Alkaline ethanolic solution, $\lambda_{max.}$ (nm)	Nickel peroxide reaction product, $\lambda_{max.}$ (nm)	Absorption change after nickel peroxide reaction	Alkaline reaction product, $\lambda_{max.}$ (nm)
Topanol OC	2,6-Di-t-butyl-4-methylphenol	277	303, 274, 257	340, 286	×8	Absorption suppressed
Binox M	Bis-(3,5-di-t-butyl-4-hydroxy-phenyl) methane	277	303, 255	428	×30	578
Ionox 330	1,3,5-Trimethyl-2,4,6-tris-(3,5-di-t-butyl-4-hydroxy-benzyl) benzene	277	303, 274	336, 304	×8	No change

This paper introduces two further stages in this procedure for extending the use of
ultraviolet spectrophotometry in the characterisation of these compounds. They consist in
(*a*) measuring the ultraviolet absorption spectrum of the stabiliser solution after reaction
with solid nickel peroxide and (*b*) re-measuring it after making the reaction products alkaline.
Cook[6] obtained the substituted stilbene quinone after reaction of 2,6-di-t-butyl-4-methylphenol
with lead dioxide, and Braithwaite and Penketh[7] have used lead dioxide for the determination
of Topanol OC in liquid paraffin. They obtained an absorption peak with $\lambda_{max.} = 420$ nm.
Stafford[8] used air for the oxidation and obtained a product with $\lambda_{max.} = 365$ nm.

Kharasch and Joshi[9] have reported on the oxidation of bis-(3,5-di-t-butyl-4-hydroxy-
phenyl) methane, carried out by pumping oxygen into an alkaline ethanolic solution of the
phenol. They obtained a dark purple solution produced by the anion of
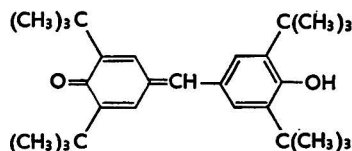
Our early work was carried out by using lead dioxide for the determination of very
small amounts of Topanol OC in extracts from polythene compounds.[10] However, when new
batches of lead dioxide were purchased we found that they did not give quantitative recoveries
of Topanol OC, and in some instances the reagent would not react at all. In 1960, twelve
samples of different grades from different suppliers were examined and shown to have differing
reactivities. Other higher oxides, including mercury(II) oxide and manganese dioxide, did
not give the reaction, but it was shown that nickel peroxide, which was first made com-
mercially available in January, 1963, was in fact more reactive than the original lead dioxide.
Nickel peroxide consists of the higher oxides of nickel, and has been used by Nagakawa,
Konaka and Nakata[11] for the oxidation of aliphatic and aromatic alcohols and, more recently,
Sugita[12] has studied the reaction of some alkyl and dialkyl phenols.

The reaction at room temperature of dilute solutions of Topanol OC, about 1 mg per
100 ml, with lead dioxide required 20 minutes' shaking time, whereas the reaction with
nickel peroxide is complete within 2 minutes and, if continued for longer periods, the absorb-
ance of the strongest band at 286 nm begins to diminish. This speed of reaction hinders

any attempt to obtain reproducible results for quantitative work with Topanol OC, although the speed of reaction varies with the antioxidant concerned. A recent batch of nickel peroxide has been found to be more reactive than usual, so that any attempts at quantitative work are, therefore, made even more difficult.

METHOD

APPARATUS—

Any ultraviolet spectrophotometer capable of measurements over the range 200 to 700 nm can be used but a recording spectrophotometer is more suitable.

REAGENTS—

Ethanol—A spectroscopic grade of absolute ethanol must be used. Distillers Co. Ltd. RR grade was used throughout this work.

Ethanolic potassium hydroxide solution—Dissolve 10 g of analytical-reagent grade potassium hydroxide in 10 ml of distilled water and dilute the solution to 100 ml with absolute ethanol.

Nickel peroxide—B.D.H. laboratory-reagent grade.
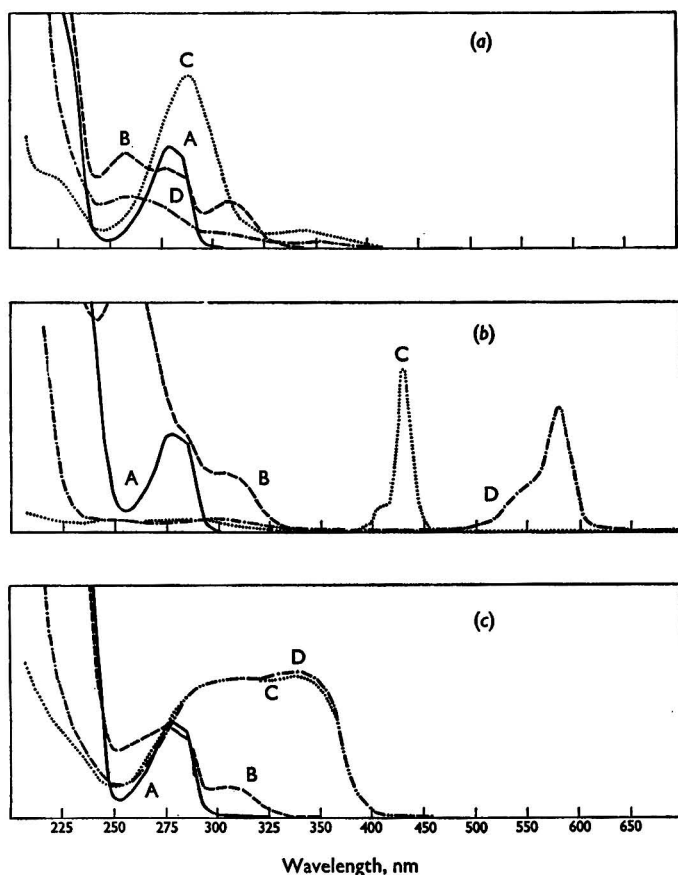
Fig. 1. Spectra for: (a), Topanol OC; (b), Binox M; and (c), Ionox 330. Curve A, ethanolic solution; curve B, alkaline ethanolic solution (10 ml of ethanolic solution *plus* 1 ml of water *plus* 2 ml of ethanolic potassium hydroxide solution); curve C, ethanolic solution after reaction with nickel peroxide; and curve D, nickel peroxide reaction product made alkaline with 2 drops of ethanolic potassium hydroxide solution

EXTRACTION PROCEDURE—

Prepare the polymer compound containing the unknown stabiliser by cutting or grinding to small pieces, and extract about 5 g by heating under reflux for 24 hours with 50 ml of ethanol. Cool the extract to room temperature and filter off the polymer. Treat a solvent blank of 50 ml of ethanol identically.

SPECTROPHOTOMETRY—

1. Measure the ultraviolet absorption spectrum of the extract over the range 250 to 450 nm, with the solvent blank in the comparison beam. Dilute the extract, if necessary, so that the absorbance of the main band is about 0·6.

2. To 10 ml of the solution used in 1, add 1 ml of distilled water and 2 ml of ethanolic potassium hydroxide solution. Mix well and measure the ultraviolet absorption spectrum of this alkaline solution over the same range against a solvent blank similarly treated.

3. Transfer 20 ml of the solution used in 1 to a 50-ml flask, add about 0·5 g of nickel peroxide, stopper the flask and shake, with a mechanical shaker, for 5 minutes. Filter the solution and measure the absorption spectrum as before, over the range 250 to 450 nm, with a solvent blank in the comparison beam. If necessary, because of the high absorption, dilute the filtered reaction products before measurement and note the dilution used. If the solutions are coloured, also measure the absorption spectrum over the range 450 to 700 nm.

4. Add 2 drops of ethanolic potassium hydroxide solution to the cell containing the solution used in 3. Mix well and re-measure the absorption spectrum over the same range as before.

RESULTS

The above procedures give four spectra for each extract, which are then compared with the spectra obtained by carrying out the procedures with known stabiliser solutions.

The sets of spectra for the three compounds previously mentioned, Topanol OC, Binox M and Ionox 330, the chemical constitutions for which are given below, are shown in Fig. 1 [(*a*), (*b*) and (*c*)]. Table I gives the wavelengths of maximum absorption for each procedure, and also some indication of the absorbance change, *i.e.*, the dilution required, after nickel peroxide reaction. The initial concentration of the stabilisers was 10 mg per 100 ml of ethanol.

Topanol OC

Binox M

Ionox 330
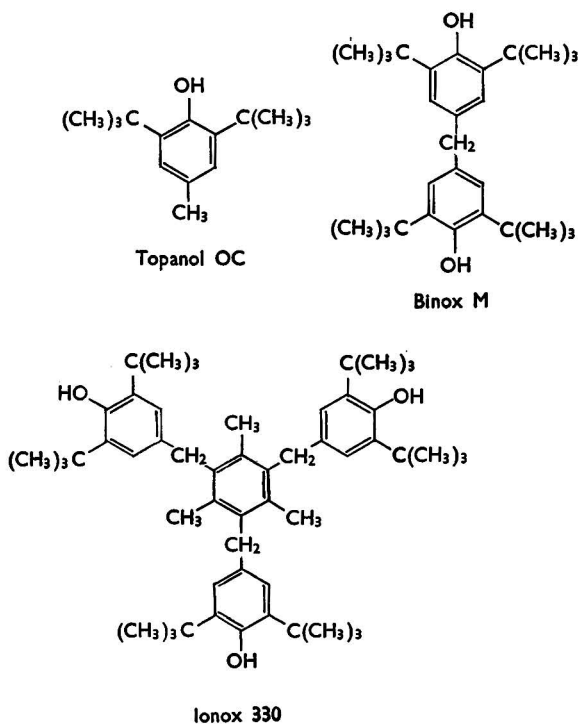
The reaction product of Topanol OC has $\lambda_{max.} = 285$ nm, and is probably the intermediate product obtained by Cook,[6] that is, 1,2-bis-(3,5-di-t-butyl-4-hydroxyphenyl) ethane. The highly coloured substituted stilbene quinone is obtained at room temperature only after reaction of much more concentrated solutions.

The colour obtained on making the nickel peroxide reaction product of Binox M alkaline was similar to that obtained by Kharasch and Joshi.[9]   The band gave $\lambda_{max.} = 578$ nm and was presumably caused by the anion mentioned previously.

The nature of the reaction products of the other stabiliser is not known.

Although only three sets of spectra are given here, it will be appreciated that a collection of such sets of spectra can be acquired for all of those antioxidants and ultraviolet absorbers encountered during day-to-day work.

The components of mixtures of ultraviolet-absorbing stabilisers cannot usually be identified by carrying out this nickel peroxide reaction, and such mixtures must first be separated by thin-layer chromatography.   The separation of the residue on evaporation of the extract is carried out by a method similar to that previously described for the examination of plasticisers from poly(vinyl chloride) compositions.[13]   The fractions are scraped from the plate, the stabilisers extracted from the powder with ethanol and the nickel peroxide reaction carried out on these solutions.

The high sensitivity of this ultraviolet procedure enables the identification to be made with much smaller samples than would be required for infrared examination.   Mixtures of Topanol OC with Ionox 330, and of Topanol OC with a substituted benzophenone-type ultraviolet absorber, have been identified by this method.

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The Absorptiometric Determination of Silicon in Water

Part VII.* Improved Method for Determining the Total Silicon Content of High-purity Water

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A method is described for determining the total silicon content of high-purity waters; this method allows more precise results to be obtained than the method given in Part III of this series. Silicon in the water is initially concentrated on a mixture of finely ground cation and anion-exchange resins, which are then ignited and fused with sodium carbonate. The resulting melt is dissolved in water and silicate is determined absorptiometrically as the reduced β-molybdosilicic acid. The standard deviation of analytical results for 1-litre samples containing between 0 and 100 μg of silica was about 3 μg of silica. Ten analyses and the necessary blank determinations can be carried out in 8 hours.

MORRISON and Wilson[1] have described reasons why the total silicon content of high-purity waters (*e.g.*, boiler feed-water and make-up water) is of interest in the chemical control of modern power stations. They have described[2] a method that was developed primarily for a preliminary investigation of the occurrence of "non-reactive" silicon† in make-up waters. This method consisted of evaporation of a sample to dryness, fusion of the residue with sodium carbonate and absorptiometric determination of silicate in the melt. When 20-ml samples were analysed, a standard deviation of about 0·015 p.p.m. of silica was obtained, the dominant sources of error being contamination and heterogeneous distribution of silicon in the sodium carbonate. Such precision is inadequate for the analytical control of high-purity water, which may often contain 0·02 p.p.m., or less, of silica. Accordingly, ways of obtaining better precision were considered.

Three methods for converting "non-reactive" into "reactive" forms of silicon were considered, namely—

(*a*) Treatment of the sample with sodium hydroxide at high temperatures and pressures in a steel bomb.[3]

(*b*) Treatment of the sample with hydrofluoric acid.[3,4]

(*c*) Fusion with sodium carbonate.[2,3,5]

Method (*a*) was rejected because it was considered too inconvenient for normal use. In addition, little evidence was available for the efficiency of the technique for different forms of "non-reactive" silicon. Method (*b*) appeared most attractive because of its relative simplicity, as compared with method (*c*), but the latter appeared[2,4,5] more likely to enable all possible forms of "non-reactive" silicon to be determined. Accordingly, it was decided initially to concentrate on the fusion technique. It is possible that high-purity waters can be analysed satisfactorily after treatment by method (*b*). It is hoped, therefore, in subsequent work to compare results obtained by methods (*b*) and (*c*).

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* For details of earlier parts of this series, see reference list, p. 120.

† "Non-reactive" silicon is defined as those compounds of silicon which do not react with ammonium molybdate in 10 minutes under the conditions described by Webber and Wilson.[6]

The main source of random error in the previously reported[2] fusion technique was considered to arise from the evaporation and fusion procedures. The magnitude of errors from the fusion could be simply reduced by increasing the volume of sample evaporated. However, to obtain a worthwhile improvement, long evaporation times would be required, and would, therefore, make the method more cumbersome, as well as leading to proportionally greater danger of contamination of the samples. Thus, we sought an alternative to evaporation as a means of concentrating the silicon in the sample. Preliminary tests were made of the possibility of co-precipitating silicon with metal hydroxides (iron, aluminium and thorium were used), but the metal contents of the solutions obtained by dissolution of the precipitates markedly affected the precision of the absorptiometric determination. Attention was, therefore, turned to the use of ion-exchange resins.

Wickbold[7] has described a method in which an anion-exchange resin was used for concentrating the silicon present in high-purity waters. The sample is first treated with hydrofluoric acid to form fluorosilicic acid, which is strongly absorbed by the resin. This technique was rejected for the same reason that hydrofluoric acid was rejected as a means of converting "non-reactive" silicon into "reactive" forms. The approach finally adopted was to adsorb silicon on a mixture of cation and anion-exchange resins, which were then ignited. The residue was fused with sodium carbonate and the melt analysed absorptiometrically.

Morrison and Wilson[2] used α-molybdosilicic acid for the absorptiometric determination of silicon in the fusion melt. This compound was used in preference to the more usual β-molybdosilicic acid because it was thought that it would allow better precision. However, the dominant errors did not arise in the absorptiometric procedure, and the use of the β-molybdosilicic acid is more convenient for routine analysis. It was, therefore, decided to use the absorptiometric procedure described by Webber and Wilson.[6]

The development of this method for total silicon and the tests made of its performance are described below.

EXPERIMENTAL

APPARATUS, REAGENTS AND TECHNIQUE—

The procedure given under Method was used for the work described in this section, except when indicated otherwise. Optical-density measurements were made in 4-cm cuvettes at 810 nm with a Hilger Uvispek spectrophotometer, distilled water being used to fill the reference cuvette.

Distilled water from a Manesty still was used for most of the tests, but for certain tests specially purified water was prepared by passing distilled water through a bed of "Powdex" resins (see below).

Standard solutions of "reactive" silicon, polymeric silicic acid and dilute suspensions of clay were prepared as described previously.[8,1,2]

For all of the statistical significance tests applied in this work, the 95 per cent. confidence level was used.

"POWDEX" RESINS—

"Powdex" is the trade name used by the Graver Water Conditioning Company (U.S.A.) in their Graver Powdex process for water purification. This process was designed[9] for the purification of condensate in power stations, and involves the use of a mixture of finely divided, strongly acidic cation and strongly basic anion-exchange resins (in the hydrogen and hydroxyl forms, respectively) as a pre-coat on a specially designed filter support. This mixture of finely divided resins produces a heavy flocculant type of precipitate that allows rapid filtration. The process thus provides an adsorbent with a large surface area, and yet allows a rapid flow-rate through the pre-coat. Duff and Levendusky[9] have reported that this process was efficient in removing all forms of silicon and, accordingly, the experiments described below were made to test whether it was suitable for analytical application.

PRELIMINARY TESTS—

A solution was prepared containing about 1 p.p.m. of "reactive" silica and 1·8 p.p.m. of "non-reactive" silica (as bentonite clay). A 200-ml portion of this solution was stirred for 50 minutes with a mixture of 0·5 g* of "Powdex" anion-exchange resin and 0·25 g of

* Weights of resin refer to the "as received" material containing 50 to 60 per cent. of water.

cation-exchange resin in a polythene centrifuge bottle. The solution was then centrifuged for 10 minutes at 2500 r.p.m., and 20-ml aliquots of the supernatant solution were analysed for "reactive"[6] and total silicon.[2]

A further 100 ml of the original solution were passed through a bed of mixed resins (the weights used being the same as for the previous test) supported on a Millipore filter (pore size, $0.8\,\mu$) in a Perspex filter-stick; the flow-rate was 20 ml per minute, and 20-ml aliquots of the filtrate were taken for analysis.

The results of the analyses showed that in both tests the total silicon contents of the treated waters were less than 0.02 p.p.m. of silica, and the "reactive" silicon contents less than 0.005 p.p.m. of silica. The use of these resins, therefore, appeared to offer a suitable means of concentrating the silicon from the water, and the main problem would be to determine the silicon in the mixture of resins. The development of a suitable procedure is described below.

DETERMINATION OF SILICON IN THE RESIN MIXTURE—

Effect of sodium sulphate—Neutralisation with sulphuric acid of the sodium carbonate used for the fusion leads to the presence of sodium sulphate in the solution for analysis. Morrison and Wilson[2] reported that this salt decreased both the sensitivity and precision of the absorptiometric method when using the reduced β-molybdosilicic acid. However, although our tests confirmed a slight loss of sensitivity, no significant decrease in precision was found when 4 ml of a 10 per cent. solution of sodium carbonate and 8 ml of 1.0 N sulphuric acid were added to standard solutions of "reactive" silicon. Accordingly, the absorptiometric method of Webber and Wilson[6] was used in all of the subsequent work.

Effect of the resins—The effect of any residue left after ignition of the mixture of resins was tested by igniting a large bulk of the two resins and fusing the residue with sodium carbonate. After dissolution of the melt in water, the solution was neutralised with sulphuric acid and made up to a known volume. Aliquots of this solution, each containing the equivalent of 0.4 g of anion-exchange resin, 0.08 g of cation-exchange resin, 4 ml of a 10 per cent. solution of sodium carbonate and 4 ml of 2.0 N sulphuric acid, were added to a series of solutions containing standard amounts of silicon. After diluting each solution to 100 ml, the amount of silicon present in each was determined. At the same time, standard silicon solutions containing only sodium carbonate and sulphuric acid were analysed. The results of these determinations, given in Table I, show that the resins had no marked effect on the recovery of silicon, although the optical densities of the blank determinations were increased by the resins.

Subsequently, it was found that when the resins were ignited and fused under the conditions recommended in the Method, the resultant solutions were sometimes turbid and smelt of hydrogen sulphide. These effects were accompanied by the formation of a blue colour in the solution immediately the ammonium molybdate - sulphuric acid reagent was added. These observations indicated incomplete oxidation of the resins and, in an attempt to eliminate this effect, hydrogen peroxide was added to the cooled melt in the platinum

TABLE I

EFFECT OF RESIN RESIDUES ON THE ABSORPTIOMETRIC DETERMINATION OF SILICON

| | Optical density* | |
Concentration of silicon added, p.p.m. of silica	Pure solution	Solution containing resin residues
0	0.062 ± 0.0025	0.336 ± 0.0025
0.1†	0.138 ± 0.004	0.138 ± 0.004
0.5†	0.683 ± 0.005	0.692 ± 0.0055

* Mean of four results.
† Optical densities corrected for the appropriate blank.

crucible; any excess of peroxide was destroyed by gentle warming. This treatment facilitated the subsequent dissolution of the melt and reduced any errors caused by the above effects to less than 0.001 p.p.m. of silica. Other tests indicated that this addition of hydrogen peroxide decreased the slope of the calibration graph by about 4 per cent. The explanation

of this effect is not known with certainty, but it may be caused by the depressive effect[10] of residual hydrogen peroxide on the formation of the molybdosilicic acid. No measurements of residual peroxide were made, and the effect was not studied further because it is allowed for adequately by the method of calibration given under Method.

