AUGUST, 1964 Vol 89, No. 1061

THE ANALYST

Analytical Methods Committee

REPORT PREPARED BY THE PROPHYLACTICS IN ANIMAL FEEDS SUB-COMMITTEE

The Determination of Amprolium in Animal Feeding Stuffs

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

REPORT

The Sub-Committee was set up in 1963 to continue the work of the Prophylactics Panel of the Additives in Animal Feeding Stuffs Sub-Committee; its composition was Dr. R. F. Phipers (Chairman), Mr. N. C. Brown, Mr. P. J. Cooper, Mr. G. Drewery, Mr. A. W. Hartley, Mr. R. S. Hatfull, Mr. A. Holbrook (resigned November, 1963), Mr. D. H. Mitchell, Mr. H. E. Monk (resigned November, 1963), Mr. S. G. E. Stevens, Mr. J. A. Stubbles and Mr. D. C. Thomas, with Miss V. Lewis as Secretary.

INTRODUCTION

The determination of amprolium [1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride] has been described by the Association of Official Agricultural Chemists[1] and the Sub-Committee has investigated the process on a collaborative basis in order to assess its merits and applicability.

The method involves extraction of the amprolium from the ground feed with a 2 + 1 mixture of methanol and water, chromatographic purification of the extract on alumina and a colorimetric determination with a reagent containing 2,7-naphthalenediol, potassium ferricyanide, potassium cyanide and sodium hydroxide in methanol. The resulting colour has an absorption maximum at 530 mμ, and the colour can be matched against a standard solution of amprolium.

A preliminary trial of the method gave recoveries ranging between 95 and 105 per cent. of the theoretical, and a collaborative study was therefore made on (*i*) wheatfeed, (*ii*) normal broiler feed and (*iii*) high-fat feed, all medicated at a level of 0·0125 per cent. w/w of amprolium. The results are shown in Table I.

The Sub-Committee agreed that the results on the normal broiler and high-fat feeds confirmed the suitability of the process for the purpose for which such information is required. The low results in respect of the medicated wheatfeed called for further investigation.

For wheatfeed it is possible that the volume of eluate discarded before the collection of the 5 ml recommended in the determination influences the result. This was confirmed by tests showing that 16 ml of eluate had to be discarded before an expected concentration of amprolium was present in the volume of eluate collected. It was further shown that when only 1 ml of eluate was discarded, still lower recovery values were obtained when wheatfeed that had been previously extracted with methanol, dried and subsequently medicated with amprolium was used.

These findings are in agreement with the fact that a proportion of amprolium is retained on the alumina when simple amprolium solutions are used. It is possible that in the broiler and high-fat feeds, materials are extracted that influence the retention of amprolium by alumina.

The wheatfeed sample did not represent the multiple ingredient feeds of commerce, and the results are considered to have only informative value.

Further studies on various feeds supplied by two manufacturers were undertaken. These feeds had been medicated with a proprietary pre-mix previously assayed as containing

22·5 per cent. w/w of amprolium. The recoveries given in Table II tended to be less than the theoretical value. The heterogeneous nature of the feedstuffs, the low levels of drug incorporated and the difficulty in obtaining final solutions containing only amprolium all present hazards. The value of 22·5 per cent. w/w of amprolium in the pre-mix and on which the recoveries were based must also be subject to some tolerance.

TABLE I

RECOVERY OF AMPROLIUM FROM MEDICATED MIXTURES

The amprolium was added at a concentration of 0·0125 per cent.

Laboratory	Wheatfeed		Normal broiler feed		High-fat feed	
	Found, %	Recovery, %	Found, %	Recovery, %	Found, %	Recovery, %
A	0·0117	91	0·0125	103	0·0134	106
	0·0113		0·0125		0·0135	
	0·0112		0·0131		0·0127	
			0·0134		0·0134	
B	0·0099	80	0·0114	94	0·0123	98
	0·0100		0·0120		0·0126	
C	0·0091	88	0·0144	115	0·0129	105
	0·0105		0·0144		0·0122	
	0·0118		0·0146		0·0131	
	0·0127		0·0143		0·0132	
D	0·0128	98	0·0129	101	0·0128	101
	0·0124		0·0125		0·0124	
E	0·0097	74	0·0130	107	0·0128	103
	0·0090		0·0167		0·0132	
	0·0088		0·0122		0·0136	
			0·0117		0·0116	
F	0·0104	82	0·0138	110	0·0137	107
	0·0101		0·0136		0·0135	
	0·0102		0·0137		0·0132	
	0·0101		0·0141		0·0130	

