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THE ANALYST

The So-called Phenylthiohydantoic Acid: Its Structure and Application to the Gravimetric Determination of Cobalt*

BY A. BASHAR AND A. TOWNSHEND

(Chemistry Department, The University, P.O. Box 363, Birmingham 15)

The compound previously known as phenylthiohydantoic acid is shown to be carbaminothioglycollic acid anilide. The precipitate produced when this reagent is added to cobalt solutions is tris(thioglycollic acid anilido) cobalt(III), formed by hydrolysis of the carbamino compound. This precipitate is not formula-pure, however, and cannot be weighed directly. It must be ignited to tricobalt tetra-oxide, Co_3O_4 , for the determination of cobalt.

THE reagent known as phenylthiohydantoic acid was first recommended for the detection of cobalt by Pozzi-Escot¹ because of the red - brown precipitate produced when it reacted with aqueous cobalt solutions. Later, Willard and Hall² investigated the gravimetric determination of cobalt with this reagent. Precipitation was quantitative in ammoniacal solutions, and cobalt could be separated from numerous metals, including nickel, iron and manganese. Unfortunately, the cobalt precipitate was not quite sufficiently reproducible in its composition, so it had to be ignited, thus making the conversion factor much less favourable. Also, the precipitate was extremely bulky, and apparently only less than 25 mg of cobalt could be handled.

It was thought that the application of precipitation from homogeneous solution to this system would retain the selectivity and completeness of the precipitation of cobalt, and at the same time reduce the bulk of the precipitate (as, for example, with nickel dimethylglyoximate^{3,4}), so that larger amounts of cobalt could be dealt with. It could also improve the stoichiometry of the precipitate (as occurred with the cobalt 1-nitroso-2-naphthol precipitate⁵), so that the precipitate could be weighed after drying. Finally, any co-precipitation would be minimised.⁶

In 1877, Jaeger⁷ reacted together monochloroacetic acid, ammonium thiocyanate and aniline and identified the product (which he was unable to hydrolyse) as phenylthiohydantoic acid, I. Later, Rizzo⁸ and Beckurts and Frerichs⁹ reported that the compound hydrolysed readily to give thioglycollic acid anilide, III, and thus concluded that Jaegers' compound was carbaminothioglycollic acid anilide, II. These observations seem to have been completely overlooked by Willard and Hall,² by Cuvelier,¹⁰ who also investigated the gravimetric method for cobalt, and by Duval and Duval,¹¹ who recorded a thermogravimetric curve for the precipitate.



We have confirmed the earlier observations^{8,9} on the facile hydrolysis of Jaeger's compound, which is apparent even during its crystallisation, for the melting-points of successive

* Part of this work was previously summarised in *Chem. Commun.*, 1967, 901.

samples fall, and the NH band at 3380 cm^{-1} gradually disappears. Hydrolysis to thioglycollic acid anilide was completed in 40 minutes. A single recrystallisation of authentic carbaminothioglycollic acid anilide from water gave a product shown by elemental analysis to contain 23 per cent. of **III**; this increased to 65 per cent. after three "recrystallisations." Continued refluxing produced dithioglycollic acid anilide, **IV**, as previously noted by Rizzo.⁸ Attempts to form ethyl and 2-hydroxypropyl esters of Jaeger's compound failed. A band at 1700 cm^{-1} caused by the C=O stretch in a carboxylic acid is absent. This and the failure to undergo cyclisation to give phenylpseudothiohydantoin in the presence of mineral acids¹² again point against structure **I** and favour **II**.

TABLE I
SOME INFRARED FREQUENCIES, CM^{-1}

	$\nu\text{-NH}$ (primary amide)	$\nu\text{-NH}$ (secondary amide)	$\nu\text{-SH}$	$\nu\text{-C=O}$ (amide)	C_6H_6	Amide
Carbaminothioglycollic acid anilide	3380	3275	—	1660	1605	1540
Thioglycollic acid anilide	—	3240	2530	1640	1600	1545
Cobalt precipitate with thioglycollic acid anilide	—	3230	—	1640	1595	1550
Cobalt precipitate with carbamino- thioglycollic acid anilide.. ..	—	3320	—	1635	1590	1550

Infrared spectra confirm these observations (Table I). Nuclear magnetic resonance spectra recorded at 60 Mc/s in dimethylsulphoxide (D_6) gave singlets at -0.15τ (1H), 7.3τ (2H) and 6.6τ (2H), all against tetramethylsilane, with a multiplet at 2.0 to 3.2τ given by the phenyl group. As the resonance at -0.15 could have arisen either from a carboxylic acid or from a secondary amine group these results do not distinguish between **I** and **II**. However, a comparison of the resonances of the compound studied, its *N*-methyl derivative and thioglycollic acid anilide in acetone (D_6), which are summarised in Table II, confirms that the compound is carbaminothioglycollic acid anilide, **II**.

TABLE II
NUCLEAR MAGNETIC RESONANCE FREQUENCIES IN ACETONE (D_6)
Shifts in p.p.m. against tetramethylsilane

Number of H atoms	1	5	2	3	2	1
Carbaminothioglycollic acid	—	—	—	—	—	—
<i>N</i> -methylanilide	—	2.4 (m)	6.4 to 6.6 (s)	6.7 (s)	7.2 (s)	—
Carbaminothioglycollic acid anilide	0.5 (s)	2.3 to 2.6 (m)	6.3 (s)	—	6.95 (s)	—
Thioglycollic acid anilide	0.6 (s)	2.1 to 2.8 (m)	6.6 (d)	—	—	7.6 (t)
Assignment	NH	C_6H_6	CH_2	CH_3	NH_2	SH

s = singlet, d = doublet, t = triplet, m = multiplet.

POLAROGRAPHY—

The alleged phenylthiohydantoinic acid gave an anodic wave ($E_1 = 0.0$ volt against an S.C.E.) at pH 4.0. Such a wave can be readily attributed to a thiocarbonic acid derivative, **II**, but would not be expected for a thio-ether, **I**. At pH 6.8 the wave was unchanged after 1 hour, but at higher pH values hydrolysis produced a well developed anodic wave ($E_1 = -0.5$ volt against an S.C.E. at pH 7.2), identified as being due to thioglycollic acid anilide, **III**. The gradual formation of this wave is shown in Fig. 1. By using this wave, the rate of hydrolysis was found to be instantaneous in 0.1 M sodium hydroxide or in a borate buffer (pH 9.2); in a phosphate buffer (pH 7.8), the plot of log (diffusion current) against time was linear for the first 11 minutes of the reaction and suggested a first order reaction.

Hydrolysis at pH above 7 would be expected¹³ for the cleavage of the $-\text{S}-\text{CO}-$ grouping of **II** but not for an *S*-substituted thiourea, such as **I**.

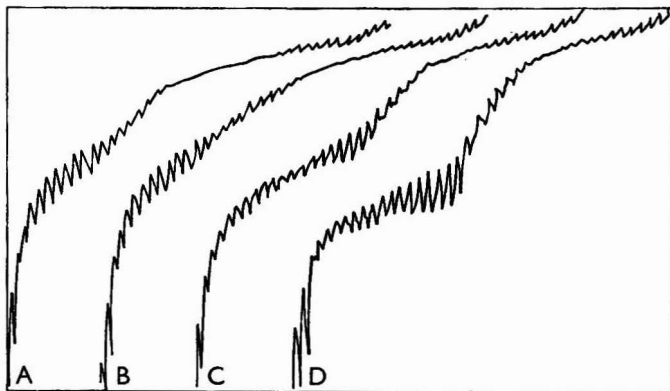


Fig. 1. Polarographic anodic waves of carbaminothioglycolic acid anilide (2×10^{-4} M) in a phosphate buffer solution (pH 7.2) containing 2 per cent. of ethanol. Measurements were made at about 20° C after A, 0 minute; B, 11 minutes; C, 43 minutes; and D, 93 minutes (this is the wave of thioglycolic acid anilide). All curves go from -1.0 to -1.1 volts *versus* an S.C.E. Capillary characteristics: drop time is 3.7 seconds; mercury flow is 1.53 mg per second

PRECIPITATION OF COBALT—

When cobalt(II) was precipitated with carbaminothioglycolic acid anilide by using the procedure of Willard and Hall,² the ligand involved in the precipitate was found to have only one nitrogen atom. As the precipitation occurred at pH 7 from boiling solution, it was reasonable to expect that this ligand was thioglycolic acid anilide, III. Indeed, thioglycolic acid anilide gave precipitates with cobalt that were identical (colour, elemental analysis and infrared spectra) with those produced by Willard and Hall's method. In fact, thioglycolic acid anilide has been recommended as a reagent for the detection of cobalt because of the voluminous blood-red precipitate it gives with cobalt.^{14,15} In all instances, irrespective of whether cobalt(II) or cobalt(III) was initially present in the solution, the analytical results for the precipitate agreed most closely with a formula of CoL_3 .

Thus carbaminothioglycolic acid anilide is merely a source reagent that generates the actual precipitant, thioglycolic acid anilide, in solution. The procedure of Willard and Hall, therefore, is essentially one of the earliest examples of precipitation from homogeneous solution; no other example of this type of generating reaction has previously been reported.

It was possible, therefore, that the original aims of this investigation could be accomplished by a detailed study of the use of carbaminothioglycolic acid anilide for precipitating the cobalt - thioglycolic acid anilide complex from homogeneous solution.

CONVENTIONAL PRECIPITATION OF COBALT WITH THIOGLYCOLIC ACID ANILIDE—

To evaluate the procedure that is developed for precipitation from homogeneous solution, it must be compared with both the original procedure of Willard and Hall² and also the direct precipitation with thioglycolic acid anilide. Bersin¹⁴ found that the latter reagent was suitable for the detection of cobalt in the presence of iron(III), aluminium and chromium(III). He assumed that the precipitate was $\text{CoL}_3 \cdot 4\text{H}_2\text{O}$, which changed on heating to Co_2L_3 . This form was not suitable for weighing, however, and had to be converted into a form that was suitable. This is the only recorded investigation of thioglycolic acid anilide as a gravimetric reagent. However, Misra and Sircar¹⁵ have investigated other thioglycolic acid amides as precipitants for cobalt, and found that the precipitates can be weighed after drying at 125° to 130° C. The complexes were formulated as $\text{CoL}_{3.1}$. Thionalide, the 2-naphthyl analogue of thioglycolic acid anilide, is the best known of the thioglycolic acid anilide derivatives.¹⁶

When cobalt(II) (25 mg) was precipitated with thioglycolic acid anilide, according to the method used with other thioglycolic acid amides,¹⁵ the voluminous red - brown precipitates

had a wide range of weights after drying at 125° to 130° C; all of the weights were excessive for CoL_3 chelates. At least part of the excessive weight was caused by co-precipitated reagent, which could be seen as white particles. This could be reduced by decreasing the digestion time from 3 to 4 hours to 30 minutes. The application of thioglycollic acid anilide in the Willard and Hall procedure² gave similar results, and both were similar to the results obtained by using the original Willard and Hall procedure. All of these results are summarised in Table III.

TABLE III
PRECIPITATION OF COBALT (25 mg) WITH THIOLYCOLLIC ACID ANILIDE

Method	Weight of precipitate, mg*	Drying temperature
Misra and Sircar ¹⁵	266.7 to 291.8†	125° to 130° C
Misra and Sircar ¹⁵	283.3 (3)‡ 239.4 to 265.7§ 251.6 (3)	125° to 130° C
Willard and Hall, ² with thioglycollic acid anilide ..	242.8 to 327.1 292.7 (3)	125° to 130° C
Willard and Hall, ² with carbaminothioglycollic acid anilide	235.3, 268.5	Room temperature, over silica gel

* CoL_3 would weigh 236.5 mg.

† Range of precipitate weights.

‡ Mean weight and number of determinations.

§ After only 30 minutes' digestion.

PRECIPITATION FROM HOMOGENEOUS SOLUTION—

None of the methods described above provided a satisfactory means for the gravimetric determination of cobalt by the direct weighing of the complex. Although Willard and Hall's original method meets some of the requirements of a procedure for precipitation from homogeneous solution, the voluminous nature of the precipitate formed by reaction with cobalt(II) indicates an extremely rapid precipitation process. Thus the hydrolysis, in which the thioglycollic acid anilide is formed, is so fast as to preclude a slow, homogeneous generation of precipitant in the solution. It was possible to slow down this hydrolysis sufficiently by using a reaction solution containing more than 20 per cent. v/v of ethanol. In this instance, the precipitate produced is compact and dark red. Even then, however, the weights of the dried precipitates were still high and variable.

Other methods of precipitation from homogeneous solution were attempted, *viz.*, urea hydrolysis⁶ and precipitation from mixed solvents.¹⁷ In these instances, there was no significant reduction in the bulk of the precipitates, and their weights were equally variable.

TABLE IV
IGNITION OF PRECIPITATES
Cobalt taken = 25.05 mg

Weight of oxide found, mg	Cobalt recovery		Cobalt in filtrate, mg*	Cobalt in washings, mg*
	Calculated as Co_2O_3 , mg	Calculated as Co_2O_4 , mg		
34.40	24.45	25.26	0.008	0.140
34.10	24.23	25.05	0.014	0.094
34.80	24.77	25.55	0.000	0.034
34.50	24.52	25.53	0.018	0.078
34.40†	24.45	25.26	0.007	0.082
34.60†	24.59	25.41	0.008	0.120
34.20†,‡	24.30	25.17	0.003	0.026
34.80†,‡	24.77	25.55	0.009	0.041
Mean	24.57	25.35	0.009	0.077

* Determined by the nitroso-R-salt method,²¹ after destruction of organic matter.

† After only 2 hours' digestion on water-bath.

‡ Precipitation in 20 per cent. ethanol.

IGNITION OF THE PRECIPITATES—

Direct weighing of the dried precipitates was, therefore, impossible, even with the new knowledge of the precipitant. One controversy remained, however. Willard and Hall² found that if the precipitate is ignited to oxide at 850° C, better recoveries are obtained if the ignited substance is considered to be Co_2O_3 , rather than Co_3O_4 , although the results are high for both. Duval and Duval¹¹ also identify the oxide as Co_2O_3 in their gravimetric studies. This is surprising, because Co_2O_3 is converted¹⁸ into Co_3O_4 above 265° C. Repetition of the experiments of Willard and Hall, including experiments in which more than 20 per cent. of ethanol was used, gave the results shown in Table IV. They show that more accurate results are obtained if the oxide is considered to be Co_3O_4 , although there is little to choose between either oxide if solubility losses are also considered. Identical results were obtained by using thioglycollic acid anilide as the precipitant. In all instances, precipitation of cobalt is quantitative, and most of the cobalt not recovered is lost during the washing of the precipitate.

EXPERIMENTAL

SYNTHESIS OF REAGENTS—

Carbaminothioglycollic acid anilide—This was prepared by Jaeger's method⁷ for "phenylthiohydantoic acid." The product was recrystallised three times from ethanol to give white crystals, m.p. 146° to 150° C. Value given in literature,⁷ 148° to 152° C. Found: C, 51.5 per cent.; H, 5.0 per cent.; N, 13.45 per cent.; S, 15.2 per cent. $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_2\text{S}$ requires C, 51.4 per cent.; H, 4.8 per cent.; N, 13.3 per cent.; S, 15.25 per cent.

Carbaminothioglycollic acid N-methylanilide—This was prepared similarly, but by using N-methylaniline (214.3 g) in place of aniline (189 g). After refluxing the reaction mixture for 24 hours, the product was twice recrystallised from ethanol to give white crystals, m.p. 138° to 140° C. Value given in literature,⁹ 147° C. Found: C, 53.5 per cent.; H, 5.4 per cent.; N, 12.4 per cent.; S, 14.0 per cent. $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_2\text{S}$ requires C, 53.5 per cent.; H, 5.4 per cent.; N, 12.5 per cent.; S, 14.3 per cent.

Thioglycollic acid anilide—This was prepared by reported methods^{9,15} and recrystallised from aqueous ethanol to give white crystals, m.p. 105° to 108° C. Value given in literature,⁹ 111° to 112° C.

REACTIONS OF THE REAGENTS—

Attempted esterification—Treatment of "phenylthiohydantoic acid" with ethanol in the presence of sulphuric acid and with propylene oxide, by an adaptation of the method of Haggis and Owen,¹⁹ yielded only starting material.

Attempted cyclisation—Only starting material was recovered after treatment of "phenylthiohydantoic acid" with 20 per cent. hydrochloric acid, according to Mouneyrat's procedure.²⁰

Hydrolysis—Pure thioglycollic acid anilide was obtained by refluxing an aqueous solution of carbaminothioglycollic acid anilide for 90 minutes. The product was white crystals, m.p. 104° to 108° C. Value given in literature,⁹ 111° to 112° C. Found: C, 57.7 per cent.; H, 5.45 per cent.; N, 9.0 per cent.; S, 19.3 per cent. $\text{C}_8\text{H}_9\text{NOS}$ requires C, 57.5 per cent.; H, 5.4 per cent.; N, 8.4 per cent.; S, 19.2 per cent.

Oxidation to dithioglycollic acid anilide—Refluxing an aqueous solution of carbaminothioglycollic acid anilide for 48 hours in a vessel open to the air gave a white crystalline product, m.p. 156° to 158° C. Value given in literature,⁸ 160° to 161° C. Found: C, 58.5 per cent.; H, 4.5 per cent.; N, 9.1 per cent.; S, 18.5 per cent. $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2$ requires C, 57.8 per cent.; H, 4.9 per cent.; N, 8.4 per cent.; S, 19.3 per cent. All melting-points were uncorrected.

Thin-layer chromatography—Chromatograms were run on silica gel G, 250 μ thick, activated at 110° C for 1 hour. The solvent system was xylene - glacial acetic acid (60 + 40 v/v). The spots were located by iodine adsorption. The R_F values obtained (relative to a solvent-front movement of 10.0 cm) were as follows: carbaminothioglycollic acid anilide, 0.34, 0.52 (faint); thioglycollic acid anilide, 0.49; carbaminothioglycollic acid anilide, after partial hydrolysis, 0.34, 0.53; pure hydrolysis product, 0.53; "phenylthiohydantoic acid" (British Drug Houses Ltd.), 0.33, 0.50 (faint), 0.57; and dithioglycollic acid anilide, 0.52.

The R_F values were not reproducible to better than ± 0.05 .

Polarography—The studies were carried out in a Kalousek-type cell, with a separated standard calomel electrode, and the polarograms recorded on an Evershed-Tinsley or a Radiometer PO₄ polarograph. Solutions were de-aerated by bubbling nitrogen through

them. All of the buffer solutions were prepared from analytical-reagent grade reagents. Freshly prepared 0.01 M solutions of carbaminothioglycollic acid (in 20 per cent. ethanol) and thioglycollic acid anilide (in water or ethanol) were used. Hydrolysis rates were measured by following the change in the diffusion current at -0.3 volt (against an S.C.E.).

Infrared studies—All spectra were recorded by using potassium bromide discs on a Perkin-Elmer, Model 23, spectrophotometer.

Nuclear magnetic resonance—Spectra were recorded on a Perkin-Elmer R10 spectrometer, operating at 60 Mc/s.

PRECIPITATION OF COBALT—

A stock solution of analytical-reagent grade cobalt nitrate containing 1 mg of cobalt per ml was used. The precipitation procedures used were those previously published, or simple modifications thereof, as described in the text. The precipitant (0.7 g) was added as a solution in water or ethanol (30 ml). The precipitates were filtered through No. 4 sintered-glass crucibles, washed with hot water, and then dried, either by heating at temperatures within the range 110° to 140° C or at room temperature in a vacuum desiccator, to constant weight. Precipitations of cobalt(III) were carried out after oxidising cobalt(II) with hydrogen peroxide in a potassium hydrogen carbonate solution.²

Precipitates produced from cobalt(III) solutions were more compact than when cobalt(II) was initially present. Elemental analyses for various precipitates that appeared to be little contaminated were as follows—

Cobalt(II) precipitated by the original Willard and Hall procedure²: found C, 51.5 per cent.; H, 4.3 per cent.; N, 7.3 per cent.; S, 16.7 per cent.

Cobalt(II) precipitated similarly, but with thioglycollic acid anilide: found C, 51.4 per cent.; H, 4.4 per cent.; N, 6.5 per cent.; S, 16.2 per cent.

Cobalt(III) precipitated similarly, with thioglycollic acid anilide: found C, 51.1 per cent.; H, 3.9 per cent.; N, 6.9 per cent.; S, 17.4 per cent.

$\text{Co}(\text{C}_8\text{H}_8\text{NOS})_3$ requires C, 51.7 per cent.; H, 4.3 per cent.; N, 7.5 per cent.; S, 17.2 per cent.

Ignition of precipitates was carried out after filtering on to Whatman No. 40 filter-paper, in porcelain crucibles, at 850° C.

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Spectrophotometric Determination of Niobium in Zirconium, Titanium and Other Metals with 4-(2-Pyridylazo)resorcinol

BY D. F. WOOD AND J. T. JONES

(Imperial Metal Industries Limited, Kynoch Works, Witton, Birmingham 6)

A reaction based on the formation of a purple-coloured complex between niobium and 4-(2-pyridylazo)resorcinol (PAR) in a tartrate - ethylenediamine-tetra-acetate (EDTA) solution has been successfully applied to the direct spectrophotometric determination of niobium, in the range 50 p.p.m. to about 1 per cent., in zirconium, zirconium alloys, hafnium, tungsten and molybdenum. The absorbance is measured at 550 m μ .

By modification, this direct procedure can be used for the determination of down to 200 p.p.m. of niobium in titanium and its alloys.

Following an extractive concentration, amounts of niobium down to about 10 p.p.m. can be determined in titanium, zirconium, and their alloys, and in hafnium, molybdenum and iron. For tungsten (and molybdenum), a preliminary collection of niobium on zirconium hydroxide is recommended.

The effects of common alloying elements, and likely impurities, have been investigated.

The absorption of the tantalum complex is only about one eighth of that of the niobium - PAR complex.

THE level of niobium in zirconium, zirconium alloys and hafnium is usually below 100 p.p.m. Other analytical requirements involve the determination of niobium at levels below about 500 p.p.m. in titanium, titanium alloys and certain other metals, and these factors necessitate the provision of reliable analytical procedures for determining small amounts of niobium in all of these materials.

The formation of coloured compounds of niobium with selective chromogenic reagents is generally accepted as the most satisfactory basis for determining small amounts of niobium. Reagents widely used for this purpose include hydrogen peroxide,¹ pyrogallol,² 8-hydroxyquinoline³ and thiocyanate,⁴ but none of these reagents is suitable for the direct determination of niobium below about 100 p.p.m., because of inadequate sensitivity, poor selectivity, or instability of the complex.

In recent years increasing attention has been given to the spectrophotometric determination of small amounts of niobium, and several new reagents have been proposed; these include xylenol orange,⁵ tribromopyrogallol,⁶ 4-(2-pyridylazo)resorcinol (PAR),^{7,8,9} and bromopyrogallol red.¹⁰

From published results it appeared that PAR and bromopyrogallol red in the presence of ethylenediaminetetra-acetate (EDTA), which was used to mask interfering ions, offered advantages in respect of selectivity and sensitivity, and these two reagents were selected for further examination.

Initial tests indicated that zirconium and titanium also formed coloured complexes with bromopyrogallol red, and the interference of 100 mg of either of these metals could not readily be prevented. Preliminary tests with PAR, however, were more promising, and a more detailed examination of this reagent was made.

EXPERIMENTAL

APPARATUS—

A Unicam SP600 was used in the experimental work, and absorbance measurements were made at 550 $m\mu$ (4-cm cells). All pH adjustments were made with a direct-reading pH meter.

PREPARATION OF CALIBRATION GRAPH—

A standard solution of niobium was prepared from 0.1 g of high-purity niobium, which was fused in potassium hydrogen sulphate. The cooled melt was dissolved in 50 ml of 20 per cent. tartaric acid solution, and the solution was diluted to 500 ml in a calibrated flask. To a 25-ml aliquot of this solution were added 50 ml of 20 per cent. tartaric acid solution, and the solution was diluted to 500 ml so that 1 ml of solution contained 10 μg of niobium.

Aliquots of this standard solution ranging from 1.0 to 5.0 ml were transferred into a series of 100-ml beakers; each solution, and a blank (water only), was diluted to about 10 ml. The characteristic colour was developed by using conditions similar to those recommended by Belcher, Ramakrishna and West,⁷ viz., to each solution, 10.0 ml of 1 per cent. EDTA solution were added, the solution was adjusted to pH 6.0 with dilute ammonia solution (1 + 2), then 10.0 ml of 0.03 per cent. PAR solution and 5 ml of the ammonium acetate buffer solution⁷ were added. All of the solutions were allowed to stand for 1 hour, then transferred into separate 100-ml calibrated flasks and diluted to the mark. Absorbances were measured against the blank solution. After the absorbance of the reagent blank (0.035) had been deducted, the graph (absorbance against niobium) was a straight line that passed through the origin; an absorbance value of 0.55 corresponded to 50 μg of niobium. A further series of tests showed that addition of the acetate buffer solution, as recommended by Belcher, Ramakrishna and West,⁷ was unnecessary, provided that the pH of the test solution was adjusted to 6.0, immediately before, and after, addition of the PAR solution.

Tests were made next in the presence of zirconium. Aliquots of the standard niobium solution (1 ml of solution \equiv 10 μg of niobium) ranging from 1.0 to 5.0 ml were added to 0.1-g samples of high-purity zirconium; an additional sample of zirconium was used as a "blank." The zirconium was dissolved in 10 ml of dilute sulphuric acid (1 + 49), 1.0 ml of fluoroboric acid solution and 2.0 ml of 50 per cent. tartaric acid solution; the presence of tartaric acid prevents the subsequent hydrolysis of zirconium salts when the niobium - PAR complex is developed.

The solutions were cooled, the niobium - PAR complex was developed in the absence of the buffer solution, as before, and absorbances were measured. These were much higher than those obtained in the absence of zirconium, and this was attributed to the formation of a zirconium - PAR complex. In subsequent tests, the interference by zirconium was overcome by increasing the amount of EDTA. For example, with 10.0 ml of 5 per cent. EDTA solution, the graph of absorbance (corrected for a blank of 0.04) against niobium was a straight line that passed through the origin; an absorbance value of 0.58 corresponded to 50 μg of niobium. In the latter tests, a period of 2 hours was allowed for the colour to develop before the absorbances were measured, because it was observed by the previous authors⁷ that an increase in the amount of EDTA increases the time required for the reaction to reach equilibrium.

The graph obtained was applicable to the determination of niobium in the range 50 to 500 p.p.m. in zirconium. Further tests showed that this upper niobium limit could be extended up to at least 1 per cent. by taking a suitable aliquot of the test solution and maintaining the volume (2.0 ml) of 50 per cent. tartaric acid solution in the final solution in which the colour is developed.

CONCENTRATIONS OF THE REAGENTS—

PAR—In tests with 50 μg of niobium and 10.0 ml of 0.03, 0.04 and 0.05 per cent. solutions of PAR, absorbance values (*minus* blank) were 0.58, 0.64 and 0.69, respectively; corresponding reagent blank values were 0.03, 0.05 and 0.075. An explanation of this progressive increase in absorbance with increase in concentration of the reagent is that the niobium - PAR complex is partially dissociated, and the degree of dissociation decreases as the amount of PAR is increased. Because the absorbance of the reagent blank also

increases as the amount of PAR is increased, a compromise was made to obtain a reasonably high sensitivity, together with an acceptable (low) blank value, and in all of the subsequent tests 10.0 ml of 0.04 per cent. PAR solution were used.

When PAR solutions prepared from different batches of reagent were used, widely different sensitivities were obtained. The reagent used in the previous tests, in which an absorbance value of 0.64 was obtained, was supplied by British Drug Houses Ltd. Absorbance values obtained for 50 μg of niobium with PAR from two other sources were 0.55 and 0.74. Only a limited amount of the most sensitive of these reagents (supplied by Professor T. S. West) was available, and all of the subsequent tests were made with the British Drug Houses' reagent.

EDTA—The calculated amount of EDTA required to form a complex with 0.1 g of zirconium is about 0.41 g; 10.0 ml of 5 per cent. EDTA solution, as used in the previous tests, are, therefore, sufficient to provide a reasonable excess and for traces of interfering ions.

Decrease in the concentration of EDTA below the theoretical amount required to react with 0.1 g of zirconium caused an increase in absorbance, as unchelated zirconium species react with PAR to form a strongly coloured complex. When the concentration of EDTA was increased above the equivalent of 10.0 ml of a 5 per cent. solution, absorbances progressively decreased; in the presence of 2 g of EDTA, an absorbance value of 0.50 was obtained for 50 μg of niobium, compared with 0.64 in the presence of 0.5 g of EDTA. This effect is probably caused by an increase in competition of the EDTA for the niobium ions. In subsequent tests 10.0 ml of 5 per cent. EDTA solution were used.

Sulphuric acid and fluoroboric acid solutions—Satisfactory dissolution of 0.1 g of zirconium is effected with 1 ml of fluoroboric acid solution and 10 ml of dilute sulphuric acid (1 + 49). Variation in the amount of dilute sulphuric acid (1 + 49) used from 8 to 12 ml had no significant effect on the final absorbance. Increase in the volume of fluoroboric acid solution from 1 to 2 ml had no significant effect on the absorbance, but when the volume of this reagent exceeded 2 ml, absorbances progressively decreased; with 50 μg of niobium, an absorbance value of 0.55 was obtained in the presence of 5 ml of fluoroboric acid solution, compared with 0.64 in the presence of 1 to 2 ml of the reagent.

Tests on solutions containing no zirconium and variable amounts of fluoroboric acid, with other conditions the same as those used in the presence of zirconium, showed that an increase in the amount of fluoroboric acid solution of up to 1 ml produced a significant increase in absorbance. In the absence of fluoroboric acid, an absorbance value of only 0.40 was obtained for a solution containing 50 μg of niobium, compared with 0.66 in the presence of 1 ml of fluoroboric acid solution.