Fusion procedure—The fusion procedure was similar to that used by Morrison and Wilson.[2] The sodium carbonate was added as a solution, rather than as a solid, so that a coating of solid carbonate would be left on the resins after evaporation; this was thought likely to reduce the possibility of losses of silicon during the ignition and fusion procedure. Addition of a solution of sodium carbonate has the added advantage that it removes the errors introduced if the solid sodium carbonate contains heterogeneously dispersed silicon.

Tests of this procedure showed that the recoveries of sodium silicate added to resins in a platinum crucible varied between 98 and 102 per cent. Further tests showed that rapid ignition of the resins in a fierce flame (in preference to the gentle heating prescribed under Method) resulted in the loss of about 2 per cent. of a known amount of silicate added to the resins. The ignition procedure is, therefore, not critical.

SAMPLING AND SAMPLE TREATMENT—

Two methods were considered for the collection of silicon on the resins. One was to pass the sample through a small column of the mixed resins, and the other was to shake the sample with the mixed resins, with subsequent recovery of the resins by filtration. Assuming that both methods give 100 per cent. retention of silicon on the resins under ideal conditions, the choice of the method is governed by practical considerations such as sampling technique, contamination and operator time. The latter method was adopted because detailed consideration indicated that it is likely to be more robust. The tests carried out to prove its applicability are described under Results.

Amount of sample necessary to obtain the required precision—Morrison and Wilson[2] obtained a standard deviation of about 0·015 p.p.m. of silica when 20-ml samples of water were analysed absorptiometrically after fusion with sodium carbonate. Thus, errors from these operations could be made negligible if a much larger initial volume of sample could be analysed. The most likely source of imprecision in the present method appeared to be the heterogeneous distribution of silicon in the ion-exchange resins. Therefore, the magnitude of this error was determined so that the necessary volume of sample for adequate precision could be calculated.

For this purpose, 10 samples of resins (each containing 1 and 0·2 g of the anion and cation-exchange resins, respectively) were ignited, fused with sodium carbonate and the melts analysed. The results showed that the standard deviation of the difference between two individual results was about 2 μg of silica. Thus, a 1-litre sample of water should give a standard deviation of about 0·002 p.p.m. of silica. This error was considered sufficiently small, and 1-litre samples were, therefore, used in further tests.

Removal of silicon from water—A series of tests was carried out to determine the parameters affecting the removal of "reactive" silicon, polymeric silicic acid and clay from water. These forms of silicon were chosen as being reasonably representative of the forms likely to occur in high-purity waters. Except when stated otherwise, samples were analysed exactly as described under Method.

To check the amount of resin required to remove silicon from water, a solution containing about 0·04 p.p.m. of "reactive" silica and 0·15 p.p.m. of silica of both polymeric silicic acid and clay was prepared. Portions of this solution were analysed, and the "reactive" silicon and polymeric silicic acid contents in the filtrates also determined. Three different amounts of resins were used, four determinations being made for each amount; two of these four determinations were made by gently shaking the sample with the resins for 20 minutes, the other two being set aside for the same length of time. The mean results obtained are given in Table II and show that 1 g of the anion exchanger was a suitable amount. The effect of different weights of the cation exchanger was then investigated by using 1 g of anion exchanger for all of the tests. Duplicate portions of the same solution were analysed, and the mean recoveries of total silicon were 97·2, 98·9 and 99·9 per cent. for 0·1, 0·2 and 0·4 g of cation exchanger, respectively. The weight of cation exchanger was not of crucial importance, and it was decided to use 1 g of anion exchanger and 0·2 g of cation exchanger in subsequent work.

TABLE II

EFFECT OF THE AMOUNT OF ION-EXCHANGE RESINS ON THE REMOVAL OF
SILICON FROM SOLUTION

Amount of resin	Shaking time, minutes	"Reactive" silicon removed, per cent.	Polymeric silicic acid removed, per cent.	Clay removed,* per cent.	Total silicon removed, per cent.
Anion (0·2 g) + cation (0·1 g)	0	23·3	38·9	34·8	36·3
	20	2·4	86·1	100·0	82·0
Anion (0·5 g) + cation (0·25 g)	0	51·1	73·2	45·6	57·6
	20	89·7	88·5	78·2	82·6
Anion (1·0 g) + cation (0·5 g)	0	71·3	73·8	69·4	70·0
	20	97·2	98·6	97·5	97·2

* These results were obtained by deducting the amounts of "reactive" silicon and polymeric silicic acid removed from the total amount of silicon recovered from the resins.

The results in Table II indicated the desirability of shaking the sample with the resins. To check this point, eight portions of the same solution as that used in the preceding tests were analysed, four portions being shaken for 5 minutes and the other four for 20 minutes; 10-ml aliquots of the solutions, after the fusion stage, were analysed, and the mean optical densities were 0·503 ± 0·018 and 0·497 ± 0·013 for 5 and 20 minutes' shaking time, respectively, with an over-all mean recovery of 100 ± 3 per cent. It was concluded that a shaking time of 10 minutes was satisfactory.

To check the effect of the size of the resin beads, each resin was sieved to give fractions of 72 to 200, 200 to 325 and >325 mesh size. Portions of each fraction were used to analyse a solution containing about 0·04 p.p.m. of "reactive" silica and 0·14 p.p.m. of silica as polymeric silicic acid. In all instances, at least 99 per cent. of the "reactive" silicon was removed, and at least 98 per cent. of the polymeric acid was removed for the two smaller mesh sizes. When the 72 to 200-mesh resins were used, about 7 per cent. of the polymeric silicic acid was not retained by the resins. The mesh size of the resins did not, therefore, appear to be critically important.

METHOD

APPARATUS—

Evaporating hood—An inverted 8-inch polythene funnel, placed about 0·25 inch above the surface of a hot-plate, forms a suitable hood that will accommodate twelve 30-ml platinum crucibles. Pass a gentle stream of air, filtered through two Whatman No. 542 filter-papers (or suitable alternatives), through the stem of the funnel to reduce condensation on the inside of the funnel.

Platinum crucibles—Platinum crucibles (30 ml) are suitable. Clean them thoroughly by repeated fusions of sodium carbonate until satisfactorily precise results are obtained when 4 ml of the 10 per cent. sodium carbonate reagent are fused and the melts analysed as described under Procedure.

This treatment did not remove all traces of silicon from all of the crucibles, and it was necessary to keep certain crucibles filled with molten sodium carbonate for up to 16 hours before satisfactory results could be obtained. Store the clean crucibles by inverting them on clean filter-paper in a dust-free atmosphere, and use them only for silicon determinations. They should be handled with platinum-tipped tongs.

Polythene bottles for collecting samples—These bottles should be capable of holding about 1250 ml of water, and should have plastic screw-top stoppers that do not leak; the necks should be shaped so that the entire contents can be poured out. To enable samples to be collected free from contamination, a modified stopper, with inlet and outlet tubes sealed into it, can be used. These bottles are also used for blank determinations, and are suitable for storing reagents.

Clean the bottles by washing them with water and then allowing them to stand overnight filled with water to which 0·5 g† of anion exchanger and 0·1 g† of cation exchanger have been added. Finally, wash them thoroughly with water.

† The weights of resin used refer throughout the remainder of this paper to resins that have been dried overnight at 110° C.

Polythene bottles, 8-oz capacity—These bottles are required for the absorptiometric determinations and should be thoroughly washed with water. Their suitability for use should be determined by carrying out blank determinations (absorptiometric stage only) in them.

Filtration apparatus—The resins on which the silicon is concentrated are recovered from the sample by collection on a Millipore (or other suitable membrane) filter disc (47-mm diameter, 0.8-μ pore size) by filtration under reduced pressure. The filter disc is supported on a porous polythene plate (0.1 inch thick) held in a Perspex holder. A diagram of the apparatus is shown in Fig. 1.
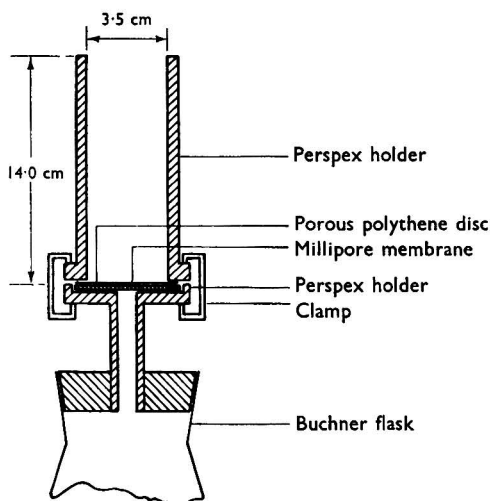
Fig. 1. Apparatus used for filtration

This apparatus should be cleaned initially by immersing it overnight in 5 N sodium hydroxide solution, and then washing it thoroughly with water. It should be stored in a dust-free atmosphere and rinsed with water immediately before use.

REAGENTS—

All reagents should be of analytical-reagent grade unless otherwise stated.

Water—Distilled water from a Manesty still, and stored in polythene, was found suitable, and usually contained less than 0.005 p.p.m. of silica as "reactive" silicon.

Sodium carbonate solution—Dissolve 100 g of anhydrous sodium carbonate (microanalytical grade) in about 800 ml of water. Dilute with water to 1 litre in a polythene measuring cylinder, and store in a polythene bottle.

Sulphuric acid, 2 N—Add 56 ml (± 0.5 ml) of 98 per cent. sulphuric acid, cautiously, to about 800 ml of water. Allow the solution to cool, and dilute with water to 1 litre in a polythene measuring cylinder; store in a polythene bottle.

Hydrogen peroxide, 100 volumes.

Acidified molybdate solution—Dissolve 89 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in about 800 ml of water at room temperature. Add 63 ml of 98 per cent. sulphuric acid, cautiously, to 100 ml of water, with stirring, and allow the mixture to cool. Add the acid to the molybdate solution, cool it to room temperature, and dilute to 1 litre with water. This solution has been found to be adequately stable for at least 6 months.

Tartaric acid solution, 28 per cent. w/v—This solution has been found to be adequately stable for at least 6 months.

Reducing agent solution—Dissolve 2.4 g of sodium sulphite, $Na_2SO_3.7H_2O$, and 0.2 g of 1-amino-2-naphthol-4-sulphonic acid (purest grade available) in about 70 ml of water. Add 14 g of potassium metabisulphite, shake well until dissolved, and dilute to 100 ml. This reagent should be freshly prepared each week.

Standard solutions of silica—Fuse 1·000 g of pure dry silica with 5 g of anhydrous sodium carbonate in a platinum crucible at red heat. When cool, dissolve in water, and dilute to exactly 1 litre. This solution contains 1000 p.p.m. of silica. Prepare, by dilution, a solution containing 10 p.p.m. of silica. The solutions containing 1000 p.p.m. were stable, within $\pm 0\cdot 5$ per cent., for at least 2 years in polythene bottles, and those containing 10 p.p.m. for at least 1 year.

The most suitable silica for this purpose is probably transparent Spectrosil rod (Thermal Syndicate Ltd.), which has metallic impurities of less than 1 p.p.m. and is not appreciably hygroscopic.

"Powdex" resins*—Place at least 250-g amounts of the anion and cation exchangers (in the hydroxyl and hydrogen forms, respectively) separately into air-tight polythene containers. Determine their water contents by weighing 1-g samples on to watch-glasses, drying overnight at 110° C, cooling and re-weighing.

The efficiency with which "reactive" silicon is retained by the resins is decreased if the anion exchanger absorbs appreciable amounts of carbon dioxide. It is essential, therefore, to store the resin in air-tight containers.

PROCEDURE—

Before attempting any determinations of silicon in samples, all of the apparatus should be cleaned as described above, and a series of blank determinations should be carried out to ensure that adequate precision is achieved.

Sample collection—Weigh out the equivalent of 0·5 g† ($\pm 0\cdot 005$ g) of anion-exchange resin and 0·1 g ($\pm 0\cdot 005$ g) of cation-exchange resin, and transfer directly to a dry, clean, pre-weighed polythene sample bottle. Collect 1 litre (± 50 ml) of sample into the bottle, avoiding contamination. Re-weigh the bottle and calculate the weight of sample taken.

During the filtration and fusion stages described below, care must be taken to avoid contamination of the sample by air-borne particles. The immediate area in the laboratory should be wiped clean with a damp cloth before a batch of determinations is started.

Sorption and filtration stage—Shake the sample vigorously for 10 minutes (a mechanical shaker is convenient). Allow the resins to settle for at least 5 minutes, and then decant about 800 ml of the sample through the filter. Make a slurry of the resins with the remaining 200 ml of solution, and transfer it quantitatively on to the filter.

Fusion stage—Place the filter disc with the resins in a 30-ml platinum crucible. Add 4 ml ($\pm 0\cdot 1$ ml) of sodium carbonate solution (a Perspex or polythene pipette should be used), and place the crucible on a hot-plate under the evaporation hood. Warm gently (avoiding spitting) until the contents of the crucible are dry.

Place the crucible on a silica triangle and heat gently with a burner to vaporise the resin. If ignition occurs remove the burner. When all of the volatile matter has been removed, increase the heating until the sodium carbonate melts, and continue heating until a clear melt is obtained. Rotate the crucible in the flame so that the melt touches all parts of the inside of the crucible to within about 3 mm of the rim. Replace the crucible in the triangle, cover it with a platinum lid, and continue heating strongly for 1 to 2 minutes. Allow to cool.

To the contents of the crucible, add 0·5 ml ($\pm 0\cdot 05$ ml) of 100-volume hydrogen peroxide and, after evolution of gases has ceased, add a further 0·5 ml ($\pm 0\cdot 05$ ml). Warm the solution gently until no more gas is evolved and continue warming for a further 5 minutes, taking care that the solution does not boil. Nearly fill the crucible with water (from a polythene wash-bottle), and heat gently to dissolve the melt. Allow to cool.

Add 4 ml ($\pm 0\cdot 1$ ml) of 2 N sulphuric acid to a clean, pre-weighed, 8-oz polythene bottle, and quantitatively transfer the contents of the crucible through a polythene funnel into the bottle; pour out the solution from the crucible in one continuous stream, and rinse the crucible in an inverted position with a jet of water from a polythene wash-bottle. Dilute the contents of the bottle with water to 100 g ($\pm 0\cdot 2$ g). (It was found more convenient to dilute solutions by weight than by volume.) The solution can be left overnight, if necessary, before starting the absorptiometric stage.

* Obtainable from William Boby & Co. Ltd.

† The absolute weight of resins used is not critical and should be calculated to the first decimal place. However, within a batch of resin, the weight of resin used should not vary by more than ± 1 and ± 5 per cent. for the anion and cation exchangers, respectively.

Absorptiometric stage—Transfer 25 ml (± 0.2 ml) of the above solution to another clean, pre-weighed, 8-oz polythene bottle and dilute with water to 100 g (± 0.2 g). If the silicon content of this aliquot is beyond the range of the calibration graph, a smaller aliquot can be taken (see Sources of error below). Add 2.5 ml (± 0.1 ml) of acidified molybdate solution and mix; after 10 minutes (± 1 minute), add 2.5 ml (± 0.1 ml) of tartaric acid solution and mix. After a further 5 minutes (± 1 minute), add 2.0 ml (± 0.1 ml) of reducing agent solution and mix. Between 20 and 60 minutes later, measure the optical density of the solution at 810 nm, in 4-cm cuvettes, against distilled water. Subtract the optical density of the blank determination (see below) from that of the sample, and read off the concentration of silicon from the calibration graph.

Blank determination—Shake 1.2 litres of water with about 0.8 g of anion exchanger and 0.2 g of cation exchanger in a polythene sample bottle. Filter 1 litre (± 50 ml) of this solution through a filter directly into another sample bottle containing 0.5 g (± 0.002 g) of anion exchanger and 0.1 g (± 0.005 g) of cation exchanger. (It is assumed that the filtrate is silicon-free water.) Treat this water as for a sample.

Preparation of calibration graph—To each of a series of polythene sample bottles, add 0.5 g (± 0.005 g) of anion exchanger, 0.1 g (± 0.005 g) of cation exchanger and 1 litre (± 20 ml) of water. To these bottles add 0, 5 and 10 ml of a standard silicon solution containing 10 p.p.m. of silica; these volumes correspond to 0, 0.05 and 0.1 p.p.m. of silica, respectively. Repeat the procedure given for samples, and plot a graph of the optical densities (corrected for the blank) against concentration of silicon added. Repeat these determinations until the calibration graph is defined with the required precision.

SOURCES OF ERROR—

Turbidity in final solutions—As described under Experimental, the addition of hydrogen peroxide reduced errors caused by turbidity to negligible proportions. It is conceivable that the magnitude of these errors depends on the precise technique used during the ignition of the resins. It is desirable, therefore, to check this effect occasionally. This can be achieved by analysing an additional 25-ml aliquot of the sample and blank exactly as described above, but adding the tartaric acid before the molybdate and reducing-agent solutions. The optical densities of these solutions will be due to any turbidity or colour, or both, resulting from incomplete destruction of the resins. If these optical densities are appreciable, they should be subtracted from the normal determinations.

Size of aliquot—The calibration graph is prepared by using 25-ml aliquots of the solution obtained after the fusion stage of the procedure. If an aliquot of different volume is taken, the concentration of sodium sulphate in the final solution will differ from that in the solutions used to define the calibration graph. The effect of sodium sulphate is small, *e.g.*, when no sodium sulphate is present the slope is about 2 per cent. greater than for the conditions recommended above. Correction for this effect should be made if this error is unacceptable.

Other waters—The method has not been tested for waters containing high concentrations of dissolved solids, *e.g.*, raw waters, but it seems probable that there would be incomplete sorption of silicon on the resins in the presence of large amounts of other anions.

Carbon dioxide—When resins that had been exposed to the atmosphere for many months were used, it was found that "reactive" silicon was not quantitatively retained on them. This effect is thought to be caused by the sorption of carbon dioxide by the anion exchanger. However, it was shown that polymeric silicic acid and clay were retained, and it was possible to determine the "reactive" silicon in the filtrate from the resins. It is recommended, therefore, that the filtrates from the resins should be checked occasionally for the presence of "reactive" silicon, and new batches of resin should be used whenever appreciable concentrations of silicon are found in the filtrate.

RESULTS

PRECISION—

On each of five occasions, the calibration procedure was carried out with concentrations of 0, 0.02 and 0.1 p.p.m. of silica, each in triplicate. "Reactive" silicon was used in these tests, as it was thought that this form of silicon would be the most difficult of the three forms investigated to remove from solution. A summary of the results is given in Table III.

TABLE III

PRECISION OF ANALYTICAL RESULTS

Sample	Optical density	Optical density per 0·01 p.p.m. of silica	Standard deviation,* p.p.m. of silica		
			Within batch	Between batch	Total†
Water	0·123₅	—	0·0020	—	—
Water + 0·02 p.p.m. of silica ..	0·182	0·029₃	0·0020	N.S.	0·0025
Water + 0·10 p.p.m. of silica ..	0·436	0·031₃	0·0028	N.S.	0·0036
Absorptiometric standard (0·5 p.p.m. of silica)‡ ..	0·666	0·033₃	0·0018	N.S.	0·0018

* Each batch of within and between-batch standard deviations has 10 and 4 degrees of freedom, respectively.

N.S. means not statistically significant at the 5 per cent. probability level.

† The total standard deviation is the estimate of the standard deviation of any one result in any one batch.

‡ One-hundred millilitres of this solution were analysed by the absorptiometric stage only. The optical density in the second column has been corrected for the appropriate blank, and the value in the third column calculated on the basis of the equivalent concentration of silicon in a 1-litre sample analysed by the full procedure.

On the second and fifth occasions, the filtrates were analysed for "reactive" silicon. In the filtrates from the solutions, to which either no or 0·02 p.p.m. of silica had been added, less than 0·0003 p.p.m. of silica were detected; in the solutions to which 0·1 p.p.m. of silica had been added, 0·001 ± 0·0003 p.p.m. of silica were detected.

The total standard deviation for the 0·5 p.p.m. of silica standard (to which 1 ml of 10 per cent. sodium carbonate solution and 1 ml of 2 N sulphuric acid were added) agrees well with the value of 0·0016 p.p.m. of silica previously reported by Webber and Wilson.[6]

EFFECT OF OTHER SUBSTANCES—

Three solutions containing different substances were prepared and analysed in triplicate exactly as described under Method. The tests were repeated at another concentration of "reactive" silicon.

The substances added were as follows (all concentrations refer to a sample of 1 litre).

Solution 1—0·1 p.p.m. each of iron(III), copper(II) and nickel(II), 1·0 p.p.m. each of ammonia and hydrazine, 0·5 p.p.m. of sulphate and 0·2 p.p.m. of chloride.

Solution 2—0·1 p.p.m. each of tungsten(VI), aluminium(III), molybdenum(VI), cobalt(II), zinc(II), vanadium(V), manganese(II), chromium(III), magnesium(II), tin(II), titanium(IV), fluoride and phosphate, 0·2 p.p.m. of sodium, 0·4 p.p.m. of potassium, 0·05 p.p.m. of ammonia, 0·06 p.p.m. of chloride and 2 p.p.m. of sulphate.