TABLE II

RECOVERY OF AMPROLIUM FROM A RANGE OF MEDICATED FEEDS

Sample	Amprolium* found, per cent., in laboratory—							
	A	B	C	D	E	F	G	H
Breeders mash (0·0125%)	0·0105 (84)	0·0107 (85) 0·0110 (88)	0·0110 (88)	0·0118 (94)	0·0120 (96)	0·0128 (102)	0·011 (88)	0·0122 (98)
Broiler diet (0·0125%)	0·0103 (82)	0·0103 (82) 0·0103 (82)	0·0105 (85)	0·0113 (90)	0·0105 (85)	0·0117 (93)	0·0110 (88)	0·0114 (91)
Broiler starter chick diet (0·0125%)	0·0110 (88)	0·0105 (84) 0·0107 (85)	0·0115 (92)	0·0122 (97)	0·0119 (95)	0·0116 (93)	0·0120 (96)	0·0124 (99)
Grain balance mash (0·0100%)	0·0089 (89)	0·0098 (98) 0·0107 (107)	0·0095 (95)	0·0091 (91) 0·0091 (91)	0·0089 (89)	0·0112 (112)	0·010 (100)	0·0098 (98)
Baby chick mash (0·0100%)	0·0105 (105)	0·0107 (107) 0·0108 (108)	0·0125 (125)	0·0125 (125) 0·0125 (125)	0·0123 (123)	0·0139 (139)	0·0110 (110)	0·0127 (127)
Intensive growers mash (0·0100%)	0·0089 (89)	0·0093 (93) 0·0093 (93)	0·0095 (95)	0·0093 (93) 0·0090 (90)	0·0093 (93)	0·0116 (116)	0·010 (100)	0·0098 (98)

* The figures in parenthesis are the percentage recoveries.

As many feeds are sold either pelleted or crumbed, it was thought that the method might be applied to a product before and after processing in this fashion. Since preparation of crumbs involves an additional stage after pelleting, this type was investigated. The results given in Table III show slightly lower recoveries on the crumbs than on the mash from which it had been made.

TABLE III

RECOVERY OF AMPROLIUM FROM CHICK MASH AND CHICK CRUMBS MEDICATED WITH 0·005 PER CENT. OF DRUG

Sample	Amprolium* found, per cent., in laboratory—				
	B	C	E	G	H
Baby chick mash ..	0·0047	0·0046	0·0047	0·0048	0·0045
	(94)	(92)	(94)	(96)	(90)
	0·0049	0·0048		0·0047	0·0044
	(98)	(96)		(94)	(88)
	0·0047	0·0048		0·0050	
	(94)	(96)		(100)	
Baby chick crumbs	0·0041	0·0046	0·0054	0·0043	0·0046
	(82)	(92)	(108)	(86)	(92)
	0·0049	0·0044		0·0045	0·0046
	(98)	(88)		(90)	(92)
	0·0046	0·0045		0·0044	
	(92)	(90)		(88)	

* The figures in parenthesis are the percentage recoveries.

Appendix I

RECOMMENDED METHOD FOR DETERMINING AMPROLIUM IN ANIMAL FEEDS

PRINCIPLE OF METHOD—

The amprolium is extracted from the ground feed with diluted methanol, the extract is purified chromatographically and the determination is completed colorimetrically by reference to a standard solution of amprolium.

APPLICABILITY—

The method is applicable to animal feeding stuffs, of the type marketed at the time this Report was prepared, containing amprolium as the only coccidiostat.

REAGENTS—

Methanol, anhydrous—Analytical-reagent grade.

Methanol, diluted—Mix two volumes of anhydrous methanol with one volume of distilled water. Cool to room temperature before use.