The niobium - PAR complex has been shown to be anionic,⁷ and it is likely that it contains tartrate. It is possible, therefore, that this increase in sensitivity with increase in fluoroboric acid is caused by replacement of tartrate in the complex by fluoroborate (or fluoride) ions, thus producing a complex that is more strongly co-ordinated.

Tartaric acid—Tartaric acid is added to prevent the hydrolysis of zirconium and niobium salts. Tests showed that the minimum amount of tartaric acid necessary to prevent this hydrolysis completely (at pH 6) is about 0.5 g; a reasonable safety margin is provided in the presence of 2.0 ml of a 50 per cent. solution of the reagent. Increase in the amount of tartaric acid to 5 g caused a decrease in absorbance of about 30 per cent.; this is probably caused by increased competition of tartrate for the niobium ions.

OTHER VARIABLES—

pH—By using the best conditions established in the previous tests, absorbance was shown to be independent of pH over the range 6.0 to 6.4. In subsequent tests, the pH was adjusted to 6.2 immediately before, and after, addition of the PAR solution. Tests showed that if the PAR is added before adjusting the pH, low and erratic results are obtained.

Temperature and colour development time—Tests on typical solutions showed that at 15° C the colour developed fairly rapidly during the first 15 minutes, but more slowly during the next 90 minutes; thereafter, it was constant for at least 3 hours. Similar tests at 20° and 30° C followed the same pattern, except that during the first 15 minutes the rate at which the colour developed increased with temperature. The final absorbance, measured after 2 hours, was the same in each series of tests. In subsequent tests, the colour was allowed to develop for 2 hours at 20° C before the absorbance was measured.

Other metals—Tests showed that the procedure used in the presence of zirconium was equally applicable to the determination of niobium in the presence of 0.1 g of hafnium. Satisfactory results were also obtained in the determination of 10 to 50 μg of niobium in the presence of 0.1 g of tungsten. With 0.1 g of molybdenum, the absorbance was suppressed slightly, but the effect was not significant; larger amounts of molybdenum, however, could not be tolerated.

Details for preparing solutions of these metals to enable the proposed method to be extended are given in Method I.

Based on a 0.1-g sample, no significant interference was introduced by the presence of up to 25 per cent. of tin, 10 per cent. of aluminium, 5 per cent. of titanium, 1 per cent. of copper, 0.5 per cent. of iron, chromium, manganese or nickel, or 0.1 per cent. of vanadium. Larger amounts of iron up to about 5 per cent. could be tolerated by using 10 ml of a 5 per cent. solution of the disodium salt of 1,2-diaminocyclohexanetetra-acetate (CDTA) instead of the EDTA solution, but CDTA was less effective than EDTA in suppressing the interference of titanium. The effect of CDTA in the presence of other interfering elements was not examined, and in subsequent tests EDTA was used as the complexing agent.

When present in excess of the limiting concentrations specified above, copper interfered by forming a coloured complex with EDTA; iron, nickel and vanadium interfered by forming coloured complexes with PAR, and manganese suppressed development of the niobium - PAR complex. The effect of chromium and titanium was 2-fold; these metals reacted with PAR to form coloured complexes and also suppressed development of the niobium - PAR complex.

Under conditions similar to those established for determining niobium in zirconium, tantalum also reacts to form a coloured complex. The molar extinction coefficient of the tantalum - PAR complex is about 8000, whereas that of the niobium - PAR complex is about 30,000 but, because of their relative atomic weights, absorbance of the tantalum complex is about one eighth of that of the niobium complex. For most practical purposes, up to about 50 p.p.m. of tantalum can be tolerated in the determination of 50 to about 200 p.p.m. of niobium; proportionately more can be tolerated if the niobium content of the sample exceeds 200 p.p.m. The tantalum content of zirconium, hafnium, tungsten and molybdenum is usually less than 20 p.p.m., hence the effect of tantalum in the determination of niobium in these metals is not significant.

Belcher, Ramakrishna and West⁷ have shown that the interference of tantalum can be minimised by increasing the tartrate concentration, and subsequent tests in the present investigation confirm this observation. According to Elinson, Pobedina and Rezova,⁸ the interference of tantalum is eliminated by developing the niobium - PAR complex in the presence of a large excess of tartrate at pH 4.5 to 4.8. Under these conditions, however, the sensitivity of the reaction is considerably reduced, and their method is restricted to niobium contents above 0.1 per cent.

EXTENSION OF THE PAR PROCEDURE TO TITANIUM AND TITANIUM ALLOYS—

Earlier tests had shown that titanium in amounts above 5 per cent., based on a 0.1-g sample, interferes in the direct determination of niobium, and tests were designed to establish, more precisely, the extent of this interference, and, if possible, provide a suitable modification that would overcome the interference.

The procedure developed so far was applied to solutions containing (a) 0.1 g of titanium and (b) 0.1 g of titanium and 50 μg of niobium. Absorbance values obtained in these tests were (a) 0.33 and (b) 0.38; corresponding values obtained in similar tests in which zirconium replaced titanium were 0.05 and 0.69, respectively. These absorbance values show the extent to which titanium forms a coloured complex with the PAR and to which it suppresses development of the niobium - PAR complex.

The effects of titanium were shown to be considerably reduced when the amounts of EDTA and tartaric acid were increased to 1 and 5 g, respectively; solutions similar to (a) and (b) containing these increased amounts of EDTA and tartaric acid gave absorbance values of (a) 0.04 and (b) 0.42. In further tests under similar conditions, the graph (absorbance against niobium) over the range 20 to 100 μg was a straight line that passed through the origin. However, because of the decrease in sensitivity caused by the increased amounts of these two reagents, the direct procedure (Method I) is only suitable for determining niobium in amounts above 200 p.p.m.

Tests showed that by increasing the amount of tartaric acid to 5 g, interferences from tantalum and vanadium were reduced, and up to 500 p.p.m. of tantalum, or 5 per cent. of vanadium, had no significant effect on the determination of niobium in amounts above 200 p.p.m. The effects produced by increasing the amount of tartaric acid or EDTA, or both, in the presence of other interfering metals were not examined.

To make the fullest use of the inherent sensitivity of the proposed method for determining lower levels of niobium in titanium-based samples, a method of separation, based on extraction of the niobium from a hydrofluoric acid - sulphuric acid - ammonium fluoride solution into isobutyl methyl ketone¹¹ was investigated. This extraction procedure was applied to solutions containing 0.1 g of titanium and amounts of niobium ranging from 10 to 50 μg ; a solution containing 0.1 g of titanium was used as a blank. The separated organic phase was back-extracted with a solution of hydrogen peroxide, and niobium was subsequently determined with PAR, as in the direct procedure for the determination of niobium in zirconium.

Absorbance values obtained in these tests, compared with those obtained by a direct application of the PAR method to solutions that contained niobium alone, showed that recoveries of niobium were between 94 and 99 per cent. The absorbance value of the blank solution (0.046) indicates that no significant amount of titanium is extracted with the niobium. Recoveries of 10 to 50 μg of niobium from solutions containing 0.5 g of titanium were equally satisfactory. This extraction procedure is, therefore, suitable for determining down to about 10 p.p.m. of niobium in titanium, and tests on solutions containing 0.5 g of titanium in the presence of certain other metals showed that the extraction also overcomes the interference of at least 5 per cent. of iron, copper, vanadium, chromium, manganese or nickel in the determination of between 10 and 50 μg of niobium. As tantalum is also extracted with isobutyl methyl ketone, the extraction stage does not overcome the interference by tantalum.

In further tests incorporating the extraction procedure, complete recoveries of 10 to 50 μg of niobium were obtained from 0.5 g of iron.

Similar tests on solutions containing 10 to 50 μg of niobium in the presence of 0.5 g of zirconium or hafnium showed that the extraction enabled the determination of niobium in these metals to be extended down to about 10 p.p.m.

In tests on solutions containing 10 to 50 μg of niobium and 0.5 g of molybdenum, the separation of molybdenum from niobium was not complete, but the amount of molybdenum extracted with the niobium did not significantly affect development of the niobium - PAR complex, and recoveries of niobium down to about 10 p.p.m. were satisfactory.

In similar tests on solutions containing 0.5 g of tungsten, a large proportion of the tungsten was extracted with the niobium, and difficulties were introduced by the precipitation of hydrated tungsten trioxide at later stages in the procedure, but recoveries from solutions containing only 10 mg of tungsten were satisfactory.

In subsequent tests, the niobium was precipitated from an ammoniacal solution in the presence of added zirconium, which provided a zirconium hydroxide carrier; the precipitate was filtered off, calcined and then fused with sodium hydrogen sulphate before applying the niobium - PAR reaction. These tests showed that 10 to 50 μg of niobium could be separated from at least 1 g of tungsten.

This procedure was shown to be equally applicable to solutions containing up to at least 1 g of molybdenum; it is preferable to the extraction procedure, because a complete separation of niobium can be achieved from a relatively large amount of molybdenum. The extraction procedure, however, has the advantage that it is less susceptible to interference from certain alloying constituents.

METHOD I

This method is suitable for the direct determination of from 50 p.p.m. up to about 1 per cent. of niobium in zirconium, zirconium alloys (ZR20 and ZR30), hafnium, tungsten and molybdenum. It can also be applied to titanium if the niobium content is more than about 200 p.p.m. (Note 1).

REAGENTS—

Fluoroboric acid solution—To 280 ml of 40 per cent. hydrofluoric acid (maintained at 10° C) add, in small amounts, 130 g of boric acid. Store in a polythene bottle.

Standard niobium solution—Transfer 0.1 g of high-purity niobium (powder or millings) into a 100-ml conical flask, add 5 g of potassium hydrogen sulphate, heat the flask over a Meker burner until the metal has dissolved, then cool. Add 50 ml of 20 per cent. tartaric acid solution, warm gently until the melt has dissolved, then cool. Transfer the solution into a 500-ml calibrated flask and dilute to the mark.

Transfer 25 ml of this solution into a 500-ml calibrated flask, add 50 ml of 20 per cent. tartaric acid solution, and dilute to the mark.

1 ml of solution \equiv 10 μ g of niobium.

EDTA solution, 5 per cent.—Dissolve 50 g of the disodium dihydrate salt of ethylenediaminetetra-acetic acid (EDTA) in water, and dilute the solution to 1 litre.

PAR solution, 0.04 per cent.—Dissolve 0.4 g of the disodium salt of 4-(2-pyridylazo)-resorcinol (PAR) in water, and dilute the solution to 1 litre.

PROCEDURE FOR NIOBIUM CONTENTS 50 TO 500 P.P.M. (NOTE 2)—

Determine a reagent blank with each batch of samples and apply the procedure to two reference solutions, each containing 2.5 ml of the standard niobium solution (1 ml of solution \equiv 10 μ g of niobium). In the preparation of the reference solutions for use in the analysis of zirconium alloys, tungsten, molybdenum or titanium, add the standard niobium solution *after* the fuming stage with sulphuric acid, otherwise the tartaric acid in the standard niobium solution decomposes during fuming with the sulphuric acid and discolours the solution.

PREPARATION OF SAMPLE SOLUTION—

Zirconium and hafnium—Transfer a 0.1-g sample into a 50-ml beaker, add 2.0 ml of 50 per cent. tartaric acid solution, 10 ml of dilute sulphuric acid (1 + 49) and 1.0 ml of fluoroboric acid solution. Heat gently until the sample has dissolved, then cool the solution.

Zirconium alloys (ZR20 and ZR30)—Transfer a 0.1-g sample into a small platinum dish, add about 5 ml of water, then 40 per cent. hydrofluoric acid, dropwise, until the sample has dissolved. Add a few drops of nitric acid (sp.gr. 1.42) (to dissolve precipitated copper or tin), cool and add 1 ml of sulphuric acid (sp.gr. 1.84). Evaporate the solution to fumes of sulphuric acid, fume for about 3 minutes, then cool. Add 2.0 ml of 50 per cent. tartaric acid solution, 5 ml of water, then transfer the solution into a 50-ml beaker with a minimum amount of water and add 1.0 ml of fluoroboric acid solution.

Tungsten and molybdenum—Transfer a 0.1-g sample into a small platinum dish, add 2 ml of nitric acid (sp.gr. 1.42), dropwise, then 40 per cent. hydrofluoric acid, dropwise, until the sample has dissolved, and cool the solution. Add 1 ml of sulphuric acid (sp.gr. 1.84), evaporate to fumes of sulphuric acid, fume almost to dryness (about 10 minutes), then cool. Add 2 g of sodium hydrogen sulphate, heat gently over a Meker burner until a clear melt is obtained, then cool.

Add 2.0 ml of 50 per cent. tartaric acid solution and about 5 ml of water; heat gently to dissolve any precipitated tungstic or molybdic acid, then cool. Transfer the solution into a 50-ml beaker with a minimum amount of water and add 1.0 ml of fluoroboric acid solution.

Titanium—See Note 1.

COLOUR DEVELOPMENT—

Proceed with the reagent blank, reference and sample solutions as follows.

Add 10.0 ml of the 5 per cent. EDTA solution, then dilute ammonia solution (1 + 1), dropwise, until the pH of the solution is 6.2 ± 0.1 (use a pH meter). Add 10.0 ml of the 0.04 per cent. PAR solution. If necessary, adjust the pH to 6.2 with dilute ammonia solution (1 + 4) or dilute sulphuric acid (1 + 9) as required, and allow the solution to stand for 2 hours. Transfer the solution into a 100-ml calibrated flask, dilute to the mark, mix, then measure the absorbance at a wavelength of 550 μ m, with 4-cm cells.

Deduct the blank value, and calculate the niobium content of the sample from the niobium recoveries obtained on the reference solutions.

NOTES—

1. Based on a 0.1-g sample, no significant interference is caused by the presence of up to 25 per cent. of tin, 10 per cent. of aluminium, 5 per cent. of titanium, 1 per cent. of copper, 0.5 per cent. of iron, chromium, manganese or nickel, 0.1 per cent. of vanadium or 50 p.p.m. of tantalum.

For samples that contain alloying levels of copper or tin, or both, use the dissolution procedure recommended for zirconium alloys.

The materials for which the method is recommended normally contain less than 20 p.p.m. of tantalum, but up to 500 p.p.m. of tantalum can be tolerated by increasing the volume of 50 per cent. tartaric acid solution to 10.0 ml; a similar amount must be added to the blank and reference solutions, because tartaric acid lowers the sensitivity of the niobium - PAR reaction.

This direct procedure can be applied to the determination of 200 to 1000 p.p.m. of niobium in titanium (0.1 g), provided that the sample is dissolved as described for zirconium alloys, the tartaric acid solution (50 per cent.) is increased to 10.0 ml,* and 1 g of EDTA is added (the EDTA will not dissolve completely until the pH of the solution is adjusted to about 6.2). Higher concentrations of niobium in titanium, up to about 1 per cent., can be determined in an aliquot of the sample solution (containing not more than 100 μg of niobium) by adjusting the tartaric acid concentration to the equivalent of 10.0 ml of a 50 per cent. solution before developing the coloured complex. A blank, and reference solutions containing 5.0 ml of the standard niobium solution (1 ml of solution \equiv 10 μg of niobium), must be examined in the same way as the sample solution; in the preparation of the reference solutions, add the standard niobium solution *after* the fuming stage with sulphuric acid.

For determining lower levels of niobium in titanium, see Method II.

2. For niobium contents below 50 p.p.m., use a larger weight of sample, and make a preliminary separation of niobium (see Methods II and III).

For niobium contents from 500 p.p.m. up to about 1 per cent. in zirconium, zirconium alloys and hafnium, use a 0.1-g sample and prepare the solutions as described under Zirconium alloys,† but add 4.0 ml of 50 per cent. tartaric acid solution. Add 5 ml of water, dilute the solution to 100 ml in a calibrated flask, and transfer an aliquot not exceeding 50 ml and containing not more than 50 μg of niobium into a 100-ml beaker. If necessary, add more of the 50 per cent. tartaric acid solution to bring the total amount present to 2.0 ml, add 1.0 ml of fluoroboric acid solution, and continue as described under Colour development.

For niobium contents from 500 p.p.m. up to about 1 per cent. in tungsten and molybdenum, prepare solutions as described for these metals, but add 4.0 ml of 50 per cent. tartaric acid solution. Heat gently to dissolve any tungstic or molybdic acid, then cool. Transfer the solution into a 100-ml calibrated flask, dilute to the mark, and continue as described above in this Note for determining from 500 p.p.m. to 1 per cent. of niobium in zirconium.

METHOD II

This extraction procedure enables the PAR method to be extended down to about 10 p.p.m. of niobium in titanium, zirconium, hafnium, iron and molybdenum; it also enables niobium, up to about 500 p.p.m., to be determined in samples that contain alloying metals in excess of the limiting concentrations specified in Method I. (See Notes 3 and 4.)

SPECIAL REAGENTS—

In addition to those described for Method I.

Isobutyl methyl ketone (equilibrated)—To 40 ml of 40 per cent. hydrofluoric acid contained in a 250-ml polythene beaker add, cautiously, 33.5 ml of sulphuric acid (sp.gr. 1.84), then cool. Add 8.0 g of ammonium fluoride, stir until the reagent has dissolved, then cool and dilute the solution to 100 ml. Transfer the solution into a 500-ml polythene bottle, add 300 ml of isobutyl methyl ketone, shake the mixture for 2 minutes, then allow the organic and aqueous phases to separate.

Use 25-ml aliquots of the upper organic layer, as required, in the following procedure.

PROCEDURE FOR NIOBIUM CONTENTS 10 TO 100 P.P.M. (NOTE 4)—

Determine a reagent blank with each batch of samples and apply the procedure to two reference solutions, each containing 2.5 ml of the standard niobium solution (1 ml of solution \equiv 10 μg of niobium). In the preparation of the reference solutions, add the standard niobium solution after the fuming stage with sulphuric acid.

Transfer a 0.5-g sample into a small platinum dish, add about 5 ml of water, then 40 per cent. hydrofluoric acid (Note 5), dropwise, until the sample has dissolved. Add about 3 ml of nitric acid (sp.gr. 1.42) and 17.0 ml of dilute sulphuric acid (1 + 1), evaporate the solution to fumes of sulphuric acid, fume for 5 minutes, then cool. Add 10.0 ml of 40 per cent. hydrofluoric acid, mix, then cool the solution and transfer it into a 100-ml polythene separating

* Transfer the sample solution into a 100-ml beaker before adding the tartaric acid solution.

† This procedure ensures complete dissolution of these higher concentrations of niobium.

funnel. Rinse the dish with 6.5 ml of water, and transfer the washings into the separating funnel. Add 2.0 g of ammonium fluoride, shake gently until the reagent has dissolved, then add 25.0 ml of the equilibrated isobutyl methyl ketone (Note 6). Shake the funnel (on a mechanical shaker) for 10 minutes, then shake vigorously, by hand, for 2 minutes. Allow the organic and aqueous phases to separate, and discard the aqueous (lower) layer.

To the organic extract, add 25 ml of hydrogen peroxide solution (5 volume) and shake the mixture, as before. Allow the organic and aqueous phases to separate, then run off the aqueous (lower) layer into a small platinum dish. Add 2 ml of dilute sulphuric acid (1 + 1), evaporate the solution to fumes of sulphuric acid, fume for 5 minutes [if the solution darkens during evaporation, cool, add 2 ml of nitric acid (sp.gr. 1.42), and continue the evaporation], then cool. Add 2.0 ml of 50 per cent. tartaric acid solution and 5 ml of water, then transfer the solution into a 50-ml beaker with a minimum amount of water, and add 1.0 ml of fluoroboric acid solution.

Add 10.0 ml of the 5 per cent. EDTA solution, and continue as described under Colour development, Method I.

NOTES—

3. Up to at least 25 per cent. of tin, 10 per cent. of aluminium, 5 per cent. of copper, vanadium, chromium, manganese or nickel, 2 per cent. of tungsten or 20 p.p.m. of tantalum do not cause a significant interference. Up to about 100 p.p.m. of tantalum can be tolerated by increasing the tartaric acid concentration (see Note 1).

This extraction procedure can also be applied to the determination of niobium in molybdenum, but the procedure described in Method III provides a more efficient method of separating niobium from relatively pure molybdenum, and enables a larger weight of sample to be used. If alloying constituents are present, however, the solvent-extraction procedure is recommended.

4. When applying this procedure to samples containing alloying constituents and 100 to 500 p.p.m. of niobium, use a 0.1-g sample.

5. In the determination of niobium in iron (or molybdenum), cautiously add the 3 ml of nitric acid, dropwise, before adding the 40 per cent. hydrofluoric acid.

6. Partial hydrolysis of the zirconium salts occurs at this stage, but this does not affect the recovery of niobium; the insoluble material is subsequently discarded with the aqueous layer after extracting the niobium with the isobutyl methyl ketone.

METHOD III

This hydroxide-separation procedure enables the PAR method to be extended to levels of niobium from about 50 down to about 5 p.p.m. in tungsten and molybdenum (Note 7).

SPECIAL REAGENTS—

As described for Method I.

PROCEDURE FOR NIOBIUM CONTENTS 5 TO 50 P.P.M.—

Determine a reagent blank with each batch of samples, and apply the procedure to two reference solutions, each containing 2.5 ml of the standard niobium solution (1 ml of solution \equiv 10 μ g of niobium). In the preparation of the reference solutions, add the standard niobium solution *after* the fuming stage with sulphuric acid.

Transfer a 1-g sample and 10 mg of high-purity zirconium into a small platinum dish. Add 5 ml of water, 5 ml of nitric acid (sp.gr. 1.42), dropwise (Note 8), and about 3 ml of 40 per cent. hydrofluoric acid, dropwise. After the sample has dissolved, cool the solution, and add 5 ml of sulphuric acid (sp.gr. 1.84). Evaporate the solution to fumes of sulphuric acid, fume for about 10 minutes, cool, add 10 ml of water, then cool again. Add dilute ammonia solution (1 + 1), dropwise, until the precipitated tungstic acid (or molybdic acid) has dissolved, and continue to add the ammonia solution until the test solution is alkaline to litmus. Allow the solution to stand for 3 hours (or overnight).

Filter the precipitated hydroxides of zirconium and niobium on a No. 540 Whatman filter-paper, and wash the filter-paper and precipitate with dilute ammonia solution (1 + 99). Transfer the filter-paper and contents to a small platinum dish, dry and char, with the usual precautions, heat at 700° C for about 30 minutes, then cool. Fuse the residue with 2 g of sodium hydrogen sulphate and 1 ml of sulphuric acid (sp.gr. 1.84), then cool. Add 2.0 ml of 50 per cent. tartaric acid solution and 10 ml of water, warm gently until the melt has dissolved, then cool. Transfer the solution into a 50-ml beaker with the minimum amount of water, then add 1.0 ml of fluoroboric acid solution.

Add 10.0 ml of the 5 per cent. EDTA solution, and continue as described under Colour development, Method I.

NOTES—

7. Based on a 0.1-g sample, no significant interference is caused by the presence of up to 1 per cent. of tin or aluminium, 0.5 per cent. of titanium, 0.1 per cent. of copper, 0.05 per cent. of iron, chromium, manganese or nickel, 0.01 per cent. of vanadium or 10 p.p.m. of tantalum. Up to about 50 p.p.m. of tantalum and 0.5 per cent. of vanadium can be tolerated by increasing the tartaric acid concentration (see Note 1).

8. In the dissolution of finely powdered molybdenum, add the nitric acid cautiously to prevent the reaction becoming too vigorous.

EXAMINATION OF SAMPLES

The results of the tests described below are given in Table I.

TABLE I
DETERMINATION OF NIOBIUM IN VARIOUS MATERIALS

Material	Niobium added, p.p.m.	Niobium determined, p.p.m.		
		Method I	Method II	Method III
Hafnium	Nil	40, 45	41, 39, 42	—
	Nil	30, 30	27, 27, 26	—
	300	335, 340	—	—
Zirconium	Nil	45, 40	38, 40, 40	—
	75	110, 120	—	—
	500	525, 535	—	—
ZR20 (a)	Nil	40, 30	37, 35	—
	50	90, 90	—	—
ZR30 (b)	Nil	50, 45	42, 45	—
	100	140, 135	—	—
Commercially-pure titanium ..	Nil	—	10, 11	—
	Nil	—	8, 10	—
	50	—	55, 57	—
	200	*(200, 205)	—	—
Titanium 318A (c)	Nil	—	15, 18	—
	100	—	120, 123	—
Titanium 684 (d)	Nil	—	12, 15	—
	200	*(200, 220)	—	—
Titanium 679 (e)	Nil	—	21, 24	—
	200	*(230, 220)	—	—
Molybdenum	Nil	—	10, 12	14, 14
	50	55, 60	61, 63	60, 65
Tungsten	Nil	120, 110	—	100, 105
	100	230, 220	—	210, 206

(a) Zirconium + 1.5 per cent. Sn, 0.12 per cent. Fe, 0.10 per cent. Cr, 0.05 per cent. Ni.

(b) Zirconium + 0.5 per cent. Cu, 0.5 per cent. Mo.

(c) Titanium + 6 per cent. Al, 4 per cent. V.

(d) Titanium + 6 per cent. Al, 5 per cent. Zr, 1 per cent. W, 0.3 per cent. Si.

(e) Titanium + 11 per cent. Sn, 2.25 per cent. Al, 5 per cent. Zr, 1 per cent. Mo, 0.3 per cent. Si.

* Tests made in the presence of 5 g of tartaric acid and 1 g of EDTA.

The direct procedure (Method I) was applied to samples of hafnium, zirconium and zirconium alloys (ZR20 and ZR30) containing about 40 p.p.m. of niobium, and recoveries obtained were all reproducible to within about 5 p.p.m.

Previous experience has shown that the acid mixture used for dissolving zirconium and hafnium does not dissolve alloying amounts of tin in ZR20, or copper in ZR30. To ensure complete dissolution of these alloys, therefore, a mixture of hydrofluoric and nitric acids was used.

Samples of zirconium, hafnium, titanium, tungsten and molybdenum, to which had been added between 50 and 500 p.p.m. of niobium, were also examined by the direct procedure, and recoveries were satisfactory.

The direct procedure was also applied to samples of tungsten containing about 0.5 and 1.5 per cent. of niobium, and recoveries compared satisfactorily with those obtained with a procedure that involves the separation of niobium by hydrolytic precipitation of sodium niobate¹² and subsequent colorimetric evaluation of the niobium with hydrogen peroxide.^{13,14} These results are shown below.

Samples	Niobium, per cent.		
	Present	Determined by Method I	Determined by hydrolytic precipitation - H ₂ O ₂ method
Tungsten	0.5	0.53, 0.52	0.55, 0.58
	1.5	1.48, 1.52	1.56, 1.59

The extraction procedure (Method II) was applied to samples of commercially pure titanium and titanium alloys containing about 10 p.p.m. of niobium, and the recoveries were reproducible. The method was also applied to samples of titanium, to which had been added 50 and 200 p.p.m. of niobium, and good recoveries were obtained. Samples (0.5 g) of zirconium, zirconium alloys and hafnium, each taken from the same bulk material as the 0.1-g samples analysed by the direct procedure, were examined by the extraction procedure; recoveries obtained by both methods were of the same order, but better reproducibilities were obtained by the extraction procedure.

Samples of tungsten and molybdenum were examined by the hydroxide-precipitation procedure (Method III), and reproducible recoveries were obtained.

CONCLUSIONS

Tests have shown that a direct procedure, based on spectrophotometric measurement of the coloured complex formed between niobium and PAR in a tartrate solution containing EDTA, can be satisfactorily applied to the determination of 50 to 500 p.p.m. of niobium in 0.1-g samples of zirconium, zirconium alloys (ZR20 and ZR30), hafnium, tungsten and molybdenum.

This procedure can also be applied to 0.1-g samples of titanium and titanium alloys, provided that higher concentrations of tartrate and EDTA are used to suppress development of the titanium - PAR complex, but this modification leads to a reduction in sensitivity and limits its application to the determination of niobium in amounts above 200 p.p.m.

Higher levels up to about 1 per cent. of niobium can be determined in all of these materials by dilution of the sample solution and examination of a suitable aliquot.

Lower levels down to about 10 p.p.m. of niobium can be determined in titanium, zirconium, hafnium, molybdenum and iron by a procedure that involves a preliminary extraction of the niobium from a hydrofluoric acid - sulphuric acid - ammonium fluoride solution into isobutyl methyl ketone, back-extraction of the organic phase with hydrogen peroxide solution and subsequent development of the niobium - PAR complex. Up to at least 25 per cent. of tin, 10 per cent. of aluminium, 5 per cent. of copper, chromium, vanadium, manganese or nickel, or 2 per cent. of tungsten, based on a 0.5-g sample, do not interfere.

The PAR procedure can also be satisfactorily applied to the determination of down to at least 10 p.p.m. of niobium in tungsten, provided that niobium is first separated by precipitation of the niobium (as hydroxide) from an ammoniacal solution, with a zirconium hydroxide carrier. The same procedure is equally applicable to the determination of niobium in molybdenum; separation of these two metals in this way is more efficient than by the extraction procedure, and it enables a larger weight of sample to be used. Solvent extraction is advantageous, however, when certain alloying constituents are present.

Because of the close chemical similarity of niobium and tantalum, these metals cannot be individually identified, either by incorporating an isobutyl methyl ketone extraction stage, or following a hydroxide precipitation. Like niobium, tantalum also forms a coloured complex with PAR under the specified conditions, but the sensitivity of the tantalum - PAR complex is only about one eighth of that of the corresponding niobium complex; hence, the effect of tantalum in the determination of 50 to 200 p.p.m. of niobium, based on a 0.1-g sample, is only likely to be significant when tantalum is present in excess of 50 p.p.m. The effect of tantalum can be reduced by increasing the concentration of tartaric acid; a similar amount of tartaric acid must also be present in the reference solution, because an increase in tartrate concentration also causes a decrease in the sensitivity of the reagent to niobium.