Solution 3—1·0 p.p.m. of cyclohexylamine, 2·0 p.p.m. of morpholine, 0·1 p.p.m. each of octadecylamine and alkylaryl sulphonate and 1·0 p.p.m. of ammonia.

The mean results of these tests are given in Table IV; they indicate that at the concentrations likely to be present in feed and make-up waters, these substances do not cause any appreciable bias. For solution 2, the final solution after fusion was slightly turbid, and gave an optical density about 0·015 greater than the blank value; this effect was eliminated by correcting for the turbidity as described under Sources of error.

TABLE IV

EFFECT OF OTHER SUBSTANCES

Substances added	Apparent* silicon content, p.p.m. of silica, at silicon concentrations of—	
	0·000 p.p.m. of silica	0·030 p.p.m. of silica
Solution 1	−0·001₂	0·030
Solution 2	0·001₂	0·036₃
Solution 3	0·003₀	0·033₃

* The 95 per cent. confidence limits for these determinations are ±0·004 p.p.m. of silica.

DISCUSSION

ION-EXCHANGE RESINS—

We used only "Powdex" resins in this work as they were the only resins of such fine mesh size known to us that were readily available in the hydrogen and hydroxyl forms. After this work was finished, we learned that the corresponding Dowex resins are also available in these ionic forms with sizes of 200 to 400 mesh. We would expect that any other manufacturers' resins would also be satisfactory, provided the size and ionic form were suitable.

The silicon content of the "Powdex" resins is higher than is desirable, and the results obtained indicate that the amounts of resin used in the analysis of a sample contain about 40 μg of silica. We understand that Dowex resins with a silicon content of less than 1 p.p.m. of silica are available; the use of resins of this purity would markedly decrease the optical density of blank determinations and also improve the precision attainable.

PRECISION—

The results in Table III show that the total standard deviation of analytical results was about 0·0025 p.p.m. of silica at a concentration of 0·02 p.p.m. of silica; the standard deviation of 0·1 p.p.m. was slightly, but not significantly, greater. This precision is considered adequate, and represents a considerable improvement over that reported by Morrison and Wilson.[2] The criterion of detection[11] (taken as 2·326 times the within-batch standard deviation of the blank determinations) was about 0·005 p.p.m. of silica (95 per cent. confidence level).

The within-batch standard deviations of the results are largely independent of the concentrations of silicon. The most likely cause of this imprecision is the heterogeneous distribution of silicon in the resins used, and it is, therefore, essential to test each batch of resin used to ensure that adequate precision is obtained. The most fruitful approach for improving precision appears to be by the use of resins with a much smaller silicon content than the resins used by the authors.

In the present method a much greater volume of sample is used than that suggested for the previous method of Morrison and Wilson[2] and, consequently, the precision of results with the present method is much less sensitive to contamination.

BIAS—

The results obtained indicate that 100 per cent. recovery of silicon was not obtained; the mean recoveries at concentrations of 0·02 and 0·1 p.p.m. of silica were 88 ± 10 per cent. and 94 ± 3 per cent., respectively. These recoveries are based on the optical density of a standard "reactive" silicon determination in which the concentration and fusion stages of the procedure were omitted. The results given earlier indicated that the ignition and fusion stages caused the slope of the calibration graph to decrease by about 4 per cent. The mean recoveries are consistent with an expected value of 96 per cent., and no further study of the apparent bias was made. It is concluded that the effect can be allowed for adequately by preparing a calibration graph as recommended under Method.

TIME REQUIRED FOR ANALYSIS—

The method is time consuming, but a batch of twelve analyses, including a blank and control standard can be carried out in about 8 hours, of which 5 hours are operator time.

Baker and Farrant[12] have recently published details of an adaptation of Morrison and Wilson's method.[2] They have taken great precautions to reduce random errors caused by contamination (for example, by working in a specially designed laboratory), and obtained appreciably better precision than that reported by Morrison and Wilson.[2] Baker and Farrant's paper is of value in showing the precision that can be achieved with their approach. However, laboratories meeting their specification will often not be readily available; the main advantage of the method in the present paper is that it is not as easily affected by contamination.

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NOTE—References **1, 2, 6** and **8** are to Parts VI, III, IV and II of this series, respectively.

An Improved Technique for Transferring Fractions from a Gas Chromatograph to a Mass Spectrometer

By W. D. WOOLLEY

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A simple technique is outlined for the collection, storage and mass-spectrometric analysis of small amounts of volatile components separated by a gas - liquid chromatograph. Mass spectra obtained with an A.E.I. MS10c2 mass spectrometer are shown, indicating a collection efficiency of about 95 per cent. A useful modification to the inlet system of the mass spectrometer for the analysis of small samples is described, giving a sensitivity increase of up to thirty times the standard inlet sensitivity. This is demonstrated by a mass spectrum of 0·005 μl (3·5 μg) of hexane collected from a chromatograph. A maximum working sensitivity for the system is about 0·1 μg.

THE recent introduction of relatively inexpensive low resolution mass spectrometers has considerably widened the use of the mass spectrometer as a general analytical instrument. Unfortunately, these instruments are usually slow-scanning and, without sophisticated interrupted elution techniques,[1] cannot be coupled directly to a gas chromatograph.

The analytical system outlined in this paper was developed for the indirect mass spectrometry of chromatographic fractions in a study of the possible toxic combustion products of building materials, especially plastics, involved in fires. A high analytical sensitivity was required for the analysis of minor components, particularly volatile oxygenated species, from burning plastics.

In preliminary work, chromatographic fractions were collected in short tubes filled with column packing attached to the exit port of the chromatograph. Samples could be collected satisfactorily in this way but the over-all collection and re-elution into the mass-spectrometer inlet proved to be rather laborious.

The heated line and refrigerated trap technique described in this report appears to be extremely simple to operate, and applicable to a wide range of materials. There is usually visual indication of trapped material in the glass trap during collection, and the traps are coupled directly to the mass spectrometer for analysis.

Preliminary experiments in the analysis of the combustion products of poly(vinyl chloride) showed that many of the materials separated by gas chromatography were close to the detection limit of the A.E.I.* MS10c2 mass spectrometer. Details are given of a modified inlet unit for the mass spectrometer such that the normal batch injection system is coupled to the analyser tube by a new and much faster leak. Thus a substantial increase in sensitivity has been obtained without altering the normal performance of the instrument and without using relatively complex direct injection techniques.[2,3]

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EXPERIMENTAL

COLLECTION SYSTEM—

The collection unit is shown diagrammatically in Fig. 1.   A length of thin-walled stainless-steel tubing with 1·25-mm o.d., supplied by Research and Industrial Instruments Co., is attached to the exit port of the gas chromatograph to direct the effluent gas into a glass collection trap, which is cooled with a refrigerant.   Another stainless-steel tube running parallel to the collection line in the trap is silver-soldered to the tip, as shown, acts as an electrical conductor and provides, via hole C, an exit path for the carrier gas from the trap. The tube is heated electrically by applying a low voltage power supply across the points A and B.
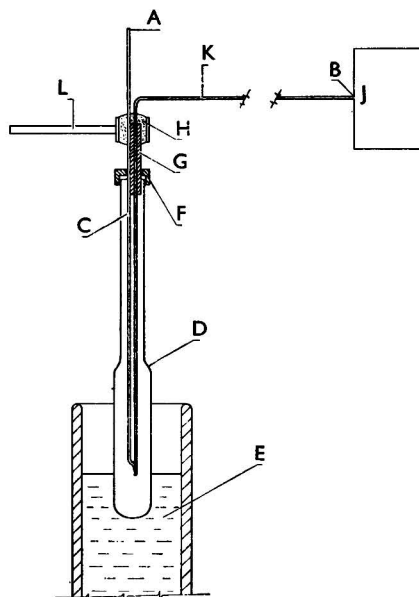
A and B = Stainless-steel tubes   G = Ceramic insulator
C = Hole in tube   H = Araldite
D = Glass collection trap   J = Exit port of gas chromatograph
E = Liquid nitrogen   K = Collection line
F = Silicone rubber cap   L = Clamp

Fig. 1.   Details of the collection unit

The two stainless-steel tubes are electrically insulated at the head of the trap by a 50-mm length of ceramic tubing of about 3·5-mm o.d. inserted over the main collecting tube, as shown.   The unit is held together and fixed into a small clamp with Araldite.

The Pyrex collecting tubes are held in place by a silicone rubber cap, cut out in the centre to fit over the ceramic tube.   The collection tubes are about 130-mm long with 7·5-mm o.d., enlarging to 10-mm o.d. near the collection zone to ensure that the collection tip does not touch the cold sides of the trap.

For the collection of volatile material from the chromatograph, the lower part of the trap is cooled with liquid nitrogen.   It is usually adequate to raise the liquid nitrogen container until the liquid surface is level with the tip of the collection tube.   Further immersion into the liquid nitrogen may cool the collection line and promote condensation in the tip.

After collection the trap is removed, quickly sealed with a silicone rubber cap and stored in liquid nitrogen.   Samples collected in this way have been stored for several days without any apparent loss.

The collection line has a maximum working temperature of about $300°$ C.   Relatively high boiling materials are collected by using less severe refrigerants, such as a solid carbon dioxide bath at $-78°$ C, or an ice-bath.

For mass-spectrometric analysis the sample tubes are coupled directly to the MS10c2 inlet, as shown in Fig. 2. For preliminary work the glass traps were simply inserted into a short length of PVC tubing attached to a brass adaptor on the inlet pipe, as shown in Fig. 2 (A). This gave an adequate vacuum seal but was later replaced by fixing the trap to a modified adaptor with a suitable O-ring, as in Fig. 2 (B). This latter method allowed the whole inlet system to be operated at elevated temperatures.
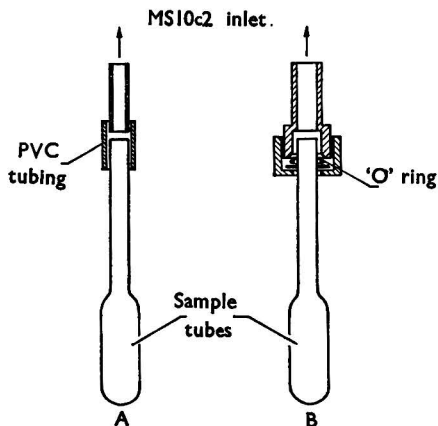
Fig. 2. Collection tubes coupled to the mass spectrometer

Fig. 3 (a) shows a mass spectrum of ethyl acetate collected from a 1-μl injection into the chromatograph, fitted for test purposes with a 1:1 collector - detector split. For comparison purposes a mass spectrum of 0·5 μl of ethyl acetate injected directly into a cold collection tube with a syringe is shown in Fig. 3 (b). The two spectra indicate a collection efficiency of almost 95 per cent. The spectra were recorded on different days, and this may account for the slight variations in the general cracking pattern. The peaks at 44$^+$ in the spectra arise from carbon dioxide frozen from the atmosphere into the cold tubes when the caps are removed before fitting to the mass spectrometer, but if the tubes are opened and fitted quickly to the inlet this peak is virtually eliminated. However, on certain occasions the 44$^+$ peak provided a useful mass marker in the spectrum.
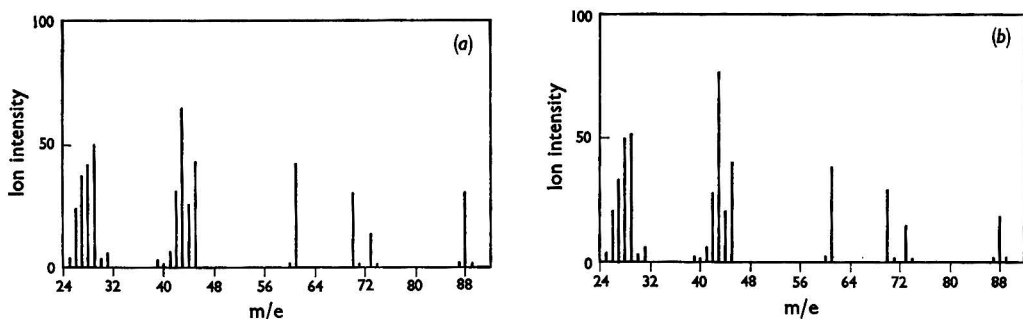
Fig. 3. Mass spectra of 0·5 μl of ethyl acetate. In (a) the sample is collected from a gas chromatograph and is compared with a direct mass spectrum in (b). Ion intensities are given as percentages of full-scale recorder output on amplifier range 10

MODIFIED MASS-SPECTROMETER INLET—

The A.E.I. MS10c2 mass spectrometer contains a stainless-steel inlet block fitted with five valves for the pumping, handling and injection of samples into the main analyser tube. For the analysis of small amounts of material, the sample is introduced into the inlet block only and then into the analyser tube via a porous plug leak. The reservoir and pressure gauge cannot be used because of their large volumes.

The mass spectrum in Fig. 3 (*b*) indicates the general order of sensitivity of the mass spectrometer for materials injected in this way. The spectrum was recorded on amplifier range 10. A further magnification of ten times is available (range 1), but a clean background is then more difficult to obtain and the pumping out time between samples increases.

Various methods were investigated for increasing the sensitivity of the instrument. An inlet pipe inside the analyser tube to feed the sample directly into the ion source gave only a small increase in sensitivity. A reduction in the pumping speed of the main tube gave a further increase, but the pumping speed was soon returned to the original 2 litres per second because of the increased pumping out time between analyses.

To maintain a constant pressure in the mass-spectrometer tube during an analysis, the inlet leak is designed so that only a small fraction of the sample leaks into the mass spectrometer. Experiments showed that even a small sample would remain in the inlet block for several hours during an analysis, and it was considered that a sensitivity increase could be obtained by fitting a new and faster leak.

To fix the new leak to the mass spectrometer without altering the normal performance of the instrument, the inlet pressure gauge was removed, and a new inlet line and leak bolted from the vacant gauge position to the spare port on the analyser tube. This gave an inlet system that could be operated as before, but now with the choice of a standard or fast leak.
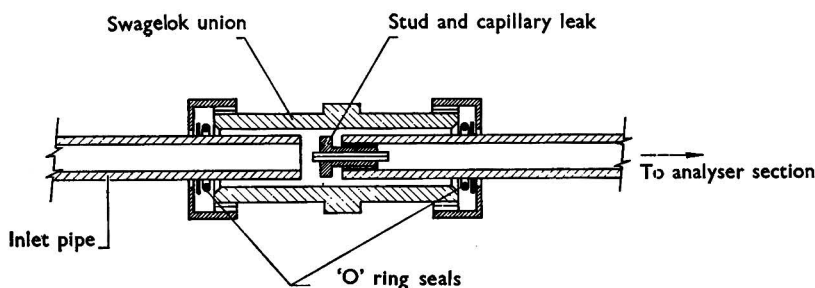
Fig. 4. Leak unit

The inlet pipe consists of a length of thick-walled stainless-steel tube, 6·35 mm o.d., shaped to match the existing standard inlet line. The two inlet pipes are strapped together and heated by the same heating tape for operation at temperatures up to 150° C. The leak itself is a 20-mm length of stainless-steel capillary tubing, 0·15-mm i.d. and 1·58-mm o.d., fitted into the inlet line near the analyser tube, as shown in Fig. 4. The inlet pipe is cut and rejoined with a modified stainless-steel Swagelok union by using rubber O-rings in place of the normal metal ferrules. The capillary leak is soldered with a silver - 15 per cent. manganese soldering alloy, obtained from Johnson Matthey Metals Ltd., into a special stud, which, in turn, screws into the inlet pipe; the end of the inlet pipe and the face of the stud
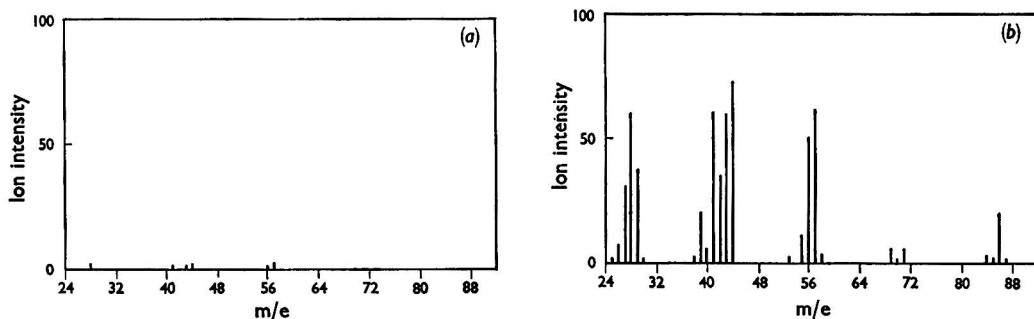
Fig. 5. Mass spectra of 0·005 µl of hexane collected from a gas chromatograph recorded on (*a*), the standard leak, and (*b*), the fast leak. Ion intensities are given as percentages of full-scale recorder output on amplifier range 10

are machined so no gasket is required.   The leak unit is versatile because the leak can be readily removed simply by unbolting the Swagelok union.   The inlet pipe is fixed to the pressure gauge port with an O-ring seal and the other end soldered into the analyser section.

Fig. 5 (*a*) shows a mass spectrum, obtained when the standard leak was used, of hexane collected from a 1-μl injection into the chromatograph of a solution of 1 per cent. hexane in toluene.   When the 1:1 split is used this corresponds to a collection of 0·005 μl (3·5 μg) of hexane.   After analysis the fast-leak valve was opened and the new spectrum, shown in Fig. 5 (*b*), recorded.   As can be seen the ion intensities are increased considerably and, based on more accurate measurements, a magnification of about thirty times is obtained with the new leak.

However, at this rate of injection into the analyser tube, the ion currents show a decrease of about 10 per cent. in the 15 minutes required for a complete scan, but to overcome this difficulty it is usual, after a scan with a fast leak, to repeat the scan immediately.   From a comparison of the first major peaks in each scan the rate of decrease is readily found.   The mass spectrum in Fig. 5 (*b*) has been corrected to compensate for this error.

As yet, little work has been done, with the collection system outlined in this report, on samples smaller than about 1 μg.   However, by using the most sensitive range of the mass spectrometer (range 1) and a more favourable ratio of chromatographic collection to detector split (*e.g.*, 20:1), a 0·1-μg sample of hexane injected into the chromatograph should give an acceptable mass spectrum.

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The Use of Thin-layer Chromatographic Techniques for the Determination of Breakdown Products of Additives to Plating Solutions

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The combination of conventional thin-layer chromatographic techniques with a novel form of column chromatography has enabled seventeen derivatives of coumarin in used nickel-plating solutions containing this additive to be isolated. Ten derivatives were identified, as mono-, di- and trihydroxy-coumarins and dihydrocoumarin, melilotic acid, o-coumaric acid and umbellic acid. R_F values and colour reactions of twenty typical coumarin derivatives are also reported.

ORGANIC additives are frequently added to electroplating solutions to modify the properties of the electro-deposit.
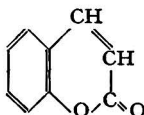
A common additive to nickel-plating solutions is coumarin. This is added primarily to give an even deposit, but it is also used to promote ductility, which is particularly required in motor-car components (bumpers, etc.).

During electroplating, electrode reactions cause the formation of other compounds from coumarin. As these build up in solution, they counteract the beneficial effect of the additive. Identification of the breakdown products might lead to knowledge about the mechanism of their formation and possibly to ways of their prevention.

Thin-layer chromatography was considered a more rapid and sensitive technique for identification than paper chromatography, and it had the added advantage that acidic plating solutions could be examined directly and compared with concentrated extracts of plating solutions, which were obtained from plating solutions adjusted to pH 1 by extraction with chloroform.

Direct chromatography of the plating solutions and the extracts (thick, oily substances) proved difficult, because of masking effects and "tailing" by the additive itself and one breakdown product, identified as melilotic acid (o-hydroxyphenylpropionic acid).
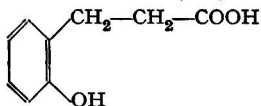
Column chromatography was tried to improve separation, but a solid, open column, cast from thin-layer adsorbent slurry, which could be developed like an ordinary thin-layer chromatogram, provided a more convenient means. This was followed by a further separation of the minor constituents on thin-layer chromatographic plates.

EXPERIMENTAL

PREPARATION OF COLUMNS—

Cellophane dialysis tubing (60 cm long × 20 mm o.d.) was sealed at one end by a knot and filled with silica gel G or aluminium oxide G slurry, as made for normal thin-layer chromatographic plates. The tube was then suspended vertically and the bottom perforated by a few holes (to allow excess water to drain off). After 20 minutes, the tube was

cut, with a razor blade, into sections of 20-cm length, which were then placed in an oven for 25 minutes at 110° C, when the cellophane could be removed. The columns thus obtained were activated for 60 minutes at 110° C.

PREPARATION AND STORAGE OF PLATES—

Good quality glass plates were coated with a 250-μ thick layer of water - silica gel G (2 + 1) or water - aluminium oxide G (4 + 3) slurry, with a home-made spreader. After being left to set for 15 minutes, the plates were activated for 30 minutes at 130° C and subsequently stored in an empty desiccator under vacuum.

SAMPLE APPLICATION—

Amounts of 1 to 10 μl of solution were applied at 2 cm from the bottom edge of the plates by repeated application of 1-μl drops.