Potassium ferricyanide solution—Dissolve 200 mg of analytical-reagent grade potassium ferricyanide in 100 ml of distilled water. The solution kept tightly stoppered is stable for 2 weeks.

Potassium cyanide solution—Dissolve 1·0 g of analytical-reagent grade potassium cyanide in 100 ml of distilled water. The solution kept tightly stoppered is stable for 2 weeks.

Sodium hydroxide solution—Dissolve 2·25 g of analytical-reagent grade sodium hydroxide in 200 ml of distilled water.

Methanolic sodium hydroxide solution—Dilute 15·0 ml of the sodium hydroxide solution with sufficient anhydrous methanol to make 200 ml. Stopper the container, and mix well.

Naphthalenediol solution—Dissolve 25 mg of 2,7-naphthalenediol in 1000 ml of anhydrous methanol.

Colour developing reagent—Transfer 90 ml of the naphthalenediol solution into a 250-ml glass-stoppered flask, add 5 ml of potassium ferricyanide solution, and mix well. Then add 5 ml of the potassium cyanide solution, stopper the flask, and mix well. Set aside for 30 to 35 minutes, and then add 100 ml of the methanolic sodium hydroxide solution. Mix well. Use within 75 minutes, filtering through a medium-porosity sintered-glass filter just before use.

Reference standard amprolium—Recrystallised amprolium, 100 per cent. pure, available from Agricultural Products Department, Merck Sharp & Dohme Ltd., Hoddesdon, Herts.

Reference standard amprolium solution—Accurately weigh 25·0 mg of the reference standard amprolium into a 50-ml calibrated flask, dissolve it in sufficient diluted methanol to make 50 ml, and mix well. Transfer 5·00 ml to a 100-ml calibrated flask, dilute to the mark with diluted methanol, and mix. Each millilitre of this solution contains 0·0250 mg of amprolium. The solution is stable for 1 week. (This solution should not be chromatographed on an alumina column, but should be used directly as described below under "Colorimetry.")

Aluminium oxide, suitable for chromatographic adsorption—This material should pass the following test. Shake vigorously for at least 2 minutes 10 g of the aluminium oxide with 100 ml of water in a glass-stoppered 250-ml flask. Allow the slurry to settle, decant off the liquid, and determine its pH electrometrically. The pH should be 9·5 to 10·5.

To prepare the aluminium oxide for use, transfer 100 g to a suitable container, add 500 ml of distilled water, and stir mechanically for 30 minutes. Collect the slurry on filter-paper in a Buchner funnel. Wash the aluminium oxide on the filter with three 50-ml portions of methanol, and suck it dry. Dry it for 2 hours at 100° C in a vacuum oven. The aluminium oxide thus prepared should be free-flowing. Store it in a stoppered bottle.

PROCEDURE

EXTRACTION—

Grind a representative portion of the mash or pellets to a fine powder in a suitable laboratory mill, and mix it thoroughly. Weigh a sufficient amount of the ground feed equivalent to 3·0 to 5·0 mg of amprolium (see Note 1). Transfer the weighed sample to a glass-stoppered 250-ml flask, and add 200 ml of diluted methanol. Stopper the flask, and continuously stir the contents magnetically or shake on a mechanical shaker for 60 minutes. Filter the liquid through a Whatman No. 42 filter-paper, and collect 25 to 40 ml of the clear filtrate, rejecting the first 10 to 15 ml (see Note 2).

CHROMATOGRAPHY—

Preparation of columns—The coloumn should be of 9-mm internal diameter glass tubing, 40 to 50 cm high with an opening 4 to 5 mm in diameter at the lower end (see Note 3). Insert a small plug of glass-wool in the lower end of the clean dry tube, and compress the plug firmly with a glass rod so that a thickness of about 2 to 3 mm is obtained. Weigh 5 g of the prepared aluminium oxide, and transfer it to the dry column. Pack by gentle tapping against the side of the tube. Prepare a separate column for each sample.

Chromatography of feed extract—Transfer approximately 10 ml of the clear extract to the column, and allow the liquid to pass through the column bed by gravity. Reject the first 1·0 ml of eluate and collect the next 5·0 ml for subsequent colour development (see Note 4).