Because variations in the concentrations of the reagents and the quality of the PAR have a significant effect on the absorbance of the niobium - PAR complex, it is recommended that the final absorbance of the sample solution be related to absorbance obtained on standard solutions of niobium that have been simultaneously examined by the entire procedure.

With the direct procedure, a batch of about twelve determinations can be completed in a normal working day; when a preliminary separation involving either extraction or precipitation of the niobium is necessary, the number of determinations is reduced to about eight.

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The Spectrophotometric Determination of Vanadium(V) with 3,3'-Dimethylnaphthidine

BY LORNA G. BANNARD AND J. D. BURTON

(Department of Oceanography, The University, Southampton)

An investigation has been made of the factors concerned in the reaction of vanadium(V) with 3,3'-dimethylnaphthidine. Some probable causes of previous difficulties in the application of the reaction in spectrophotometric analysis have been elucidated. A simple procedure is given for the spectrophotometric determination of vanadium in the range from 0.08 to 2 p.p.m.; with 0.32 p.p.m. the coefficient of variation ranged from 0.60 per cent. for analyses at one time to 1.8 per cent. for analyses over a period of 3 months. Small, but significant, deviations from adherence to Beer's law occur. The limit of detection is about 0.004 p.p.m. of vanadium. The method is selective; the effects of numerous foreign ions have been examined, and interfering effects were found only with chromium(VI), iron(II), iron(III), cerium(III) and cerium(IV).

THERE is conflicting evidence concerning the value of 3,3'-dimethylnaphthidine (3,3'-dimethyl-4,4'-diamino-1,1'-binaphthyl) as a reagent for the spectrophotometric determination of vanadium(V). Introduced as a spot-test reagent by Belcher, Nutten and Stephen,¹ it was later applied to the analysis of vanadium in alloys by Milner and Nall,² who found that in a solution containing sulphuric and orthophosphoric acids, the reaction was rapid, and the colour of the oxidation product was stable for more than 30 minutes. Under apparently identical conditions, Scholes³ found that the colour was unstable. Although the stability could be improved by varying the acid concentrations, the reaction was still erratic and calibration graphs prepared at intervals were widely divergent; he rejected the use of the reagent for precise determinations. Similarly conflicting reports have been made of the reaction in solutions containing perchloric and orthophosphoric acids. Forrester and Jones⁴ described conditions under which the colour was stable for about 1 hour, but their findings were not confirmed by Macmillan and Samuel.⁵

These contradictory reports suggested that the influences of various conditions on the reaction were inadequately understood. As the reagent has considerable advantages in its selectivity and high sensitivity for vanadium (it is, for example, about five times as sensitive as 3,3'-diaminobenzidine⁶), it seemed worthwhile to examine its use more fully. The results of an investigation into the factors concerned in the reaction are presented in this paper.

EXPERIMENTAL

Preliminary experiments were commenced by using the conditions recommended by Forrester and Jones.⁴ To a solution containing 5 ml of dilute orthophosphoric acid (1 + 1, with 90 per cent. w/w H₃PO₄), 5 ml of dilute perchloric acid (1 + 1, with 72 per cent. w/w HClO₄) and 8 μg of vanadium(V), 2.5 ml of a 0.1 per cent. w/v solution of 3,3'-dimethylnaphthidine in glacial acetic acid were added. The solution was diluted to 25 ml and measurements of optical density were made at 550 mμ in a 4-cm cell, at intervals. The results (Fig. 1) confirmed the findings of Macmillan and Samuel⁵ that the colour was not stable. The experiment was repeated with reagent solutions of various other concentrations (*viz.*, 0.003, 0.01 and 0.03 per cent. w/v). The results (Fig. 1) showed that colour development was markedly retarded at the lowest concentration, but that with the 0.01 per cent. w/v reagent

there was a period of about 25 minutes during which the colour was stable; this reagent concentration, which was used by Milner and Nall² in solutions containing sulphuric and orthophosphoric acids, was used in all subsequent work. Investigations in which sulphuric acid was used, instead of perchloric acid, were not pursued because the rate of colour development was found to be slow under these conditions.

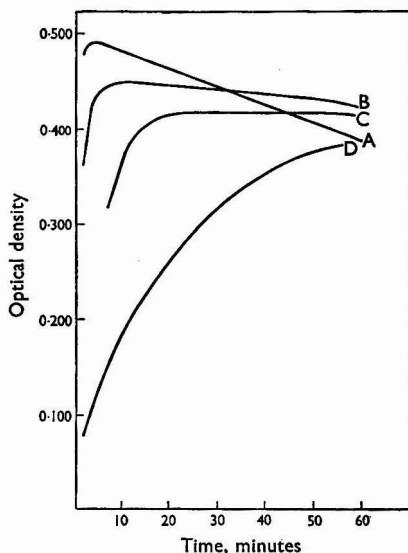


Fig. 1. Effect of concentration of 3,3'-dimethylnaphthidine on the rate of colour development in solutions containing perchloric and orthophosphoric acids. Each solution contained 8 μg of vanadium(V) and 2.5 ml of reagent solution in glacial acetic acid. Concentrations of 3,3'-dimethylnaphthidine solution: curve A, 0.1 per cent.; curve B, 0.03 per cent.; curve C, 0.01 per cent.; curve D, 0.003 per cent. Optical densities are uncorrected for reagent blanks (about 0.010)

The effects of varying the amounts of acid were studied next, but erratic results were frequently obtained. It was found that these were attributable to three causes. Firstly, the reaction is sensitive to sunlight; the optical density of a solution exposed directly to natural light during colour development was about 30 per cent. lower than that of an identical solution that was kept in darkness or subdued light during the development period. Secondly, there can be variations in sensitivity and rate of colour development between different batches of solid reagent. This effect was noted with two batches (from one supplier), one of which was noticeably darker than the other in the solid state. The response of both batches to changes in conditions was similar, despite the difference in rate of reaction. This question is discussed further below. The third cause of variable results was that, in some experiments, solutions containing vanadium, orthophosphoric and perchloric acids were allowed to stand for appreciable periods before colour development. Under these circumstances low results are obtained, presumably because some reduction of vanadium takes place. A reduction in optical density of 5 per cent. was found to occur when the delay in adding the reagent was 1 hour, and when the period was extended to 3 hours the reduction was 9 per cent.; allowing the solution to stand for longer periods had little further effect.

In further experiments, the procedure was modified to take account of these factors, and the effect of varying the concentration of each component in turn was examined, while keeping the concentration of the other components constant. These determinations were

made with 8 μg of vanadium(V) in a final volume of 25 ml; optical densities were measured at 550 $m\mu$ in 4-cm cells and corrected for reagent blanks, which were generally about 0.010.

The effect of varying the amount of acetic acid added with the 3,3'-dimethylnaphthidine reagent was examined first; the solutions contained 5 ml of dilute orthophosphoric acid and 5 ml of dilute perchloric acid. The results are given below.

Volume of glacial acetic acid, ml	..	0.25	0.625	1.25	2.5	5.0	7.5
Optical density (less blank)	..	0.402	0.400	0.405	0.410	0.406	0.404

Variations in the concentration of acetic acid in this range thus had no considerable effect on the reaction. In subsequent experiments, 2.5 ml of a reagent solution in 25 per cent. v/v acetic acid were added. This reagent solution is oxidised only slowly and shows no loss of sensitivity for 36 hours.

The effect of varying the amount of dilute perchloric acid was studied next; each solution contained 5 ml of dilute orthophosphoric acid. The following results were obtained.

Volume of dilute perchloric acid, ml	..	0	1	2	3	4	5	7.5	10
Optical density (less blank)	..	0.353	0.374	0.387	0.404	0.404	0.401	0.392	0.381

With solutions containing 5 ml of dilute perchloric acid, the effect of varying the amount of dilute orthophosphoric acid was investigated, with the following results.

Volume of dilute orthophosphoric acid, ml	..	0	2.5	5	7.5	10	
Optical density (less blank)	0.155	0.358	0.390	0.400	0.393

As the omission of perchloric acid had a comparatively small effect on the reaction, the possibility of using orthophosphoric acid alone was examined. The rate of colour development was measured first, 10 ml of dilute orthophosphoric acid being used. In Fig. 2,

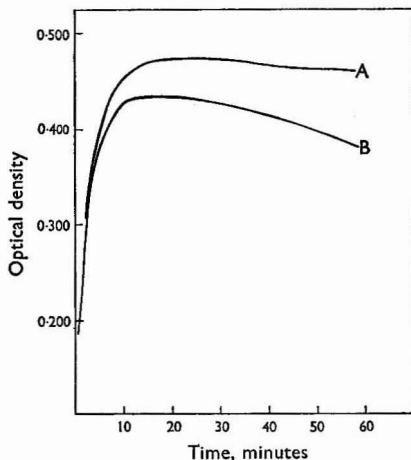


Fig. 2. Rate of colour development in orthophosphoric acid solution with different batches of solid 3,3'-dimethylnaphthidine. Each solution contained 8 μg of vanadium(V); the reagent was added as an 0.01 per cent. solution in 25 per cent. v/v acetic acid. Curve A, with reagent used for results shown in Fig. 1; curve B, with different batch of 3,3'-dimethylnaphthidine under identical conditions. Optical densities are uncorrected for reagent blanks (about 0.010)

the results are shown for the two batches of 3,3'-dimethylnaphthidine referred to previously. Curve A was obtained with the lighter-coloured reagent that was used to obtain the results shown in Fig. 1. Comparing this curve with curve C in Fig. 1, it is seen that the reaction occurs rather more rapidly in orthophosphoric acid solution than in the mixed solution, and a slight increase in sensitivity is obtained; the period of stability of the complex is somewhat

reduced. With the second batch of reagent, the colour was more fugitive, but there was still an adequate period of stability of 10 minutes (curve B, Fig. 2); there was some reduction in sensitivity. A similar change in the rate of colour development occurred when the same reagent was used in solutions containing perchloric and orthophosphoric acids. Except for the results in Fig. 1 and curve A, Fig. 2, the values reported in this paper were obtained with the less satisfactory batch of reagent, measurements being made within the period of stability of the colour, according to the solution used.

The effect of varying the concentration of orthophosphoric acid, while omitting perchloric acid, was investigated next, and the results are shown below.

Volume of dilute orthophosphoric acid, ml	0	1	2.5	5	7.5	10	15
Optical density (less blank)	0.009	0.090	0.157	0.291	0.388	0.425	0.456

In the conditions finally adopted for the determination, perchloric acid was omitted and 10 ml of dilute orthophosphoric acid were added. The absorption curve of the oxidation product was unchanged. There was no significant effect of temperature on the reaction in the range 10° to 25° C.

REPRODUCIBILITY, BEER'S LAW AND LIMIT OF DETECTION—

The reproducibility of the method was tested by making twelve measurements under the conditions of the recommended procedure and using 8 μ g of vanadium(V). A coefficient of variation of 0.60 per cent. was obtained. Similar measurements were made in groups of up to four determinations, at intervals over 3 months; for 135 such determinations, the coefficient of variation was 1.8 per cent. Replicate determinations of various amounts of vanadium(V), in the range 0.25 to 50 μ g, were made to investigate whether Beer's law was obeyed. The results (Table I) show that there was significant deviation from linearity, the response being reduced with lower concentrations; the effect was especially pronounced at concentrations below 0.08 p.p.m., which may be taken as the lower end of the useful working range of the method.

TABLE I
DETERMINATION OF VANADIUM(V)

Amount of vanadium(V), μ g per 25 ml	Mean optical density (less blank) in 4-cm cell	Optical density per μ g present	Deviation from linearity, relative to highest concentration, per cent.
0.25	0.010	0.0400	26.2
1	0.047	0.0470	13.3
2	0.101	0.0505	6.8
4	0.208	0.0520	4.1
6	0.315	0.0525	3.1
8	0.424	0.0530	2.2
10	0.527	0.0527	2.8
25	1.344*	0.0538	0.7
50	2.708*	0.0542	—

* Measured in 1-cm cell and calculated for 4-cm cell.

The average value for the optical density of the blank in a 4-cm cell was 0.008, with a standard deviation of less than 0.001. If the response was linear below a concentration of 0.01 p.p.m., then, by using Wilson's definition⁷ of the limit of detection, there would be a high probability of detection with 0.002 p.p.m. of vanadium. Because there is deviation from linearity, a somewhat higher limit of detection must apply. It was found experimentally that 0.004 p.p.m. of vanadium could be detected with a high degree of certainty.

EFFECTS OF FOREIGN IONS—

The effects of various foreign ions were examined by carrying out determinations of 8 μ g of vanadium(V) in the presence of each ion; measurements were also made in the absence of added vanadium. The results (Table II) show the reagent to be selective for vanadium(V). Cerium(IV) and chromium(VI) interfere by oxidising 3,3'-dimethylnaphthidine; cerium(III) has a suppressing effect on the reaction with vanadium. The most important interfering ion likely to be encountered in significant amounts is iron. Iron(II) interferes strongly, presumably by reducing vanadium; iron(III) also interferes, although to a lesser extent.

TABLE II
EFFECTS OF FOREIGN IONS

Ion added	Amount, μg	Optical density in absence of vanadium*	Recovery of 8 μg of vanadium(V), per cent.	Ion added	Amount, μg	Optical density in absence of vanadium*	Recovery of 8 μg of vanadium(V), per cent.
Sodium ..	10,000	0.002	101.5	Cadmium ..	100	0.001	100.0
Magnesium ..	1000	0.000	102.0	Tin(II) ..	100	0.006	99.3
Aluminium ..	100	0.000	99.5	Tin(IV) ..	100	0.002	99.8
Potassium ..	1000	0.001	100.0	Antimony(III)	100	0.000	100.7
Calcium ..	1000	0.001	100.6	Barium ..	100	0.000	99.8
Titanium(IV)	100	0.000	100.5	Cerium(III)	100	0.001	81.8
Chromium(III)	100	0.001	101.0	Cerium(IV)	50	0.691	107.6
Chromium(VI)	40	0.316	>400	Tantalum ..	100	0.001	99.6
Manganese(II)	100	-0.001	99.8	Tungsten(VI)	100	0.001	100.5
Iron(II) ..	5	-0.001	48.9	Lead ..	100	-0.004	101.0
	10	0.000	<1	Thorium ..	100	0.000	100.5
Iron(III) ..	50	0.000	56.0	Uranium(VI)	100	0.002	100.5
	100	0.000	12.5	Chloride ..	1000	0.001	100.6
Nickel ..	100	0.000	99.5	Nitrate ..	1000	0.000	98.8
Copper ..	100	0.000	100.5	Sulphate ..	1000	0.000	101.5
Zinc ..	100	-0.002	100.8	Phosphate ..	1000	0.001	98.8
Arsenic(V) ..	100	0.000	100.0	Fluoride ..	1000	-0.002	99.1
Selenium(VI)	100	0.001	99.8	Oxalate ..	1000	0.001	98.8
Niobium ..	100	0.001	100.2	Citrate ..	1000	0.000	99.5
Molybdenum(VI)	100	-0.002	100.5				

* Optical densities measured in 4-cm cell and corrected for reagent blank.

Experiments in this laboratory on the application of the reagent to the analysis of vanadium in natural waters have shown that small amounts of vanadium are readily separated from iron(III) by anion exchange in concentrated hydrochloric acid solution. After any preliminary separation, the vanadium must finally be converted into the quinqui-positive oxidation state. This may be achieved by evaporation with perchloric acid. During the present work it has been found that when solutions containing microgram amounts of vanadium are evaporated alone, with concentrated nitric or perchloric acids, or with dilute ammonia solution, the vanadium is not subsequently dissolved quantitatively in water or in dilute perchloric or orthophosphoric acids. The residues are completely dissolved, however, in a 1 per cent. w/v solution of sodium hydroxide.

METHOD

APPARATUS—

Measurements of optical density were made with a Unicam SP500 spectrophotometer against water in the compensator cell. Absorption curves were plotted directly from a Beckman DB spectrophotometer.

REAGENTS—

3,3'-Dimethylnaphthidine reagent—Dissolve 0.01 g of 3,3'-dimethylnaphthidine in 25 ml of glacial acetic acid and 5 ml of water, and dilute the solution to 100 ml with water. Discard the solution after 36 hours.

Dilute orthophosphoric acid (1 + 1)—Mix equal volumes of analytical-reagent grade 90 per cent. w/w orthophosphoric acid and water.

Standard vanadium(V) stock solution—Dissolve 0.4592 g of analytical-reagent grade ammonium metavanadate in water and dilute to 100 ml.

1 ml of solution \equiv 2 mg of vanadium(V).

Standard vanadium(V) dilute solution—Prepare, as needed, by appropriate dilution of the standard stock solution.

1 ml of solution \equiv 2 μg of vanadium(V).

RECOMMENDED PROCEDURE—

Ensure that the vanadium is present in the quinquipositive oxidation state. To the test solution, containing 2 to 50 μg of vanadium, add 10 ml of dilute orthophosphoric acid and sufficient water to dilute the solution to about 20 ml. Without delay, add 2.5 ml of 3,3'-dimethylnaphthidine reagent and dilute the solution to 25 ml. Keep the solution in the dark for 15 minutes and measure its optical density at 550 $m\mu$. Determine the reagent blank in a similar way, omitting the sample. Prepare a calibration graph by using the standard vanadium(V) dilute solution. Run duplicate standards of one concentration at least daily. Check the rate of colour development whenever a new batch of solid 3,3'-dimethylnaphthidine is used.

CONCLUSIONS

The reaction of vanadium(V) with 3,3'-dimethylnaphthidine provides a sensitive and selective method for the spectrophotometric determination of the element. Previous difficulties experienced with the reagent may have been caused by failure to take account of the effects of sunlight on the reaction, variations in the behaviour of different batches of the solid reagent and changes in the reactivity of vanadium on allowing it to stand in strongly acidic solutions. The use of too high a concentration of reagent has also probably been a cause of unsatisfactory results in some work. Colour development in orthophosphoric acid is as satisfactory as that in a mixture of orthophosphoric and perchloric acids. The colour forms rapidly and is stable for an adequate period. The reagent solution is stable for 36 hours and has a low extinction at the relevant wavelength.

Under the conditions given in the recommended procedure the reagent may be used for precise determinations in the range from 0.08 to 2 p.p.m. of vanadium. With 0.32 p.p.m., the coefficient of variation was 0.60 per cent. for replicates analysed at one time; over a period of 3 months the corresponding value was 1.8 per cent. There are small, but significant, deviations from adherence to Beer's law, but accurate values are obtained by the use of a calibration graph. The limit of detection is about 0.004 p.p.m. of vanadium. Of the numerous foreign ions investigated, only chromium(VI), iron(II), iron(III), cerium(III) and cerium(IV) had appreciable interfering effects.

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Investigations on Long-path Absorption Tubes in Atomic-absorption Spectroscopy

BY IVAN RUBEŠKA AND BEDŘICH MOLDAN

(*Geological Survey of Czechoslovakia, Prague 7, Kostelní 26, Czechoslovakia*)

Although it is known that the use of long tubes in atomic absorption brings considerable increase in sensitivity, little is known about various factors affecting the sensitivity. In this work the rôle of fuel flow-rate for several elements (copper, silver, gold, cadmium, tin, lead, antimony, bismuth, manganese and rhodium) is studied and the results are discussed. A heated, 45-cm long, alumina tube is used, the burner being a Beckman type fed by air and hydrogen.

THE use of absorption tubes in atomic-absorption spectroscopy has two major advantages, the first being the considerable increase in the sensitivity of determination for several elements, and the second the improvement in the geometric stability of the flame gases in relation to the optical axis. This is particularly important when measuring lines near 2000 Å where the flame gases absorb.¹ It may, therefore, be expected that the flame-in-tube technique will be more widely used. To make full use of the possibilities offered by absorption tubes, investigations were carried through to find what factors affect the sensitivity of the elements in absorption tubes.

EXPERIMENTAL

APPARATUS—

The experimental arrangement has been described previously.² It consists of a 45-cm long, ceramic tube heated in an electric furnace and placed in the optical axis. Tubes of 17, 11 and 9-mm i.d. are used. At one end of the tube an air - hydrogen flame from a Beckman burner is introduced. Light from the source is focused at the entrance of the tube and is collected at the exit by a spherocylindrical lens and focused on the monochromator slit. The molecular spectra are scanned with a hydrogen lamp and a Zeiss G1B1 recorder. Because a simple d.c. measuring device was used our investigations were confined to the wavelength region below 3300 Å.

RESULTS

SENSITIVITY AND FUEL FLOW—

It has already been observed that the sensitivity of elements in absorption tubes depends markedly on the fuel flow-rate.³ For all the elements investigated, *i.e.*, copper, silver, gold, cadmium, tin, lead, antimony, bismuth, manganese and rhodium, a relatively steep absorbance increase was observed at a definite value of the hydrogen-to-air flow ratio. For most of the elements, namely copper, silver, lead, antimony and bismuth, the dependences had the form reproduced in Fig. 1. It was found that for a fuel-lean flame the small absorbance value suddenly increased, after which it remained constant. The critical value at which this change takes place depends, primarily, on the tube diameter. The greater the diameter of the tube the more ambient air is entrained, and the higher the necessary hydrogen flow. To a lesser extent it also depends on the burner position. The more the burner is tilted the more air is entrained, and the higher the required hydrogen flow.

The sensitivity obtained is generally higher the smaller the inclination of the burner from the vertical. Evidently less air is entrained and the flame gases are less diluted. However, to secure a laminar flow through the tube the angle between the burner and the optical axis cannot exceed a definite value. This was about 60° for the 17-mm tube, 45° for the 11-mm tube and 25° for the 9-mm tube. With this inclination the critical hydrogen-to-air flow ratios were 2.8, 1.9 and 1.5, respectively.

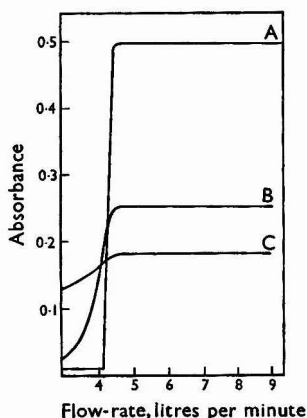


Fig. 1. Absorbance of curve A, 1 p.p.m. of copper; curve B, 1 p.p.m. of bismuth; and curve C, 0.2 p.p.m. of silver, with increasing hydrogen flow. Air flow, 2.5 litres per minute; corundum tube, 11-mm diameter

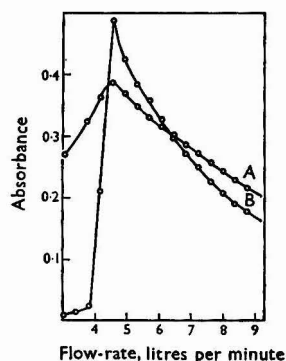


Fig. 2. Absorbance of curve A, 1 p.p.m. of gold; and curve B, 2 p.p.m. of rhodium. Conditions as in Fig. 1

For gold and rhodium the absorbance increase was followed by an absorbance fall again, as seen in Fig. 2. For manganese the sudden sensitivity increase was followed by a further slight increase up to a maximum value, shown in Fig. 3, and for tin the sensitivity increase was slow and shifted considerably towards higher hydrogen flow values, as shown in Fig. 4. Whereas for all other elements the curves for different tube diameters were simply shifted on the fuel-flow scale, with only minor effects on the maximum sensitivities, for tin a much higher sensitivity was found for small diameter tubes.

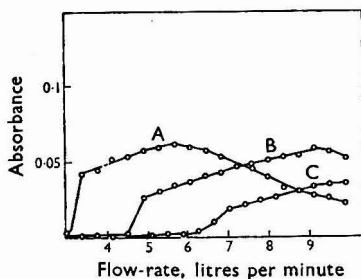


Fig. 3. Absorbance of 0.1 p.p.m. of manganese: curve A for corundum tube 9-mm diameter; curve B, for 11-mm diameter; curve C, for pyrolyan tube 17-mm diameter

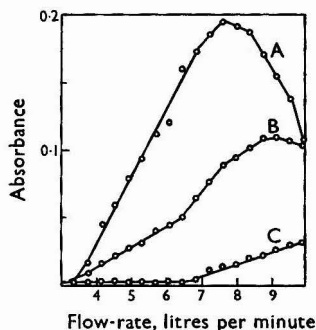


Fig. 4. Absorbance of tin. Same conditions as in Fig. 3

DISCUSSION OF THE RESULTS

The relatively high increase in absorbance at a critical hydrogen-to-air ratio suggests that a basic change in the experimental conditions takes place. Most probably the mixture of hydrogen and total air, *i.e.*, including the entrained air, becomes fuel-rich. By shielding the flame gases from the surrounding atmosphere a split flame, with the primary reaction zone at the entrance end and the secondary reaction zone at the exit end of the tube, results. The interconal gases then fill the whole tube length. Because the flame is split along a horizontal axis a considerable sensitivity increase is possible.

To confirm the existence of hydroxyl radicals along the whole tube length the absorption of the hydroxyl band head at 3089 Å was measured for different hydrogen flows (see Fig. 5). The dependence is similar to that of the previously mentioned elements, *i.e.*, with an increase

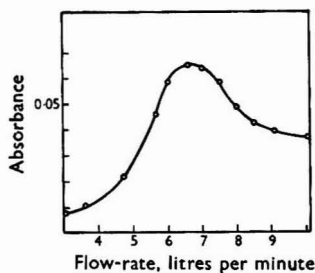
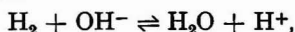


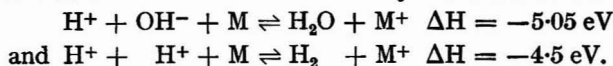
Fig. 5. Absorbance of OH radicals with increasing hydrogen flow. Pyrolan tube 17-mm diameter. Band head 3098 Å measured

at the critical values. The decrease of the hydroxyl concentration when increasing the hydrogen flow beyond the critical value is evidently caused by the formation of hydrogen radicals according to the reaction—



which proceeds immediately.⁴ The maximum absorbance value was about twenty times higher than that found for a hydrogen flame burning freely in air, *i.e.*, without the tube and combusted solely with entrained air. Because the concentration of hydroxyl radicals cannot vary considerably the high absorbance is evidently caused by the longer absorption path length with the absorption tube.

The energy available in the tube comes mainly from the re-combination reactions—



In both reactions M represents a third body capable of absorbing the energy released by the re-combination.⁴ It may be a water molecule but any other molecule, for instance, the oxide of the metal being determined, has the same effect. Oxides of all the elements investigated, with the exception of tin, have a smaller dissociation energy than the energy released by the re-combination of the hydrogen and hydroxyl radicals (see Table I). Any energy transfer may, therefore, result in their dissociation.

TABLE I

COMPARISON OF ENHANCEMENT FACTOR, DISSOCIATION ENERGY OF THE OXIDES AND VAPOUR PRESSURE

	Absorbance (maximum) Absorbance with fuel-lean flame	Dissociation energy, eV	Pressure, torr T = 1200° K
Silver	1.4	1.4	1.23×10^{-5}
Cadmium	1.7	3.8	> 760
Gold	2.2	—	4.69×10^{-7}
Bismuth	15	4.0	6.03*
Rhodium	34	—	< 10^{-10}
Antimony	45	3.8	6.54†
Manganese	60	4.0	3.61×10^{-5}
Lead	65	4.1	4.47×10^{-1}
Copper	~200	4.9	8.15×10^{-6}
Tin	~200	5.7	1.95×10^{-5}

* $p_{\text{Bi}} = 6.03$; $p_{\text{Bi}_2} = 1.74$.

† $p_{\text{Sb}} = 9.3 \times 10^{-3}$; $p_{\text{Sb}_2} = 1.02$; $p_{\text{Sb}_4} = 5.51$.

The absorbance increase at a critical value of the hydrogen flow is in correlation with the dissociation energy of the particular oxide. In Table I the elements are arranged in order of the observed enhancement factor (*i.e.*, the ratio of the maximum absorbance to absorbance with a fuel-lean flame), with their dissociation energies and vapour tensions. This correlation holds, with the exception of antimony, which is, however, predominantly present in the vapour phase as a dimer and tetramer,⁵ and the concentration of free antimony atoms is thus lowered. The existence of antimony molecules (Sb_2) was confirmed by the absorption spectrum,⁶ as shown in Fig. 6.

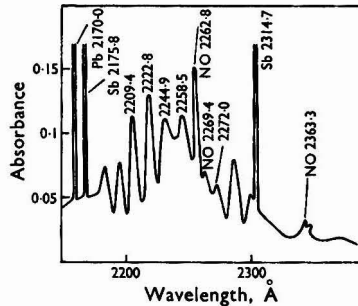


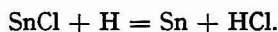
Fig. 6. Absorption spectrum of antimony(II). The solution sprayed contained 5000 p.p.m. of antimony, 1 g of tartaric acid, 6 ml of nitric acid per 100 ml (see NO bands in the spectrum). Lead was present as an impurity in antimony

The slow absorbance increase of manganese following the sudden increase is probably caused by the formation of $MnOH^7$ (dissociation energy = 3.15 eV), which dissociates readily as the OH concentration in post-flame gases decreases with increasing hydrogen flow. The absorption decrease observed for gold is probably caused by the formation of AuH (dissociation energy = 3.1 eV). For rhodium no values for the hydride are available. However, rhodium is the only element for which the saturation point of its vapour pressure is exceeded,³ so that the observed curve may be influenced by condensation of rhodium with decreasing temperature.