For columns, 200 to 500 μl of solution were applied to the circumference at 2 cm from the bottom edge by allowing the solution, contained in a short piece of glass tubing, to flow through a capillary on to the rotating column.

DEVELOPMENT—

Plates were developed in a tank (23 × 23 × 8 cm) fitted with a ground-glass lid and lined with filter-paper for uniform saturation.

Columns were developed either in cork-stoppered, flat-based test-tubes or, alternatively, in a shallow Petri dish filled with solvent and covered with a beaker to provide an air-tight seal. Plates were developed 15 cm and columns 10 cm.

SOLVENTS—

The three solvents, toluene - ethyl acetate - formic acid (5 + 4 + 1); hexane - ethyl acetate (3 + 1); and toluene - ethyl formate - formic acid (5 + 4 + 1), were tried. Although the second and third gave good separation of monohydroxy derivatives of coumarin on silica gel G, the third gave improved separation on aluminium oxide G and was used throughout the later work; results reported are confined to the third solvent.

DETECTION—

Columns were examined under ultraviolet light (253 nm) only; detected bands were cut from the column, extracted with purified ethanol, and the concentrated extract applied to a normal thin-layer chromatographic plate.

Plates were examined under ultraviolet light before and after spraying with either diazotised sulphanilic acid in 5 N sodium hydroxide solution, diazotised o-dianisidine in 5 N sodium hydroxide solution, p-nitrobenzenediazonium fluoroborate in water or N sodium hydroxide solution.

COLOUR PHOTOGRAPHY—

Plates, 20 × 15 cm, were recorded on 35-mm Agfacolor CT18 film under ultraviolet light (253 nm); the exposure was 10 minutes at f 11. Two standard photographic ultraviolet filters were used to exclude reflected ultraviolet light.

RESULTS AND DISCUSSION

Table I gives R_F values and colour reactions of some synthesised coumarin derivatives and related compounds.

The relationship between R_F value and structure is clear, especially the decrease in R_F value as the substituent moves from the 3- to the 5- and 7-positions, or from the 4- to the 6- and 8-positions, and when a second and third hydroxyl group is introduced.

In plating solutions only 7-hydroxycoumarin and o-coumaric acid were detected, in addition to coumarin and melilotic acid.

The chloroform extractions provided the breakdown products in the plating solution in a much more concentrated form and hence the number of identified compounds is much greater; it increases from two, in an extract from a relatively new solution, to seventeen, in an extract from a spent solution. Melilotic acid, o-coumaric acid, 4-hydroxycoumarin, 6-hydroxycoumarin, 7-hydroxycoumarin, 6,7-dihydroxycoumarin, 4,6,7-trihydroxycoumarin

TABLE I

COUMARIN DERIVATIVES SEPARATED BY THIN-LAYER CHROMATOGRAPHY

Solvent, toluene - ethyl formate - formic acid; layer thickness, 250μ

Derivative	R_F value on Al₂O₃ G	R_F value on silica gel G	Ultraviolet light (253 nm) no spray	Ultraviolet light (253 nm) aqueous sodium hydroxide	Diazotised sulphanilic acid spray visible	Diazotised sulphanilic acid spray ultra-violet	Diazotised o-dianisidine spray visible	Diazotised o-dianisidine spray ultra-violet	p-Nitrobenzene-diazonium fluoroborate spray visible	p-Nitrobenzene-diazonium fluoroborate spray ultra-violet	Found in plating solution	Found in CHCl₃ extract of plating solution
3-Hydroxycoumarin	0·85	0·55	26	26	71	40	5	44	59	10		×
4-Hydroxycoumarin	0·71	0·40	26	26	—	26	—	26	2	44		×
5-Hydroxycoumarin	0·78	0·42	7	7	nd	nd	56	7	nd	nd		×
6-Hydroxycoumarin	0·73	0·37	34	34	—	72	—	3	58	65		
7-Hydroxycoumarin	0·75	0·38	33	33	58	33	26	27	59	33	×	×
8-Hydroxycoumarin	0·66	0·35	65	65	8	65	nd	nd	nd	nd		
Coumarin	0·89	0·54	65	40	8	40	13	40	—	65	×	×
Dihydrocoumarin	0·91	0·61	—	—	6	13	17	—	3	65		×
Melilotic acid	0·75	0·46	65	—	7	61	64	14	57	65	×	×
o-Coumaric acid	0·72	0·48	16	47	8	47	18	40	57	65	×	
Umbellic acid	0·59	0·35	32	44	7	51	25	33	9	23		
3,6-Dihydroxycoumarin	0·55	nd	34	7	nd	nd	nd	nd	nd	nd		
4,7-Dihydroxycoumarin	0·46	nd	26	26	5	26	18	26	58	44		
5,6-Dihydroxycoumarin	0·51	nd	44	7	nd	nd	nd	nd	nd	nd		
5,7-Dihydroxycoumarin	0·53	nd	33	65	nd	nd	65	65	nd	nd		
6,7-Dihydroxycoumarin	0·47	nd	33	39	48	40	56	44	57	44		×
7,8-Dihydroxycoumarin	0·59	nd	44	44	nd	nd	57	nd	nd	nd		
4,5,7-Trihydroxycoumarin	0·34	nd	26	26	7	65	23	25	62	65		
4,6,7-Trihydroxycoumarin	0·46	nd	32	32	71	71	57	71	18	27		×
4,7,8-Trihydroxycoumarin	0·44	nd	26	26	57	70	56	70	56	23		

nd = not determined.   — = not detectable.

Colour code (appearance as compared with a Derwent colour pencil chart)

2	Lemon Cadmium	23	Imperial Purple	51	Olive Green
3	Gold	25	Dark Violet	56	Raw Umber
5	Straw Yellow	26	Light Violet	57	Brown Ochre
6	Deep Cadmium	27	Blue Violet Lake	58	Raw Sienna
7	Naples Yellow	32	Spectrum Blue	59	Golden Brown
8	Middle Chrome	33	Light Blue	61	Copper Beech
9	Deep Chrome	34	Sky Blue	62	Burnt Sienna
10	Orange Chrome	39	Turquoise Blue	64	Terra-cotta
13	Pale Vermilion	40	Turquoise Green	65	Burnt Carmine
14	Deep Vermilion	44	Water Green	70	French Grey
16	Flesh Pink	47	Grass Green	71	Silver Grey
17	Pink Madder Lake	48	May Green	72	White
18	Rose Pink				

and umbellic acid were identified positively, and 5-hydroxycoumarin tentatively.   The R_F values and colour reactions of other compounds could not be related to any available derivative.

Melilotic acid was always detectable in all samples, including extracts.   Both o-coumaric acid and 7-hydroxycoumarin appeared at a fairly early stage in the life of the plating solution, and their concentration increased gradually throughout the life of the plating solution.

Many reaction paths are possible for the various compounds produced.   Melilotic acid appears to be formed by cathodic hydrogenation of coumarin to 3,4-dihydrocoumarin, followed by rapid hydrolysis at pH 4.   For the formation of o-coumaric acid, Ashurst[1] has suggested that the reaction might be analogous to the oxidation of cyclohexane.   6-Hydroxy-coumarin and 6,7-dihydroxycoumarin could be formed by the well known persulphate oxidation.[2]   The persulphate formation in the plating solution according to the reaction

$$2SO_4^{2-} - 2e^- \longrightarrow S_2O_8^{2-}$$
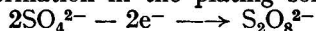
is quite feasible.   However, a random attack of the coumarin molecule by active oxygen is the most likely explanation.

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Gas-chromatographic Determination of Acetyl and Trimethylsilyl Derivatives of Alkyl Carbamates and their *N*-Hydroxy Derivatives

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Microgram amounts of mixtures of alkyl carbamates, and of urethane and *N*-hydroxyurethane, as their trimethylsilyl derivatives, and similar mixtures of alkyl *N*-hydroxycarbamates, as their trimethylsilyl and acetyl derivatives, have been analysed by gas chromatography on SE30 columns. With a programmed temperature rise, the elution temperatures varied linearly with the number of carbon atoms in the alkyl side-chain of a homologous series; the isobutyl analogues were eluted at lower temperatures than the corresponding butyl analogues. When the corresponding isobutyl derivatives were used as internal standards, the ratios of peak heights (test to standard) varied linearly with the concentration of urethane and *N*-hydroxyurethane.

THE alkyl carbamates and their *N*-hydroxy derivatives are of interest in carcinogenesis and in many other chemical and biological studies.[1,2,3] These substances have been determined by various paper-chromatographic[4] and colorimetric procedures,[5,6] and several carbamates have been qualitatively analysed by gas chromatography.[7] This paper describes (*i*) the quantitative analysis by gas chromatography of urethane and *N*-hydroxyurethane, as their acetyl or trimethylsilyl derivatives, and (*ii*) the qualitative analysis of mixtures of alkyl carbamates or their *N*-hydroxy analogues, containing from one to six carbon atoms in the alkyl group in a homologous series, and one branched chain, the isobutyl group.

METHOD

APPARATUS—

The analyses were performed on a Perkin-Elmer, Model 800, dual column, gas chromatograph, incorporating a dual flame-ionisation detector and a Honeywell "Electronik" continuous balance recorder with a range of from 0·25 to 2·5 mV. The dual chromatographic columns consisted of 1-m stainless-steel, coiled tubes of $\frac{1}{8}$ inch o.d., packed with 1·5 per cent. silicone gum rubber (SE30) on a solid support of 80 to 100-mesh, HMDS-treated Chromosorb W.

OPERATING CONDITIONS—

The conditions used were: hydrogen pressure, 15 lb per inch²; air pressure, 30 lb per inch²; nitrogen flow-rate through both columns, 30 ml per minute; and injector block temperature, about 160° C (dial setting 4). The rate of temperature rise in both columns in all of the experiments was 5° C per minute. The columns were used after equilibration for 24 hours at an oven temperature of 200° C, and all recordings were made at the basic chart speed of 15 inches per hour.

REAGENTS—

Pyridine.
Triethylamine.
Diethyl ether.
Trimethylchlorosilane.
Hexamethyldisilazane.
All were dried and distilled before use.

MATERIALS—

Methyl, ethyl, propyl and butyl carbamates were of commercial origin. Isobutyl and pentyl carbamates were prepared from the corresponding chloroformates and ammonia solution. All were recrystallised to constant melting-points and dried overnight in an evacuated desiccator over phosphorus pentoxide before use. The alkyl N-hydroxycarbamates and some of their O-acetyl and NO-diacetyl derivatives were prepared as previously described.[8,9]

Trimethylsilyl-N-hydroxyurethane—A 10-ml portion of trimethylchlorosilane was added, dropwise, during 15 minutes, to a stirred mixture of 5 g of N-hydroxyurethane, 20 ml of hexamethyldisilazane and 50 ml of pyridine. The mixture was heated at 75° C for 2 hours, diluted with 100 ml of ether, stored at 4° C for 16 hours, filtered and the residue washed with 50 ml of ether. The ethereal solutions were combined, washed with two 20-ml portions of water, dried over anhydrous sodium sulphate and distilled, giving 8 g (95 per cent. yield) of trimethylsilyl-N-hydroxyurethane as a colourless oil with a fruity odour (b.p. 45° to 49° C at 0·04-mm pressure of mercury). The composition of the oil is given below.

Element	C	H	N
Found, per cent.	41·28	9·12	7·50
$C_6H_{15}NO_3Si$ requires ..	40·66	8·53	7·90

The compound was miscible with all of the common organic solvents and immiscible with water; it gave an immediate purple colour with 1 per cent. w/v iron(III) chloride solution, and immediately reduced ammoniacal silver nitrate solution. These properties are consistent with those expected from an N-substituted derivative, but do not provide conclusive evidence of structure, which was not further investigated.

Bis-(trimethylsilyl)-N-hydroxyurethane—A mixture of 9·6 g of N-hydroxyurethane and 100 ml of pyridine was introduced into a 1-litre, round-bottomed flask, equipped with a mercury-sealed stirrer, a dropping funnel and a reflux condenser with exit guarded by a calcium chloride drying tube. The apparatus was flushed with dry nitrogen, 25 ml of triethylamine introduced and 30 ml of trimethylchlorosilane added, dropwise, with stirring, during 30 minutes. The mixture was heated under reflux for 3 hours, allowed to cool, diluted with 300 ml of ether, filtered after being allowed to stand for 16 hours at 4° C and the residue washed with 100 ml of ether. The ethereal solutions were combined and distilled, giving 18 g (79 per cent. yield) of bis-(trimethylsilyl)-N-hydroxyurethane as a colourless liquid (b.p. 42° to 44° C at 0·1-mm pressure of mercury) with a fruity odour. The composition of the oil is given below.

Element	C	H	N
Found, per cent.	43·78	9·44	6·14
$C_9H_{23}NO_3Si_2$ requires ..	43·33	9·29	5·62

The compound had properties similar to those described for the mono-trimethylsilyl derivative, except that the coloration with iron(III) chloride solution and the reduction of ammoniacal silver nitrate solution occurred more slowly.

PROCEDURES—

1. *Preparation and chromatography of trimethylsilyl derivatives*—The carbamates and N-hydroxycarbamates were dissolved in 0·5 ml of pyridine, and 0·2 ml of hexamethyldisilazane, 0·1 ml of trimethylchlorosilane and 0·2 ml of triethylamine then added. After being allowed to stand for 4 hours at 50° C, the mixtures were centrifuged, and 1 μl of the supernatant liquor was analysed as described under Operating conditions.

2. *Preparation and chromatography of acetyl derivatives*—The N-hydroxycarbamates were dissolved in 0·5 ml of pyridine, to which were then added 0·2 ml of acetic anhydride and 0·3 ml of triethylamine. After being allowed to stand for 4 hours at 23° C, a 1-μl portion was analysed as described under Operating conditions.

The concentrations of the test substances in the final mixtures obtained by Procedures 1 and 2 were such that 1 μl contained the amounts determined, as shown in Figs. 1 to 6.

RESULTS AND DISCUSSION

Zielinski and Fishbein[7] described the qualitative analysis of several alkyl carbamates by gas chromatography on two polar columns [Carbowax 20M, a poly(ethylene glycol) resin, and Versamid 900, a polyamide resin] and on one non-polar column (SE30, a methylsilicone resin). In the present study, 1 μl of a 0·1 M solution of urethane in ether was not detectable under the conditions described (see legend, Fig. 1); 1 μl of a 1·0 M solution, under similar conditions, gave a broad elution peak, with a mean retention time of 3·8 minutes, and a sharp peak, with a retention time of 1·0 minute when the temperature programme was changed to 100° to 120° C, other conditions being unchanged. This agrees with the 1·2 minutes reported by Zielinski and Fishbein,[7] who used isothermal conditions (100° C) and a 10 per cent. w/v SE30 coating on 60 to 80-mesh Chromosorb W.
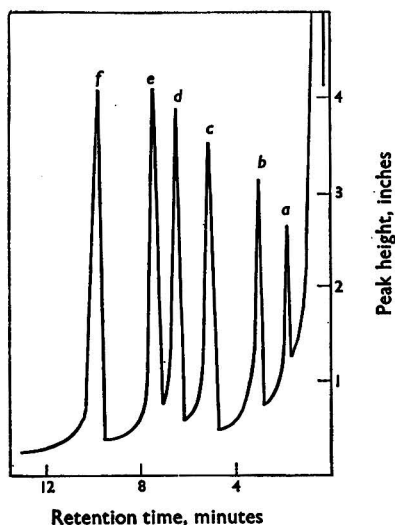
Fig. 1. Gas chromatogram of alkyl carbamates (Procedure 1). Temperature programme: 35° to 100° C; and attenuation × 200. Amount of each carbamate analysed, 5 × 10⁻⁸ moles. Alkyl groups: (*a*), methyl; (*b*), ethyl; (*c*), propyl; (*d*), isobutyl; (*e*), butyl; and (*f*), pentyl
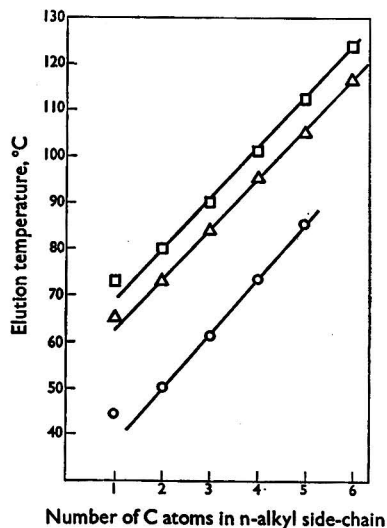
Fig. 2. Relationship between alkyl chain length and elution temperature in the gas chromatography of n-alkyl carbamates and N-hydroxycarbamates by Procedures 1 and 2. ○, n-Alkyl carbamates (Procedure 1). Conditions as for Fig. 1. △, n-Alkyl N-hydroxycarbamates (Procedure 1). Temperature programme: 35° to 130° C; and attenuation × 200. □, n-Alkyl N-hydroxycarbamates (Procedure 2). Temperature programme: 50° to 140° C; and attenuation × 20

The generally more volatile and less polar derivatives formed when active hydrogen atoms are substituted by trimethysilyl groups have been extensively used in the gas-chromatographic determination of many substances, including pesticidal carbamates,[10] ureas,[10,11] steroids,[12,13] fatty acids,[14] amino-acids,[15,16] biological amines[17,18] and carbohydrates.[19] The elution temperatures of the trimethylsilyl derivatives of the homologous series of alkyl carbamates (from methyl to pentyl), and of the trimethylsilyl and acetyl derivatives of the alkyl N-hydroxy-carbamates (from methyl to hexyl), under the conditions described, were directly proportional to the number of carbon atoms in the alkyl side-chains, except for the methyl derivatives, which gave somewhat longer retention times (Fig. 2); the elution temperatures were also approximately proportional to the respective boiling-points of the parent compounds (Table I). Comparison of the butyl and corresponding isobutyl derivatives shows that chain branching reduced the retention time (Figs. 1, 3 and 4) in spite of the higher boiling-point of isobutyl carbamate (Table I). The same effect of chain branching on retention time has been observed

TABLE I

GAS CHROMATOGRAPHY OF ALKYL CARBAMATES AND ALKYL N-HYDROXYCARBAMATES
BY PROCEDURES 1 AND 2

	Compound	Boiling-point, °C/mm pressure of mercury	Reference†	Relative elution*	
				Trimethylsilyl derivative	Acetyl derivative
A.	ROCONH₂				
	R = Methyl	177/760	23	0·60	N.D.
	Ethyl	184/760	23	1·0	N.D.
	Propyl	195/760	23	1·73	N.D.
	Isobutyl	206/760	23	2·20	N.D.
	Butyl	203/760	23	2·53	N.D.
	Pentyl	56‡	7	3·33	N.D.
B.	ROCONHOH				
	R = Methyl	50 to 51‡	8	0·79	0·77
	Ethyl	86 to 88/0·6	8	1·0	1·0
	Propyl	90 to 92/0·6	8	1·29	1·33
	Isobutyl	41‡	9	1·45	1·53
	Butyl	100 to 102/0·8	8	1·58	1·70
	Pentyl	115 to 118/0·04	9	1·84	2·07
	Hexyl	42‡	9	2·13	2·43

* Relative to the retention time of the corresponding derivative of the ethyl analogue. For the trimethylsilyl derivatives of urethane and N-hydroxyurethane, and for the acetyl derivative of N-hydroxyurethane, the retention times under the conditions described were 3·0, 7·6 and 6·0 minutes, respectively.
† Refers to sources of values given in second column.
‡ Melting-point, °C.
N.D.—Not determined.