COLORIMETRY—

Mark separate centrifuge tubes X, S and B. In tube X place 4·00 ml of the clear eluate from the column, in tube S place 4·00 ml of the reference standard amprolium solution and in tube B place 3·00 ml of diluted methanol. Add 10·0 ml of the colour-developing reagent to the contents of each tube, stopper the tubes, and mix well. Set the stoppered tubes aside for 20 minutes, then clarify the coloured solution by spinning it in a centrifuge for 2 to 3 minutes at 1500 r.p.m. (see Note 5).

Decant the clear solutions (see Note 6) into 1-cm absorption cells, and insert covers (see Note 7). Measure the optical densities of the solutions in tubes X and S in a suitable spectrophotometer or colorimeter at 530 mμ. Use the solution in tube B as reference, and read the optical density within 20 to 45 minutes of adding the colour-developing reagent.

CALCULATION—

The percentage of amprolium in the feed is given by the expression—

$$\frac{5\,A_{X}C}{A_{S}W}$$

where A_X = optical density at 530 mμ of the feed sample extract,
A_S = optical density at 530 mμ of the standard solution,
C = weight of amprolium (mg) in 4·00 ml of standard solution (0·100 mg),
W = original weight in grams of feed sample.

NOTES—

1. For most samples, **30** g is usually preferable.

2. The filtrate should be clear. Re-filter if necessary through fresh filter-paper; alternatively, transfer the mixture to centrifuge tubes and spin until clear.

3. The opening at the lower end is obtained by simply rotating the tube in a hot flame.

4. These volumes should be measured in small graduated centrifuge tubes or cylinders. It is important to collect no more and no less than the indicated volumes.

5. Do not filter through paper as the colour is absorbed on filter-paper.

6. If, on careful inspection, the solutions are not clear and free from suspended particles after centrifugation, decant them into the cells through a small plug of dry glass-wool.

7. It is important to use cell covers to avoid errors owing to possible precipitation on exposure to air.

REFERENCE

1. Szalkowski, C. R., and Schulz, E. P., *J. Ass. Off. Agric. Chem.*, **1961**, **44**, 5.

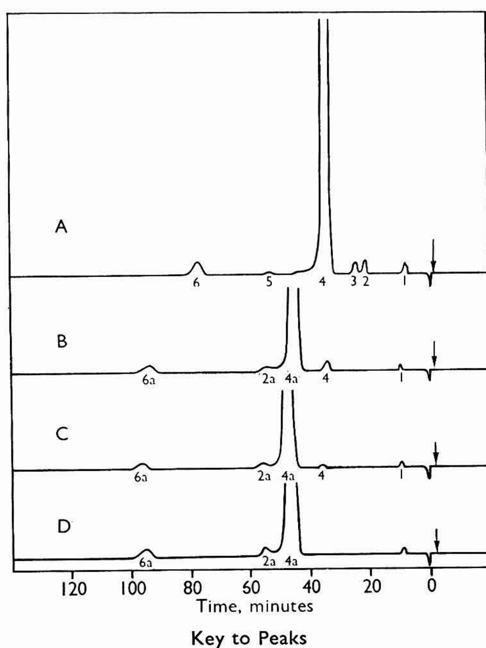
Analytical Methods Committee

ESSENTIAL OILS SUB-COMMITTEE

The Determination of Linalol in Essential Oils: Amendment

HOLNESS'S[1] investigation into the acetylation of linalol by the acetyl chloride - dimethylaniline method originated by Fiore[2] was accepted in 1960 by the Analytical Methods Committee[3] as the recommended procedure for determining linalol in essential oils.

Workers in many laboratories have since found that the specified 3-hour period of acetylation may be inadequate for complete esterification unless the analytical-grade reagents and especially the acetyl chloride, are freshly distilled immediately before use. It has also been found that longer periods of acetylation, extending even to 40 hours, ensure its completion without detrimental side-effects and permit reagent-grade acetyl chloride, not necessarily freshly distilled, but stored with precautions to exclude moisture, to be used as a more convenient and equally reliable reagent.