Tin presents the most intriguing problem and at this point our conclusions are rather speculative. It has already been observed that the dissociation of tin(II) oxide in a hydrogen-air flame is anomalously high and that a hydrogen-air flame provides a much higher concentration of free tin atoms than the oxy-hydrogen flame.⁸ The presence of nitrogen does not cause this difference because Gibson, Grossman and Cooke⁹ found, by using a Beckman burner, that no great change in absorbance of tin occurred when nitrogen was replaced by argon. They suggest, therefore, that the higher concentration of tin atoms in a hydrogen-air flame with a slower burning velocity is caused by a longer stay of the tin(II) oxide molecules in the reaction zone where reduction of tin oxide takes place.

In absorption tubes this reducing zone may be extended by increasing the hydrogen flow. The hydrogen flow required for maximum sensitivity is, therefore, considerably higher than for the elements with smaller dissociation energies. The reduction of tin may probably involve a reaction with hydrogen molecules, because tin(IV) oxide is easily reduced by hydrogen, even at temperatures below 800° C.

For elements other than tin no great difference in sensitivity is found when nitric acid or hydrochloric acid solutions are used, whereas for tin nitric acid has a slight depressive effect and hydrochloric acid a strong enhancing one. This is possibly because of the preferential formation of tin(II) chloride in the presence of hydrochloric acid and the reduction facilitated by the exothermal reaction—



The decrease of sensitivity with increasing tube diameter could then be attributed to a decrease of the partial pressure of hydrochloric acid in the flame gases because of a somewhat greater dilution by ambient air.

CONCLUSIONS

From these experiments it may be concluded that the best sensitivities are achieved when working under fuel-rich conditions, *i.e.*, when the primary and secondary reaction zones are at the entrance and exit ends of the tube, respectively. The sensitivity enhancement for different elements, when changing over from a normal to the split flame, increases with increasing dissociation energy of the metal oxide.

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The Nitrous Oxide-Hydrogen Flame in Spectroscopic Analysis

By R. M. DAGNALL, K. C. THOMPSON AND T. S. WEST

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

A high temperature flame, burning hydrogen supported by nitrous oxide, is described as an atom reservoir for thermal emission spectroscopy of elements such as aluminium. The flame has a low burning velocity and can be supported on most conventional burners supplied for use with air - acetylene in emission or absorption studies. Emission from aluminium at 3962 and 3944 Å shows a 2:1 intensity ratio and extends throughout the entire length of the flame. A detection limit of 4 p.p.m. at 3962 Å was obtained with a 1.5×10^{-2} -mm slit and a linear calibration graph between 20 and 200 p.p.m. Zinc, cadmium and lead, which show negligible emission in the air - hydrogen flame, emit strongly in nitrous oxide supported hydrogen. Calcium shows strong emission, even in the ionic doublet at 3934 and 3968 Å, particularly at low concentrations.

RECENT trends in spectroscopic analysis have been directed towards producing pre-mixed high temperature flames, which have the effect of increasing sensitivity in thermal-emission studies and of eliminating the depressive matrix effects produced by elements that form refractory oxides in atomic-absorption spectroscopy. The nitrous oxide - acetylene flame finds considerable application in this technique,¹ while oxy-acetylene is perhaps one of the most versatile flames in emission spectroscopy.²

One of the greatest general disadvantages of these flames is that they nearly all exhibit high background emission (the oxy-hydrogen flame being an exception, except over the OH band region). The background is not usually stable and hence leads to less precision and high limits of detection in both emission and absorption measurements, regardless of the method of amplifying the photomultiplier signal. In addition to this general disadvantage, most high temperature flames present other problems, *e.g.*, the high burning velocity of the nitrous oxide - acetylene flame makes burner design difficult, and the explosive nature of some mixtures such as oxy-cyanogen and oxy-acetylene renders them unsatisfactory for routine use. An equally important consideration is the anomalously high electronic excitation that occurs in some pre-mixed flames when metal salts are present,³ and is particularly strong in pre-mixed flames of hydrocarbons with air or oxygen and especially so with acetylene. As the oxy- and air - hydrogen flames that have favourable background radiations unfortunately seem to be completely free from this anomalous behaviour there appears to be no one flame at present that is completely acceptable as an atom reservoir for either thermal-emission or atomic-absorption studies. The present interest in atomic-fluorescence and thermal-emission spectroscopy makes the need for alternative high temperature, low background flames even more pressing.

It is the purpose of this paper to illustrate some of the advantages to be gained from the use of a pre-mixed nitrous oxide - hydrogen flame in the above areas. Firstly, this flame has a low burning velocity, unlike oxy-acetylene or oxy-hydrogen, and thus the burner design and gas mixtures are not critical. In fact, we have found that this flame can be burned on practically any commercially available burner head whether it is designed for emission or absorption measurements. In addition, the risk of explosion is no greater with this flame than with any other commonly in use.

The background radiation is also much lower with the nitrous oxide - hydrogen flame than with any hydrocarbon flame, because there is no possibility of emission from carbon species. The only background emission obtained under normal operating conditions results from OH bands at about 3000 to 3200 Å (present in all hydrogen or hydrocarbon-based flames), the NH band at about 3360 Å, and from the NH₂ radical or the reaction between O and NO in the visible region.³ In spite of this, the temperature of this flame is not thought to be any lower than the usually recognised high temperature flames, such as nitrous oxide - acetylene, oxy-hydrogen, etc.

Finally, the anomalously high electronic excitation that has been found in pre-mixed, oxygen-supported hydrocarbon flames is also marked in the nitrous oxide - hydrogen flame.³

In theory it would seem that this flame is well suited to the requirements of thermal-emission, atomic-absorption and atomic-fluorescence⁴ measurements.

At this time evidence is presented to support its use in thermal-emission studies.

EXPERIMENTAL

BURNER UNIT—

Initially the flame was burned on the 7 × 1.5-cm long, air - acetylene emission burner head supplied as a standard item with the Unicam SP900A thermal-emission - atomic-absorption flame spectrophotometer. This burner has a 1-cm square series of 13 holes near one end, and is particularly suitable for use with this instrument as a steady flame condition could be maintained by nebulising on 15 p.s.i. of nitrous oxide and supplying between 1.5 to 2.5 p.s.i. of hydrogen. The nitrous oxide was supplied to the burner via the normal instrumental nebulising system, but the hydrogen was supplied via an external gauge and was led into the burner through the jet fitted in the base of the burner stem.

Below 1.5 p.s.i. of hydrogen the flame struck back, and above 2.5 p.s.i. the primary reaction cones became unstable. This flame gave good emission signals for zinc (2139 Å) and cadmium (2288 Å) solutions, but relatively poor signals for aluminium (3944 and 3962 Å), which required a more fuel-rich flame (about 3 p.s.i.). Good aluminium emission signals were obtained by using the circular air - propane burner head supplied with the Unicam SP900A, and steady flame conditions were achieved with 15 p.s.i. of nitrous oxide and between 2.5 and 3.5 p.s.i. of hydrogen.

In the early stages of this work, the flame was lit with a small volume of nitrogen flowing through an auxiliary jet in the burner base⁵ to eliminate any danger of flash-back. When the required pressures of nitrous oxide and hydrogen had been supplied, the nitrogen flow was turned off. The reverse procedure was carried out when the flame was extinguished but this precaution is not essential, however, as only a comparatively weak "pop" is experienced when a flash-back occurs.

FLAME SPECTRUM—

The flame background of the nitrous oxide - hydrogen flame burning on the circular air - propane Unicam emission head showed above the yellow primary cones only strong OH band emission between about 3000 to 3200 Å, weak NH band at about 3360 Å and a steady continuum from 3500 to 6000 Å, which is partly caused by the reaction³ between NO and O. The primary cones, in addition, exhibit in the visible region the ammonia α-band system, which is now known to be caused by the NH₂ radical.³

The air - acetylene flame background, measured under conditions as similar as possible and also above the primary cones, was about four to five times greater than that of the nitrous oxide - hydrogen flame at about 4000 Å. Measurements in the primary cones showed an even greater difference in background radiation.

APPLICATION TO THERMAL EMISSION OF ALUMINIUM—

Thermal emission from aqueous solutions of aluminium at the resonance lines 3944 and 3962 Å was observed throughout the entire length of the nitrous oxide - hydrogen flame, but reached a maximum about 3 to 4 cm above the top of the burner head. Optimum emission was obtained by using the Unicam circular air - propane burner head with a slightly fuel-rich flame. A typical spectral plot is shown in Fig. 1. In agreement with the observations of other workers,⁶ who used different flames, the emission at 3962 Å was found to be about twice that at 3944 Å.

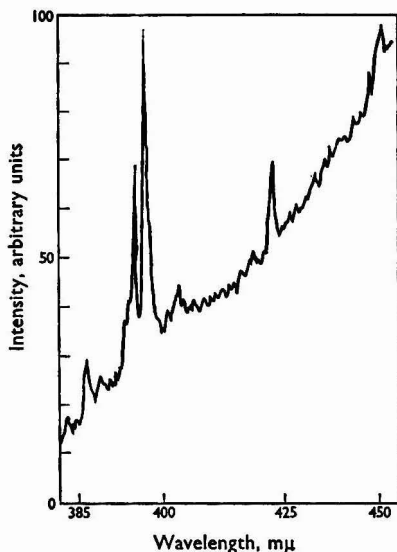


Fig. 1. Flame-emission spectrum of aluminium, obtained by nebulising a 1000 p.p.m. aluminium solution with a slit width of 0.008 mm, gain 3.5, band width 2, 15 p.s.i. nitrous oxide and 3.5 p.s.i. hydrogen

The limit of detection (signal-to-noise ratio of 1) measured at 3962 Å with a slit width of 0.015 mm was about 4 p.p.m. of aluminium. The calibration graphs plotted over the range 20 to 500 p.p.m. showed that between 20 to 200 p.p.m. they were linear, but above this a slight curvature developed and above 1000 p.p.m. of aluminium the log - log slope was about 0.56, indicating appreciable self-absorption.

The above limit of detection could be lowered by diluting the aqueous phase with organic solvents, for example, a solution in 20 per cent. isopropyl alcohol doubled the response of an equivalent concentration of aluminium in aqueous solution. This illustrates further advantages of this flame, *i.e.*, the ability to handle solutions containing high concentrations of inflammable organic solvents without risk or without need to modify the operating conditions.

Solutions containing 10 and 20 per cent. of glycerol, on the other hand, caused a signal decrease of 12 and 21 per cent., respectively. The differences in the nebulisation rates largely account for these effects.

PREPARATION OF CALIBRATION GRAPH FOR ALUMINIUM

REAGENTS—

Standard aluminium solution—Dissolve 1 g of aluminium foil in 40 ml of 1 + 1 hydrochloric acid (analytical-reagent grade) and dilute to 1 litre with distilled water to give a solution containing 1000 p.p.m. of aluminium.

Nitrous oxide and hydrogen from cylinders.

APPARATUS—

The equipment used in this work was a Unicam SP900A thermal-emission - atomic-absorption spectrophotometer (coupled to a Servoscribe 0 to 100-mV recorder), with an air - propane burner head. The normal E.M.I. 9529B photomultiplier supplied with the instrument was replaced by an E.M.I. 9601B photomultiplier, which is more sensitive in the ultraviolet region.

PROCEDURE (FOR 20 TO 2000 P.P.M. OF ALUMINIUM)—

Pipette 2 to 20 ml of the standard aluminium solution into 100-ml conical flasks and dilute to volume with distilled water. Nebulise aliquots of the solutions with 15 p.s.i. of nitrous oxide and measure the emission at 3962 Å with a hydrogen pressure of 3.5 p.s.i., slit width 0.015 mm, gain 3, 10, band width 3, and with the top of the burner head 3 to 4 cm below the centre of the monochromator slit.

OTHER EMITTING SPECIES—

Preliminary measurements indicate that this flame can also be used with advantage in thermal-emission studies of elements that do not form refractory oxides. For example, zinc, cadmium and lead, which normally show negligible emission in air - hydrogen flames,⁶ are readily excited in the nitrous oxide - hydrogen flame. The emission from these elements extends throughout the flame and is usually strongest about 2 cm above the top of the burner head. Comparable emission signals for zinc and cadmium in an air - acetylene flame can only be obtained by taking measurements from the primary reaction zone about 2 cm above the top of the burner head. The emission from lead atoms in the nitrous oxide - hydrogen flame is about twice that obtained in the primary reaction zone of an air - acetylene flame.

TABLE I
THERMAL-EMISSION STUDIES IN THE NITROUS OXIDE - HYDROGEN FLAME

Element	Concentration, p.p.m.	Wave-length, Å	Slit width, mm	Gain	Emission signals					
					Air - hydrogen		Air - acetylene		Nitrous oxide - hydrogen	
					<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
Zn	2000	2139	0.1	3,10	0	0	28	0	25	17
Cd	1000	2288	0.05	3,10	4	0	168	3	92	60
Pb	200	4057	0.01	3,5	4	4	42	23	62	78
Ca	40	3934	0.01	2,0	0	0	Background	0	86	20
	40	3968	0.01	2,0	0	0	too high	0	46	10
Al	1000	3962	0.012	3,5	0	0	↓	0	45	90

a corresponds to the emission in primary reaction zone.

b corresponds to the emission 3 cm above the top of the burner head.

Easily excited elements such as calcium can also be measured with advantage in the nitrous oxide - hydrogen flame. The ionic calcium doublet lines at 3934 and 3968 Å are quite strong and exhibit an intensity ratio of 2:1. Emission signals at these lines cannot normally be obtained either in air - hydrogen or air - acetylene flames. Table I shows a comparison of the emission signals of the above elements in normal nitrous oxide - hydrogen, air - hydrogen and air - acetylene flames on the Unicam SP900A spectrophotometer. Measurements were made in the primary reaction zone and about 3 cm above the top of the burner head.

The calcium emission from the ionic doublet at 3934 and 3968 Å was quite strong and at low calcium concentrations (less than 0.05 p.p.m.) was more intense than that from the 4277 Å resonance line. Table II shows the 4227:3934 Å intensity ratio for various calcium concentrations.

TABLE II
CALCIUM INTENSITY RATIO MEASUREMENTS

Calcium concentration, p.p.m.	Emission intensity ratio, 4227:3934 Å
40	29
16	21
4	8
2	5
0.4	3.3
0.1	1.4
0.05	1

Hence at low calcium concentrations appreciable ionisation must occur in this flame. The extent of the ionisation at these wavelengths is known to be sensitive to traces of the other easily ionised elements, *e.g.*, potassium, rubidium and caesium, and hence emission at these wavelengths is not normally used analytically. However, in their absence, *e.g.*, in distilled water, a more sensitive determination of calcium becomes possible in the nitrous oxide - hydrogen flame.

CONCLUSIONS

The nitrous oxide - hydrogen flame shows considerable promise as a strongly reducing, high temperature flame of low background for use in thermal emission or atomic absorption, even for elements such as aluminium that form refractory oxides. Other elements, such as cadmium, lead and zinc, which do not form such oxides, but which are not easily excited in an air - hydrogen flame, show strong atomic emission and absorption throughout the body of the flame. Calcium shows strong ionic emission, particularly at low concentrations. The above experiments show that the nitrous oxide supported hydrogen flame acts as a most efficient atom reservoir for a wide range of metals and is worthy of further examination.

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Determination of Low Levels of Sodium in Water by Using a Sodium-ion Responsive Glass Electrode

BY D. HAWTHORN AND N. J. RAY

(Central Electricity Generating Board, Midlands Region, Regional Research and Development Department, Hams Hall Power Station, Lea Marston, Sutton Coldfield, Warwickshire)

An E.I.L. sodium-ion responsive glass electrode has been tested under laboratory conditions.

The electrode responded linearly to sodium-ion concentrations over the range 0.25 to 25 p.p.m. of sodium, and the slope of the potential - concentration curve approximated to the theoretical value calculated from the Nernst equation. By correcting for the level of sodium estimated to be present in the pure water used to prepare the standards, a linear relationship could be obtained covering the range down to the level of sodium in the pure water. High precision could then be achieved for measurements made over the 0.004 to 25 p.p.m. range of sodium.

At low sodium levels the electrode is capable of detecting extremely small changes in sodium concentration. The possible errors involved in measuring sodium levels below 0.025 p.p.m. would tend to give results biased slightly high with respect to the true concentration. For plant control purposes this is generally preferable to readings biased low.

A sodium monitor would be capable of responding quickly to the changes in sodium concentration likely to be encountered in the water - steam circuit of a power plant.

A RELIABLE, continuous method of measuring sodium in the 0.005 to 25 p.p.m. range is required in the power industry.

In recent years, glass electrodes have been developed that are capable of responding selectively to sodium ions. Basically, the instrument used is a pH meter, but a sodium-responsive glass electrode is used in place of a pH glass electrode in a novel cell assembly. The manufacturers claim that sodium-ion concentrations in the 0.001 to 25 p.p.m. range can be accurately measured. Analysers have now been developed that appear to be suitable for both laboratory and plant applications, and these would, obviously, be of value in determining and controlling the level of sodium in the water - steam circuit of a modern power plant. This report describes the work carried out under laboratory conditions with apparatus borrowed from Electronic Instruments Ltd.

APPARATUS

ELECTRODE SYSTEM—

During the past few years, various types of glass have been formulated that are primarily responsive to sodium ions, with suppression of other cation response.¹ The theoretical equation for glass electrodes responding solely to sodium ions is—

$$E = E_0 + \frac{2.3026 RT}{F} \log a_{\text{Na}^+}$$

where a_{Na^+} is the sodium-ion activity.

At low levels of sodium, the activity coefficient can be maintained at a constant value by controlling the environment around the electrode, e.g., excess ammonium ions, and therefore the above equation can be written—

$$E = E_0 + \frac{2.3026 RT}{F} \log KC_{\text{Na}^+}$$

where C_{Na^+} is the concentration of sodium ions in gram ions per litre and K is a constant for the environment selected.

As with pH measurements, potential measurements can be made with reference to a conventional calomel electrode with a potassium chloride salt bridge.

Industrial, unscreened sodium-responsive glass electrodes manufactured by E.I.L., and designated GEA 28, were used throughout the work. The glass used in the fabrication of the sensitive bulbs is designated B.H.104. No specific details of the composition of the glass could be obtained from the manufacturers, but it is thought to be a sodium - lithium - aluminium - silica glass, possibly containing some boric oxide, the level of aluminium oxide or boric oxide, or aluminium oxide and boric oxide being the important factor determining selectivity to sodium ions. The inner reference system of the electrode comprised a silver - silver chloride electrode dipping into a hydrochloric acid - sodium acetate solution.

Before the tests were started, the electrode was activated by allowing the sensitive membrane to stand in 0.1 M sodium chloride for 72 hours (the manufacturers recommend at least 24 hours' immersion). Over the three months' period of the tests, no drifting or sluggishness of the electrode occurred and therefore no "re-activation" was necessary.

An E.I.L. R.J.23 calomel electrode, with a remote liquid junction of saturated potassium chloride, was used as the reference electrode.

BUFFER SYSTEM—

Mattock² has shown that with solutions buffered to a pH above 8.0 with triethanolamine, the sodium-responsive electrode gives a linear response over the range 0 to 4 pNa (23,000 to 2.3 p.p.m. of sodium), but some curvature is obtained from 4 to 5 pNa (2.3 to 0.23 p.p.m. of sodium). The presence of sodium impurities in the triethanolamine could have been responsible for this departure from linearity. In an attempt to overcome this difficulty, E.I.L. now recommend that ammonia vapour be used instead of a liquid buffer. In this work, ammonia vapour was produced by bubbling air through ammonia solution, and the ammonia vapour was subsequently absorbed by the solution being tested (see Fig. 1). By this method, the test solution could be buffered to a pH of about 11.0, and this further increased the possibility of hydrogen-ion interference.

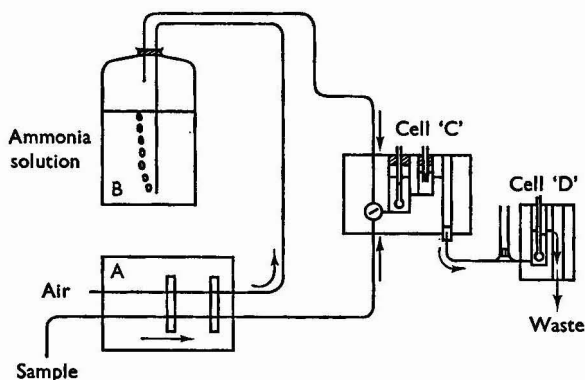


Fig. 1. General arrangement of apparatus

GENERAL ARRANGEMENT—

Figs. 1 and 2 show a general arrangement of the apparatus and details of the cell used to measure sodium-ion concentration. To minimise contamination from glassware, the cell assemblies were made in Perspex and connecting tubing was flexible PVC.

A Technicon peristaltic pump, A, was used to deliver 2.8 ml of sample per minute and 1.85 ml of air per minute into the apparatus. The air was bubbled through about 20 per cent. w/v ammonia solution in a Winchester quart and ammonia vapour was carried through to the cell, C, through inlet, F, and into chamber, G, where it was absorbed by the sample entering from E. A Perspex-covered magnetic stirrer was rotated in the chamber to ensure adequate mixing of vapour and liquid. The alkaline solution was passed over the sodium-responsive glass electrode, L, and then on to the porous plug of the salt bridge of a calomel.

reference electrode, M. Excess of ammonia vapour and air were removed through hole, H. To minimise the back-diffusion of potassium ions from the salt bridge into the measuring cell, the head of potassium chloride was never higher than 1 inch above the overflow, O, and the tip of the remote liquid junction just touched the surface of the solution as it overflowed from the cell. The fine bore of the flow path, J, also helped to restrict back-diffusion of potassium chloride.

The electrodes used to determine sodium concentration were connected to a high impedance electrometer (E.I.L. Vibron 39A), which in turn was connected to a Kent "Dynamaster" recorder.

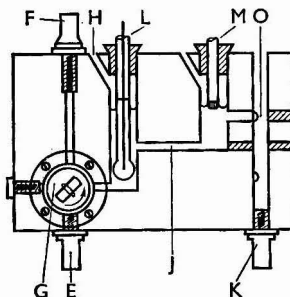


Fig. 2. Details of cell "C"
(Lettered parts of the apparatus are referred to in the text)

On leaving cell, C, via K, the solution was passed into a second cell, D, which was used to monitor the pH. The cell was comprised of an E.I.L. pH glass electrode G.H.S.23 and an E.I.L. calomel reference electrode R.J.23. The pH electrodes were connected to an E.I.L. 28A pH meter and a Cambridge D.E. recorder. Slight variations in pH were observed when the porous plug of the reference electrode was placed downstream of the glass electrode. When the positions were reversed, however, a much steadier pH record was obtained.

The pump, cells and ammonia solution were kept in a cabinet, which was fitted with a thermostatically controlled heater. No provision was made for cooling, however, and the temperature of the cabinet varied slightly (27° to 30° C) with changes in ambient conditions.

EXPERIMENTS AND RESULTS

PREPARATION OF STANDARD SOLUTIONS—

A 1000 p.p.m. sodium stock solution was prepared from sodium chloride, and standard solutions down to 0.025 p.p.m. of sodium were prepared by progressive dilution with distilled water. The presence of traces of sodium in pure distilled water and from contamination arising from apparatus during handling makes the preparation of accurate standards below 0.025 p.p.m. extremely difficult. During this work, therefore, standards below 0.025 p.p.m. were considered to be water containing a small unspecified amount of sodium, to which controlled additions of sodium had been made. The amounts of sodium added were progressively decreased by factors of 10 until additions equivalent to 0.00025 p.p.m. of sodium were reached. Distilled water subjected to an identical handling procedure, but without an addition of sodium, suffered an increase in sodium content, and is therefore referred to in this paper as "contaminated" distilled water. A single batch of distilled water was used for preparing the above solutions.

The same experimental procedure was adopted when water treated by powdered ion-exchange resins was used to make up the solutions in an attempt to obtain an over-all lower level of contamination than with distilled water.

PREPARATION OF CALIBRATION GRAPHS—

The standard solutions were pumped into the instrument, as previously described, in increasing order of strength, starting with distilled water and finishing with a 25 p.p.m. standard solution, and the potential reading was noted for each addition. These results were treated as follows to obtain a calibration graph.

A plot of observed potential against concentration of sodium added to distilled water followed the form described by Mattock² and is shown in Fig. 3. Practically, the effect on the 0.25, 2.5 and 25 p.p.m. standards of traces of sodium in distilled water, and of the slight contamination arising from apparatus, is negligible; consequently they can be considered as "true" standards. A line of "best fit" was calculated from these potential-concentration values and was extrapolated to the potential line for contaminated distilled water.

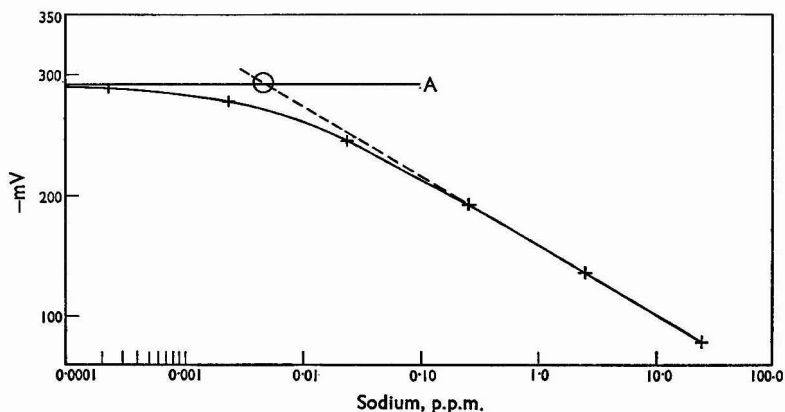


Fig. 3. Plot of observed results and first approximation to sodium in "contaminated" pure water: A, "contaminated" pure water

The concentration at the intersection of these lines was taken as the first approximation to the sodium content of the contaminated distilled water. All the solutions had been prepared identically, and therefore their total concentrations would be higher than the known sodium additions by an amount equal to that in the contaminated distilled water (0.005 p.p.m.). All the points were corrected on this basis, and re-plotted against potential. The best straight line was calculated and is shown graphically in Fig. 4. The intersections of this line with the lines representing potential readings of distilled and glass-contaminated distilled water then gave second approximations to the sodium concentrations of these waters.

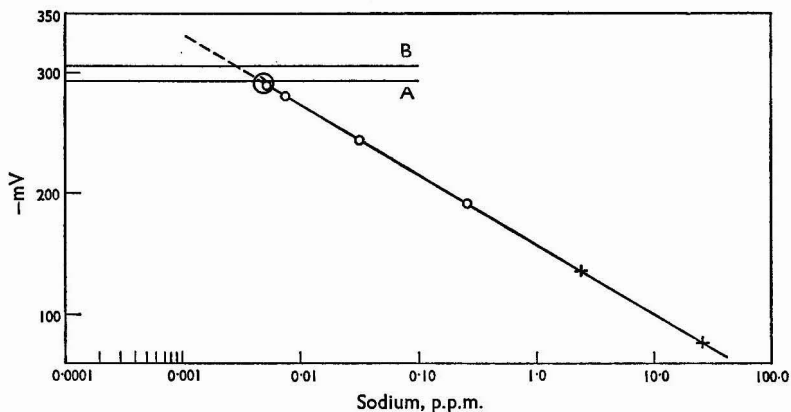


Fig. 4. Plot of previous results corrected for the effect of contamination of samples: A, "contaminated" pure water; B, pure water: O, corrected points; X, original points

A similar procedure was adopted with the results obtained when de-ionised water was used to prepare the standard solutions (see Fig. 5). It can be seen that this water had a much lower sodium content than the distilled water previously used.

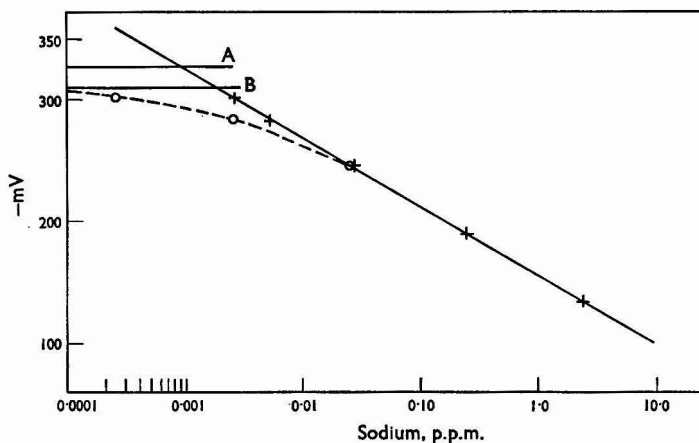


Fig. 5. Calibration graph obtained with water treated by powdered ion-exchange resins: A, water treated by ion-exchange resins; B, "contaminated" de-ionised water: O, observed results; X, corrected results

PRECISION TESTS—

Starting with a 25 p.p.m. standard solution, distilled water was "spiked" to give increases of 0.00025, 0.0025, 0.025, 0.25 and 2.5 p.p.m. of sodium, as described previously.

The potentials of this range of solutions were measured on ten different occasions over a period of 7 days, with a fresh batch of solutions for each series of measurements. A batch of measurements was always made starting with the lowest and finishing with the highest concentration level. Previously, it had been noticed that on isolated occasions at low sodium concentrations, some interference associated with the operation of the pump was obtained on the recorder and, as a result, the trace appeared as a band 8 mV wide. As earlier work had established that identical results could be obtained with either static or flowing solutions,

TABLE I
OBSERVED RESULTS OF PRECISION TESTS

Batch No.	pH	Temperature, °C	Potential, mV							
			Stock distilled water	Con-tamin-ated distilled water	Additions of sodium to distilled water, p.p.m. of sodium					
					+0.00025	+0.0025	+0.025	+0.25	+2.5	+25.0
1	10.95	27.8	311	305	304	289	257	201	145	84
2	11.00	28.6	314	308	304	294	253	198	138	78
3	10.95	28.5	309	303	299	296	248	194	137	77
4	11.00	29.8	309	303	299	287	251	197	139	79
5	10.95	28.7	311	303	299	289	254	199	141	81
6	10.95	30.0	308	302	300	290	255	201	143	82
7	10.90	29.2	310	302	297	288	253	198	139	80
8	11.00	28.5	313	305	298	290	256	202	142	83
9	10.85	28.5	314	307	300	293	259	199	144	81
10	10.95	29.4	306	299	293	281	249	195	136	76
11	10.90	28.3	306	299	294	285	252	195	136	77
12	10.95	29.3	315	306	298	290	255	201	141	81
Mean ..	10.95	28.89	310.5	303.5	298.8	288.5	253.5	198.3	140.1	79.9
Standard deviation		0.65	3.1	2.8	3.3	3.5	3.2	2.3	3.1	2.5

it was decided to take readings under static conditions to avoid the possibility of interference from the pump during the precision tests; accordingly, the following procedure was adopted. When a steady trace was obtained after the introduction of each fresh solution, the pump was stopped for 30 seconds and the potential noted; two further readings were obtained during $\frac{1}{2}$ -minute stops after runs of $2\frac{1}{2}$ minutes. The three readings of potentials were always found to be the same.