when O-alkyl carbamates[7] and their N-alkyl derivatives[20] were determined directly on polar and non-polar columns. When the corresponding isobutyl derivatives were used as internal standards, urethane (by Procedure 1) and N-hydroxyurethane (by Procedures 1 and 2) gave peak heights that were directly proportional to the concentration of the corresponding test substance (Fig. 5). Fig. 6 shows the elution of a mixture of urethane and N-hydroxyurethane, each at a concentration of 0·1 M in pyridine and determined by Procedure 1.
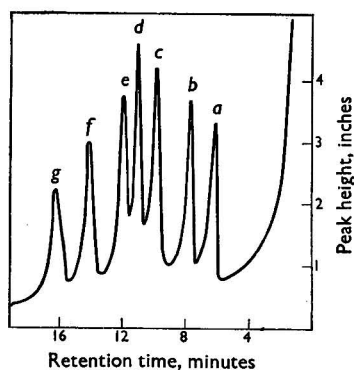
Fig. 3. Gas chromatogram of alkyl N-hydroxycarbamates (Procedure 1). Temperature programme: 35° to 130° C and attenuation × 200. Amount of each hydroxycarbamate analysed, 4·25 × 10⁻⁸ moles. Alkyl groups: (a) to (f) as for Fig. 1; and (g), hexyl
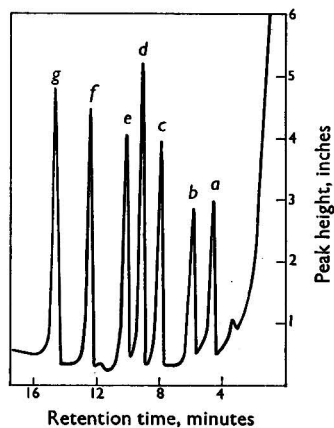
Fig. 4. Gas chromatogram of alkyl N-hydroxycarbamates (Procedure 2). Temperature programme: 50° to 140° C; and attenuation × 20. Amount of each hydroxycarbamate analysed, 1·25 × 10⁻⁸ moles. Alkyl groups as for Fig. 3
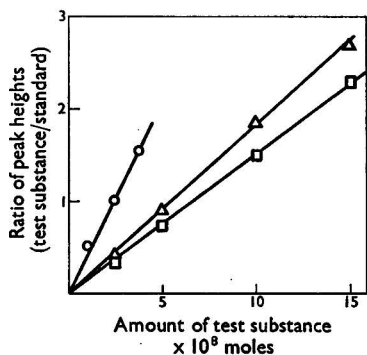
Fig. 5. Calibration graphs for urethane (Procedure 1) and N-hydroxyurethane (Procedures 1 and 2). □, Urethane. Temperature programme: 35° to 95° C; and attenuation × 200. Amount of isobutyl carbamate in each assay, 5×10^{-8} moles. △, N-Hydroxyurethane (Procedure 1). Conditions as for urethane, except that isobutyl N-hydroxycarbamate was used as internal standard. ○, N-Hydroxyurethane (Procedure 2). Temperature programme: 50° to 110° C; and attenuation × 20. Amount of isobutyl N-hydroxycarbamate in each assay, $1\cdot25 \times 10^{-8}$ moles
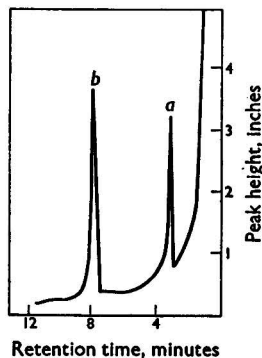
Fig. 6. Gas chromatogram of a mixture of urethane and N-hydroxyurethane (Procedure 1). Temperature programme: 35° to 90° C; and attenuation × 200. Amount of each substance analysed, 5×10^{-8} moles. (*a*), Urethane; and (*b*), N-hydroxyurethane

The structures of the products formed after trimethylsilylation or acetylation of N-hydroxyurethane, by Procedures 1 and 2, were ascertained by comparison of their retention times with those of the synthetic mono- and disubstituted derivatives (see Materials). Trimethylsilyl- and O-acetyl-N-hydroxyurethanes were first formed by Procedures 1 and 2, respectively, with retention times of 8·0 and 6·2 minutes; after being allowed to stand for 4 hours, these were quantitatively converted into the corresponding disubstituted derivatives, retention times for the di-(trimethylsilyl) and diacetyl derivatives being 7·6 and 6·0 minutes, respectively (Figs. 3 and 4, and Table I). These results show that, for N-hydroxyurethanes, the species that are analysed after 4 hours (by Procedures 1 and 2) are the di- and not the monosubstituted derivatives. The other alkyl N-hydroxycarbamates probably formed analogous derivatives, as no change in the retention times, obtained after 4 hours, occurred after longer reaction times (up to 8 hours). No attempt was made to establish the structures of the trimethylsilylcarbamates, but they were probably the NN-bis(trimethylsilyl) derivatives, as acetamide reacts analogously,[21] and the retention times of the derivatives formed were not changed after up to 18 hours.

The results shown (Figs. 1 to 6 and Table I) were reproducible on repeat determinations, providing the conditions described were unchanged. Each experiment was performed at least five times, and standard deviations varied between ±0·005 and ±0·021; larger variations sometimes occurred, but these were due to malfunctioning of the instrument, *e.g.*, to partial clogging of the injector port or columns. The results were obtained by starting with known amounts of the various unsubstituted carbamates or N-hydroxycarbamates, forming the relevant derivatives (see Procedures 1 and 2), and analysing 1 μl of the corresponding supernatant solutions. For N-hydroxyurethane alone, the quantitative relationships shown in Fig. 5 were also obtained by direct analysis of known amounts of the pre-formed NO-disubstituted acetyl and trimethylsilyl derivatives. No attempt was made to establish a linear peak height - concentration relationship, except for urethane and N-hydroxyurethane (Fig. 5).

This work resulted from an attempt to study, by gas chromatography, the metabolic interconversion[22] of urethane and N-hydroxyurethane by rodent tissues. Organic solvent extracts of de-proteinised rat or mouse liver homogenates, when analysed by Procedures 1 and 2, contained interfering substances that gave ambiguous results when the homogenates v є є incubated with urethane or N-hydroxyurethane. Attempts to eliminate the interference

by using ether, benzene, chloroform or methylene chloride as the solvent for extraction, or trichloroacetic acid, ammonium sulphate, zinc sulphate - sodium hydroxide or ethanol as the protein precipitant, were unsuccessful.

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A Method for the Analysis of Cereals and Groundnuts for Three Mycotoxins

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A method is proposed for the analysis of samples for three mycotoxins, aflatoxin, ochratoxin and sterigmatocystin, by suitable treatment of a single sample extract. Based on the subjective evaluation of thin-layer chromatograms of the extract, results can be reproduced with an accuracy of ±20 per cent. The method is considered to be satisfactory for the purposes of a field survey when the determination of the approximate level of mycotoxin contamination of cereals and groundnuts in the shortest possible time is of prime importance. Problems encountered with samples that have high oil contents or that are darkly pigmented are dealt with by appropriate modifications of the method.

THE discovery of the aflatoxins has led to much research in the field of mould contamination of food and feeds. It has already been established that many moulds elaborate metabolites that are toxic or carcinogenic, or both, to laboratory animals. While direct evidence linking aflatoxin, ochratoxin and sterigmatocystin with diseases in man is lacking, the possibility cannot be excluded that the presence of these materials in food constitutes a grave threat to human health. It has, therefore, become essential for the safety of both man and animals that the extent to which our food and feed crops are contaminated by mycotoxins should be determined.

Assaying for mycotoxins, for which analytical procedures have been developed, is expensive and time consuming, especially in respect of the preparation from the samples of extracts containing the toxins. It would, therefore, be of great advantage if a method could be developed to assay for a number of mycotoxins in the same sample extract.

The following paper deals with work carried out to determine whether aflatoxin, ochratoxin and sterigmatocystin contents could be determined on one extract of a sample. As a result, a method is proposed that gives satisfactory results when applied to samples of maize and sorghum. Problems caused by high fat content of the extract (as is the case with groundnuts) and by fluorescent pigments (extracted from some varieties of sorghum) were overcome by modifying the basic method.

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS FOR PRELIMINARY ANALYSIS

PREPARATION OF MYCOTOXIN-CONTAINING SAMPLES—

The main South African crops liable to contamination by mycotoxins are maize, groundnuts and sorghum. It was, therefore, decided that each of the three mycotoxins being investigated should be produced separately on media prepared from each of the three food crops referred to. After determining the mycotoxin contents of these materials, samples containing varying but known concentrations of all three mycotoxins were prepared by mixing the media in suitable proportions. These samples were used as raw material for the proposed investigation.

Separate samples (250 to 300 g) of the three media, crushed and adjusted to a moisture content of 30 per cent., were sterilised in 5-litre Erlenmeyer flasks and inoculated with the appropriate mould spores (*Aspergillus flavus*, *A. ochraceous* and *A. nidulans*). The cultures were incubated at 28° C ($\pm$3° C) in a darkened room for 11 days in the case of *A. ochraceous*, and for 17 to 18 days for the other moulds. The cultures were then dried in a forced-draught oven at 40° C before being ground in a hammer mill. Each medium was thus cultured to produce, separately, each of the three mycotoxins under investigation.

The aflatoxin contents of the respective samples were determined according to the procedure prescribed by the IUPAC Sub-Commission on trace substances.[1] The method of Steyn and van der Merwe,[2] with modifications suggested by Scott and Hand,[3] was used to determine ochratoxin contents, and the procedure proposed by Vorster and Purchase[4] was used for sterigmatocystin. Each culture was checked qualitatively to ensure that it was not contaminated by the other mycotoxins under investigation.

PREPARATION OF QUANTITATIVE AND QUALITATIVE STANDARD SOLUTIONS—

Samples of several toxins known to be produced by the three species of fungi under consideration, with the notable exceptions of aspertoxin[5] and nidulol,[6] were obtained either in pure form or as mixtures for use in reference solutions.

Aflatoxins—Pure aflatoxins B_1 and G_1 were dissolved separately in analytical-reagent grade benzene[7] to give estimated concentrations of 10 μg ml^{-1}. The procedure described in Appendix A of the IUPAC bulletin[1] was followed to determine the exact concentration of the respective toxins in the two solutions. A qualitative standard solution was prepared by dissolving sufficient amounts of the four major, and all of the available minor, aflatoxins in benzene so that 10 μl of the solution would produce readily visible spots of each toxin when separated by thin-layer chromatography.

Ochratoxins—Chloroform solutions of ochratoxins A and C (ethyl ester of A) containing an estimated 40 μg ml^{-1} were prepared (allowing for benzene content of crystalline A) and standardised by the procedure described for the aflatoxins, substituting the following values[8] in the calculations—

Ochratoxin A: $\epsilon^{333}_{chloroform} = 2400$; molecular weight $= 402\cdot5$

Ochratoxin C: $\epsilon^{333}_{chloroform} = 7000$; molecular weight $= 430\cdot5$

As ochratoxin B and its methyl and ethyl esters have been reported to be non-toxic,[9] it was considered that their determination would be unnecessary. Consequently, no standard solutions of them were prepared.

Sterigmatocystin—Pure sterigmatocystin was dissolved in analytical-reagent grade chloroform to give an estimated concentration of 200 μg ml^{-1}. The optical density at 327 nm was measured and the concentration calculated ($\epsilon^{327}_{chloroform} = 16,220$ and molecular weight $= 324$).

DETERMINATION OF THE LIMIT OF VISUAL DETECTABILITY

The subjective evaluation of chromatograms is considered to be more accurate when fluorescent spots at, or just above, the limit of visual detectability are compared with spots of about the same intensity derived from a standard solution. Consequently, the limit of visual detectability for each of the mycotoxins in question was determined. This was done by spotting different amounts of each standard solution on an activated silica chromatoplate, starting with aliquots that were known to produce readily visible spots and decreasing the amount, stepwise, until a point was reached at which the developed chromatogram would no longer contain a visible fluorescent spot. Each plate was then developed with an eluant suitable for the particular toxin. After evaporation of the solvent, the plate was examined under long wave (peak emission 360 nm) ultraviolet light or a combination of long and short wave (peak emission 254 nm) ultraviolet light. The smallest detectable amount was thus determined and noted as being the limit of visual detectability for each particular toxin. For sterigmatocystin this value could be reduced to about one quarter by lightly spraying the chromatogram with 20 per cent. potassium hydroxide solution. The dull red fluorescence

of the toxin is thereby changed to greenish yellow, which is easier to detect (Note 1). The following limits of visual detectability values were determined under 5×20-watt Philips fluorescent tubes (TL20W/08), positioned 30 cm above the developed chromatoplate—

Aflatoxin B_1	..	..	..	0·0004	μg
Aflatoxin G_1	..	..	..	0·0003	μg
Ochratoxins A and C		..	..	0·002	μg
Sterigmatocystin	..	..	..	0·04	μg (brick red)
				0·01	μg (yellow)

NOTE 1—

    The technique of spraying a developed chromatoplate with potassium hydroxide solution is found to be very useful during the initial evaluation of chromatograms of a crude extract. The fluorescence of ochratoxins is changed from green to light blue without decrease in intensity, while the dull fluorescence of sterigmatocystin becomes more readily visible and that of interfering pigments is often reduced considerably. The fluorescence intensity of the aflatoxins is, however, decreased by this treatment. These colour changes have been shown to be reversible.

CHOICE OF SOLVENT FOR EXTRACTION

As a starting point in the development of the method the solvents known to be most suitable for aflatoxin extraction were examined, *viz.*, chloroform (dampened sample), chloroform - methanol and aqueous acetone. All three solvents were used to extract separate samples of the three media, which contained only ochratoxin or only sterigmatocystin. Each sample was macerated with the solvent in an explosion-proof blender for 3 minutes, followed by filtration and re-extraction with fresh solvent for 1 minute.

The extraction efficiencies of the solvents were compared by thin-layer chromatography of the concentrated extracts. It was shown that the most effective extraction (more than 90 per cent.) occurred when chloroform - methanol $(8 + 2)$ was used. It was, therefore, decided to use this solvent, with the addition of a small proportion of hexane to facilitate the removal of lipids with which the mycotoxins seem to be associated physically, for the investigation of mixed toxin extraction.

Samples of each of the three media were compounded so that three composite samples were obtained with the following mycotoxin contents, expressed as $\mu g \ kg^{-1}$ of final mixture—

					Sample No.				
Mycotoxin				**1**		**2**		**3**	
Aflatoxin	..	..	..	high	(500)	medium (50)		low	(10)
Ochratoxin ..		..	..	low	(50)	high	(2000)	medium (500)	
Sterigmatocystin	..	..	medium (500)		low	(200)	high	(2000)	

These samples were analysed according to the following method and modifications.

METHOD

REAGENTS—

All solvents should be of recognised analytical-reagent grade.
Chloroform.
Methanol.
Hexane.
Benzene.
Light petroleum, boiling range 30° to 60° C.
Diethyl ether, anhydrous.
Acetic acid, glacial.
Formic acid.
Toluene.
Ethyl acetate.
n-Propanol.
Trichloroethylene.
Sodium sulphate, anhydrous powder.
Potassium hydroxide, pellets.
Silica gel—Suitable for column chromatography, 0·05 to 0·2 mm; and for thin-layer chromatography, Macherey-Nagel G–HR or Camag D–5.

APPARATUS—

Chromatographic columns—These were 22 × 300 mm, and fitted with Teflon stopcocks.
Filter-paper, Whatman No. 12, 18·5 cm.
Rotary evaporator.
Blender, explosion-proof.
Centrifuge, with 4 × 250 head.
Thin-layer chromatographic apparatus.

PREPARATION OF THIN-LAYER PLATES—

Prepare several thin-layer chromatographic plates (10 × 20 cm) as described in the Official Method of the A.O.A.C.,[10] with silica gel. Activate the plates for 90 minutes in an oven at 105° C after air-drying them for 30 minutes in a dust-free atmosphere.

EXTRACTION OF SAMPLES—

Extract 50 g of finely ground sample with 200 ml of solvent (chloroform - methanol - hexane, 8 + 2 + 1, v/v) for 3 minutes at high speed in an explosion-proof blender. Transfer the resulting suspension to a 250-ml centrifuge tube and centrifuge at 2000 r.p.m. for 5 minutes. Filter the supernatant liquid through fluted filter-paper. Break up the sediment in the tube with a glass rod and rinse into the blender flask with 100 ml of fresh solvent. Blend for 1 minute, repeat the centrifugation and filtration and combine the filtrates. Concentrate the clarified extract to 5 ml under slightly reduced pressure.

PRELIMINARY THIN-LAYER CHROMATOGRAPHY—

Spot 10 μl of the concentrated extract and 10 μl of each of the qualitative standard solutions on an imaginary line, 3 cm from the bottom of an activated chromatoplate. Develop the plate in an unequilibrated tank, containing toluene - ethyl acetate - 90 per cent. formic acid (5 + 4 + 1, v/v), to about 12 cm above the origin. Remove the plate from the tank, allow the solvent to evaporate and examine it under an ultraviolet source. The mycotoxins contained in the three reference standards should all be clearly resolved. Observe whether there are any fluorescent spots at the comparable R_F values in the sample chromatogram. Spray the chromatoplate with 20 per cent. potassium hydroxide solution and immediately examine it again under ultraviolet light.

Any samples that definitely do not contain detectable amounts of the mycotoxins can be eliminated at this stage, and the results can be reported as containing less than 4 μg kg^{-1} of aflatoxin B$_1$; less than 3 μg kg^{-1} of aflatoxin B$_2$; less than 20 μg kg^{-1} of ochratoxin A; less than 20 μg kg^{-1} of ochratoxin C; and less than 100 μg kg^{-1} of sterigmatocystin. If a positive or doubtful result is obtained, the extract is processed as detailed below.

COLUMN CLEAN-UP OF EXTRACT—

Place a ball of glass-wool or non-absorbent cotton-wool loosely into position at the constriction of a chromatographic column, which is half filled with light petroleum. Drain off a portion of the light petroleum to ensure that all air bubbles are removed. Add about 5 g of anhydrous sodium sulphate to form an even base for 10 g of silica gel, which is subsequently poured slowly into the column. Allow the silica to settle evenly by drawing off the light petroleum until the level is about 5 cm above the silica, and add about 10 g of anhydrous sodium sulphate. Draw off the light petroleum to just above the top of the sodium sulphate and transfer the concentrated sample extract quantitatively to the column, with the smallest volume of light petroleum required to effect the transfer. Drain the extract into the column and elute with a mixture of 75 ml of light petroleum and 25 ml of anhydrous diethyl ether. Adjust the flow-rate to about 15 ml minute^{-1}. Collect the eluate as Fraction I. Continue the elution of the column with 100 ml of chloroform - methanol (97 + 3, v/v), collecting the eluate as Fraction II. Finally, elute with 100 ml of benzene - acetic acid (9 + 1, v/v). Allow the column to drain completely and collect the acidic eluate as Fraction III. Evaporate the three fractions separately to dryness under reduced pressure in a rotary evaporator. Fraction III will be found to require increased vacuum, and a few millilitres of toluene added

to the acetic acid residue will improve the distillation rate. Cool the flasks and immediately dissolve the residues in 5 ml of benzene. Stopper the flasks and store them in a dark cupboard while preparing for thin-layer chromatography.

THIN-LAYER CHROMATOGRAPHY OF THE EXTRACT—

The extent of dilution required to produce satisfactory results from the thin-layer chromatography of a positive sample extract can often be assessed as a result of the preliminary thin-layer chromatography. Much time can thus be saved by suitable dilution of the various fractions with benzene, before attempting the quantitative determination of the toxins.

Follow the generally accepted procedure by spotting a series of aliquots from the sample extract and from the relevant standard solution calculated to produce fluorescent spots with intensities at, or just above, the limit of visual detectability. Develop the plate in any one of the following eluants (all proportions are v/v).

For sterigmatocystin (Fraction I)—

 (*i*) chloroform - methanol, 98 + 2;

 (*ii*) toluene - ethyl acetate - formic acid (90 per cent.), 5 + 4 + 1;

 (*iii*) trichloroethylene - n-propanol - acetic acid, 90 + 9 + 1;

 (*iv*) benzene - n-propanol - acetic acid, 86 + 10 + 4.

For the aflatoxins (Fraction II)—

 (*i*) chloroform - methanol, 96 + 4;

 (*ii*) chloroform - acetone, 9 + 1.

For the ochratoxins (Fraction III)—

 (*i*) benzene - acetic acid, 9 + 1;

 (*ii*) toluene - ethyl acetate - formic acid (90 per cent.), 5 + 4 + 1.

Remove the plate from the tank, allow the solvent to evaporate and examine the fluorescent pattern on the plate under long wave ultraviolet light. Compare the fluorescent intensities caused by the toxin in the sample extract with those of the standard spots. If the sample appears to be negative for sterigmatocystin, spray the plate lightly with 20 per cent. potassium hydroxide solution. Re-examine the plate immediately. The sterigmatocystin standard will appear as an intensely fluorescent yellow spot and the sample chromatogram may also show up the presence of sterigmatocystin. The extracts from highly contaminated samples may have to be diluted and re-chromatographed before the spots can be matched with the standards.

Calculate the concentration of the toxins in the sample as follows—

$$\mu g \text{ of toxin per kg of sample} = \frac{S_S \times C_S \times FV}{X \times W}$$

where S_S is the volume of standard solution spotted to give fluorescence equal to X μl of sample extract, μl;

 C_S is the concentration of standard solution, μg ml^{-1};

 FV is the final volume of sample extract, μl;

 X is the volume of sample extract spotted to give fluorescence equal to S_S, μl; and

 W is the weight of sample extracted, g.

PROBLEMS ENCOUNTERED IN APPLYING THE METHOD TO THE ANALYSIS OF
GROUNDNUTS AND CERTAIN VARIETIES OF SORGHUM

A significant percentage of the oil content of groundnuts is extracted by the solvent used for mycotoxin extraction. This oil causes problems when assaying for sterigmatocystin and the ochratoxins. It is impracticable to separate oil from sterigmatocystin by column chromatography. Davies, Kirkaldy and Roberts[11] proposed the purification of sterigmatocystin from liquid culture extracts by separation on a column of heavy magnesium oxide

by elution with chloroform. However, no clear separation of oil and sterigmatocystin can be obtained by this procedure, and inconveniently large volumes of solvents are required to recover all of the sterigmatocystin. The use of 1 g of magnesium oxide above the silica in the column was tried, but complete recovery was not achieved. The effects of thin-layer chromatography of the extract, by using layers of magnesium oxide or alumina with various solvent systems, were determined, but again, no clear separation was obtained.