The pH of the solution leaving the cell was continuously recorded, and the temperature inside the cabinet was measured when each batch of samples was analysed.

TABLE II

RESULTS OF PRECISION TESTS CORRECTED, ASSUMING A CONSTANT POTENTIAL AT +0.25 P.P.M. OF SODIUM

	Stock distilled water	Con-tamin-ated distilled water	Addition of sodium to distilled water, p.p.m. of sodium					
			+0.00025	+0.0025	+0.025	+0.25	+2.5	+25.0
Mean potential, mV ..	312.1	305.3	300.5	290.2	255.2	200	141.8	81.6
Standard deviation ..	2.5	2.6	3.0	2.7	1.5	0	1.6	1.0
Equivalent concentration of standard deviation, p.p.m. of sodium ..	0.0003	0.0004	0.0005	0.006	0.0015	0	0.1	1.0

Table I shows the results obtained during these precision tests. The results given in Table II have been derived by correcting the observed values of potential to a constant-potential reading (200 mV) at the 0.25 p.p.m. level. This is equivalent to standardising the instrument with a 0.25 p.p.m. solution before the analysis of each batch of solutions. Slopes of lines of best fit were calculated from these values of potential by using all of the points, after making an allowance for the level of sodium present in the contaminated distilled water, and the 0.25, 2.5 and 25 p.p.m. points. E_0 values were calculated from the slopes given by all of the points. The theoretical slope at the temperature of the cabinet was calculated from the Nernst equation. The results obtained are given in Table III, in which details are given of the levels of sodium estimated to be present in contaminated distilled and distilled waters, respectively, as calculated from intercepts of the appropriate lines.

TABLE III

VALUES OF CALCULATED SLOPES AND ESTIMATED LEVEL OF SODIUM IN DISTILLED WATER

	Sodium, p.p.m.			mV per 10-fold change in concentration			
	Distilled water	Con-taminated distilled water, 1st approximation	Con-taminated distilled water, 2nd approximation	Slope calculated from all of the corrected points	Slope calculated from the 0.25, 2.5 and 25.0 p.p.m. points only	Theoretical slope	E_0 + mV
Mean	0.0032	0.0043	0.0040	58.71	59.25	59.53	91.7
Standard deviation	0.0003	0.0005	0.0004	0.51	0.50	0.13	2.4

RESPONSE—

A similar series of tests was carried out to determine the response time of the system to various changes in sodium concentration in the 0.005 to 25 p.p.m. range. A 90 per cent. response was achieved in less than 3 minutes, and steady-state conditions were reached in less than 5 minutes. Essentially, these times are those required to displace and rinse the previous solution from the system. The electrode appeared to respond instantaneously to changes in concentration within the range studied.

DISCUSSION

PREPARATION OF CALIBRATION GRAPHS—

In the preparation of the calibration graphs shown in Figs. 3, 4 and 5, the assumption was made that the electrode gives a response of constant slope down to the level of sodium present in the water used to prepare the "spiked" solutions.

The two main factors likely to cause departure from linearity are changes in activity coefficient with concentration of sodium ions and the influence of hydrogen or other ions on the selectivity of the glass electrode for sodium ions at low concentrations. Changes in activity coefficient at low sodium levels would be expected to be extremely small when a constant ionic background was used, and under the experimental conditions used, therefore, the ratio of hydrogen (or possibly ammonium) to sodium-ion activity is the major factor that could affect the response of the electrode. E.I.L.³ state that interference occurs when the ratio a_{H^+}/a_{Na^+} exceeds a critical value (about 10^{-4}) and that the response of the electrode to changes in sodium will decrease; consequently the slope of the calibration graph at low sodium levels would decrease. Readings taken from a calibration graph constructed on the basis of linear response at low levels of sodium could, therefore, give high values with respect to the true sodium concentrations, which, for power station applications, is preferable to readings biased low.

The method of extrapolation shows that the maximum bias cannot be greater than the estimated level of sodium in the "contaminated" water. During this work, water from powdered ion-exchange resins gave the lowest level of sodium in the "contaminated" water (0.0018 p.p.m.), and demonstrated that bias must be within this level under these conditions. The true value of bias might be even lower, but a much purer water than was available during these investigations would be required to establish this point.

The calibration graphs show clearly that at low levels of sodium slight changes in sodium concentration (0.00025 p.p.m.) can be detected.

PRECISION TESTS—

The results given in Table I show that a high level of precision could be achieved, even under unfavourable operational conditions. For example, the variations in potential observed over several days included errors arising from (a) electrometer drift, (b) change of slope with temperature, (c) slight day-to-day variations in the sodium contents of the stock, "contaminated" and "spiked" solutions, and (d) changes in ammonia solution concentration and pH caused by variations in the rate of evolution of ammonia with temperature (this is not an equilibrium condition).

In practice, the monitor now supplied by E.I.L. can be calibrated either manually or automatically with a standard solution of sodium, and a correction will be applied automatically to compensate for any electrometer drift. In addition, a fixed theoretical slope-to-temperature relationship is assumed and changes in temperature will be compensated for automatically. Variations caused by (a) and (b) will therefore be negligible. A relatively strong solution will be used (0.1 to 0.2 p.p.m. of sodium) for standardising the instrument so that variations from (c) will be minimised. The instrument will be thermostatically controlled, hence variations caused by (d) would not be expected to arise.

Results in Table II were obtained by applying a correction based on an assumed constant potential of 200 mV at the 0.25 p.p.m. sodium level. From Table III it can be seen that the mean slope calculated from the 0.25, 2.5 and 25 p.p.m. points was only slightly lower than the mean theoretical slope. The mean slope calculated from all of the points, however, was somewhat lower, even after both corrections had been applied, being 97.8 per cent. of the theoretical. This indicates that there is a slight decrease in response at low sodium levels. An instrument calibrated on the basis of a theoretical slope would therefore tend to give values biased slightly high with respect to values obtained from a practically derived slope. From the results obtained when water treated by powdered ion-exchange resin was used the maximum bias possible from the basic assumption of linear response can be fixed.

CONCLUSIONS

The sodium-sensitive glass electrode responds linearly to changes of concentration over the range 0.025 to 25 p.p.m. with solutions buffered from pH 10.8 to 11.0, and the slope of potential against concentration approximates to that calculated from the Nernst equation.

After corrections have been applied below this range for the sodium present in the "pure" water used to prepare "spiked" solutions of sodium, the linear calibration graph can be extended and used down to the level of sodium in the pure water (say 0.001 p.p.m.). The maximum bias introduced by using this method would be +0.0018 p.p.m. of sodium.

At low sodium levels, the electrode is capable of detecting slight changes in sodium concentration. For example, at the estimated 0.005 p.p.m. level a change in concentration of 0.00025 p.p.m. could be detected, corresponding to an observed potential difference of 5 mV.

By using the commercial E.I.L. instrument, which operates on a fixed theoretical slope - temperature relationship, it should be possible to monitor sodium levels in the range of 0.005 to 25 p.p.m. to a high level of precision. At levels below 0.025 p.p.m. possible errors may arise from the assumption, which is made when calibrating the instrument, that a constant theoretical slope of potential - concentration applies throughout this concentration stage. Results would be biased slightly high relative to the true concentrations.

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Automated Procedure for the Simultaneous Determination of Phosphorus and Nitrogen in Plant Tissue

By W. D. BASSON, D. A. STANTON AND R. G. BÖHMER

(South African Co-op Citrus Exchange, P.O. Box 14105, Lyttelton, Pretoria, South Africa)

An automated technique for determining phosphorus in plant tissue in an aliquot of the acid-digested sample issuing from the AutoAnalyzer Kjeldahl Analyzer digester unit, by an adaptation of the molybdovanadophosphoric yellow-colour procedure, is described. Optimum reaction conditions have been established, and results indicate that the phosphorus determination can be made within the limits of accuracy normally acceptable for similar determinations.

In this laboratory, Technicon AutoAnalyzer instrumental systems are used for determining nitrogen and phosphorus in plant tissue. Nitrogen is determined with the automated Kjeldahl Analyzer,¹ and phosphorus by the molybdovanadophosphoric yellow-colour colorimetric procedure after dry ashing. If, however, the two systems could be coupled and the determinations made simultaneously, the rate of obtaining analytical results would be increased and a considerable saving in manpower made possible.

This paper describes the work carried out to develop a technique that could be used to determine phosphorus in an aliquot of the acid-digested sample issuing from the AutoAnalyzer digester unit. For this purpose, the molybdovanadophosphoric yellow method developed by Kitson and Mellon² was decided upon for adaptation, because of its simplicity and relative freedom from interference by various anions and cations.

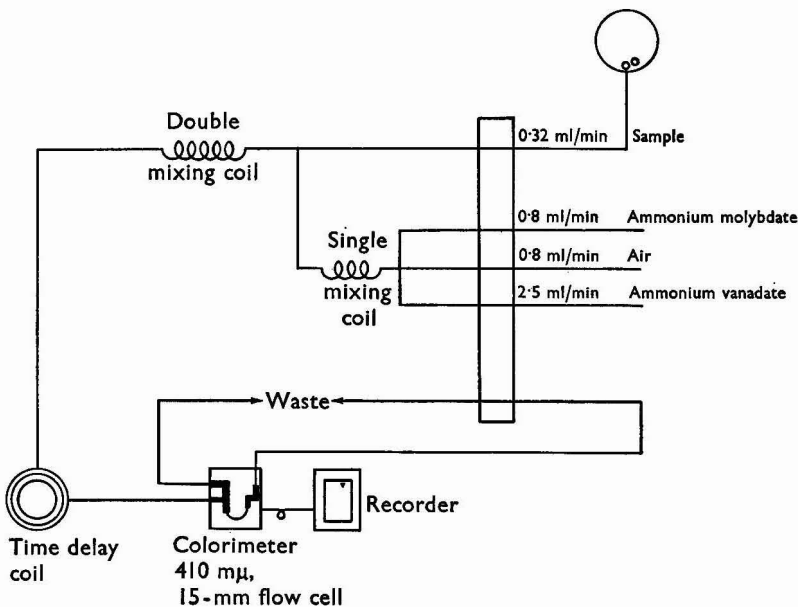


Fig. 1. Flow diagram of apparatus for flow system I

This study was based on the assumption that no volatilisation of phosphorus compounds occurred^{3,4,5,6} during the digestion stage, or, alternatively, that if it did occur, it would be of little consequence because of the identical conditions to which standards and unknowns were subjected. Subsequent comparisons showed that the values obtained by the technique developed and conventional methods are in good agreement.

EXPERIMENTAL

Studies to establish reactant conditions at different concentrations were carried out with the simplified flow system shown in Fig. 1. This obviated the comparatively lengthy step of passing samples through the digester unit. Subsequently, the flow system shown in Fig. 2 was used to determine the phosphorus content of the plant digest issuing from the digester unit.

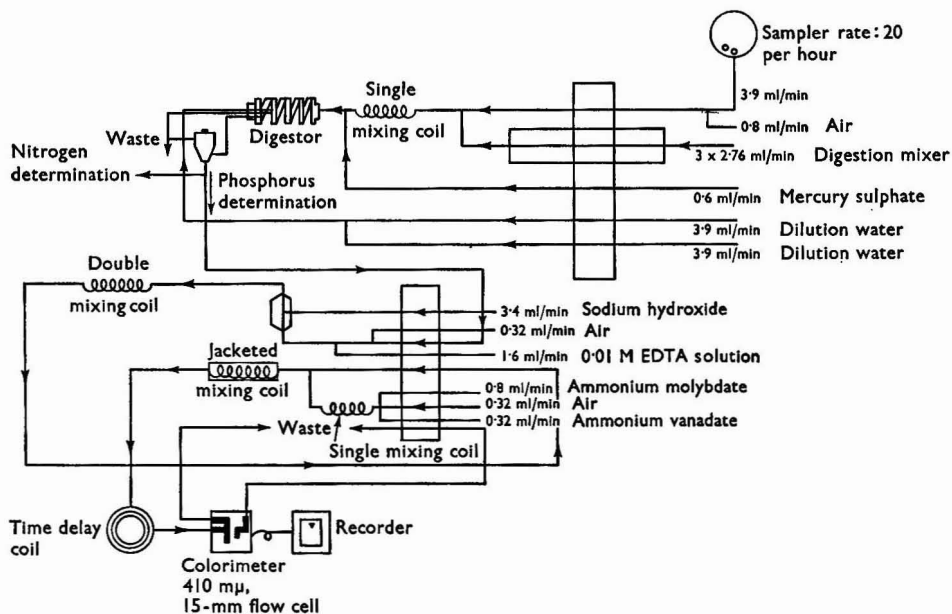


Fig. 2. Flow diagram of apparatus for flow system II

In both of the flow systems the ammonium vanadate and ammonium molybdate solutions are introduced separately into the system. Initially the determination was attempted with a system similar to that developed by Varley,⁷ in which the solutions are pre-mixed during preparation and delivered through one tube into the system. With such a system, phosphorus determinations are neither accurate nor reproducible, and a constant drift in the base-line was observed during a 3 to 4-hour period. Ferretti and Hoffman⁸ observed a similar drift, and attributed this to incomplete clearing of the flow cuvette between samples. They overcame the problem by devising a flow scheme whereby additional water was pumped into the system between samplings. With flow system I, it was found that introducing the vanadate and molybdate reagents separately into the flow system effected a more satisfactory and less complicated solution. The appearance of the AutoAnalyzer chart recording when the reagents are introduced separately is shown in Fig. 3. No base-line is discernible. When flow system II was coupled to the digester system, base-line drift could not be completely eliminated by introducing the vanadate and molybdate reagents separately into the flow system. (This is probably due to pump surging and incomplete clearing of the flow cuvette because of the high salt content prevailing.) By introducing standard phosphorus samples more often in any series of determinations, this effect can, however, be effectively controlled.

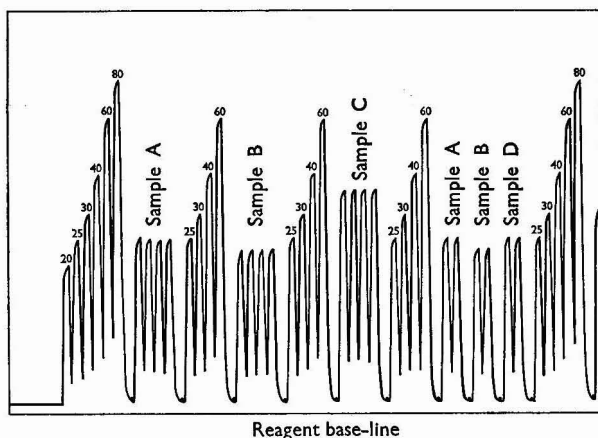


Fig. 3. The AutoAnalyzer chart recording for the separate introduction of the ammonium vanadate and ammonium molybdate reagents; numbered peaks are standards in p.p.m. of phosphorus

REAGENTS FOR FLOW SYSTEM I—

Ammonium metavanadate solutions—Prepare a series of solutions containing 0.025, 0.05, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 g of analytical-reagent grade ammonium vanadate in 1 litre of distilled water.

Ammonium heptamolybdate solutions—Prepare a series of solutions containing, 15, 25, 30, 35, 45, 55, 60 and 70 g of analytical-reagent grade ammonium heptamolybdate in 1 litre of distilled water.

Standard phosphorus solutions (i)—Prepare a series of five standard solutions containing 10, 20, 40, 80 and 100 p.p.m. of phosphorus from analytical-reagent grade sodium dihydrogen orthophosphate.

Standard phosphorus solutions (ii)—Prepare a series of fourteen solutions each containing 40 p.p.m. of phosphorus and volumes of hydrochloric acid to give concentrations of 0.04, 0.09, 0.13, 0.17, 0.22, 0.27, 0.31, 0.35, 0.40, 0.44, 0.53, 0.62, 0.71 and 0.82 M when diluted to 100 ml with distilled water.

REAGENTS FOR FLOW SYSTEM II—

On the basis of results described in the first section of the Results and discussion, the following vanadate and molybdate concentrations are used.

Ammonium metavanadate solution—Dissolve 4 g of ammonium metavanadate in 1 litre of distilled water.

Ammonium heptamolybdate solution—Dissolve 70 g of analytical-reagent grade ammonium heptamolybdate in 1 litre of distilled water.

Digestion mixture—Dissolve 6 g of selenium dioxide in a little water and add 1.8 litres of concentrated sulphuric acid, followed by 40 ml of 70 per cent. perchloric acid.

Mercury(II) sulphate solution—Dissolve 35 g of mercury(II) sulphate in 1 litre of a 10 per cent. sulphuric acid solution.

Solution A—Slowly add 910 ml of the digestion mixture to a flask containing 845 ml of distilled water, followed by 66 ml of the 3.5 per cent. mercury(II) sulphate solution and 170 ml of concentrated sulphuric acid.

Standard phosphorus solutions (i) and (ii)—To determine the combined effects of the mercury(II) sulphate, selenium dioxide and perchloric acid on the determination, the following two series of standard solutions containing 10, 20, 40 and 60 p.p.m. of phosphorus, respectively, are prepared.

(i) Sufficient stock solution is placed in a 100-ml calibrated flask to obtain the desired phosphorus concentrations, water is added to give a total volume of 11.40 ml and this solution diluted to 100 ml with solution A. This solution represents conditions after completion of

the digestion stage as far as acidity, perchloric acid, selenium dioxide and mercury(II) sulphate concentrations are concerned.

(ii) Sufficient stock solution is placed in a 100-ml calibrated flask to obtain the desired phosphorus concentrations, and sulphuric acid is added to obtain a solution about 8 M with regard to sulphuric acid.

SAMPLE PREPARATION FOR FLOW SYSTEM I—

Weigh 3 g of a dried-leaf sample into a flat-bottomed silica dish and place it in a furnace for $2\frac{1}{2}$ hours at 490°C . Cool the dish, add 5 ml of concentrated hydrochloric acid and evaporate the mixture to dryness on a water-bath; continue heating for half an hour. Add 5 ml of concentrated nitric acid, followed by 10 ml of distilled water and warm the solution for a few minutes on a water-bath to aid dissolution. Filter into a 100-ml calibrated flask, wash with warm water and dilute to volume.

SAMPLE PREPARATION FOR FLOW SYSTEM II—

Weigh 0.5 g of plant material into a 50-ml Pyrex beaker. Introduce 10 ml of concentrated sulphuric acid, followed by the slow addition of 15 ml of distilled water. Cover the beaker with a watch-glass and allow it to stand for 20 minutes. This suspension is then placed into the sampler cups for the determination of phosphorus.

RESULTS AND DISCUSSION

ESTABLISHMENT OF REACTANT CONDITIONS—

The influence of variation in the total acid concentration (sample plus vanadate solution) and in ammonium molybdate concentrations, on the colour development, when using flow system I with a standard phosphorus solution containing 40 p.p.m. of phosphorus and maintaining the ammonium vanadate concentration constant at 0.50 g per litre, is shown in Fig. 4.

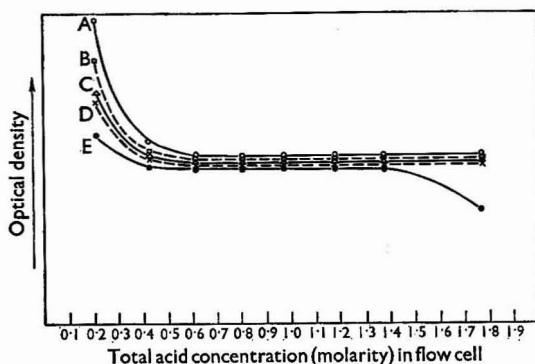


Fig. 4. The effect of molybdate concentration on the optical density of a 40 p.p.m. phosphorus solution with 0.50 g per litre of ammonium vanadate and different acid concentrations: curve A, 55 g; curve B, 45 g; curve C, 35 g; curve D, 25 g; and curve E, 15 g of ammonium molybdate per litre

For all molybdate concentrations a sharp increase in optical density is observed as the acid concentration decreases below 0.3 M, this increase being less significant at lower molybdate concentrations.

For molybdate concentrations less than 25 g per litre, the optical density is observed to decrease as the acid concentration increases above 0.7 M. This effect is not manifested at molybdate concentrations above 25 g per litre.

The results obtained by maintaining the ammonium molybdate concentration constant at 55 g per litre, while varying the vanadate and acid concentrations for a series of 40 p.p.m.

of phosphorus solutions, are shown in Fig. 5. A sharp decrease in optical density is observed at the higher acid concentrations for ammonium vanadate concentrations of less than 0.60 g per litre. This effect becomes more pronounced as the vanadate concentration decreases.

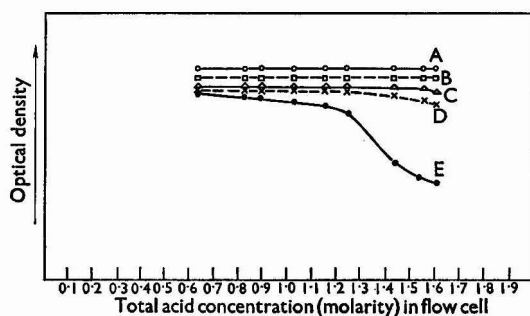


Fig. 5. The effect of vanadate concentration on the optical density of a 40 p.p.m. phosphorus solution with 55 g per litre of ammonium molybdate and different acid concentrations: curve A, 1.20 g; curve B, 0.80 g; curve C, 0.60 g; curve D, 0.40 g; and curve E, 0.20 g of ammonium vanadate per litre

We concluded from these results that, when using flow system I, a final acid concentration of about 0.7 M, and ammonium vanadate and ammonium molybdate concentrations of 1.00 and 50 g per litre, respectively, would provide the most satisfactory results.

Leaf samples were now prepared as previously described for flow system I, and the solutions obtained used to test the reproducibility of peak heights. The results for three samples, *viz.*, A, B and C, are shown in Fig. 3.

TABLE I
THE PHOSPHORUS CONTENT OF PLANT-TISSUE SAMPLES DETERMINED
BY VARIOUS PROCEDURES

Laboratory reference number	Phosphorus, per cent.		
	Acid extract of dry-ashed sample, flow system I	Sample digested on AutoAnalyzer, flow system II	Acid extract of dry-ashed sample, manual molybdovanadophosphoric yellow colorimetric procedure
1	0.124	0.131	0.133
2	0.106	0.112	0.103
3	0.087	0.093	0.092
4	0.123	0.130	0.124
5	0.104	0.113	0.108
6	0.114	0.111	0.119
7	0.164	0.167	0.174
8	0.145	0.138	0.144
9	0.126	0.119	0.123
10	0.109	0.114	0.102
11	0.126	0.134	0.124
12	0.137	0.126	0.132
13	0.105	0.116	0.102
14	0.089	0.094	0.082
15	0.115	0.123	0.125
16	0.154	0.144	0.134
17	0.192	0.183	0.175
18	0.124	0.118	0.132
19	0.215	0.206	0.214
20	0.085	0.084	0.075
21	0.234	0.216	0.224
22	0.105	0.113	0.102
23	0.085	0.094	0.083

Identical peak heights for each duplicated standard or sample were obtained, and a constant return to the original reagent base-line between samples or standards over the range 0 to 80 p.p.m. is also observed. The phosphorus values obtained for a series of plant-tissue samples are compared with the values obtained by the manual molybdophosphoric yellow method,⁹ in Table I. It will be observed that the results compare favourably.

DETERMINATION OF PHOSPHORUS CONTENT IN PLANT MATERIAL AFTER DIGESTION ON THE AUTOANALYZER DIGESTOR SYSTEM—

The acid digest issuing from the digester unit is about 8 M. It is, therefore, necessary to neutralise the digest substantially to obtain the desired acid concentration previously decided upon. In order to investigate the feasibility of such a step, the flow system shown in Fig. 2, but uncoupled from the digester unit, was used. Ammonium molybdate and ammonium vanadate concentrations were kept constant and the sodium hydroxide concentration was varied.

At sodium hydroxide concentrations below 280 g per litre, the results were non-reproducible and irregularities in the read-out curves occurred at the peak heights, arising from the high acid concentration still prevailing. At concentrations higher than 350 g per litre, a low degree of reproducibility was obtained, and blockages occurred as a result of crystallisation from the standard sodium sulphate solution. A sodium hydroxide concentration of 300 g per litre gave the most constant and reproducible results (Fig. 6).

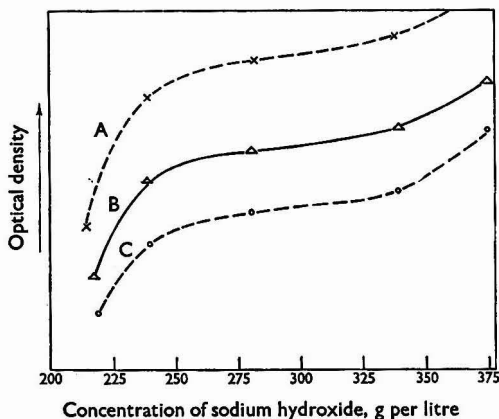


Fig. 6. The effect of sodium hydroxide concentration on the optical density at constant vanadate and molybdate concentrations: curve A, 30 p.p.m.; curve B, 20 p.p.m.; and curve C, 10 p.p.m. of phosphorus solution

In the digestion of plant tissue for the nitrogen determination, mercury sulphate, selenium dioxide, perchloric and sulphuric acids are used. To determine the combined effect of these substances on the phosphorus determination, the two series of standard phosphorus solutions prepared under Reagents for use with flow system II were compared. No difference in the percentage transmission for equivalent phosphorus concentrations was observed. This indicates that the perchloric acid, selenium dioxide and mercury sulphate used in the digestion stage had no effect on the determination.

Flow system II, coupled to the AutoAnalyzer digester system, was then used to digest and determine the phosphorus content of plant-tissue samples suspended in sulphuric acid (Fig. 3), as previously described.

Initially, irregularities occurred in the reagent base-line. This was, however, improved by substituting a 0.01 M EDTA solution for the diluting water.

Typical recorder charts obtained are shown in Figs. 7 (a) and (b). Fig. 7 (a) shows the reproducibility of replicate determinations on one sample suspension. The reproducibility of determinations on six independently prepared suspensions of sample, H, are shown in Fig. 7 (b). The peak heights V, S, P and T in Fig. 7 (b) represent 0.10, 0.08, 0.13, 0.16 per cent. of phosphorus, respectively.

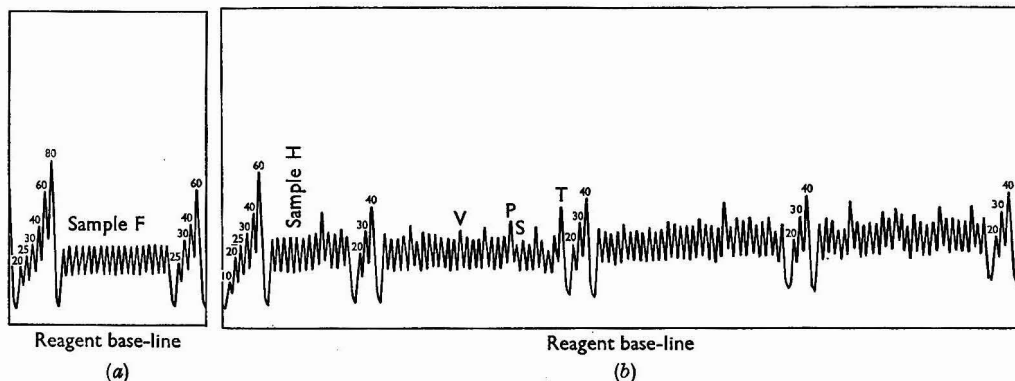


Fig. 7. Typical chart recordings: (a) the reproducibility of replicate determinations on one sample suspension; (b) the reproducibility of determinations on six independently prepared suspensions: numbered peaks are standards in p.p.m. of phosphorus: peak heights V, S, P and T represent 0.10, 0.08, 0.13 and 0.16 per cent. of phosphorus, respectively

The percentage phosphorus values obtained by using this method are compared with values obtained by the manual method in Table I. No significant differences between the results are apparent.

CONCLUSION

The results obtained in these experiments indicate that phosphorus and nitrogen may be determined simultaneously on the digest issuing from the AutoAnalyzer digester unit. The phosphorus determination can be made within the limits of accuracy normally acceptable for similar determinations.

We gratefully acknowledge the assistance of Mr. N. C. Basson in various aspects of the laboratory work.

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Thin-layer Chromatographic - Enzyme Inhibition Procedure to Screen for Organophosphorus Pesticides in Plant Extracts without Elaborate Clean-up

By C. E. MENDOZA, P. J. WALES, H. A. MCLEOD AND W. P. MCKINLEY

Research Laboratories, Food and Drug Directorate, Department of National Health and Welfare, Ottawa 3, Ontario, Canada)

A procedure is described for rapidly screening some organophosphorus pesticides in plant extracts without elaborate clean-up. Azinphos-methyl, carbophenothion, diazinon, ethion, malathion, mevinphos and parathion are extracted with acetonitrile and partitioned into hexane before analysis by the thin-layer chromatographic - enzyme inhibition technique. These pesticides, in the presence of apple, beet, carrot, lettuce, pea or potato extractives, are detected on thin-layer chromatographic plates coated with a silica-gel layer. The experiment indicates that the equivalent of only a few milligrams of the original sample is required for semi-quantitative analyses of residues present at, or above, their tolerance levels.

THE rapid procedure for screening some organophosphorus pesticides by using thin-layer chromatography and total-phosphorus determination by the molybdenum-blue reaction proposed by Abbott, Burrige, Thomson and Webb¹ was an improvement over the other procedures summarised in their paper. The analysis, although quantitative, is most suitable for plant materials with known pesticide treatment. Because the spots are not visible, the resolution of a mixture of pesticides must be greatly improved to ensure that the correct areas are scraped off for analysis. Ott, Hearth and Gunther² reported another rapid thin-layer chromatographic screening procedure, but only for parathion in canned peaches without clean-up.

In our laboratory, an enzyme-inhibition technique, in which indoxyl and substituted indoxyl acetates were used with steer-liver homogenate, was developed to detect nanograms of organophosphorus pesticide standards resolved by thin-layer chromatography.³ Because of its high degree of sensitivity, reproducibility and rapidity of detection, we developed this method to screen for organophosphorus pesticides in plant extracts without elaborate clean-up.

The loss or degradation of pesticides incurred by storing the plant extracts at room temperature, and in the cold, was determined by thin-layer chromatography. The efficiency of extracting and partitioning the pesticides into hexane was qualitatively evaluated by thin-layer chromatography in which the capacity of the plates to resolve the pesticides in the presence of plant extractives was evaluated by spotting various amounts of extracts. Various aliquots from each extract were concentrated and analysed by thin-layer chromatography to find the most convenient and simple step for screening organophosphorus pesticide residues.

EXPERIMENTAL

PESTICIDE STANDARDS⁴—

Purity is expressed in percentages and Canadian tolerance levels⁵ in p.p.m. A standard solution was prepared containing each of the following pesticides:

- Azinphos-methyl (Guthion)—93.0 per cent., 1.0 p.p.m.
- Carbophenothion (Trithion)—94.6 per cent., 0.50 p.p.m.
- Diazinon—95.8 per cent., 0.10 p.p.m.
- Ethion—95.0 per cent., 0.10 p.p.m.
- Malathion—99.5 per cent., 8.00 p.p.m.
- Mevinphos α -isomer (Phosdrin)—60.0 per cent., 0.25 p.p.m.
- Parathion—98.8 per cent., 1.00 p.p.m.

The mixed pesticide standard solution was prepared with hexane, with co-solvent acetone used for initial dissolution of azinphos-methyl only; 1 ml of this solution when added to a

50-g plant sub-sample gave the tolerance levels⁵ of the seven organophosphorus compounds. Concentrations (w/v) were not corrected for impurities* in the standards.

A typical chromatogram of the pesticide standard mixture is shown in Fig. 1; spots were white on a blue background.

GENERAL PROCEDURE—

*Extraction*⁶—Extract a 50-g sub-sample with 150 ml of acetonitrile in a food blender at about 6000 r.p.m. for 5 minutes. Repeat the extraction, then filter the homogenate through coarse sintered glass under vacuum and rinse the glassware with acetonitrile. Evaporate the acetonitrile in the filtrate and rinsings with a rotary evaporator at 35° C until only the water phase remains. If frothing occurs, vigorously shake the filtrate with 50 to 100 ml of hexane and continue the evaporation.

*Partition*⁶—Shake the water phase vigorously with 50 ml of hexane in a 250-ml separating funnel for 30 seconds; repeat the partition step twice. If three layers appear, evaporate this 3-layered mixture with the rotary evaporator; repeat the partition, continuing only when two layers separate. Combine the three 50-ml hexane fractions in a 200-ml volumetric flask; make up to volume with hexane and thoroughly mix the contents. Discard the water layer.

Development of the thin-layer chromatographic plates—The previous paper³ outlined the procedures for preparing the liver homogenate and the 5-bromoindoxyl acetate spray solution.

Spot the concentrates and standard mixtures on thin-layer chromatographic plates coated with a 450- μ thick Kieselgel G-HR^(R) layer. Chromatograph the plates with 20 ml of acetone diluted to 100 ml with hexane. Remove the plates when the solvent front reaches the 15-cm height previously marked. Air-dry the plates for 5 minutes, then evenly treat them with bromine vapour. Allow at least 20 minutes for the excess bromine to dissipate, then spray the plates with steer-liver homogenate diluted with 0.05 M Tris buffer, pH 8.32, until the gel is just thoroughly wet; leave them for 20 minutes at room temperature. Similarly, spray the plates with 5-bromoindoxyl acetate spray solution, and within a few minutes the sites of inhibited enzymes appear as white spots on a blue background.

OUTLINE OF EXPERIMENT—

Apple, beet, carrot, lettuce, pea and potato samples were divided into 50-g sub-samples, some of which were fortified with the seven organophosphorus pesticides at tolerance levels, and the analyses were carried out on sub-sample extracts refrigerated at about 9° C and on those left at room temperature, 23° to 26° C.

Aliquots from the 200-ml hexane fractions were concentrated 20 times. The 4 and 10-ml aliquots of the extracts stored at room temperature were concentrated just before analysis. The 100-ml aliquots were concentrated soon after the partition step and were stored in the cold or at room temperature.

The experiment on refrigerated, concentrated 100-ml aliquots, which was replicated once, consisted of control sub-samples, C, and sub-samples fortified after the partition step, F. For each plant sample, one C and two F sub-samples were analysed on duplicate thin-layer chromatographic plates 2 days after extraction.

The experiment on extracts and on concentrated 100-ml aliquots left at room temperature consisted of sub-samples C, F and BE (fortified before the extraction step). For each plant sample, two of each of the C, F and BE sub-samples were analysed on duplicate plates 2 days after extraction. The two C and two F sub-samples were further analysed, also on duplicate plates, 5 and 8 or 9 days after extraction.

Each sub-sample extract, without elaborate clean-up, was analysed with a gas-liquid chromatograph equipped with electron-capture detector, under the conditions used by McCully and McKinley⁷ but with 190° to 195° C column temperature.

RESULTS

DETERMINATION OF THIN-LAYER CHROMATOGRAPHIC PLATE CAPACITY—

The chromatograms of the concentrated aliquots equivalent to 100, 150 and 200 mg of samples (Fig. 2, upper plates, and Fig. 3) were slightly different from one another, but

* The impurities were detected, even although they were a small percentage of the standard, because they were probably more potent inhibitors than the parent compound. On the other hand, mevinphos gave only one spot although the other insecticidally active related compound was 40 per cent.

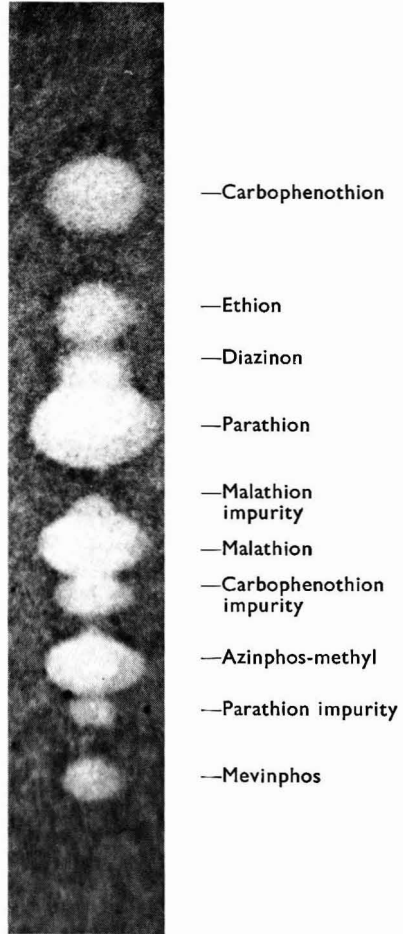
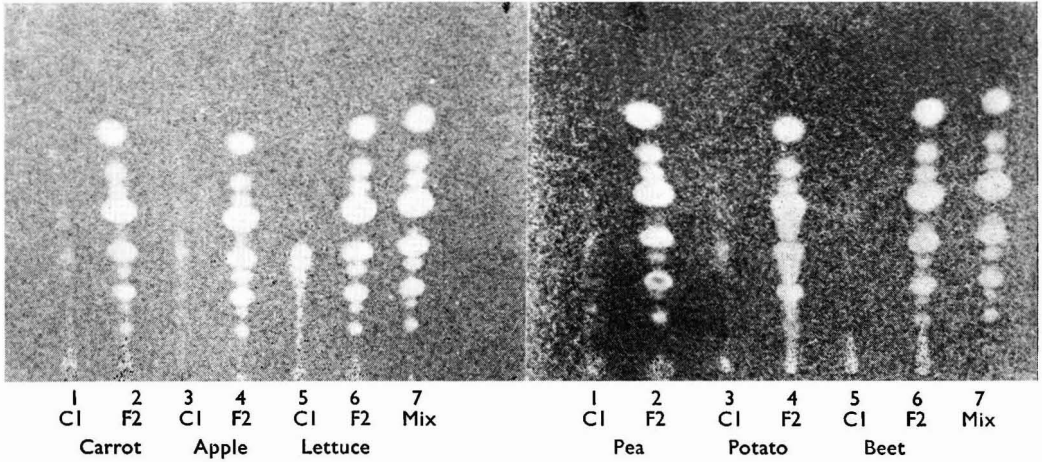


Fig. 1. A typical chromatogram of the seven organophosphorus pesticides represented by white spots on an intense blue background

150-mg sample equiv. wt.
 5-Bromoindoxyl acetate
 pH 8.32
 20% acetone in hexane



500-mg sample equiv. wt.
 5-bromoindoxyl acetate
 pH 8.32
 20% acetone in hexane

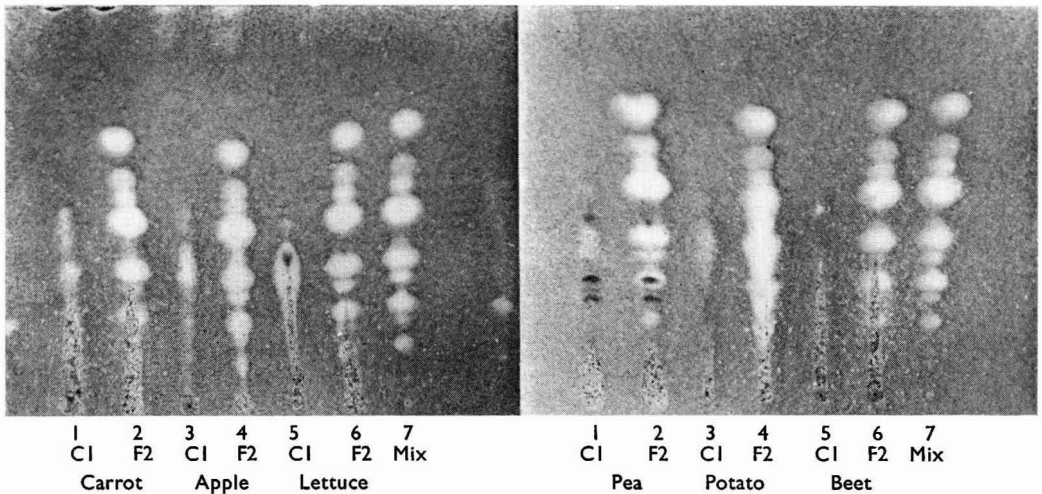


Fig. 2. Top, thin-layer chromatograms of six plant extracts (fortified and control), each spot equivalent to 150 mg of sample; extracts stored in a refrigerator 4 to 12 days before. Bottom, thin-layer chromatograms of the same six plant extracts, each spot equivalent to 500 mg of sample. Silica-gel layer, 450 μ thick; steer-liver homogenate and 5-bromoindoxyl acetate spray solutions, pH 8.32; solvent system, 20 ml of acetone diluted to 100 ml with hexane. ("Mix" means pesticide standard mixture)

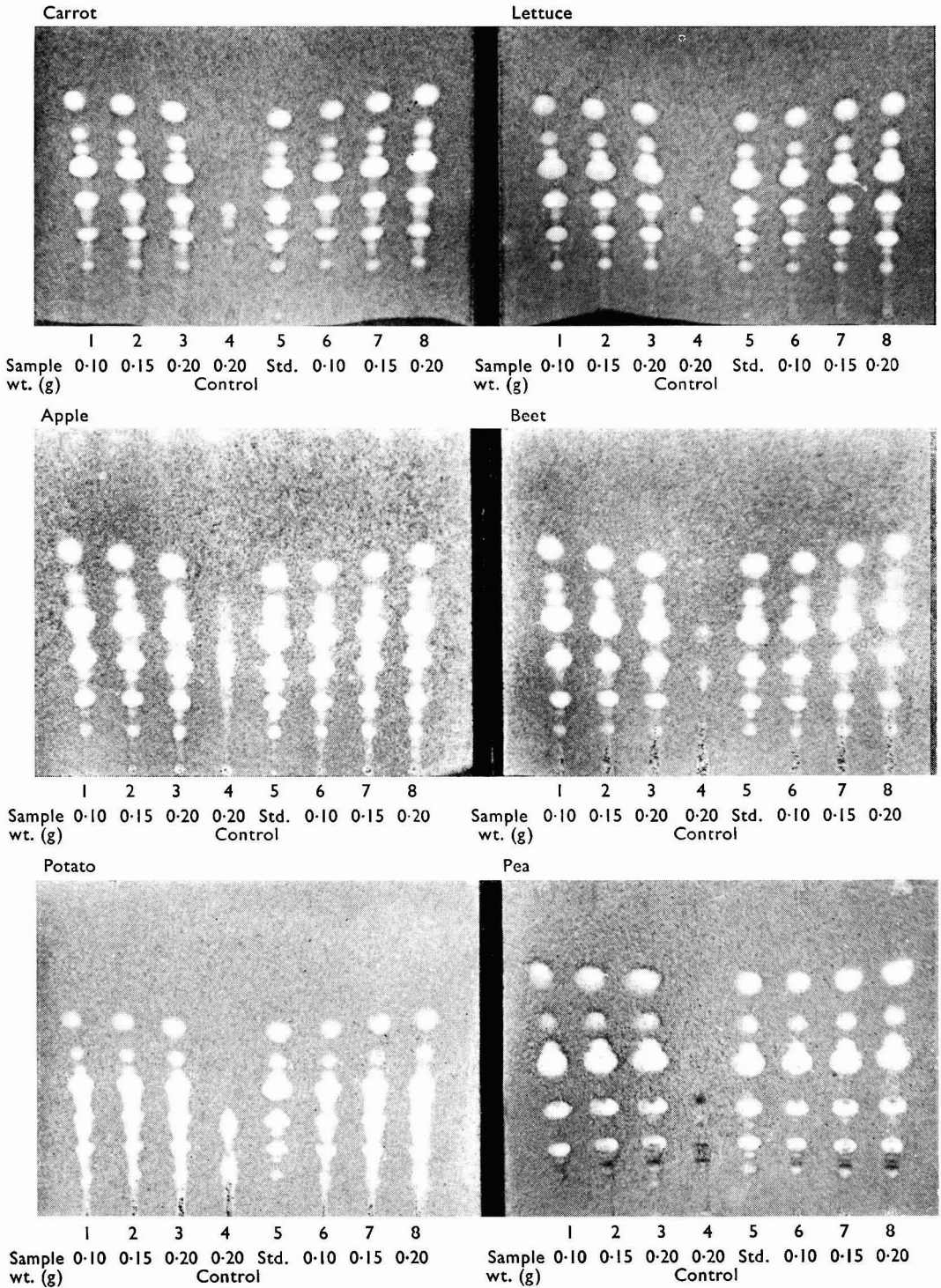


Fig. 3. Thin-layer chromatograms of six crops fortified at the tolerance levels with seven organo-phosphorus pesticides, developed plates coated with $450\text{-}\mu$ thick silica-gel layer and sprayed with steer-liver homogenate and 5-bromoindoxyl acetate solutions at pH 8.32. Solvent system was 20 ml of acetone diluted to 100ml with hexane. ("Std." means pesticide standard mixture)

markedly different from the chromatograms of the 500-mg samples (Fig. 2, lower plates). All seven organophosphorus pesticides and the enzyme-inhibiting impurities in the standards produced clearly delineated spots. The mevinphos spot from the 100-mg sample faded a few minutes after development; otherwise, there was no marked difference among the chromatograms of the 100, 150 and 200-mg samples. However, separation of the seven pesticides was seldom as distinct in the 500-mg samples (Fig. 2, lower plates) as in the 100, 150 and 200-mg samples (Fig. 3).

Pesticides added to carrot, lettuce, apple, beet and pea samples produced well defined spots on thin-layer chromatographic plates. Only carbophenothion and ethion in potato were clearly separated; spots for compounds below ethion were obscured by the streaking effect of the potato components.

In general, the spots caused by the organophosphorus pesticides were readily distinguished from those of the plant components. Some areas of enzyme inhibition in the controls were attributable to components in the plant but most disappeared a few hours after development. Most plant pigments disappeared when brominated, and the remainder did not interfere with the detection of pesticides.

In some chromatograms, the gel layer was weakened along the path travelled by plant components; the gel was blown off when sprayed with the homogenate and substrate solutions. Extracts of beet, carrot and lettuce (Fig. 2) weakened the layer most. However, the weakened area did not extend above the position for malathion, even with the 500-mg samples.

EFFECT OF STORAGE—

The chromatograms of the 2-day old concentrated aliquots left at room temperature (23° to 26° C) did not differ from those of aliquots stored in the cold (about 9° C) for various lengths of time and from the corresponding standard solutions. Storage periods were 30 and 43 days for apple, 26 and 32 days for beet, 23 and 29 days for carrot, 31 and 46 days for lettuce, 30 and 36 days for pea, and 31 and 52 days for potato. Degradation of some plant pigments occurred but did not affect the detection of the added pesticides.

Extracts stored in the original and concentrated states at room temperature gave similar thin-layer chromatograms 2, 5 and 8 or 9 days after extraction. The quality of detection of pesticides remained the same, indicating no apparent change in the concentration of pesticides during the storage, although carrot, lettuce and pea extracts showed some pigment degradation.

SELECTION OF ALIQUOT SIZE—

The chromatograms of 30 μ l from concentrates of 4, 10 and 100-ml aliquots of each of the crop extracts (equivalent to 1, 2.5 and 25 g of the crop) did not show any apparent difference among aliquots; the quality of the spots corresponding to the added pesticides did not vary from that of the standards.

The 10-ml aliquot was the most convenient; the volume of the solvent required to re-dissolve the residue of the 4-ml aliquot was small and difficult to handle, and the several evaporating and transferring steps required for the 100-ml aliquot were tedious.

EFFICIENCY OF EXTRACTION—

The chromatograms of BE and F sub-samples suggested no loss of organophosphorus pesticides during the extraction with acetonitrile, flash-evaporation of acetonitrile and partition into hexane.

GAS - LIQUID CHROMATOGRAPHY, WITH ELECTRON-CAPTURE DETECTOR, OF EXTRACTS WITHOUT FURTHER CLEAN-UP—

An equivalent of 0.25 mg of each plant sample (5 μ l taken from the 200-ml hexane extract) gave the chromatogram shown in Fig. 4. The pesticides at concentrations simulating tolerance level residues in plant materials gave peaks too disproportionate for simultaneous screening by gas - liquid chromatography. Malathion, carbophenothion and parathion showed as distinct peaks but the malathion peak was off-scale. The peaks for ethion and azinphos-methyl were too low for proper measurement; mevinphos and diazinon were not detected. In addition, constituents or contaminants in some plant extracts interfered with the detection.

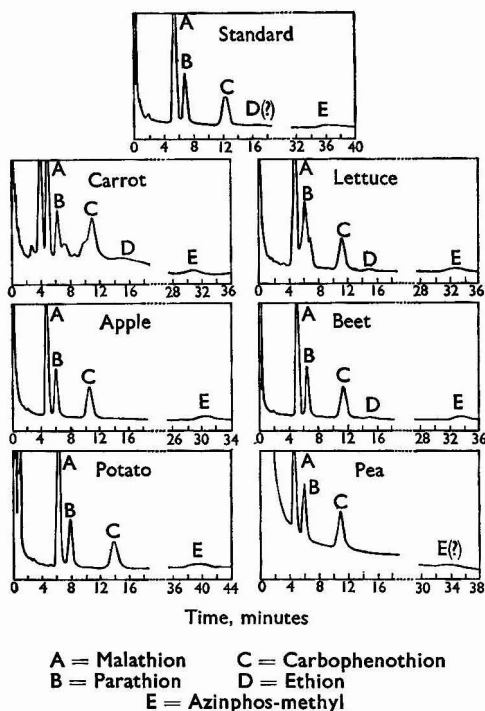


Fig. 4. Chromatograms (gas - liquid chromatograph equipped with electron-capture detector) of pesticide standard mixture and 6 fortified plant extracts. (Mevinphos and diazinon peaks are not showing and others are very disproportionated)

DISCUSSION AND CONCLUSION

A sensitive and reproducible detection of the seven organophosphorus pesticides in samples equivalent to 150 mg was obtained by using thin-layer chromatographic plates with a 450- μ thick silica-gel layer. The plates were sprayed with steer-liver homogenate, followed by 5-bromoindoxyl acetate spray solution, both at about pH 8.32. The sample, equivalent to 150 mg, used for thin-layer chromatographic analysis was most conveniently obtained from a 10-ml aliquot of the extract in hexane concentrated to 0.5 ml.

From the chromatograms of the seven organophosphorus pesticides in the presence of plant extractives, gas - liquid chromatography with electron-capture detector was shown, under the conditions used, to be unreliable for screening purposes.

GENERAL COMMENTS—

To a certain extent, the application of this method to the screening for pesticides in crops of unknown history is perhaps based on whether the enzyme inhibitor is present or not (see pea control), whether the amount of the inhibition may be attributed to the pesticides or not (see apple and potato controls), and whether the intensity of the inhibition may be interpreted as caused by the presence of pesticides at questionable levels or not (see carrot, lettuce and beet controls; the quality of inhibition will not correspond to malathion tolerance level, if it is malathion).

In this experiment, some expediency for semi-quantitative analysis was demonstrated by spotting a fraction rather than the entire aliquot. Based on the lowest limit of detection of individual pesticides by this procedure, a fraction of an aliquot may be selected to detect only that pesticide present at, or above, the tolerance level. Thus, a pesticide tolerated at

relatively high levels in foods (*e.g.*, malathion) or a strong inhibitor (*e.g.*, parathion) can be detected by thin-layer chromatography of aliquots that are too small for the detection of other pesticides present at their tolerance levels.

This procedure is readily adaptable to routine analysis of the compounds and crops studied. As solutions can be stored for at least 8 days at room temperature, or at least 23 days in the cold, several samples may be extracted before simultaneous analysis by thin-layer chromatography; solutions showing questionable residues may then be re-analysed for confirmation.

We propose that this procedure be used to screen for any of the seven organophosphorus pesticides studied in apple, beet, carrot, lettuce, pea or potato samples. Reduction of sub-sample size to reduce operating time further and extension of the scope to cover more pesticides, crops and developing systems will be investigated.

Acknowledgement is made to Dr. K. A. McCully and Dr. T. K. Murray for review and criticism, and to Mr. H. Baird and Mr. B. Korda for photography.

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A Test-paper Method for the Determination of Toluene Di-isocyanate Vapour in Air*

By D. A. REILLY

(Imperial Chemical Industries Limited, Dyestuffs Division, Hexagon House, Blackley, Manchester 9)

A test-paper has been developed that will enable up to 0.10 p.p.m. v/v of toluene di-isocyanate (TDI) vapour to be determined in a 5-litre sample of air drawn through a circle of the paper 1 cm in diameter at the rate of 1 litre per minute. In the presence of toluene di-isocyanate a reddish grey stain is produced, development of which is complete within 15 minutes of the end of sampling. The intensity of the stain is compared with artificial standards made by coating paper with specially formulated pigment suspensions. The 2,4- and 2,6-isomers react equally.

The test-papers are produced by treating Whatman No. 1 filter-paper with a solution of sodium nitrite, 2-hydroxy-11*H*-benzo[*a*]carbazole-3-carboxy-*p*-anisidide (Brenthol GB), ammonium acetate and diethyl phthalate in methanol. They are stable for at least 3 months when stored in the dark.

This method is simpler than existing "wet" methods and can be carried out by less skilled operators.

In existing methods of determining toluene di-isocyanate (TDI) in air,^{1,2} the air sample is drawn through a bubbler containing an acid-trapping medium, in which the toluene di-isocyanate is hydrolysed to tolylenediamine. This is then diazotised and coupled with *N*-1-naphthylethylenediamine dihydrochloride to produce a reddish purple solution, which is compared visually with artificial colour standards. Although this approach is sound, a considerable degree of analytical skill is needed that is not always available to industrial users of toluene di-isocyanate. An alternative simpler method has therefore been sought.

It is an established procedure to determine traces of nitrite or nitrogen dioxide by reaction with an aromatic primary amine to produce the corresponding diazonium compound, which then couples either with the same amine or with a second component to produce a colour. An example of this is the Saltzman reagent³ for determining nitrogen dioxide in air, in which the initial diazotisation of sulphanic acid is followed by coupling in the same solution with *N*-1-naphthylethylenediamine dihydrochloride. It seemed logical to try and use this type of reaction to determine isocyanate by reaction with a mixture of sodium nitrite and a suitable coupling component. This approach has proved successful. The work described below was concerned with the choice of reagents, composition of the solutions used to prepare the test-papers, measurement of the sensitivity of the papers towards di-isocyanate, specificity and quantitative reproducibility of the indication, and with the production of artificial colour standards.

DESCRIPTION OF THE METHOD

REAGENTS AND MATERIALS—

Sodium nitrite—Analytical-reagent grade.

Ammonium acetate—Analytical-reagent grade.

Brenthol GB (recrystallised)—Recrystallise Brenthol GB, 2-hydroxy-11*H*-benzo[*a*]carbazole-3-carboxy-*p*-anisidide (see Note 1), three times from pyridine containing 20 per cent. of water. Dry at 100° C.

Methanol—Analytical-reagent grade.

Diethyl phthalate.

Whatman No. 1 filter-paper, 9 and 15-cm circles.

Monolite Fast Red 2RVS paste—See Note 1.

Monastral Fast Blue BVS paste—See Note 1.

Monolite Fast Yellow GNVS paste—See Note 1.

China clay, S.P.S. grade—Obtainable from English China Clays Ltd.

* The subject of British Patent Application No. 34835/67.

Butakon ML 505 latex—See Note 1.

Thin matt white card—An example is Zara White Card, 10 × 12 inches, reference 1/4606, obtainable from R. A. Brand Ltd. Bridge Paper Mills, Manchester 6.

Test-papers—Weigh into a 250-ml round-bottomed flask 0.5 g of recrystallised Brenthol GB. Add a mixture of 35 ml of diethyl phthalate and 115 ml of methanol, both measured carefully with graduated cylinders. Attach a water-cooled reflux condenser, and boil under reflux for 30 minutes. Cool to room temperature, detach the flask and filter its contents through a Whatman No. 1 filter-paper, folded into a cone, into a clean, dry 250-ml measuring cylinder. Wash out the flask with one or two small portions of methanol and use these, in turn, to wash down the filter-paper. Finally, dilute the contents of the cylinder to exactly 150 ml and mix well. Into a conical flask, place 2.0 g of sodium nitrite, 0.15 g of ammonium acetate and 3.0 ml of water. Swirl it gently until all of the solid has dissolved and add exactly 100 ml of the filtered Brenthol GB solution. Mix well and pour the solution into a 6-inch diameter evaporating dish.

With metal forceps, immerse Whatman No. 1 filter-papers, 9 cm in diameter, in the solution, one at a time, for 30 seconds, remove and quickly blot off surplus liquid between several thicknesses of 15-cm diameter filter-paper (at least two thicknesses above and below). Hang them up to dry for 1 hour at room temperature in darkness or subdued light in an atmosphere free from chemical fumes. From each dried circle cut three strips, 5 × 2 cm. Store in a glass bottle, either painted black on the outside or completely covered with black paper. Papers stored in this way are stable for at least 3 months.

Coated paper standards—Dilute the three pigment pastes with water to 20 per cent. of their original strength by weighing about 50 g of each into a 10-oz bottle and adding four times the weight of water, *i.e.*, 4 ml for each gram. Stopper each bottle and mix the contents well.

With 10-ml dropping pipettes, measure the volumes of the diluted pigment pastes, shown in Table I, into a 600-ml beaker fitted with a wide paddle-type stirrer. Measure the specified volumes of Butakon ML 505 latex and water in measuring cylinders to the nearest millilitre. Pour the latex into the beaker, rinsing out the cylinder with small volumes of the water. Add the remainder of the water and stir until the mixture is homogeneous. Add the china clay gradually, with stirring. When all of it has been added, stir for a further 30 minutes.

Lay a sheet of white card on a sheet of plate-glass and fasten one of the shorter ends down with adhesive tape. Place the well-type paint applicator (see under Apparatus) on the paper card at this end, close to the side, with its slot parallel to the shorter end, with the cut-away portion facing the fastened down end. Transfer, by pipette, 2 ml of the coating paste into the slot of the applicator. Place one hand on each side of the applicator and, with a gentle downward pressure, draw it steadily along the sheet and off the far end, thus spreading a layer of paste 0.003-inch thick on the card. Detach the card from the glass sheet and place it in an oven at 70° to 80° C, until dry, but for not more than 5 minutes.

Mount 2.5 × 4.5-cm strips of each of the five colour standards side by side on a card. Punch a hole 7 mm in diameter through the supporting card and the standard.