In addition, it was found impossible to recover known amounts of ochratoxin from oily extracts with a silica column. It was subsequently determined that all fractions of the eluting solvents contained the mycotoxin. This is remarkable as, in the absence of oil, ochratoxin is known to migrate only in an acidic eluant.

As a result of the problems encountered when attempting purification of the toxin extract by column chromatography, this approach was abandoned when appreciable proportions of fats were present, and it was reluctantly decided to resort to the tedious method of partitioning between various solvents.

The dark-coloured pigments extracted from some varieties of sorghum were found to interfere with the detection of sterigmatocystin when the extract was chromatographed. This was overcome by precipitating the pigments with diethyl ether before applying column chromatography.

MODIFICATIONS OF THE METHOD TO DEAL WITH THESE PROBLEMS

SAMPLES WITH HIGH FAT CONTENT—

Prepare an extract as described in the method. Shake the clarified extract with 50 ml of 0·1 M sodium hydrogen carbonate in a separating funnel. Drain off the lower phase and re-extract it with a fresh 50-ml portion of sodium hydrogen carbonate solution. Drain off the chloroform layer (A) and retain it for further processing. Combine the aqueous phases, acidify with 2 M hydrochloric acid and extract three times in a separating funnel with 40-ml portions of chloroform. Filter the combined chloroform extracts through a bed of anhydrous sodium sulphate and wash the latter with 20 ml of chloroform. Evaporate the filtrate to dryness and dissolve the residue in benzene. Transfer the solution quantitatively to a small vial and make up the volume to 5 ml (Fraction I). The above procedure should be carried out in subdued light and as quickly as possible as the ochratoxins are very susceptible to photolysis in aqueous solutions.

Evaporate the chloroform solution (A) under reduced pressure and dissolve the oily residue in 100 ml of 85 per cent. methanol. Transfer the solution to a separating funnel and rinse the flask with 50 ml of hexane, adding the washings to the funnel. Shake the funnel thoroughly and, after separation of the layers, re-extract the methanol layer with a fresh portion of 50 ml of hexane. Retain the methanol layer while extracting the combined hexane portions with an equal volume of 85 per cent. methanol. This is done to recover any sterigmatocystin that may have dissolved in the hexane. Discard the hexane and add the methanol layer to the retained portion. Add water to the methanol phase to adjust the methanol content to about 50 per cent. Extract this solution three times with 40-ml portions of chloroform. Filter the combined extracts through anhydrous sodium sulphate, wash the filter bed with about 20 ml of fresh chloroform and evaporate the combined filtrates to 5 ml (Fraction II).

Proceed with thin-layer chromatography as described in the method, with Fraction I for the determination of ochratoxin A and Fraction II for that of sterigmatocystin and the aflatoxins.

SAMPLES WITH DARK-COLOURED PIGMENTS—

Concentrate the clarified extract to about 25 ml. Add 2 g of Hyflo Supercel, or similar filter aid, and 25 ml of anhydrous diethyl ether. Continue the evaporation of the solvent until distillation ceases, and transfer the residual suspension to the column with a small volume of light petroleum. Proceed with the elution of the column as described. The first fraction, which contains the sterigmatocystin, should be free from dark-coloured pigments. The second fraction, containing the aflatoxins, may also contain some pigment, but this will not interfere with the detection of the aflatoxins.

RESULTS

Table I shows the results of the analysis of the compounded samples according to the proposed method.

TABLE I

RESULTS OF MYCOTOXIN ANALYSIS OF COMPOUNDED SAMPLES

Sample No.	Aflatoxin content, $\mu g\ kg^{-1}$		Ochratoxin content, $\mu g\ kg^{-1}$		Sterigmatocystin content, $\mu g\ kg^{-1}$	
	Calculated	Found	Calculated	Found	Calculated	Found
1	500	500	50	46	500	500
2	50	45	2000	1850	200	200
3	10	10	500	500	2000	1850
Groundnuts—						
1	500	480	50	40	500	460
2	50	38	2000	1750	200	140
3	10	8	500	460	2000	1600
Sorghum—						
1	500	500	50	42	500	450
2	50	40	2000	1800	200	160
3	10	10	500	480	2000	1700

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Determination of Mercury Residues in Potatoes, Grain and Animal Tissues Using Perchloric Acid Digestion

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A rapid method for determining mercury residues in potatoes, grain and animal tissues is presented. Digestion with nitric, sulphuric and perchloric acids is used in place of the oxidation with nitric and sulphuric acids and hydrogen peroxide, which is the method recommended by the Joint Mercury Residues Panel. Recovery of added organomercurial is about 90 per cent. Six analyses can be carried out in 1 day by one worker.

METHODS for determining mercury residues in vegetable and animal materials have recently been reviewed.[1] Four methods, which involve wet oxidation with nitric and sulphuric acids, and colorimetric determination of mercury with dithizone, have been recommended as official or standard methods.[2,3,4,5] They differ in such details as the type of apparatus in which the wet oxidation is carried out and the purification of the initial dithizone extract. The methods are all lengthy; that of the Joint Mercury Residues Panel[2] enables two to four determinations to be made in 1 day, depending on the availability of preceding or succeeding days to start or finish determinations. We have had considerable experience with this method, and felt that a shortening of the wet-oxidation stage would be advantageous. The use of perchloric acid should lead to a more complete oxidation than that of nitric and sulphuric acids alone. We have, therefore, investigated the use of mixed nitric, sulphuric and perchloric acids in wet oxidation, in conjunction with the dithizone extraction, reversion and determination procedure.

Gorsuch[6] first found that mixed nitric, sulphuric and perchloric acids could be used in determining traces of mercury, although he did not propose any particular method for specific foodstuffs. His work showed that mercury compounds are more volatile when treated with perchloric acid than when digested with nitric and sulphuric acids alone, but that if efficient condensers are used, mercury should not be lost from the system.

In 1960, the Analytical Methods Committee of the Society for Analytical Chemistry proposed the use of nitric, sulphuric and perchloric acids in wet oxidation of foodstuffs containing more than about 5 p.p.m. of mercury, observing that losses occurred when the method was used for lower levels of mercury.[7] This method is not suitable, therefore, for most residue determinations.

Hordynska, Legatowa and Bernstein[8,9] followed the approach of Gorsuch in using wet oxidation with nitric, sulphuric and perchloric acids, with a trap into which lower-boiling fractions are distilled during the digestion, thus raising the oxidation potential of the digest in the flask. They used the method for determinations on dressed grain with 96·5 ± 3·8 per cent. efficiency. The method also gave 97·7 per cent. recovery of phenylmercury 8-hydroxy quinolinate from apples. No details have been given for use of the method with other vegetables or fruits, or with animal materials.

Ward and McHugh[10] developed a method for determining the mercury content of vegetation from 0·4 p.p.m. upwards, involving wet oxidation with nitric, sulphuric and perchloric acids, followed by quantitative determination with dithizone. In their method the perchloric acid is added initially, and this could lead to uncontrolled reaction of easily oxidised substances before they have been partially decomposed with nitric acid. Hydrogen peroxide is used to complete the oxidation of the vegetable material after digestion with the mixed acids.

Epps[11] has used digestion with mixed nitric and perchloric acids for determining mercury residues in rice. The method, however, is only sensitive to the nearest 0·1 p.p.m. of mercury. Addition of sulphuric acid is desirable to control the reaction rate and also to raise the effective concentration of the perchloric acid, thus giving a more complete oxidation.[12]

Kinoshita[13] has used nitric, sulphuric and perchloric acids for determining milligram amounts of mercury compounds, but the method is not applicable to residue determinations.

Following the suggestion of Gorsuch, independently of the above workers, we propose methods for determining mercury residues in potatoes, grain, eggs, hens' muscle and lambs' livers, based on established standard methods in which wet oxidation with nitric, sulphuric and perchloric acids (without hydrogen peroxide) is used, which are more rapid than the original methods.

Fruits, vegetables and animal material that might contain mercury residues were investigated using the organomercury compounds likely to arise in commercial practice in the United Kingdom. Determinations were made at the order of residue levels that may arise, as judged by previous experience.

Workers who use the method should familiarise themselves with the "Notes on Perchloric Acid and its Handling in Analytical Work," published by the Analytical Methods Committee of the Society for Analytical Chemistry.[14]

EXPERIMENTAL

Apart from wet oxidation of the sample and the reversion step for animal tissue, the method is the same as that recommended by the Joint Mercury Residues Panel.[2] A 1-litre flask is preferred for the wet oxidation.

REAGENTS—

The following reagents are required in addition to those listed in the report of the Joint Mercury Residues Panel.

Perchloric acid, 72 per cent.—Analytical-reagent grade.

For animal material the following are also required.

Sodium nitrite, 5 per cent., aqueous.

EDTA, disodium salt, 2·5 per cent., aqueous.

Urea, 10 per cent., aqueous.

Hydrogen peroxide is not needed.

Organomercury compounds were added in 1 to 5 ml of acetone (ethanol was used with phenylmercury urea) to the foodstuff contained in the wet-oxidation flask to obtain recovery results.

POTATOES, EDIBLE RICE AND SEED GRAIN—

Potatoes should be cut into quarters, which are thoroughly mixed, an aliquot diced and a 50-g sub-sample taken for analysis. Representative samples of 25 g of edible rice or 10 g of seed grain are taken.

Place the prepared sample in the reaction flask, together with a few glass beads, and mix with 0·1 g of selenium powder. Add 25 ml of water to the rice and 10 ml of water to the seed grain.

Place the flask in the heating mantle and fit the condenser system (with water flowing rapidly through it) and the tap funnel. Add 25 ml of mixed nitric and sulphuric acids (1 + 1), slowly and intermittently, over a period of 10 minutes, taking care that the mixture at no time froths appreciably. The contents of the flask should be swirled from time to time. Then add a further 10 to 20 ml of nitric acid for potatoes and seed grain, or 50 to 70 ml for rice (to prevent charring). Switch on the heating mantle and slowly increase the rate of heating for about 30 minutes. The reaction should not be allowed to become violent. When solids disappear, add 15 ml of 72 per cent. perchloric acid and reflux for 1 hour at full heat. At the end of this time the reaction mixture should be almost colourless with potato, and pale yellow with rice and seed grains. Cool the digest and wash down the condenser with 50 ml of water.

Proceed as described in the recommended method of the Joint Mercury Residues Panel (p. 613, line 9).

ANIMAL TISSUE—

Place 50 g of a representative sample of avian or mammalian tissue in the reaction flask, together with a few glass beads, and mix with 0·1 g of selenium powder. Place the flask in the heating mantle and oxidise as above for potatoes and grain.

An additional 20 ml of nitric acid are required for eggs and livers and 10 ml for muscle. The final digests are markedly yellow.

Allow the cold, partly neutralised digest (see recommended method of the Joint Mercury Residues Panel) to stand with 10 ml of 20 per cent. hydroxylammonium chloride solution. Extract an aliquot, depending on the expected level of mercury, for 1 minute with 10 ml of stock dithizone solution and then twice each with 10 ml of dilute dithizone solution. Combine the dithizone extracts in a 100-ml separating funnel. Wash the combined extracts with 25 ml of 0·1 N hydrochloric acid and 5 ml of hydroxylammonium chloride for 1 minute. Run the dithizone layer into a third 100-ml separating funnel, together with 2 to 3 ml of chloroform used for washing the second separating funnel. Add 10 ml of 0·1 N hydrochloric acid and 1 ml of 5 per cent. aqueous sodium nitrite solution. Shake the mixture for 1 minute, separate and discard the chloroform layer. Wash the aqueous layer with 2 to 3 ml of chloroform and discard the washing. Add 1 ml of 20 per cent. hydroxylammonium chloride and allow to stand for 15 minutes, with occasional shaking. Add 1 ml of 10 per cent. urea solution, 1 ml of 2·5 per cent. EDTA (disodium salt) solution and 10 ml of dilute dithizone solution. Shake the mixture for 1 minute. Allow the layers to separate and run the dithizone solution down a 0·8 × 3 to 4-cm column of tightly packed absorbent cotton-wool into the 4-cm spectrophotometer cell. Also run some of the aqueous layer on to the column to ensure that enough dithizone solution runs into the cell for the spectrophotometer beam to pass through the solution. Read the optical density at 490 nm and refer to the calibration graph.

RESULTS

POTATOES AND GRAIN—

The recoveries of added organomercury compounds from potatoes, edible rice and barley with the perchloric acid oxidation method are given in Table I.

TABLE I

RECOVERY OF ADDED ORGANOMERCURY COMPOUNDS FROM POTATOES, RICE AND BARLEY

Mercury, µg (net), recovered from

phenylmercury chloride (5 µg of mercury) added to		phenylmercury urea (100 µg of mercury) added to barley (10 g)	phenylmercury acetate (100 µg of mercury) added to barley (10 g)	ethylmercury chloride (10 µg of mercury (added to barley (10 g)
potatoes (50 g)	rice (25 g)			
4·7	4·4	99	97	9·5
4·7	4·6	95	93	8·8
4·7	5·1	98	97	9·7
4·9	4·7	90	98	8·7
4·8	4·6	94	100	8·9
4·7	4·7	97	98	9·2
4·9	4·5	97	101	8·6
Mean—				
4·7 (5)	4·6 (5)	95	97 (·5)	8·9 (5)
(95%)	(93%)	(95%)	(97·5%)	(89·5%)
Standard deviation—				
±0·1	±0·2	±3	±3	±0·3

Total reagent and crop blanks for determinations were 0·7 and 1·0 µg of mercury for potatoes; 0·7, 0·6, 0·4 and 0·3 µg of mercury for rice; 0·1, 0·1, 0·0 and 0·5 µg of mercury for barley.

ANIMAL TISSUE—

The recovery of added organomercury compounds from eggs, hens' muscle and lambs' liver by the perchloric acid oxidation method are given in Table II.

TABLE II

RECOVERY OF ADDED ORGANOMERCURY COMPOUNDS FROM EGGS, HENS' MUSCLE
AND LAMBS' LIVERS

Mercury, μg (net), recovered from

phenylmercury acetate (5 μg of mercury) added to			ethylmercury chloride (5 μg of mercury) added to		phenylmercury chloride (5 μg of mercury) added to liver (50 g)
eggs (50 g)	muscle (50 g)	liver (50 g)	eggs (50 g)	muscle (50 g)	
4·1	4·5	4·4	4·0	4·1	4·3
4·5	4·3	4·2	3·9	4·4	4·5
4·1	4·3	4·6	4·4	4·2	4·6
4·4	4·1	4·3	4·1	4·3	4·5
4·2	4·1	4·1	4·2	4·1	4·2
4·0	4·2	4·5	3·9	4·2	4·4
4·2	4·2	4·3	3·9	4·5	4·6
Mean—					
4·0 (5) (81%)	4·3 (85%)	4·4 (88%)	4·2 (84%)	4·2 (85%)	4·3 (86%)
Standard deviation—					
±0·2	±0·2	±0·2	±0·2	±0·2	±0·2

Total reagent and crop blanks for determinations were 0·4 and 0·7 μg of mercury for eggs; 0·6 and 0·7 μg of mercury for hens' muscle; and 0·7 and 0·5 μg of mercury for lambs' livers.

DISCUSSION

POTATOES AND GRAIN—

The modified perchloric acid digestion gave about 95 ± 2 per cent. recoveries for this group of materials. In this country, phenylmercury chloride is the only organomercurial used on potatoes, and was consequently used to obtain recovery results. This compound is widely used to control rice blast (*Piricularia oryzae*) in many parts of the world, and was recovered from rice at the 0·1 p.p.m. level, as mercury residues in rice have been shown to be of this order of magnitude.[1] Organomercurials used as seed dressings on wheat and barley do not give rise to residues in harvested grain. However, it is sometimes necessary to check whether grain has been dressed with organomercurials and, if so, to what extent. The proposed method has, therefore, been tested for barley, using phenylmercury acetate, phenylmercury urea and ethylmercury chloride, the organomercurials most commonly used (in this country), as fungicidal seed dressings at the levels at which these compounds are usually present on dressed seed. Methoxyethylmercury silicate was not tested because of difficulties encountered in preparing a satisfactory standard solution for addition to the grain for recovery tests.

The modified digestion shortens the method by about 3 hours, so that six analyses can by completed by one worker in 1 day. The method, therefore, takes about half the time required for the Panel's original method. It is also shorter and considerably simpler, for potatoes and grain, than the method given by the Metallic Impurities in Organic Matter Sub-Committee of the Analytical Methods Committee of the Society for Analytical Chemistry.[3]

ANIMAL TISSUE—

The modified perchloric acid wet oxidation, and determination of mercury with dithizone by the thiosulphate reversion technique, used for potatoes and grain was not satisfactory because of excessive oxidation of the dithizone. However, the nitrite reversion stage of the Metallic Impurities in Organic Matter Sub-Committee of the Analytical Methods Committee method proved satisfactory in place of the thiosulphate reversion. The final dithizone extract is cleaned up on a short cotton-wool column; 85 ± 2 per cent. recoveries were obtained for these types of avian and mammalian tissue. The organomercury compounds chosen for study with eggs and hens' muscle are those which would arise from poultry that eat mercury-dressed grain. Although not recommended as good agricultural practice, animals occasionally graze in sprayed orchards, and livers from sheep poisoned in this way are sometimes presented for analysis.

The nitrite reversion takes 15 to 30 minutes longer than the thiosulphate reversion step, but the modified method, as applied to avian and animal materials, is still appreciably shorter than the original recommended method.

This modified method should only be applied to other materials with caution, and is not suggested for determining mercury residues in tomatoes and apples, for which the Panel's original method is more suitable.

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The Determination of Cedrol, and the Total Alcohol Content of Oils of Cedarwood, by Formylation

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The purity of cedrol, or the total alcohol content of oils of cedarwood, conventionally expressed as "percentage of cedrol," is normally determined by the formylation method described by Glichitch, for which a reaction time of 72 to 96 hours at 20° to 22° C is used. The results of formylations at higher temperatures, by using gas - liquid chromatography for investigation of the products of reaction, are reported. It is shown that at temperatures not exceeding 45° C the formylation can be substantially completed in 18 to 24 hours without any loss of accuracy.

THE total alcohol content of oils of cedarwood of differing botanical and geographical origin is conventionally expressed as "percentage of cedrol," and is normally determined by the formylation method described by Glichitch,[1] for which a reaction time of 72 to 96 hours at 20° to 22° C is used.[2] Re-investigation of this reaction has shown that by carrying out the formylation* at higher temperatures, the analysis can be completed in much less time without causing any more decomposition of the tertiary alcohol to cedrene than takes place at 20° C.

PREPARATION OF FORMYLATING REAGENT[3]—

To 1·0 mole of formic acid (98 to 100 per cent., analytical-reagent grade) add, with stirring, 1·0 mole of acetic anhydride (analytical-reagent grade), keeping the temperature below 45° C. After mixing, maintain the temperature at 45° C for 1 hour, then allow to cool and use the same day.

FORMYLATING PROCEDURE—

To 10 ml (or 10 g) of sample in a conical flask slowly add 10 ml of the formylating reagent. Maintain the stoppered flask under the prescribed conditions of temperature and time, then separate the formylated sample and determine the ester value in the usual way.[2]

RESULTS

In Fig. 1, graphs are given showing the results of the determination of total alcohol in commercial cedrol (perfumery grade, melting-point 87° C, showing only one peak when examined by gas - liquid chromatography, under the conditions given below) obtained by the normal Glichitch method and the modified method described above by using various reaction times and temperatures. It should be noted that for each point represented in the graphs a separate reaction mixture was made up. Table I gives results for oil of cedarwood (Chinese), oil of cedarwood (Kenya, rectified) and mixtures of these oils with known additions of cedrol.

* In our experiments a formylation mixture prepared according to Stevens and van Es[3] has been used. An experiment in which the normal Glichitch reagent was used at 35° C gave essentially the same result as that obtained with the former mixture.
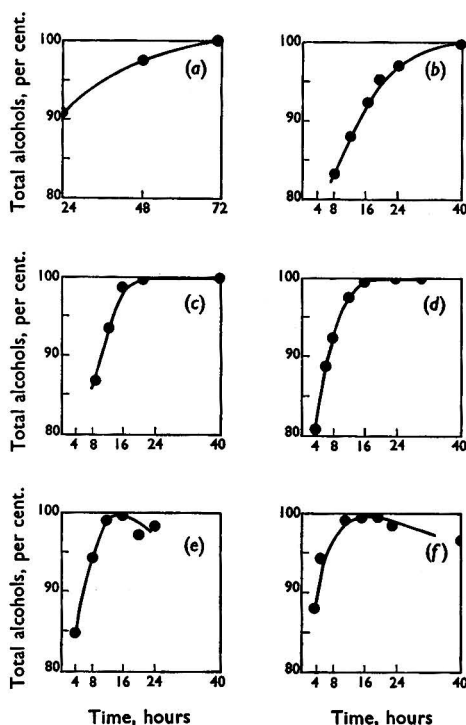
Fig. 1. Percentage of total alcohols (molecular weight 222·4) determined by using the conditions as follows: (a), Glichitch procedure at 20° C; (b) to (f), the modified procedure at the following temperatures: (b), 30° C; (c), 35° C; (d), 40° C; (e), 45° C; and (f), 50° C

TABLE I

PERCENTAGE OF TOTAL ALCOHOLS IN TWO OILS OF CEDARWOOD AND IN MIXTURES OF THESE OILS WITH CEDROL BY USING THE GLICHITCH PROCEDURE AND THE MODIFIED PROCEDURE AT 35° C FOR 24 HOURS

			Modified procedure	Glichitch procedure
Cedarwood oil (Kenya, rectified)	..	..	18·4	18·8
	mixture 1*	..	26·9	26·7
	mixture 2†	..	32·8	32·7
Cedarwood oil (China)	..	..	18·4	19·6
	mixture 1‡	..	24·6	25·7
	mixture 2§	..	31·8	32·5

* 9·21 g of cedrol and 80·48 g of oil. Total alcohols content calculated with 100·0 per cent. for cedrol and 18·4 per cent. for the oil, 26·8 per cent.; or with 18·8 per cent. for the oil, 27·1 per cent.