NOTE 1. These reagents can be obtained through Sales Offices of Imperial Chemical Industries Limited.

TABLE I
COMPOSITION OF MIXTURES FOR HAND-SPREADING OF TDI COLOUR STANDARDS

TDI in a 5-litre air sample, equivalent p.p.m. v/v	0.01	0.02	0.04	0.06	0.10
Diluted Monolite Fast Red 2 RVS paste, ml	4.0	6.6	13.9	18.9	25.5
Diluted Monastral Fast Blue BVS paste, ml	2.8	4.0	7.6	11.0	14.5
Diluted Monolite Fast Yellow GNVS paste, ml	6.2	7.8	17.2	23.8	31.2
Water, ml	220	210	200	185	170
Butakon ML 505 latex, ml	64	64	64	64	64
China clay, SPS grade, g	160	160	160	160	160

APPARATUS—

Test-paper holder—For exposing a circle of paper 1 cm in diameter to the sample stream. Suitable holders are described elsewhere,^{4,5} the latter referred to being obtainable from Siebe, Gorman Ltd., Davis Road, Chessington, Surrey.

Means of suction—This should be capable of drawing a 5-litre sample of the test atmosphere through the paper at about 1 litre per minute. The Mine Safety Appliances Company's 4-cylinder hand-cranked pump or a water-filled aspirator⁶ can be used. If the former is used the flow-rate must be adjusted before use to 1 litre per minute, with a test-paper in the holder, and a flow-meter attached to the inlet of the test-paper holder.

Well-type paint applicator—See Fig. 1.

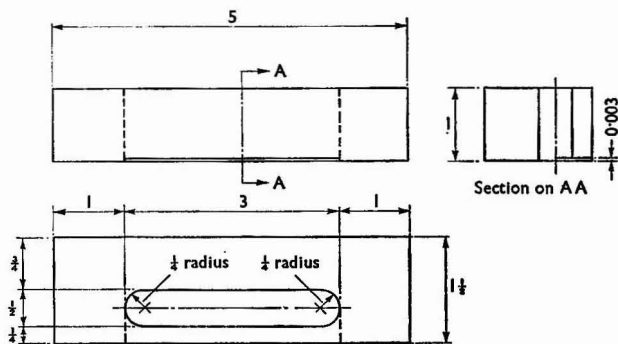


Fig. 1. Well-type paint applicator; all measurements are in inches. Material is of stainless steel, smooth finish

PROCEDURE—

Insert a test-paper in the holder in an atmosphere free from TDI, attach to the source of suction and draw 5 litres of the test atmosphere through the paper at a rate of about 1 litre per minute. If it is not convenient to have the source of suction near to the sampling point, an extension tube can be used, but this must be placed between the paper holder and the source of suction. On no account must any rubber tubing be attached to the inlet of the test-paper holder. Remove the paper, mark it for identification purposes and place it in a box or envelope. Fifteen minutes after the end of sampling, compare the stain with the five colour standards by holding it under the hole in each, in turn, and in contact with the card, so that the standard surrounds the stain. View the card at arm's length with the window or other source of light behind one. Assign the appropriate concentration to the stain, interpolating, if necessary, a value between two of the standards.

EXPERIMENTAL

PRODUCTION OF KNOWN ATMOSPHERIC CONCENTRATIONS OF TOLYLENE DI-ISOCYANATE (TDI)—

In the earlier part of the work an aspiration method was used. Pure nitrogen was bubbled at a measured flow-rate through TDI contained in a sintered-plate gas washing bottle. This was diluted with a second metered stream of pure nitrogen. A portion of this diluted mixture was metered into a stream of moistened compressed air to produce the final working concentration, which could be varied over a wide range by suitably chosen changes in the gas flows. Some typical conditions for the aspiration method were as follows. Nitrogen bubbled at 250 ml per minute through TDI at 20° C was diluted to 5 litres per minute; 1 litre per minute of this diluted stream was further diluted to 25 litres per minute. The TDI concentration produced was 0.033 p.p.m. v/v. Nitrogen bubbled at 50 ml per minute through TDI at 20° C was diluted to 5 litres per minute; 1 litre per minute of this diluted stream was further diluted to 25 litres per minute. The TDI concentration was 0.014 p.p.m. v/v.

A second approach was to inject a dilute solution of TDI in carbon disulphide or cyclohexane at a known slow rate into a metered stream of moistened compressed air by using a Palmer slow injection apparatus.⁶ When a 0.051 per cent. v/v solution of TDI in carbon disulphide was injected at 3.02 μ l per minute into an air flow of 10 litres per minute, the concentration of TDI produced was 0.013 p.p.m. v/v. With the same solution strength and

injection rate, but with an air flow of 8 litres per minute, the TDI concentration produced was 0.019 p.p.m. v/v. This was the preferred method as it gave steadier concentrations. With neither method was it possible to calculate in advance the concentration of TDI to be produced. The concentrations were always less than expected in the range 0.005 to 0.10 p.p.m. v/v. This was thought to be caused by the hydrolysis of TDI in the moist air and possibly also to the absorption on surfaces of the apparatus, which was all-glass. The desired concentrations were therefore obtained by empirical adjustment of the operating conditions. They were checked by an unpublished method, a variant of one published for the determination of di-isocyanatodiphenylmethane in air,⁶ in which the test atmosphere was drawn through an aqueous solution of sodium acetate and hydrochloric acid. The hydrolysis product was coupled with *p*-nitrodiazobenzene, and the colour produced extracted into chloroform and measured with a spectrophotometer.

CHOICE OF COUPLING AGENT—

Several potential coupling agents were examined. In each instance Whatman No. 1 filter-paper was dipped for 30 seconds in the treatment solution and then hung to dry for 1 hour at room temperature. The treatment solutions were prepared by dissolving 2 g of sodium nitrite in a saturated solution of the coupling agent in a mixture of 10 ml of diethyl phthalate and 90 ml of methanol. Each paper was tested by drawing 1 litre of air containing about 0.1 p.p.m. v/v of TDI through a 1-cm diameter circle of the test-paper in 1 minute. The intensities of the stains produced by the different papers were compared visually. The following compounds were tested in this way: 3-hydroxy-2-naphthoic acid, 2-hydroxy-1-naphthoic acid, disodium 2-naphthol-3,6-disulphonate (R-salt) and nineteen compounds from the I.C.I. Brenthol range. Three of the Brenthol range gave the most intense colours, Brenthol AS (2-hydroxy-3-naphthanilide), Brenthol MN (2-hydroxy-3-naphtha-*m*-nitro-anilide) and Brenthol GB (2-hydroxy-11*H*-benzo[*a*]carbazole-3-carboxy-*p*-anisidide). The first two gave pink stains and the third a reddish grey. Brenthol GB was chosen for further development.

METHOD OF ASSESSING STAIN INTENSITY BY USING COLOURED PAPER STANDARDS—

A series of papers of graded depth of colour was prepared, the shades of which closely matched those given by TDI on test-papers. Each coloured paper was prepared by dipping a 9-cm circle of Whatman No. 1 filter-paper in a solution of mixed dyestuff for 30 seconds, blotting quickly between several thicknesses of filter-paper and hanging up to dry at room temperature. The mixed dyestuff solutions were made from freshly prepared 0.2 per cent. w/v stock solutions of the following dyestuffs in distilled water: Chlorazol black PB 150, Durazol brown G and Chlorazol yellow G 200. Before use, equal volumes of the Chlorazol black and Durazol brown stock solutions were mixed to form a solution containing 0.2 per cent. w/v of "mixed dye." The working solutions used to prepare the coloured paper standards were made by mixing an aliquot of the "mixed dye" solution *plus* 2.0 ml of the yellow solution and diluting with distilled water to 100 ml. The shade of each coloured paper standard was characterised numerically by the percentage of "mixed dye" in the solution used to prepare it.

To facilitate comparisons of test stains with the range of coloured paper standards, a hole, 7 mm in diameter, was punched in each of the latter with a number 3 cork borer. A test stain was placed under the hole in each standard in turn, until a match was obtained when the two were held at arm's length, the operator's back being towards the light.

COMPOSITION OF THE SOLUTION USED TO IMPREGNATE THE TEST-PAPERS—

Brenthol GB—As received, this gave a rather dark coloured solution in methanol, therefore, it was recrystallised three times from aqueous pyridine and dried at 100° C. A saturated solution was prepared by refluxing 0.5 g of recrystallised Brenthol GB with the appropriate volume of diethyl phthalate diluted to 150 ml with analytical-reagent grade methanol for 30 minutes, cooling to room temperature and filtering into a 250-ml measuring cylinder. The solid on the filter-paper was washed with small volumes of methanol until the volume of filtrate and washings was 150 ml; 100 ml of this solution was mixed with the solution of sodium nitrite, ammonium acetate and water (see below) to give the final solution used to impregnate the test-papers.

Sodium nitrite and ammonium acetate—Preliminary tests suggested that by increasing the sodium nitrite content of the impregnation solution deeper stains would be produced at a given TDI concentration, but 2.0 per cent. w/v was about the greatest concentration that could be tolerated without leaving scaly deposits on the surface of the test-papers. The addition of 0.15 g of ammonium acetate to each 100 ml gave a test-paper with an appreciably paler background colour and did not appear to affect its sensitivity towards TDI. The sodium nitrite and ammonium acetate were first dissolved in 3 ml of water before adding the Brenthol GB solution, as direct dissolution in the latter was slow.

Diethyl phthalate—This was found to cause a substantial increase in the rate of trapping of TDI by the test-paper. Thirty millilitres per 150 ml of Brenthol GB solution was the most that could be used without producing a paper that was too translucent for stains caused by TDI to be seen readily. By using this proportion and hanging the papers up to dry, without blotting after impregnation, the stains obtained with TDI were rather mottled, as though the reagent was unevenly distributed on the surface of the paper. Much more even stains were obtained with papers that had been blotted between several thicknesses of filter-paper. To compensate for the slight loss of sensitivity that was caused by blotting, the volume of diethyl phthalate in each 150 ml of Brenthol GB solution was increased to 35 ml without making the papers translucent.

TIME REQUIRED FOR THE DEVELOPMENT OF STAINS—

Five-litre air samples containing TDI were drawn through 1-cm diameter circles of test-paper at 1 litre per minute. The stains produced were matched, at intervals, after the end of sampling with coloured paper standards prepared as described above. Three preparations of TDI were used, the 2,4- and 2,6-isomers and an 80 + 20 mixture of the two.

2,4-TDI—Concentrations were not checked. Results are shown in Table II.

TABLE II
RATE OF COLOUR DEVELOPMENT BY TEST-PAPERS WITH 2,4-TDI

Test No.	1			2			3			
	5	10	15	5	10	15	5	10	15	20
Time after end of sampling, minutes										
"Mixed dye" corresponding to colour standard matched, per cent.	0.050	0.065	0.065	0.095	0.100	0.100	0.040	0.050	0.060	0.060

2,6-TDI and the 80 + 20 mixture—Concentrations were checked by the method referred to above. Results are shown in Tables III and IV.

TABLE III
RATE OF COLOUR DEVELOPMENT BY TEST-PAPERS WITH 2,6-TDI

Test No.	TDI, p.p.m.	Time after end of sampling, minutes	"Mixed dye" corresponding to colour standard matched, per cent.
1	0.019	5	0.025
		10	0.040
		15	0.045
		20	0.050
2	0.019	5	0.025
		10	0.040
		15	0.045
		20	0.050
		90	0.055
3	0.048	5	0.040
		10	0.065
		15	0.080
		20	0.085
		25	0.100
4	0.048	5	0.035
		10	0.060
		15	0.080
		20	0.095

TABLE IV
RATE OF COLOUR DEVELOPMENT BY TEST-PAPERS WITH MIXED TDI

Test No.	TDI, p.p.m.	Time after end of sampling, minutes	"Mixed dye" corresponding to colour standard matched, per cent.
1	0.039	5	0.060
		10	0.080
		15	0.080
		65	0.085
2	0.020	5	0.030
		10	0.040
		15	0.040
		20	0.040
		175	0.045
3	0.031	5	0.055
		10	0.065
		15	0.065
		25	0.065
4	0.055	5	0.065
		10	0.080
		15	0.095
		20	0.095
		25	0.095

It was thus evident that with 2,4-TDI development of the test stain was complete in 15 minutes, whereas with the 2,6-isomer, 20 minutes, or more, were necessary. The effect of this slightly slower colour development with the 2,6-isomer was not apparent with the 80 + 20 mixture. Colour development was again complete in 15 minutes and the stain appeared to be stable for at least 3 hours. Qualitative inspection of test stains that had been stored in the dark suggested that the stains did not fade for several days.

EFFECT OF TOLYLENEDIAMINE—

To check the possibility of interference from any tolylenediamine that might be present with the TDI, atmospheric concentrations of 2,4-tolylenediamine were prepared by passing either 1 or 2 litres of air per minute through a bubbler containing the amine held just above its melting-point of 99° C, and diluting this to 10 litres per minute with clean air, the combined stream being passed through a silica-wool plug before sampling. Concentrations were checked by Marcali's method.² Five-litre samples were drawn through test-papers at 1 litre per minute. At the two concentrations, 0.07 and 0.10 p.p.m. v/v, no visible stains were produced on the test-papers.

EFFECT OF OTHER ISOCYANATES—

Qualitative tests with other industrially used isocyanates have shown that 4,4'-di-isocyanatodiphenylmethane (MDI), 1,5-di-isocyanatonaphthalene (NDI) and 4,4'-di-isocyanato-3,3'-dimethylbiphenyl (TODI) give stains with the test-paper similar to that given by TDI. Hexamethylene di-isocyanate gives no stain.

SIZE OF AIR SAMPLE AND SPEED OF SAMPLING—

Five litres was chosen as the sample volume because it gave a good range of readily distinguishable stains over the range 0.01 to 0.10 p.p.m. v/v of TDI. Smaller volumes gave rather pale stains in the region of the threshold limit value of 0.02 p.p.m. v/v.⁷ Larger volumes made it difficult to distinguish between the stains given by the higher concentrations in this range.

The effect of variations in sampling speed for a given total sampling volume was studied; 5-litre samples were drawn through test-papers at different rates. The TDI concentrations were held as steady as possible during each series of tests but were not checked independently. Stain intensities were measured, as before, by visual comparison with coloured paper standards 15 minutes after the end of sampling, the results being given in Table V. Stain intensity was thus largely independent of sampling rate in the range 0.5 to 2.0 litres per minute.

TABLE V
EFFECT OF VARYING RATES OF SAMPLING ON STAIN INTENSITY

Test No.	"Mixed dye" corresponding to colour standard matched, per cent.				
	0.5 litres per minute	1.0 litres per minute	2.0 litres per minute	3.0 litres per minute	4.0 litres per minute
1	—	0.070 0.070	0.060	—	0.065
2	—	0.035 0.040	0.030	0.035	—
3	0.050	0.045 0.045	0.050	—	0.035
4	0.100	0.080	0.090	—	—

STORAGE STABILITY OF TEST-PAPERS—

On exposure to bright sunlight the unused test-papers, which are initially pale yellow, rapidly acquire an orange-brown colour. Although they are still sensitive to TDI, it becomes impossible to make accurate judgments of stain intensities by comparison with the coloured paper standards. If the test-papers are stored in a glass bottle in complete darkness, they retain their pale colour and sensitivity to TDI for at least 3 months.

REPRODUCIBILITY OF STAIN INDICATION AT DIFFERENT TDI CONCENTRATIONS—

Papers from several preparations were tested with known concentrations of TDI in air, as described above, the stains being matched 15 minutes after the end of sampling with the coloured paper standards. The results are shown in graphic form in Fig. 2. The variation is about ± 20 per cent. up to 0.09 p.p.m. of TDI.

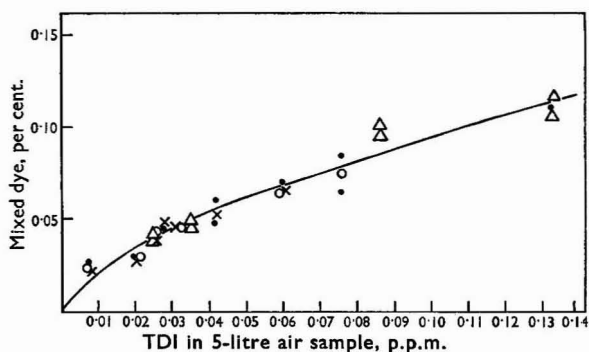


Fig. 2. Reproducibility of test-paper stains with known TDI concentrations: ●, test-paper batch 1; ○, test-paper batch 2; ×, test-paper batch 3; and △ test-paper batch 4

PRODUCTION OF PERMANENT COLOUR STANDARDS—

The practical use of any test-paper for the quantitative determination of a toxic gas or vapour in air depends on the availability of reproducible, stable colour standards, with which the test stains can be compared. The coloured paper standards described above, although adequate for use in the development of the test-paper, were not permanently stable. After 3 or 4 weeks their shades changed and test stains could no longer be matched. Alternatives were, therefore, sought.

Attempts to obtain a disc with permanent, glass colour standards were unsuccessful, as were attempts by a commercial printer to match the dyed paper standards. Success was finally achieved by coating thin paper card with aqueous suspensions of china clay and appropriately chosen amounts of red, blue and yellow dispersed pigments of high light fastness, a butadiene-styrene copolymer latex being used as a binder. By trial and error,

five standards were produced matching the stains obtained on the test-papers with 0.01, 0.02, 0.04, 0.06 and 0.10 p.p.m. v/v of TDI in a 5-litre air sample. The pigments used were Monolite Fast Red 2RVS paste, Monastral Fast Blue BVS paste and Monolite Fast Yellow GNVS paste. Full details of the preparation of these standards are given above under Description of the method. Repeated preparations of a given standard were indistinguishable from one another. Their validity was checked by comparison with stains obtained at the appropriate, known TDI concentrations. The coated standard at each concentration corresponded to the middle of the small range of variation obtained with individual test-papers.

DISCUSSION

This work has resulted in the development of a test-paper method, which is simpler to operate than earlier solution methods and, unlike them, is suitable for use by people without formal analytical training, a valuable consideration in many of the situations where TDI is produced and used. Both 2,4- and 2,6-isomers are detected with equal sensitivity, but no response is obtained from 2,4-tolylenediamine.

Although the elapsed time (about 21 minutes) for a single determination is longer than with the most rapid of the solution methods¹ (about 12 minutes), 15 minutes of this time is taken up with colour development and is available for the operator to take further air samples. By using the test-paper, considerable saving of time will be made in carrying out a multi-point survey of conditions in a plant either making or using TDI.

The test-paper is easy to produce and is stable for at least 3 months, if stored in complete darkness. The coated paper standards can be reproduced without undue difficulty. Single copies of the colour chart will be available on request from Imperial Chemical Industries Limited, Dyestuffs Division.

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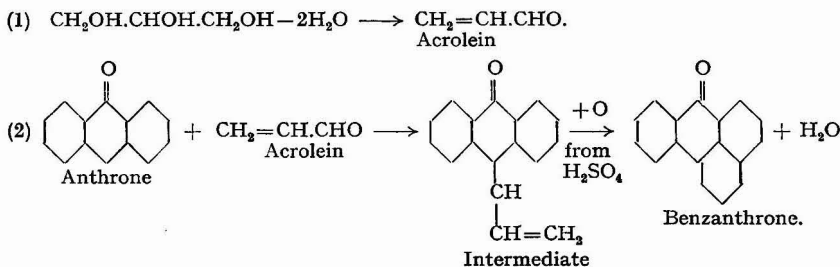
Use of Anthrone in the Determination of Trace Amounts of Glycerol

BY F. A. LYNE, J. A. RADLEY AND M. B. TAYLOR
(*J. A. Radley Research Institute, 220-222 Elgar Road, Reading, Berkshire*)

A fluorescent product is formed when a sample containing glycerol is heated with a solution of anthrone in sulphuric acid, and based on this a method for the quantitative determination of glycerol in amounts from 10 to 75 μg is described. The original concentration of glycerol is determined from the intensity of the fluorescence of this product in sulphuric acid solution, after first producing a calibration graph from known amounts of glycerol. The method is particularly useful for determining the concentration of glycerol in spots separated from interfering substances by chromatography.

THERE are several methods available for determining glycerol in milligram amounts,¹ including periodate oxidation, acetylation and Zeisel and Fanto's method.² Raveux³ outlines methods for extracting small amounts of glycerol from a mixture; this may be done by a form of steam-distillation with ethanol instead of water, which might be useful with the anthrone method of analysis. His suggestion for determining glycerol, by boiling with potassium dichromate in nitric acid for 20 minutes, followed by back-titration of the dichromate with ferrocyanide, with diphenylamine as indicator, is only suitable for amounts of glycerol in excess of 100 μg . As a qualitative test for glycerol, Schutz⁴ introduced the reaction between anthrone and glycerol in concentrated sulphuric acid solution, in which a solution showing a dull orange fluorescence in ultraviolet radiation was formed. Later this was adapted by Radley⁵ for microgram amounts of both glycerol and ethylene glycol. This latter has now been put on a quantitative basis.

The reaction involves the condensation of an α - β unsaturated aldehyde with anthrone; the formation of acrolein by dehydration of glycerol with sulphuric acid and completion of the reaction by cyclisation.⁶



EXPERIMENTAL

The excitation spectrum of benzanthrone shows that there is a wide excitation wave band between 350 and 500 $m\mu$ so that no filters were necessary in the excitation beam. With the Locarte single-sided fluorimeter Mk. 4, the maximum in the fluorescence spectrum was observed at 575 $m\mu$ and all subsequent readings were taken at this wavelength.

SOLVENTS—

The intensity of fluorescence of a solution of benzanthrone in sulphuric acid was found to increase uniformly when the concentration of acid was between 85 and 98 per cent.: fluorescence of the solution in less than 85 per cent. acid was erratic and in various organic extractants, such as benzene, carbon tetrachloride, dimethylformamide and toluene, was insufficiently intense for determination. Anthrone itself has a strong blue fluorescence in dimethylformamide because of the formation of anthranol.

When it was necessary to separate the benzanthrone from irrelevant material, it was extracted with benzene, the solvent removed and the residue dissolved in 98 per cent. sulphuric acid.

CONCENTRATION OF ACID IN REACTION MIXTURE—

The concentration of sulphuric acid and the temperature of the reaction were adjusted to give minimum decomposition of anthrone with the maximum production of benzanthrone in a reasonably short time. Rapid decomposition was caused by 98 per cent. sulphuric acid at temperatures over 100° C, and the rate of formation and the yield of benzanthrone fell rapidly at acid concentrations below 85 per cent. This concentration of sulphuric acid (85 per cent.; sp.gr. 1.783 at 16° C) was, therefore, chosen as the reaction medium.

TEMPERATURE OF REACTION—

Of the range of temperatures investigated, 120° C was chosen as the most suitable, as the production of the fluorescent compound was rapid, and little decomposition of the anthrone by the sulphuric acid occurred during the time necessary for the maximum production of benzanthrone. The development of a slight red colour in the solution did not interfere with the measurement of fluorescence intensity.

TIME OF REACTION—

In examining the development of the fluorescence with time of reaction at 120° ± 1° C, the maximum fluorescence was obtained between 15 and 20 minutes; a longer reaction time caused a decrease in the yield of fluorescent product, probably because of the destruction of the benzanthrone. The intensity of fluorescence of the blank solution containing no glycerol increased with time after 15 minutes' heating, so that this heating period was standardised at 15 minutes.

The volume of the reacting solution was standardised at 1 ml of glycerol solution and 1 ml of 0.1 per cent. w/v anthrone solution, each in sulphuric acid; this amount of anthrone theoretically allows the determination of glycerol up to about 500 µg. For determination of amounts of glycerol less than 20 µg, 0.01 per cent. w/v anthrone solutions were found to be suitable.

The method can be used to determine glycerol in the concentration range of 10 to 75 µg with an accuracy of ± 5 µg. The accuracy will be less if charred material is formed by decomposition of readily carbonisable substances present with the glycerol, in which case it is necessary to resort to the extraction technique referred to under Solvents.

In the presence of many of the interfering substances the glycerol may be separated from the sample by chromatography,^{7,8} and the amount of glycerol determined in the spot.

REAGENTS—

Anthrone—As supplied by British Drug Houses Ltd.

Glycerol—Analytical-reagent grade.

Sulphuric acid, 85 and 98 per cent.—Analytical-reagent grade.

Benzene—Analytical-reagent grade.

PROCEDURE FOR SOLUTIONS CONTAINING ONLY GLYCEROL—

Make up a 0.1 per cent. w/v solution of anthrone in 85 per cent. sulphuric acid. This solution is stable for about 14 days.

Transfer, by pipette, 1-ml portions of the anthrone solution into a series of stoppered, 15-ml Pyrex test-tubes, each containing 1 ml of a solution of glycerol in 85 per cent. sulphuric acid in known amounts from 0 to 100 µg. Heat for exactly 15 minutes in an oil-bath at

120° C. Allow to cool, dilute with 5 ml of 98 per cent. sulphuric acid and thoroughly mix the two layers. Measure the intensity of fluorescence of the solutions at 575 m μ and prepare a calibration graph of intensity of fluorescence against amount of glycerol, with a solution containing 1 μ g per ml of quinine sulphate to set the instrument sensitivity. The graph should be linear over the range of concentration 5 to 75 μ g, after which the concentration quenching effect is as shown in Fig. 1.

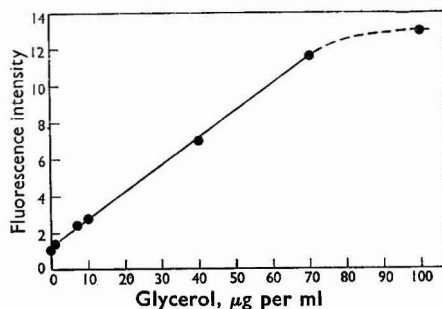


Fig. 1. Intensity of fluorescence against original concentration of glycerol

Dissolve a suitable amount of the sample in 85 per cent. sulphuric acid, then to 1 ml of this solution add 1 ml of the anthrone solution, heat and dilute, as above, before measuring the intensity of fluorescence. By reference to the calibration graph the amount of glycerol can then be determined. It is preferable to prepare the standard solutions at the same time as those containing the sample.

PROCEDURE FOR DETERMINATION OF GLYCEROL IN OTHER MATERIALS—

Many organic materials, *e.g.*, sugars, are easily carbonised by hot concentrated sulphuric acid and interfere with the anthrone reaction, so that it is usually necessary to effect a quantitative separation of the glycerol from the bulk of the interfering substances, *e.g.*, by extraction with a suitable solvent such as dry acetone, before carrying out the determination. Liquid samples may be evaporated on sand or treated with anhydrous sodium sulphate, and the mass extracted with acetone, which is subsequently removed by evaporation.

With oil or fat-containing materials the residue from the acetone extract can be taken up in light petroleum or chloroform and the glycerol recovered from this solution with water.

After removal of the solvent used to extract the original material, the glycerol residue may be sufficiently pure to proceed with its determination directly, or it may require further purification and separation by chromatography.⁹

When using the "crude" extract we find sometimes that considerable darkening occurs when the samples are heated in the reaction mixture. We have endeavoured to separate the benzanthrone from the darkened solutions that would otherwise interfere with the measurements by Tyndall scattering and the consequent variations in the adsorption of light.

To overcome this difficulty, dilute the darkened reaction mixture with 6 ml of water and add 6 ml of benzene. After thorough shaking, centrifuge the mixture, and then remove the benzene layer with a dropping pipette. Repeat the extraction twice, then evaporate off the benzene on a water-bath and re-dissolve the red-brown solid in 10 ml of concentrated sulphuric acid before measuring the intensity of fluorescence of this solution. A separate calibration graph must be drawn carrying the standards right through the extraction process. Providing gross amounts of easily charred material are not present in the extract we have found that this method gives good results.

If gross charring is encountered we have found no solvent that gives a 100 per cent. recovery of benzanthrone, perhaps because it is adsorbed on to the charred particles. Further dilution of the reaction mixture before extraction gives a greater yield of benzanthrone, and neutralisation of the acid with 10 per cent. of sodium hydroxide to give solutions of pH 2,

7 or 10 seems to have no effect, except that caused by dilution. When this occurs the original extract must be purified or the extraction repeated on the original material with a more suitable solvent.

The formation of a sulphonated benzanthrone¹⁰ may account for the loss in yield of fluorescent product with increased time described above. It may also account for the reddish brown compound produced by evaporation of the benzene.

THE EFFECT OF INTERFERING SUBSTANCES IN THE GLYCEROL EXTRACT—

The reaction between anthrone and sugars is well known, although the sensitivity of the reaction is not as high as for glycerol. Experiments were carried out to determine the extent of interference with the glycerol (50 μg) when the determination was carried out in the presence of much larger amounts (5000 μg) of sugars, sorbitol, gelatin and fat, and followed by the benzene extraction of the reaction mixture.

The figures in the "blank" column were obtained by carrying out the determination in the absence of glycerol with 5000 μg of the interfering substance.

TABLE I

EFFECT OF READILY CARBONISABLE MATERIAL ON GLYCEROL DETERMINATION

Substance added, 5000 μg	Apparent amount of glycerol, μg	Blank (interfering substances only), μg	Amount actually found, μg	Percentage recovery
Dextrose	54	16	38	76
Fructose	140	100	40	80
Sucrose	97	45	52	104
Gelatin	0	0	0	0
Sorbitol	45	0	45	90

Although gelatin completely quenched the fluorescence, glycerol can be completely extracted from it with acetone, and the anthrone reaction carried out on the residue after removal of the solvent.

Other substances mentioned by Radley⁵ as giving fluorescent products have been briefly studied under similar reaction conditions.

Formaldehyde produces a compound with a single fluorescent peak at 585 $m\mu$ when heated at 120° C for 15 minutes.

Ethylene glycol forms a green fluorescent product at a higher temperature with a peak at 540 $m\mu$.

Acetone, in about 1-mg amounts, produces a yellow - green fluorescent compound that is fluorescent in daylight with peaks at 454 and 580 $m\mu$.

Tartaric acid reacts with the anthrone solution, when heated at 150° C for 5 minutes, to form a green fluorescent product. If this is diluted with 60 per cent. sulphuric acid (5 + 1) the product can be shown to re-arrange photochemically to give a dark brown solution, which has a blue fluorescence (485 $m\mu$) after a period of about 30 seconds, with a high pressure mercury lamp as the ultraviolet source, and the green fluorescence reported by Radley⁵ is apparent during the first few seconds of observation.

Unlike the sugars, which, as shown in Table I, only interfere significantly when present in 100-fold amounts, tartaric acid, formaldehyde, ethylene glycol and acetone cause far more serious interference. Tartaric acid and formaldehyde, if present in 10-fold amounts, suppress the fluorescence of the benzanthrone to an insignificant level, ethylene glycol to a lesser but still significant extent, and acetone enhances the fluorescence. In the presence of these substances recourse would have to be made to chromatography.^{7,8,9}

The reaction mechanism may indicate other compounds that might react to form benzanthrone. Further work is in progress to investigate the reaction conditions necessary for production of fluorescent products with anthrone, and also to examine the relationship and the original concentration of the compound.

APPLICATIONS

The reaction can be used to determine milligram amounts of glycerol such as are found in spots obtained by paper chromatography.

Further work is being carried out to apply the method to the determination of small amounts of glycerol in fermented beverages, rancid fats and in the metabolic products of moulds and yeasts, as well as in a variety of other substances when the amount of sample available is restricted or the amount of glycerol is small. It is also being examined for the determination of glycerol in drugs⁶ in forensic work. The analysis of some duplicating inks and writing papers that had been in contact with hectograph "beds" was used qualitatively many years ago by J. A. Radley.

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A Sample Holder Cover for Use in the Determination of Light Elements in Liquids and Powders by Vacuum X-ray Fluorescence Spectrometry

BY K. G. CARR-BRION AND K. W. PAYNE

(Warren Spring Laboratory, Stevenage, Herts.)

A simple sample holder cover is described that permits the examination of volatile liquids and powders under vacuum in an X-ray spectrometer.

THE determination by X-ray fluorescence of light elements in volatile liquids and in powders is complicated by the need to remove the air from the X-ray path. This is usually achieved by replacing it with helium, but it lacks the convenience of vacuum operation and requires modifications to standard spectrometers. With the more convenient vacuum operation, volatile liquids will boil and a risk exists with fine powders of having them blown around the sample chamber when the air is re-admitted. A simply made cap has been devised to fit standard Philips specimen holders, which allows the safe examination of such samples under vacuum.

To make a cap, a sheet of 2-mm thick, low density polythene is suspended above a hot-plate until it clears. It is then rapidly placed on top of a sample holder, and a ring, with a diameter about 2 mm greater than the rim of the holder, pressed over it to a depth of about 1 cm. The polythene is allowed to cool and then levered off and trimmed. Finally, a pin of about 0.5-mm diameter is passed through the centre of the cap to produce a fine hole at about 45° (see Fig. 1).

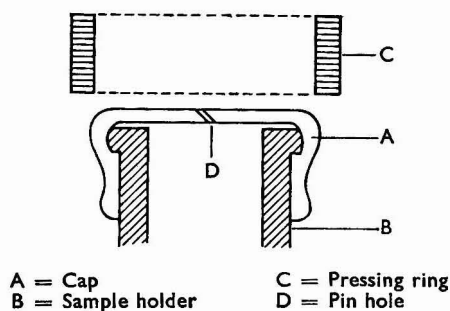


Fig. 1. Section of cap

MODE OF OPERATION—

In the case of liquids, when the vacuum is applied to the spectrometer the sample holder slowly evacuates through the pin hole until the pressure equals the vapour pressure of the liquid, when boiling should commence. However, because of the slow rate of diffusion through the pin hole, boiling is limited or does not occur. Evaporation is reduced to a level where quantitative measurement of X-ray intensity can be made on the sample. The pressure on the supporting X-ray window will be only that of the vapour pressure of the liquid. There are, obviously, limits to the volatility of liquids that can be examined, and tests should first be carried out under vacuum outside the spectrometer. For powder samples, when air is re-admitted to the spectrometer it enters the sample holder slowly, thus preventing powder from being blown around the instrument.

RESULTS

The capped holders, fitted with 12- μ Propathene X-ray windows, were used to measure sulphur in aqueous solutions and chlorine in ethanol. A Philips PW 1212 X-ray spectrometer was used, equipped with a P.E. crystal and a tungsten tube operating at 40 kV and 37 mA.

A summary of the results is given below.

Sample	K α intensity, counts per second	Exposure time for 1 per cent. change in intensity, minutes
1.0 per cent. of sulphur, as iron(II) sulphate in water	1070	>3
0.7 per cent. of chlorine, as iron(III) chloride in ethanol	1900	2

With aqueous solutions, the X-ray intensity is stable, even after several minutes' exposure to the primary X-ray beam. However, with the more volatile ethanol, appreciable evaporation occurred after 2 to 3 minutes' exposure. Thus, samples should only be exposed to the beam during the required measuring periods.

Received *June 26th, 1967*

The Determination of Molybdenum in Milk with Zinc Dithiol

BY R. E. STANTON AND ALISON J. HARDWICK

(Department of Geology, Imperial College, Prince Consort Road, London, S.W.7)

THE method of Stanton and Hardwick¹ for the determination of molybdenum in soils and sediments has been applied to milk. A 100-ml sample was subjected to a conventional wet-oxidation procedure in a 500-ml Kjeldahl flask with nitric and sulphuric acids. Five millilitres of concentrated sulphuric acid were used so that, after evaporation to copious fumes of sulphur trioxide and cooling, the solution on dilution to 10 ml with water was less than 10 N. The precipitate of calcium sulphate was left to settle overnight, and a 5-ml aliquot of the clear, supernatant solution was taken for the determination of molybdenum by the method previously described, except that standards were prepared with 5 ml of 4 M sulphuric acid instead of 6 M hydrochloric acid, and without the addition of iron.

Sulphuric acid had no effect on the determination over the concentration range of 1 to 4 M in the 5-ml aliquot, but calcium sulphate interfered when in suspension, there being visible sorption by the precipitate of the molybdenum-dithiol complex. However, recovery of molybdenum was complete in the presence of the equivalent in the milk of 0.1 per cent. of both calcium and phosphorus (added as orthophosphate), provided that the calcium sulphate was allowed to settle before the transfer by pipette of the aliquot.

When analytical-reagent grade acids were used, it was found that nitric acid (sp.gr. 1.42) contained less than 0.001 p.p.m. of molybdenum, whereas sulphuric acid (sp.gr. 1.84) contained 0.007 p.p.m.

Standard deviations were obtained on three samples of milk by replicate analyses: 24.8 ± 2.3 μg per litre (5 results), 26.8 ± 3.8 μg per litre (5 results) and 22.8 ± 4.3 μg per litre (15 results). The set of 15 replicates was mixed with routine samples.

This work formed part of the programme of the Applied Geochemistry Research Group at the Imperial College of Science and Technology under the direction of Professor J. S. Webb, and was assisted by a grant from the Natural Environment Research Council.

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Received April 13th, 1967

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Detection of Mowra Cake in Oil Cakes

BY N. G. WAGLE

(General Superintendence Co. (India) Private Ltd., Bombay, India)

THE detection of adulteration in oil cakes is a common requirement in analytical laboratories, as several international contracts specify freedom from such adulteration. In India, which is one of the largest exporters of de-oiled cakes, mowra [mahua, *Madhuca* (*Bassia*) *latifolia* Macb. Roxb.; *Madhuca* (*Bassia*) *longifolia* Koenig Macb.] is sometimes used as an adulterant.

The presence of saponins (mowrin) in mowra cake affords a convenient method for its detection provided, however, the saponins are isolated from the carbohydrates and other non-saponins in the cake, which are likely to cause interference. The haemolytic action of saponins on red blood corpuscles is specific, but this method is not suitable for adoption in all laboratories.

A quick and sensitive method, based on the well known reaction between saponin (mowric acid) and concentrated sulphuric acid, is described for the detection of mowra cake in oil cakes, such as those of groundnut, linseed and sesame.

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EXPERIMENTAL

Ten grams of the sample, after de-oiling with light petroleum (boiling range 40° to 60° C) and air-drying, are refluxed with 200 ml of 5 per cent. w/v hydrochloric acid for 30 minutes and filtered, while hot, through a Buchner funnel fitted with Whatman No. 1 filter-paper. The residue is washed several times with hot water until the washings are colourless (6 to 8 times is sufficient), followed by acetone and diethyl ether. After drying in an air-oven at 105° C, the residue and the filter-paper are transferred into a flask, 100 ml of isopropyl alcohol added, and the contents refluxed for 2 hours on a boiling water bath. The solution is filtered while hot, and evaporated to dryness on a steam-bath. The extract is boiled for 2 minutes with 10 ml of 5 per cent. w/v trichloroacetic acid solution, cooled and boiled again with 15 ml of 5 per cent. w/v sodium hydroxide for a further 2 minutes. The solution is cooled, diluted to 25 ml with water, and filtered. The filtrate is tested by adding 5 drops (0.2 ml) to about 5 ml of concentrated sulphuric acid contained in a test-tube, by allowing the drops to run down the side of the tube. The formation of a violet ring at the interface indicates the presence of mowra cake.

At concentrations of 5 per cent. of mowra cake, and above, the violet-coloured ring is formed almost instantaneously, and is intense. At higher concentrations the colour assumes a violet-red hue. At lower concentrations, the development of the colour is gradual but it is distinct (purple or reddish orange) in about 30 seconds, although less intense. The intensity gradually increases and attains a maximum within about 1 hour. With cakes free from mowra cake, the ring is almost colourless or light yellow to light yellowish brown. The intensity of the colour is almost proportional to the amount of mowra cake present and would serve as a means of determining it quantitatively.

The test proposed in this paper was always found to give a positive reaction with cakes containing as little as 0.5 per cent. of mowra cake. With suitable modifications, such as reduction of the final volume to 10 ml, instead of 25 ml, the test would respond even at lower concentrations. For a routine analytical laboratory this could be regarded as sufficiently sensitive.

First received November 28th, 1966
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Book Reviews

PARTICLE SIZE ANALYSIS. Pp. viii + 368. London: The Society for Analytical Chemistry. 1967. Price 147s. net (*plus* 5s. postage and packing); \$18.25 net (*plus* \$0.75 postage and packing).

This book records the Proceedings of a 3-day Conference on the title subject, organised by the Society for Analytical Chemistry, held at Loughborough University of Technology in September, 1966. There were over 200 delegates to the Conference from thirteen countries. Seventy-four variants of six basically different methods of size analysis were discussed in twenty-seven papers, but by far the most important part of the Conference was the excellent discussion, in which all facets of the subject were probed. A most important point is that all of those taking part in the discussions were actively engaged in some particular branch of the field under review, either as users, developers or makers of equipment.

As this review concerns the book rather than the Conference, let me say at once that the volume is extremely well printed and edited. The illustrations are uniformly produced, and very clear, and the tabulation avoids this particular reviewer's *bête noire*, tables that run on to two pages, often in reduced type-size, and sometimes printed by a different process from the main text, giving an untidy set-up, difficult to assimilate into the text. In fact, the only reduced-size printing is in the discussion, and this greatly adds to the ease with which the book can be read, as the reader is at once aware, at any point in the book, whether he is reading the main text or the discussion. With discussions that are sometimes as long as the original papers, this is a valuable advantage.

The subject matter dealt with in the 360 pages of the book covers the whole field of size analysis, including optical methods (curiously described as "manual" methods!), automatic counting, gravity and centrifugal sedimentation methods, and surface measurement. A chapter

is also included on applications of particle-size analysis, but this is regrettably short, dealing only with two rather specialised applications (in the ceramic and atomic energy fields). This is a pity as many would-be readers of the book will perhaps be mainly concerned with the ways in which accurate particle-size data can be used to improve the control and efficiency of the very many important processes that involve small particles.

The reviewer is glad to see that not too much time has been devoted to argument concerning the statistical accuracy of the various methods of size analysis (particularly microscopic counting); instead, a considerable body of accurate test data is put forward for review and criticism. It has long been the writer's view that equipment makers are too often prone to devote time and energy to elaborate instrumentation of equipment that is basically wrong (either because it operates under conditions that invalidate the fundamental physical laws under which it purports to operate, or because the makers have not been entirely clear which parameters they seek to measure). In this Conference, the makers and the assessors have come face to face, and the ensuing discussions, so admirably reported in the book, cannot but be of immense value to would-be users, either experienced or novice. The latter should not be daunted by the apparent differences of opinion expressed. The book is not a "popular" treatise on the subject; it is a reasoned presentation of scientific investigation, and, issued under the authority of the Society for Analytical Chemistry, deserves to be well regarded. Read in conjunction with the review paper on "Classification of Methods for Determining Particle Size," published in *The Analyst*, 1963, **88**, 156), it provides a very valuable summary of present day development of this important field of analytical chemistry.

The cry is raised at several points that all of this has been done before. It has—the reviewer was himself a little sad to see that some of the early "milestones" had toppled, but indeed this is progress. The very important thing is that the principles enunciated in the early work, namely, that we must define precisely what we are trying to measure, and follow up with accurate cross-checking, either by ringing the changes on the type of apparatus, or by carefully preparing multi-model test samples, have been retained.

In any case, it was time for another Conference: it is nearly 30 years since Dr. Heywood presented his masterly paper on the measurement of the fineness of powdered materials to the Institution of Mechanical Engineers (*Proc. Instn Mech. Engrs*, 1938, **140**, 257), and 20 years since the Institution of Chemical Engineers held its Symposium on Particle Size Analysis ("Symposium on Particle Size Analysis," The Institution of Chemical Engineers, London, 1947). The Proceedings of this Conference was a "best-seller" and, in fact, should still be read in conjunction with the present volume. Closely associated as the reviewer was with the early work, he is happy to say that the present book is even better. Although its price is high (7 guineas), it represents the distillation of thousands of pounds' worth of work, done under the best possible conditions, and will provide a satisfactory *vade mecum* for at least a decade, by which time one can expect that the application side will have developed to the extent that automatic determination of particle-size analysis will play an important rôle in computer control of the ever-larger unit process now becoming a common feature of our industry. This book will provide a good introduction to the techniques likely to be involved, and should be on every chemical engineer's and chemist's bookshelf.

C. J. STAIRMAND

PAPER CHROMATOGRAPHY AND ELECTROPHORESIS. Volume 1. By GUNTHER ZWEIG and JOHN R. WHITAKER. ELECTROPHORESIS IN STABILIZING MEDIA. Pp. xiv + 420. New York and London: Academic Press. 1967. Price 132s.

This is the first volume of a comprehensive two-volume work on paper chromatography and electrophoresis in stabilising media, intended to supersede the well known "Manual of Paper Chromatography and Paper Electrophoresis," by R. J. Block, E. L. Durrum and G. Zweig, the second edition of which appeared in 1958.

The rapid advances in this field since then made a complete revision and a great expansion of the text imperative. The inclusion of many stabilising media, other than paper, and the extension of applications of the technique to a much greater variety of compounds than before, largely account for the size of the present volume.

The opening chapter deals with theoretical considerations, types of stabilising media, apparatus and basic techniques, including application of sample, detection of components and quantitative

analysis. Although written in a style as economic and concise as possible, there is sufficient information on each topic to enable the reader to acquire a good understanding of the pertinent theories and facts.

The following six chapters cover all the important applications, dealing systematically with a great variety of compounds, such as amines, amino-acids, peptides, proteins, nucleic acids and derivatives, carbohydrates, organic acids, alkaloids, antibiotics and steroids.

The final chapter, describing the electrophoresis of inorganic ions, is particularly useful in drawing attention to a surprisingly little used, yet potentially very powerful, method.

The references throughout the book are well up-to-date and the indexes quite satisfactory.

It can be confidently predicted that this volume, competently written and well endowed with suitable diagrams and tables, will form a welcome addition to every chemical library.

D. GROSS

ADVANCES IN ELECTROCHEMISTRY AND ELECTROCHEMICAL ENGINEERING. Volume 5. Electrochemical Engineering. Edited by CHARLES W. TOBIAS. Pp. xii + 325. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1967. Price 116s.

The fifth volume of this series of books intended for the research worker is a worthy companion to the excellent ones already published. This volume bears the important sub-title "Electrochemical Engineering" and contains five authoritative reviews of processes and properties that are, or are likely to become, of technological value.

In the first review entitled "The Nickel Cadmium Cell," by P. C. Milner and U. B. Thomas, there is a comprehensive survey of the scientific and technological information on all aspects of this important cell. Not only do the authors discuss the charge-discharge cycles of both electrodes, but also the properties of the electrolyte, and the design and construction of both open and sealed cells. The authors could well have treated the wealth of information more critically, but nonetheless the review remains of great value.

J. Newman in "Transport Processes" deals with mass transport in electrolyte solutions in terms of the macroscopic theory of electrolytes, but although the subject is worked through in vector notation, it is well written and can be easily read and so provides a very thorough basis for the solution of important diffusion problems in dilute and concentrated electrolytes.

In their review of "The Transport Properties of Fused Salts," G. J. Janz and R. D. Reeve tabulate the most reliable data for density, electrical conductivity and viscosity, together with electrical transport and self-diffusion data for many single molten salts, some binary and a few ternary mixtures. All of the data for single salts are analysed in terms of the most generally acceptable equation for each property. The tables, which constitute the bulk of the review, are prefaced by a critical and well referenced account of methods of measurement, and a brief treatment of the basic concepts of fused-salt electrolytes.

A. Brenner, in a review entitled "Electrolysis of Non-aqueous Systems," may mislead the reader, as he deals almost exclusively with the electrodeposition of metals from organic solutions. Although the review summarises the literature, there is no attempt to support the phenomenological approach with any basic principles, and seldom is there any mention of any property apart from electrodeposition quality. This review has become a rather boring catalogue.

The final review, "Electrodeposition of Alloys," by K. M. Gorbunova and Y. M. Polukarov, is an excellent account of the theoretical basis of the subject, bearing in mind that there is already a review of the practical aspects, which was published in 1963 (A. Brenner, "Electrodeposition of Alloys"). The authors have treated the subject carefully and have drawn several interesting conclusions concerning the structure of electrodeposited alloys.

This book should prove invaluable to workers in any of the fields reviewed, but its general interest to analysts must, of necessity, be limited. Like so many specialist publications its price seems unreasonably high.

R. D. GILES

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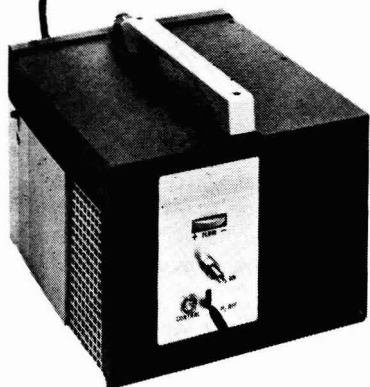
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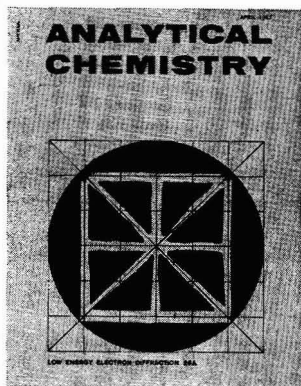
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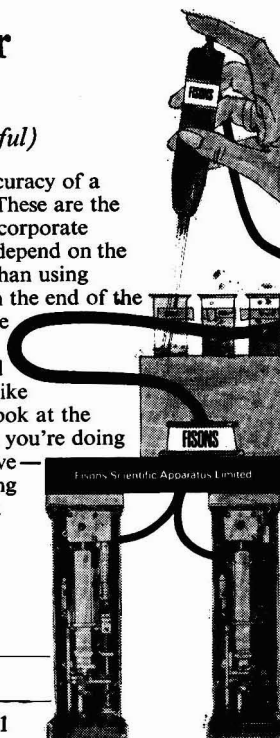


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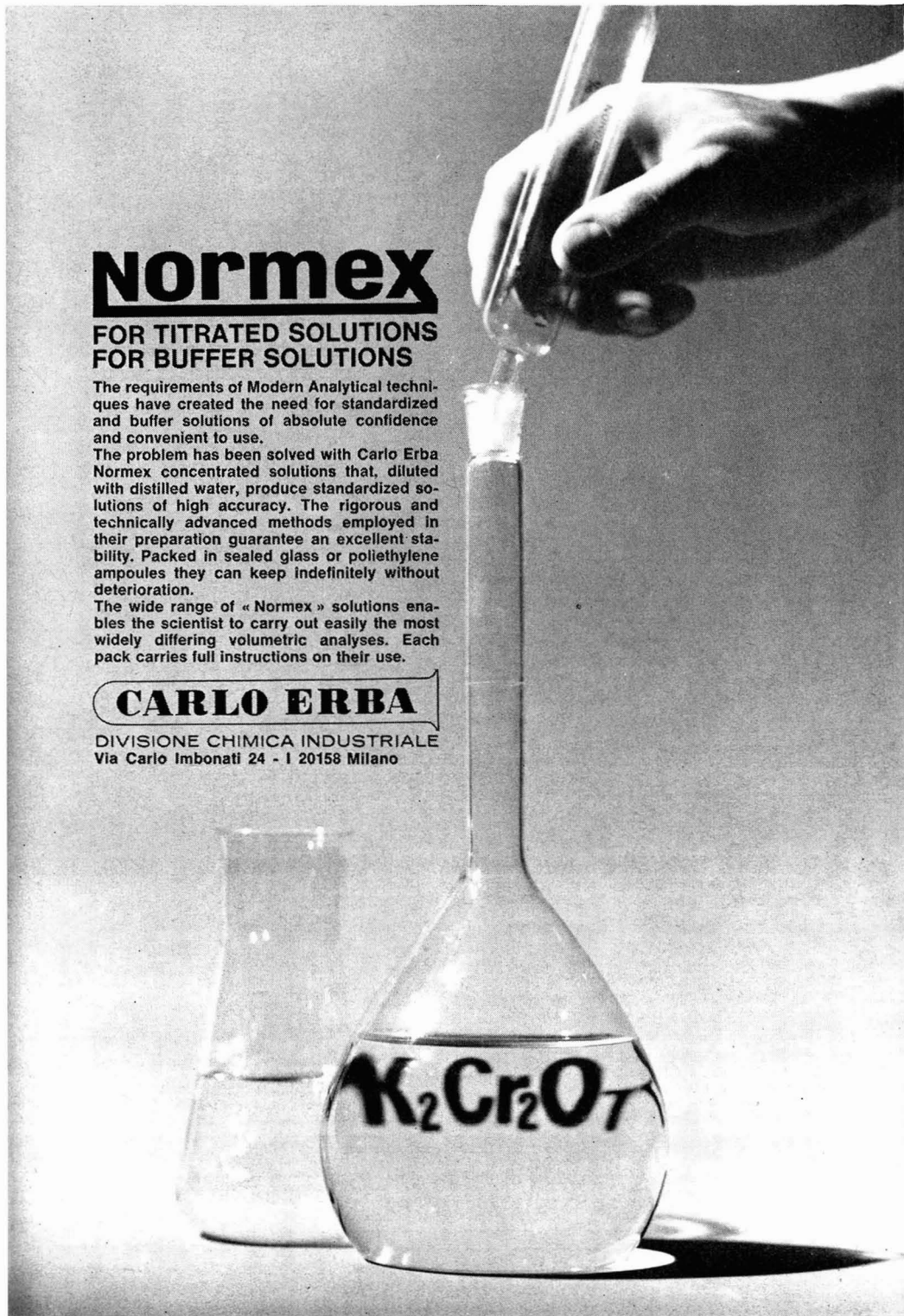
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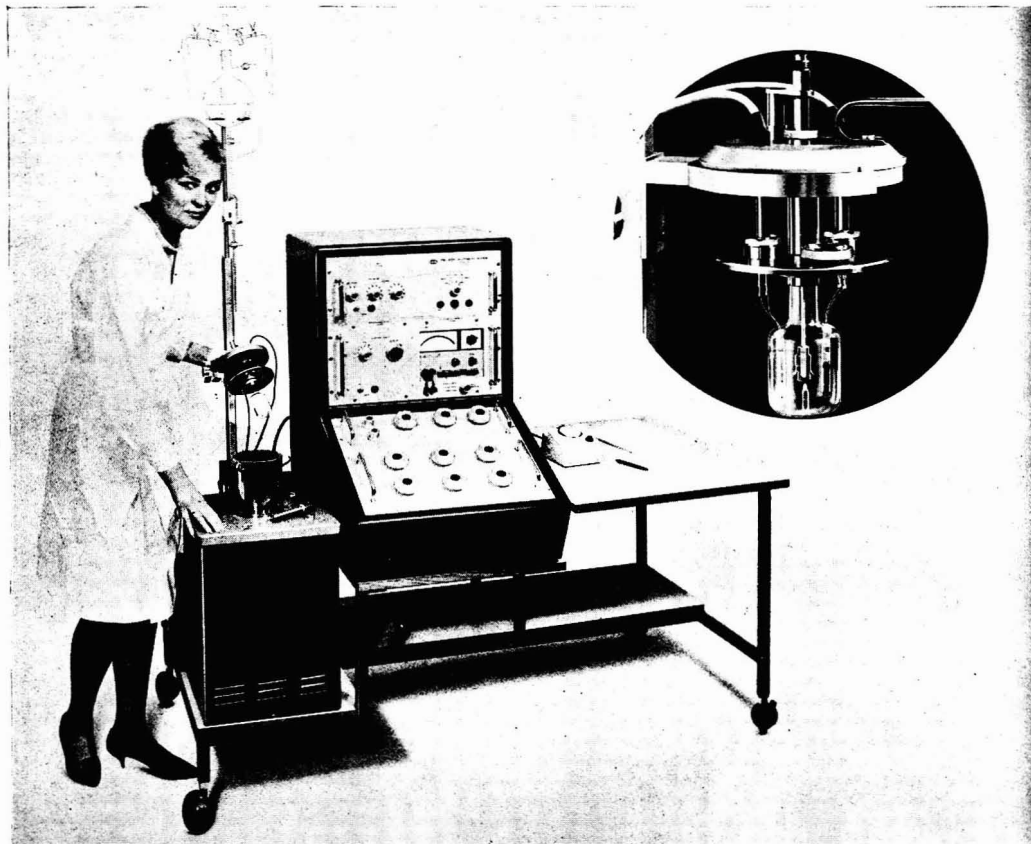
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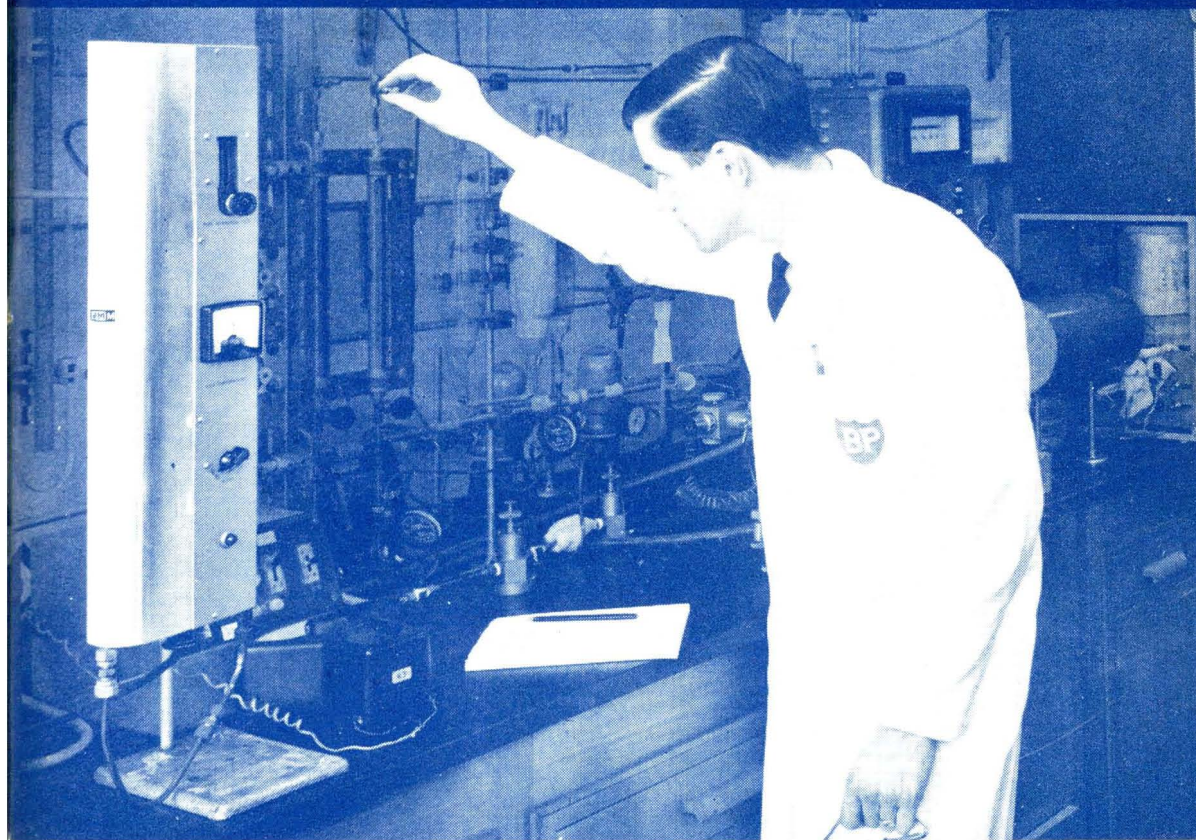
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