† 15·25 g of cedrol and 69·84 g of oil. Total alcohols content calculated with 100·0 per cent. for cedrol and 18·4 per cent. for the oil, 33·0 per cent; or with 18·8 per cent. for the oil, 33·3 per cent.

‡ 7·03 g of cedrol and 79·33 g of oil. Total alcohols content calculated with 100·0 per cent. for cedrol and 18·4 per cent. for the oil, 25·1 per cent.; or with 19·6 per cent. for the oil, 26·2 per cent.

§ 14·88 g of cedrol and 71·86 g of oil. Total alcohols content calculated with 100·0 per cent. for cedrol and 18·4 per cent. for the oil, 32·4 per cent.; or with 19·6 per cent. for the oil, 33·4 per cent.

Gas - liquid chromatograms of the products of formylation of cedrol were obtained by using the following conditions: detector, strontium-90; gas, argon; stationary phase, Apiezon L, 1 per cent.; support, glass beads, Alkaterge-washed; temperature, 150° C; and column, 120 × 0·6 cm. By adding 10 per cent. cedryl acetate to one of the products, a clear separation of cedryl acetate and formate peaks (resolution 1·0) was obtained, thus enabling the conclusion to be drawn that no acetate is formed by either method of formylation, except at the highest temperature used (50° C). Apart from the acetate, and some unidentified trace materials, the mixtures appear to contain only cedryl formate, cedrol and a mixture of α and β-cedrene (not separated under the conditions used). The peak areas of the various products visible in the chromatograms are summarised in Table II. The results given are subject to the normal limitations of quantitative gas - liquid chromatography, and do not represent actual percentage contents (w/w) because of differences in detector response to different substances. The apparent discrepancies given by the products of formylation at 50° C result from the sampling technique used in our experiments (see above), and clearly indicate the decrease in reproducibility at that temperature.

TABLE II

PEAK AREAS IN CHROMATOGRAMS OF FORMYLATED CEDROL*

Normal Glichitch procedure—

Temperature, °C	Peak		Time, hours		
			24	48	72
20	Cedryl formate	..	89·3	97·2	98·7
	Cedrol	..	10·5	2·5	0·2
	Cedrene	..	0·2	0·3	0·5
	Other	..	—	—	0·6

Modified procedure—

Temperature, °C	Peak		Time, hours								
			4	6	8	12	16	20	24	30	40
30	Cedryl formate	..	71·9	—	81·4	85·8	91·2	94·4	95·9	—	99·2
	Cedrol	..	28·1	—	18·6	14·1	8·6	5·6	3·0	—	0·5
	Cedrene	..	—	—	—	0·1	—	Trace	0·3	—	0·3
	Other	..	—	—	—	—	0·2	—	0·8	—	—
35	Cedryl formate	..	71·2	—	87·3	94·1	97·5	97·9	98·2	—	99·6
	Cedrol	..	28·0	—	12·6	5·5	2·1	1·6	1·3	—	Trace
	Cedrene	..	0·1	—	—	0·1	0·4	0·5	0·5	—	0·4
	Other	..	0·7	—	0·1	0·3	—	—	—	—	—
40	Cedryl formate	..	77·4	84·6	90·1	95·5	98·2	—	98·1	98·5	—
	Cedrol	..	22·2	14·9	9·7	4·1	0·9	—	0·8	Trace	—
	Cedrene	..	0·4	0·2	0·2	0·4	0·9	—	0·4	0·9	—
	Other	..	—	0·3	—	—	—	—	0·7	0·6	—
45	Cedryl formate	..	81·9	—	91·2	97·2	98·3	98·1	98·7	—	—
	Cedrol	..	16·2	—	7·0	1·5	0·8	Trace	Trace	—	—
	Cedrene	..	Trace	—	0·5	0·9	0·9	1·9	1·3	—	—
	Other	..	1·9	—	1·3	0·4	—	—	—	—	—
50	Cedryl formate	..	85·0	87·1	75·4†	79·9†	86·0†	86·9†	97·9	—	96·8
	Cedrol	..	14·5	5·6	5·1†	1·0†	—	—	—	—	—
	Cedrene	..	0·4	0·7	6·2†	0·6†	1·1†	1·3†	1·6	—	2·6
	Other	..	0·1	6·6	13·3†‡	18·5†§	12·9†‖	11·8†‖	0·5	—	0·6

* Expressed as percentages of the total combined area of all of the peaks. By means of calibration mixtures it was established that the detector was sufficiently linear for small percentages of cedrol and cedrene in admixture with cedryl formate.

† Results are approximate because of a shoulder to the formate peak.

Contains the following amounts of a compound with the same retention time as cedryl acetate:

‡ about 13 per cent.;

§ about 17 per cent.; and

‖ about 11 per cent.

REPRODUCIBILITY OF THE METHOD—

Ten separate determinations carried out by the same operator on commercial cedrol by using the modified procedure at a reaction temperature of 35° C for 24 hours, gave results of 100·1, 100·2, 100·0, 99·9, 100·1, 99·9, 100·2, 100·0, 100·2 and 100·2 per cent., with a mean $\bar{x} = 100\cdot08$ and standard deviation $s = 0\cdot12$.

CONCLUSIONS

Inspection of Fig. 1 and Tables I and II shows clearly that, at temperatures not exceeding 45° C, the formylation can be substantially completed under various conditions of temperature and time of reaction, with only a negligible degree of dehydration taking place.

Calculation of the percentage of cedrol from the ester value after formylation by either method apparently results in a slightly higher figure than the actual percentage of cedryl formate in the formylated mixture.

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A Rapid Method for the Determination of Manganese in Maneb and its Formulations

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A method is described for the determination of manganese in fungicidal preparations containing triphenyltin and copper. A simple back-titration technique is used in which excess of EDTA is added to an aqueous suspension of the maneb, under conditions of controlled pH. The method is non-specific but, provided the technical maneb used is analysed by the same method, it gives a ready means of controlling the maneb content of commercial fungicides.

THE colorimetric method of Willard and Greathouse[1] is usually used for the determination of relatively small amounts of manganese in organic materials. This method, when applied to maneb (manganese ethylene-1,2-bisdithiocarbamate), is somewhat lengthy for analytical control purposes and a more rapid method is required.

Titrimetric methods, involving the use of the complexone diaminoethanetetra-acetic acid (EDTA), are relatively rapid and suitable for control work, and have been successfully applied to the determination of manganese.[2,3]

Technical maneb always contains more manganese than can be accounted for by its empirical formula. The method of manufacture results in the product not only containing manganese present as the organic salt (*i.e.*, maneb) but also manganese present as an inorganic salt, probably the carbonate. Thus the total manganese content is not a true measure of the fungicidally active maneb. For control of production purposes, however, the total manganese content of a formulated product can be related to the maneb content, provided that the total manganese content of the technical maneb used in the formulation has been determined.

This paper describes the application of a complexometric titration method to the determination of maneb in fungicidal preparations containing triphenyltin and copper.

EXPERIMENTAL

The addition of a solution of a manganese salt to an aqueous solution of the disodium salt of ethylene-bis-(dithiocarbamic acid) produces the stable, insoluble complex maneb. The stability constant of this complex is not nearly as high as that for the manganese - EDTA complex. As a result, the addition of EDTA under conditions of controlled pH to an aqueous suspension of maneb will result in the quantitative replacement of the ethylene-bis-(dithiocarbamate) radical with EDTA producing the soluble manganese - EDTA complex.

Direct titration of aqueous suspensions of maneb were attempted, but difficulties in the determination of the end-point were encountered because of the slow reaction with solid maneb, and a back-titration technique was preferred, in which excess of EDTA was added to an aqueous suspension of maneb buffered at pH 10, followed by titration of the excess of EDTA with a suitable metal salt solution. The titration end-point was determined visually with Eriochrome black T indicator.

REAGENTS—

Diaminoethanetetra-acetic acid (EDTA), 0·15 M solution—Dissolve 37·21 g of EDTA in water and dilute to 1 litre in a calibrated flask. Standardise against pure, dried calcium carbonate.

1 ml of 0·1 M EDTA solution ≡ 4·008 mg of calcium.

Magnesium sulphate, 0·05 M solution—Dissolve 3·080 g of the dihydrate in water and dilute to 250 ml; standardise against the EDTA solution.

1 ml of 0·1 M EDTA solution ≡ 2·432 mg of magnesium.

Buffer solution, pH 10—Dissolve 53 g of ammonium chloride in 300 ml of ammonia solution (sp.gr. 0·88) and dilute to 1 litre with water.

Potassium cyanide, 10 per cent. w/v, aqueous.

Eriochrome black T, 0·1 per cent. solution in absolute ethanol—Prepare fresh daily.

METHOD—

Technical maneb—Weigh a sufficient amount of sample to contain about 80 mg of manganese into a conical flask and add 10 ml of water, 20 ml of 0·1 M EDTA and 20 ml of buffer solution (pH 10) and mix. Allow the mixture to stand for 5 minutes with occasional swirling until solution is complete. Titrate the excess of EDTA with 0·05 M magnesium sulphate, with Eriochrome black T as indicator. The end-point is reached when the colour changes from blue to red.

Maneb - fentin mixtures—The presence of triphenyltin salts does not interfere with the manganese titration, and the determination is carried out as above.

Maneb - copper mixtures—The interference caused by copper can be suppressed by the addition of 10 ml of 10 per cent. potassium cyanide solution. Manganese is then titrated in the same way as for technical maneb.

RESULTS AND DISCUSSION

The above methods have been used successfully for the analytical control of several different maneb formulations, and the results agree well with those obtained by the colorimetric method, when related to maneb content.

It is not possible to use this method to determine the absolute amount of manganese present in technical maneb. The technique as described gives results that are consistently higher than those obtained by the colorimetric method, which is specific for manganese. This is shown in Table I.

TABLE I

COMPARISON OF THE COLORIMETRIC AND COMPLEXOMETRIC METHODS FOR THE DETERMINATION OF MANGANESE IN DIFFERENT BATCHES OF MANEB

Manganese found by—

Batch No.	Complexometric method, per cent.	Colorimetric method, per cent.	Difference
1	19·1	18·2	+0·9
2	18·3	17·8	0·5
3	18·0	17·5	0·5
4	18·7	18·3	0·4
5	18·6	18·0	0·6
6	18·2	17·7	0·5
7	18·1	17·4	0·7
8	18·5	18·1	0·4
9	18·5	18·2	0·3
10	19·3	18·9	0·4

Mean difference + 0·5 ± 0·2

This discrepancy is almost certainly caused by the failure of the method to differentiate between the manganese and the small amounts of other metals, notably calcium, which are present in the inorganic filler used in the manufacture of technical maneb. The interference from calcium can be suppressed by the use of a suitable masking agent.[4,5] For absolute content of manganese, however, the colorimetric method mentioned earlier is recommended.[1]

As this method is primarily designed as a ready means of controlling the maneb content of fungicidal preparations then, provided the technical maneb used is analysed by the same method and blank determinations have been made on the other ingredients of the formulation, the lack of specificity can be ignored.

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Effects of Light on Glass pH Electrodes

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It has been shown that sunlight and artificial light falling on glass pH electrodes can cause a decrease in measured pH values.

This decrease does not appear to be due to any photo effect on the silver - silver chloride half-cell within the electrode, and is, therefore, attributed to increased conductivity of the glass membrane, possibly arising from the formation of F centres.

IN the course of the development of a device for controlling pH during irradiation experiments, a decrease in the measured pH value occurred when radiation from a mercury vapour lamp fell on a combined glass - calomel electrode used as a control sensor. The measured pH rose to its original value when the light source was removed.

The output of several commercial combined glass and calomel electrodes, and also of separate electrodes, immersed in borax buffer solution was measured before and during exposure to various sources of radiation. The changes caused by irradiation are shown in Table I. The measurements were made on an E.I.L. 33B Vibron electrometer, in conjunction with an E.I.L. C–33–B pH measuring unit.

TABLE I

DECREASE IN OUTPUT (mV) OF GLASS ELECTRODES IN BORAX BUFFER
ON IRRADIATION

Radiation source	E.I.L. SHDN 33 combined electrode	E.I.L. GHS 33 single electrode	E.I.L. old electrode	Pye - Ingold 405 combined electrode
Osram mercury vapour lamp, 400 watts, at a distance of 30 cm	−38·5	−20·7	−45·2	−7·0
Desaga Heidelberg T.L. illuminator, at a distance of 10 cm, 366-nm radiation	−20·0	−10·2	−29·0	−6·2
Mazda infrared lamp, 250 watts, with Wratten No. 87 filter, at a distance of 30 cm	−1·2	−6·4	−18·6	−8·2
Sunlight	−6·7	−4·5	−26·4	−2·7

After exposure of an electrode to radiation, the time taken for a stable reading to be reached varied both with the type of electrode and the source of radiation, and ranged from 5 to 9 minutes. On removal of the radiation source, the original reading was slowly regained. Fig. 1 shows a typical example, the irradiation starting at point A and finishing at point B. In one exceptional instance the time for recovery approached 3 hours.

When a separate calomel reference electrode, shielded from the radiation, was used, similar decreases in the measured pH value were observed. Thus the effect is associated with the glass electrode alone. The Osram MB/U mercury vapour and Mazda lamps gave a temperature increase of 4° to 5° C in the buffer solution surrounding the electrode. However, a rise in temperature increased the output of the cell,[1] *i.e.*, the reverse of the observed effect of radiation. This was confirmed for each electrode system by warming the buffer solution surrounding the electrode on a hot-plate to give an increase in temperature of 4° C.
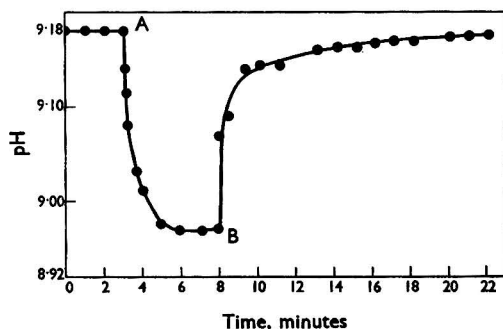
Fig. 1. Typical electrode response with time to irradiation : A, start of illumination; and B, illumination removed

Temperature coefficients ranged from $+0.14$ to $+1.5$ mV per °C. The Desaga lamp and sunlight caused an increase in temperature of only $0.2°$ and $0.8°$ C, respectively, during the course of the experiment, and the temperature effect was, therefore, negligible with these sources. The results in Table I show that ultraviolet-containing sources gave rise to the greatest effect on the glass electrode output, and it was, therefore, considered likely that the effect was photo-induced at the silver chloride coating on the silver wire within the envelope of the glass electrode. To test this a silver - silver chloride electrode was immersed in 0.1 N hydrochloric acid and, with a calomel half-cell as reference, was exposed to each source in turn. A decrease of only 1 mV was observed on irradiating with both the Desaga ultraviolet and filtered infrared sources, and a decrease of about 3 mV with the more intense Osram source. By increasing the temperature of the silver - silver chloride against calomel electrode system, the output[2] of the cell was decreased by 1 mV for every $3°$ C rise. The effect of infrared radiation on the silver - silver chloride against the calomel electrode system can, therefore, be accounted for entirely on the basis of temperature rise.

From the above results, the observed decreases in output of glass - calomel electrode systems on irradiation with ultraviolet rays and sunlight cannot be accounted for by the results obtained from similar irradiation of a silver - silver chloride electrode in 0.1 N hydrochloric acid. It is, therefore, possible that the glass membrane plays some part in the process. A possible explanation for the effect is that the electrical resistance of the glass envelope may be altered on irradiation by the creation of conducting F centres in the interstices of the Si–O lattice.[3] As it was observed that a significant decrease in pH reading was caused by sunlight falling on to the glass electrode (Table I), it is apparent that, for pH measurements of the highest precision, the electrodes must be shielded from all sources of radiation.

REFERENCES

1. Mattock, G., "pH Measurement and Titration," Heywood & Co. Ltd., London, 1961, p. 184.
2. ——, op. cit., p. 141.
3. Nott, N. F., and Gurney, R. W., "Electronic Processes in Ionic Crystals," Clarendon Press, Oxford, 1940, pp. 104 and 109.

Book Reviews

COMPREHENSIVE ANALYTICAL CHEMISTRY. Edited by CECIL L. WILSON, Ph.D., D.Sc., F.R.I.C., F.I.C.I., and DAVID W. WILSON, M.Sc., F.R.I.C., in association with the late C. R. N. STROUTS, M.A., F.R.I.C. Volume II B. PHYSICAL SEPARATION METHODS. Pp. xvi + 445. Amsterdam, London and New York: Elsevier Publishing Company. 1968. Price 170s.

The present volume deals with four subjects, each of which is given a chapter. These are Liquid Chromatography in Columns by J. F. K. Huber; Gas Chromatography by E. R. Adlard, R. Stock and B. T. Whitham; Ion Exchangers by F. C. Saville; and Distillation by G. A. Dummett, N. A. H. Holt and M. G. Royston. Six of the authors are members of industrial concerns and the other two of teaching laboratories. A chapter on paper and thin-layer chromatography, which would be suitably placed in this text, is to be included in a later volume.

The main divisions of the subjects are theory, apparatus and methods, and applications, supported by just over 1200 references to the literature. The practice, adopted in earlier volumes, of placing on alternate pages footnotes indicating the page on which references are to be found is continued. This convenience saves the reader time and is the next best thing to having references at the bottom of the appropriate pages. A general index, which passed most of the tests applied to it, completes the volume.

The theory of chromatography given in chapters 1 and 2 may not be easy reading for some analysts, and even less so to those to whom analytical chemistry is incidental to other work, but selected reading of the more general passages of the text will give a good idea of what the different forms of chromatography can do.

Chapter 1 is confined to the separation of organic substances; gas chromatography also finds its widest application to these mixtures, but an increasing number of separations of inorganic substances is being reported. The scope and power of the methods of chromatography will be a revelation to many analysts brought up on classical lines. The authors point out, for example, that gas chromatography has largely replaced the chemical and physical methods of gas analysis that were once in common use. The section dealing with the applications of this method is mainly a selective review of the literature, and it shows how numerous and versatile are the problems that can be solved by gas chromatography.

Chapter 3, the section on ion exchangers, is particularly suitable for practising chemists, especially those dealing with inorganic materials, and the comparative simplicity of the operations involved will appeal to workers unskilled in the subject. A wide selection of analytical applications, some of the more instructive being given in detail, is arranged alphabetically, and here again the range of analyses covered is wide. An appendix to this chapter gives detailed information about many exchangers, those recommended for analytical work being indicated. This table, of some 20 pages, is likely to be of considerable help to all users, and especially to the novice.

The last chapter of this book gives an account of the fundamental physico-chemical principles underlying fractional distillation, indicates the range and capabilities of the different types of apparatus available and outlines, critically, the procedures generally adopted. It seems a pity that this subject could not have been included in a volume other than this one, space thus being made for paper and thin-layer chromatography to be dealt with in a volume to which it surely belongs.

The whole text is well supported by tables, graphs and neat, clear diagrams. The printing is good and misprints are commendably few. The volume is less bulky than earlier ones and is easy to handle, but the price is high and is likely to restrict purchase to the libraries.

L. S. THEOBALD

THE MASS SPECTRA OF ORGANIC MOLECULES. By J. H. BEYNON, R. A. SAUNDERS and A. E. WILLIAMS. Pp. x + 510. Amsterdam, London and New York: Elsevier Publishing Company. 1968. Price £11 15s.

This book will be of great interest to all who are concerned with organic mass spectrometry. It opens with a highly condensed account of the principles and methods of mass spectrometry, designed for newcomers to the field, who would, however, be well advised to study Beynon's classical "Mass Spectrometry" for an account of the practical aspects of the technique. The second chapter presents a review of various types of ions encountered in the mass spectra of

organic compounds. The greater part of the book is devoted to a critical survey of the fragmentation behaviour characteristic of various classes of organic compounds under electron impact. Chapters 3 to 10 deal with hydrocarbons and with compounds containing oxygen, nitrogen, sulphur, halogens, boron, phosphorus and silicon, respectively. Structural information that can be derived from modes of fragmentation is thoroughly discussed, mainly with reference to low resolution data. The authors' extensive experience is reflected in many useful generalisations and informed comments. Line diagrams of exemplary "unknown" compounds of each class are used throughout to illustrate both the scope and deficiencies of structural correlations. The final chapter is devoted to a further group of 17 instructive examples of this kind. Appendices provide lists of commonly encountered "peaks" and of the masses and natural abundances of the more important nuclides. The references (over 500) appear to cover the literature up to 1966, and the book is completed by author and subject indexes.

The reviewer would venture one criticism. Low resolution mass spectra, in general, are most informative when considered in relation to complementary physical and chemical evidence. Apart from a curious mention of the value of the olfactory sense in identifying the lower aliphatic acids, the text makes few concessions to the utility of other techniques. A particularly simple yet powerful means of enhancing the information value of low resolution mass spectra involves the comparison of suitable derivatives of functional groups. Thus the distinction between a ketone and a hydrocarbon of the same molecular weight (discussed on p. 209) would be easily made. Trimethylsilyl ethers, which receive only brief attention, are examples of derivatives that find extensive use in the mass-spectrometric characterisation of many types of organic compound.

Several unimportant errors, mainly typographical, have been noted. Also, oleyl alcohol is 9-octadecen-1-ol, not "a C_{24} alcohol with a terminal secondary butyl group" (p. 133).

The book is printed on two different stocks, one matt and one glossy, but in other respects the production is satisfactory.

Professor Beynon and his colleagues are to be congratulated on providing yet another essential reference book for mass spectrometrists and organic chemists.   C. J. W. BROOKS

MASS SPECTROMETRY IN INORGANIC CHEMISTRY. A symposium sponsored by the Division of Inorganic Chemistry at the 152nd Meeting of the American Chemical Society, New York, N.Y., Sept. 15–16, 1966. Advances in Chemistry Series 72. Pp. viii + 329. Washington: American Chemical Society. 1968. Price $12.00.

This book presents a valuable illustration of the wide applicability of mass spectrometry to chemical problems. It does not provide a comprehensive study of all of the applications within the fields of inorganic chemistry, but it does extend its coverage to a very useful range, for example, from high-temperature vapour - solid equilibrium studies to investigations in which mass-spectrometric sampling of gaseous species at relatively high pressures is necessary.

In common with most symposia proceedings, the book suffers from being a slightly disjointed collection of not wholly related papers; but it is nonetheless a sound collection comprising, in some instances, entirely new experimental developments and in others new investigations in which established techniques are used.

There is, possibly, too much emphasis on vaporisation and thermodynamic studies, while analytical methods for inorganic solids appear to find no place in the book. Knudsen-cell effusion investigations form the subject of several of the papers presented; valuable results for the rare earth fluorides are reviewed by Zmbov and Margrave; and results for the elements zinc, cadmium, arsenic and selenium, obtained by using a uniquely modified Knudsen-cell and ion-source arrangement, are reviewed by Westmore, Fujisaki and Tickner. New vapour-equilibrium data for the noble metal oxides are reported by Norman, Staley and Bell, and for the boron sulphides by Edwards and Gilles. Additional subjects covered, of importance in high-temperature studies, are the use of pulsed techniques with Langmuir evaporation, and the examination of relative electron-multiplier gains for different metal ions.

Sampling at high pressures (a technique in no way confined to inorganic systems) is described comprehensively by Milne; and interesting investigations of ion-solvent clustering, or solvation, in the gas phase at relatively high pressures (high in mass-spectrometric terms) are reported by Kebarle. Other topics covered include chemionization, catalytic and radiolytic reactions, kinetic studies with a shock-tube coupled to the ion source, automated data acquisition and the theoretical calculation of electron-bombardment ionisation cross-sections. The chemistry of boranes receives

attention both in terms of photochemical oxidation, by Porter and Grimm, and in more general terms, together with the carboranes, with the use of mass-spectral fragmentation patterns to deduce chemical structure, by Ditter, Gerhart and Williams.

The style of presentation varies, as might be expected, from paper to paper; it extends from good to disappointing. The text is generally free from errors although a few irritating misprints have been allowed through. The general layout of the book, in particular the sub-section heading and some of the figures, could have been greatly improved, although on the credit side the book is not over expensive. D. J. FABIAN

ATOMIC ABSORPTION SPECTROSCOPY. By WALTER SLAVIN. Pp. xviii + 307. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons. 1968. Price 125s.

Since the appearance of the first book on atomic-absorption spectroscopy, by Elwell and Gidley, in 1961, the number of publications on this expanding technique of analysis has progressively increased. The author of this latest book on the subject needs no introduction to workers in this field, and a publication that collates his wealth of experience is, alone, a good enough recommendation to justify its purchase.

The theoretical aspects of atomic absorption are dealt with quite briefly in the first chapter. In the second chapter, instrumentation is covered extensively, and here the author's experience with the wide range of equipment available within his Company's laboratories is apparent. It includes discussions on various types of hollow-cathode lamps, flames and burners, and an interesting account of methods for producing atomic vapour without the use of a flame, *e.g.*, by using the sputtering chamber technique, L'vov furnace, laser sampling, plasma sources or solid propellant atomisation.

In chapter III, sensitivities, methods of calibration and the types of interference that occur in atomic-absorption procedures are discussed.

The remainder of the book, slightly more than two thirds of the total text, is divided into two sections. The first includes analytical conditions and information on interferences, etc., relating to nearly seventy elements, ranging from aluminium to zirconium. The text ends with a section on the application of atomic absorption to a wide range of miscellaneous samples, covering, for example, the fields of biochemistry, agriculture, metallurgy and mining.

The short, but adequate, index is preceded by a comprehensive list of nearly 700 references.

The book is a valuable compilation of information on atomic absorption and it is well produced, as one is entitled to expect from its rather high price. H. PUGH

PHOSPHORIC ACID. Part I. FERTILIZER SCIENCE AND TECHNOLOGY SERIES. Volume I. Edited by A. V. SLACK. Pp. xxii + 501. New York: Marcel Dekker Inc.; London: Edward Arnold (Publishers) Ltd. 1968. Price £14 5s.

Part I of a work, in two volumes, on Phosphoric Acid for the Fertilizer Science and Technology series is a well produced and informative book. The importance of phosphoric acid for the fertiliser industry has grown considerably, both in the U.K. and in other countries, over the last 10 years. Although U.K. requirements of straight phosphate fertilisers (*i.e.*, those supplying phosphate only, without additions of nitrogen and potash) are still largely supplied by basic slag, phosphate for compound fertilisers containing all three major nutrients is now very largely derived from phosphoric acid in the form of fertiliser ammonium phosphate.

An introductory chapter reviews the history and status of phosphoric acid, and is followed by a full account of the chemistry and thermochemistry of the so-called wet processes, in which phosphoric acid is produced by dissolving phosphate rock in sulphuric acid. Some fourteen variants of the wet process are then described. The final section deals with filtration plant for the separation of the by-product calcium sulphate from the phosphoric acid, and the equipment offered by five different process design contractors is described. The further concentration and purification of phosphoric acid, and the disposal of by-products and treatment of effluents, remain for treatment in the second part of the work. Production of phosphoric acid by the electric-furnace process is also deferred for treatment in Part II; in this country, of course, the dearer and purer furnace product is largely confined to non-fertiliser uses.

The chapter on chemistry of phosphoric acid brings out, in a very clear fashion, the physicochemical determinants of plant design and operating conditions, and pays particular attention to factors affecting growth of calcium sulphate crystals to a size suitable for industrial filtration,

the form of calcium sulphate produced (dihydrate, hemihydrate or anhydrite) and acid strength. This chapter also contains very useful tabulations of physical and chemical properties of materials concerned in the process.

The distinctive features of the design of all of the important phosphoric acid plant contractors are reviewed by authors who are, or have been, connected with these contractors. These include Dorr-Oliver, Prayon, Pechiney-St. Gobain, Fisons and Singmaster-Breyer for processes giving calcium sulphate in the dihydrate form, and in these cases the authors are dealing with equipment and practices of proved efficiency on large industrial installations. This cannot be said for all the processes giving calcium sulphate in the form of hemihydrate or anhydrite. In this field of technology the pioneering work of Nordengren and his colleagues in Sweden led to the construction of plants in Sweden and in Italy, but considerable difficulties were met, with corrosion and intermittent hydration of lower hydrates of calcium sulphate to dihydrate during the washing stages on the filter. In Europe and North America, hemihydrate and anhydrite processes have had little success but, as this book makes clear, the situation is very different in Japan, where lack of natural gypsum has led to interest in processes giving calcium sulphate in a form suitable for conversion into gypsum wallboard. It is claimed, in Japan, that processes giving calcium sulphate in the form of hemihydrate, or in which the calcium sulphate passes through the hemihydrate stage and is subsequently converted into dihydrate, are superior for wallboard manufacture. The Mitsubishi, Nissan and KKK processes are described, and all of these processes are in large-scale operation.

The volume concludes with an account of the filtration technology associated with the separation of calcium sulphate from the phosphoric acid. Again, full descriptions are given of commercially available designs.

In summary, this is an authoritative and well presented work of reference. It does not deal with analytical control problems, nor of course would one expect this in a work of this kind, but as a source of information on less specialised problems in phosphoric acid manufacture it can be recommended.　　　　　　　　　　　　　　　　　　　　　　　　　　　　　A. E. ROUT

ORGANIC FUNCTIONAL GROUP ANALYSIS. THEORY AND DEVELOPMENT. By GEORGE H. SCHENK. Pp. x + 297. Oxford, London, Edinburgh, New York, Toronto, Sydney, Paris and Braunschweig: Pergamon Press. 1968. Price (hard-cover) 40s.; (flexi-cover) 30s.

This is an unusual book of great interest, particularly to those engaged in analytical research in the field of organic chemistry. It consists of two parts: Part I resembles an archaeological dig into the historical and theoretical development of the current methods for determining certain specific functional groups. There are seven chapters dealing with the carbonyl group, enolic-type compounds, hydroxyl and amino groups, hydroxy and alkoxysilanes, epoxide groups, 1,3-dienes and electron-rich donor compounds. Part II can be likened to an exposition of the individual layers of the dig; it consists of thirty-one chapters, each of which is a reprint of the relevant portions of original papers discussed in Part I.

In each chapter of Part I attention is mainly concentrated on one or two types of method, the kinetics and mechanisms of which are discussed. Thus, for carbonyl groups this is the oximation method; for enolic compounds the Kurt Meyer bromination and non-aqueous titration methods; for hydroxyl and amino groups, base-catalysed acetylation; for hydroxy and alkoxysilanes, acid-catalysed acetylation; for epoxides, ring-opening methods; for 1,3-dienes, Diels-Alder addition; and for electron-rich compounds, complexing methods. For each the possibilities of instrumental analysis are discussed. The treatment, in general, is at graduate and post-graduate level.

To those with a historical bent, Part II provides some interesting panoramas (to change the metaphor). Thus the oximation method for carbonyl group determination is illustrated by seven reprints beginning with the determination of aldehydes in oil of lemon from *The Analyst* (1909) and concluding with gas chromatography, mass spectrometry and nuclear magnetic resonance of *O*-methyloximes from *Analytical Chemistry* (1965).　　　　　　　　　　　　　J. I. M. JONES

TWO PAPERS ON THE LIMIT OF DETECTION OF A COMPLETE ANALYTICAL PROCEDURE. By H. KAISER and A. C. MENZIES. Pp. viii + 59. London: Adam Hilger Ltd. 1968. Price 30s.

This little book has been produced by the translator as a tribute to Professor Kaiser on the occasion of his sixtieth birthday and contains the two papers, previously published in *Z. analyt. Chem.*, preceded by 20 pages of introduction contributed by the translator. This Introduction

describes clearly the basic principles of the statistical approach, and concisely introduces some of the ideas of the main paper in order to assist comprehension by the reader. Thus, standard deviation, relative standard deviation, normal distribution, one- and two-sided probability and limit of guarantee of purity are explained before being re-introduced in Kaiser's papers. The main paper takes the reader, after a warning that the undertaking may be "somewhat fatiguing" and demand "considerable indulgence," from the analytical calibration function, through the formulae for the limit of detection to the limit of guarantee of purity. The second paper continues the discussion to include sections on the magnitude and causes of scatter, the applicability of the term "limit of detection" and the limit of guarantee for inhomogeneous samples.

The author states that he has omitted any sentence that does not perform a necessary function of explanation but, unfortunately, this has occasionally been carried to extremes, thus making comprehension more difficult. Also there are abrupt references to earlier work when a more complete explanation would be more helpful.

Nevertheless, this is a well produced text containing a concise account of the problem, which would appeal to the serious students of analytical chemistry.   L. H. RUDDLE

MONOGRAPHS ON E.S.R. ELECTRON SPIN RESONANCE SPECTROMETERS. By T. H. WILMSHURST, B.Sc., Ph.D. Pp. viii + 280. London: Adam Hilger Ltd. 1968. Price 70s.

Dr. Wilmshurst's book "Electron Spin Resonance Spectrometers" is addressed to physicists, chemists and engineers, who wish to know more about the working principles of e.s.r. spectrometers. The first chapter is an introduction to the subject and a discussion of fundamental requirements, including, briefly, optical spectroscopy, microwave-absorption spectroscopy of gases and the development of the technique of e.s.r. spectroscopy. In the second chapter, a basic e.s.r. spectrometer is developed, by describing recording methods, such as crystal-video recording and double-modulation recording, and by treating the detector response and spectrometer-system noise factor. In chapter three, under the heading of Microwave Systems, the following types of spectrometers are analysed and compared: transmission-cavity, reflection-cavity, absorption-cavity, travelling wave, balanced mixer, twin-cavity and induction-cavity spectrometers. Chapter four is devoted to spectrometer cavities. Theoretical background and practical design for different cavity types are given, with a special emphasis on cavities for samples with high dielectric loss. Chapter five gives a short survey of superheterodyne spectrometers, and in chapter six, automatic frequency-control systems most commonly used in e.s.r. spectrometers are discussed. Chapter seven gives a very short account of modern low-noise microwave pre-amplifiers, and in chapter eight magnet systems and their stabilisation are mentioned briefly. Finally the ninth and last chapter, under the heading of electronic circuitry, is mainly a description of 100 kc and 10 kc phase sensitive detecting systems. Throughout his book Dr. Wilmshurst gives a good and easily understood description of the operating principles of e.s.r. spectrometers, taking care to emphasise many of those small points important for the more perfect operation of an e.s.r. spectrometer. For those who really wish to design a spectrometer a more complete list of references would be helpful. On the whole this book presents a definitely worthwhile contribution to the e.s.r. experimentalist's bookshelf.   J. T. SUSS

Errata

OCTOBER (1968) ISSUE, plate facing p. 693, Figs. 3 and 4. *For* "Diazinon" *read* "Carbophenothion," *and for* "Carbophenothion" *read* "Diazinon."

APPOINTMENTS VACANT

LECTURES AND COURSES

APPOINTMENT WANTED

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Determination of Mercury Residues in Potatoes, Grain and Animal Tissues Using Perchloric Acid Digestion

A rapid method for determining mercury residues in potatoes, grain and animal tissues is presented. Digestion with nitric, sulphuric and perchloric acids is used in place of the oxidation with nitric and sulphuric acids and hydrogen peroxide, which is the method recommended by the Joint Mercury Residues Panel. Recovery of added organomercurial is about 90 per cent. Six analyses can be carried out in 1 day by one worker.

N. A. SMART and A. R. C. HILL

Plant Pathology Laboratory, Ministry of Agriculture, Fisheries and Food, Harpenden, Hertfordshire.

The Determination of Cedrol, and the Total Alcohol Content of Oils of Cedarwood, by Formylation

The purity of cedrol, or the total alcohol content of oils of cedarwood, conventionally expressed as "percentage of cedrol," is normally determined by the formylation method described by Glichitch, for which a reaction time of 72 to 96 hours at 20° to 22° C is used. The results of formylations at higher temperatures, by using gas - liquid chromatography for investigation of the products of reaction, are reported. It is shown that at temperatures not exceeding 45° C the formylation can be substantially completed in 18 to 24 hours without any loss of accuracy.

G. RIEZEBOS and J. H. GREAVES

Proprietary Perfumes Limited, Ashford, Kent.

A Rapid Method for the Determination of Manganese in Maneb and its Formulations

A method is described for the determination of manganese in fungicidal preparations containing triphenyltin and copper. A simple back-titration technique is used in which excess of EDTA is added to an aqueous suspension of the maneb, under conditions of controlled pH. The method is non-specific but, provided the technical maneb used is analysed by the same method, it gives a ready means of controlling the maneb content of commercial fungicides.

A. S. HYMAN

Fisons Cambridge Division, Harston, Cambridge.

Effects of Light on Glass pH Electrodes

It has been shown that sunlight and artificial light falling on glass pH electrodes can cause a decrease in measured pH values.

This decrease does not appear to be due to any photo effect on the silver - silver chloride half-cell within the electrode, and is, therefore, attributed to increased conductivity of the glass membrane, possibly arising from the formation of F centres.

A. F. MILWARD

Department of Agriculture and Horticulture, Bristol University, Long Ashton, Bristol.

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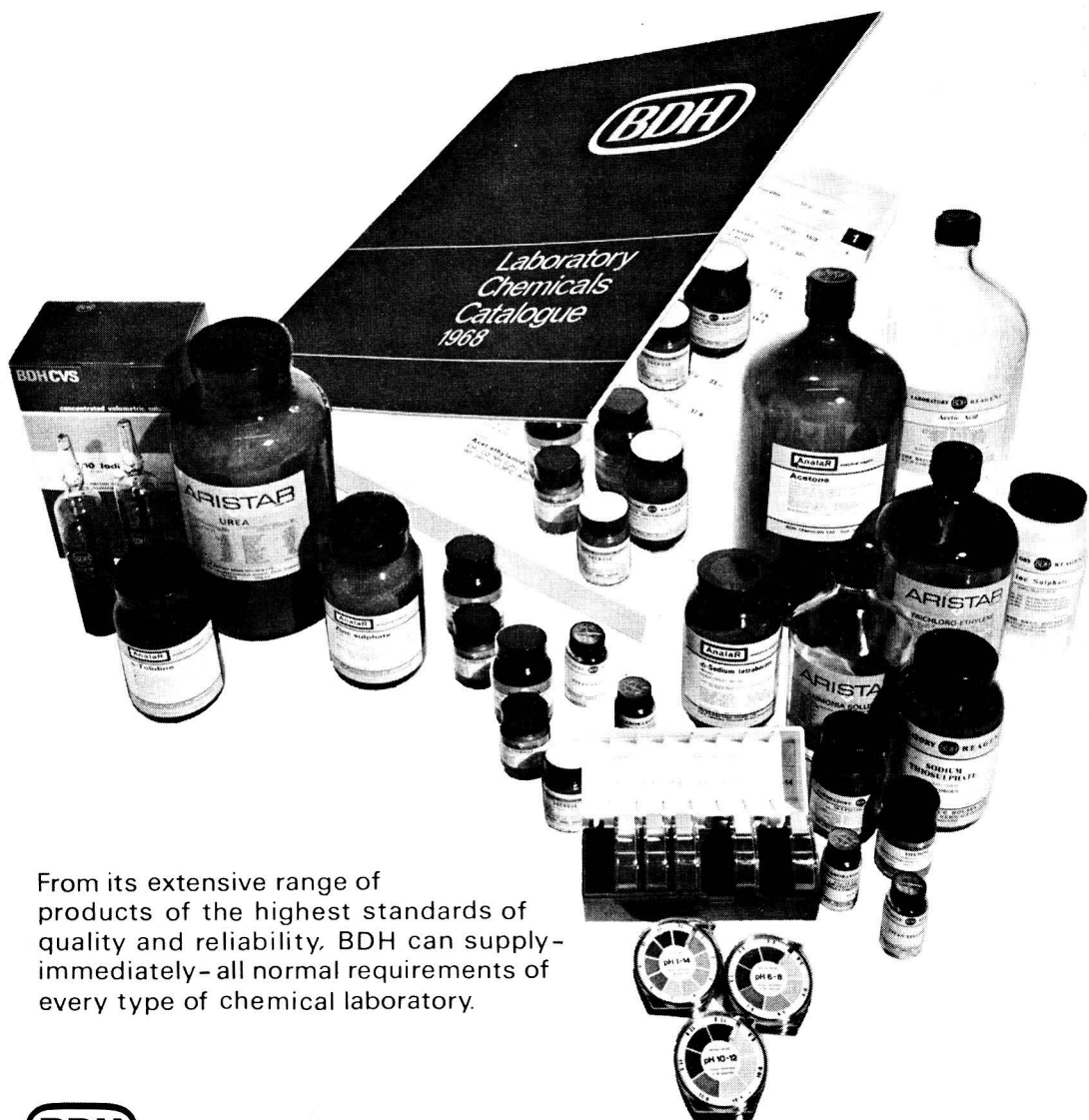
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