Key to Peaks

1	Terpenes	4a	Linalyl acetate
2	*cis*-Linalol oxide	5	α-Terpineol
3	*trans*-Linalol oxide	6	Geraniol
2a	Acetate of 2 and 3	6a	Geranyl acetate
4	Linalol		

Fig. 1. Chromatograms showing the effect of increasing the period of acetylation of a typical oil of Brazilian Rosewood by the dimethylaniline - acetyl chloride method

Chromatogram	Period of acetylation, hours	Ester value after acetylation	Total alcohols, as linalol, $C_{10}H_{18}O$, per cent.
A	0	—	—
B	3	266·6	91·6
C	6	270·2	93·2
D	16	273·3	94·5

The gas chromatograms in Fig. 1 show the effect of increasing the period of acetylation of a typical oil of Brazilian Rosewood when redistilled, but not freshly distilled, acetyl chloride is used, and support deductions based on the determination of the ester value after acetylation. Similar evidence has been provided by Johnston[4] and Walker[5] in House publications that may not be readily accessible.

Recommendation—

The Sub-Committee accordingly recommends that the period of acetylation given in its Report[6] should be amended to "not less than 6 and preferably 16 hours" in place of "3 hours" as originally specified.

References

1. Holness, D., *Analyst*, 1959, **84**, 3.
2. Fiore, A. T., *News Capsule* (Essential Oil Association of the U.S.A.), 1943, **1**, No. 15.
3. Analytical Methods Committee, *Analyst*, 1960, **85**, 165.
4. Johnston, V. D., "The Givaudanian," Givaudan-Delawanna Inc., U.S.A., May, 1962.
5. Walker, D. A., "Progress from Pinene," A. Boake, Roberts & Co. Ltd., London, 1962, No. 3, p. 3.
6. Analytical Methods Committee, *Analyst*, 1957, **82**, 325.

The Application of the Freundlich Isotherm to the Adsorption of Sugars from Solution by a Column of Charcoal

By E. A. WALKER AND PAMELA MORTON
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Wilson's theory of chromatography has been used to derive an equation for determining the lengths of adsorption bands of solute produced when a solution, whose adsorption characteristics can be expressed by the Freundlich adsorption isotherm, is passed through an adsorbing column. The practical problems posed in extending the principle to the measurement of the "breakthrough" volume have been overcome by using two columns of different length. This has led to a method for determining the adsorption isotherm by column methods and in principle to a method of determining solute concentration.

SINCE the discovery of column chromatography by Tswett, theory has made little impact on the practical technique, with the exception of gas-phase chromatography. As an example, the analysis of liquid glucose by chromatography on a carbon column has been developed entirely on an empirical basis, which is both time consuming and tedious and has prompted this study as part of a programme seeking closer links between the theory and practice of solid - liquid chromatography.

One technique in particular that has been neglected is that of frontal analysis. Introduced by Tiselius[1] in 1940, the method consists of continuously passing a solution containing one or more solutes through an adsorbing column. The concentration of the column effluent, plotted against the effluent volume, has a stepped profile in which successive steps indicate the emergence of an additional solute in the effluent liquid. This simple technique has been discussed by Kipling,[2] who related the adsorption isotherm of acetic acid to the retention ("breakthrough") time of the acetic acid on a column of charcoal, and by Claesson[3] who developed a theoretical approach for an idealised example of solutions containing more than one solute and related "breakthrough" volume to the Langmuir isotherm. However, the former technique presents numerous practical problems, and the latter theory assumes perfectly plane fronts that do not occur in practice. Glueckauf[4] has given a rigorous theoretical treatment of the process in terms of a linear isotherm, in which equations are derived allowing precise definition of the Gaussian profile of the effluent curve, but once again the practical application is limited, since linear isotherms are rare. An alternative and more empirical approach has therefore been made with an earlier theory suggested by Wilson[5] and later discussed by other workers.[6 to 11]

Wilson showed that, under equilibrium conditions, if a solution is passed through an adsorbing column, a uniform band of adsorbed solute is formed at the top of the column, and that the level of adsorption in the band could be related to the adsorption isotherm obtained by static experiments. Thus, if m is the amount of adsorbate per unit length of column, c_0 is the initial concentration of the solution entering the column, w is the weight per unit length of adsorbent and $f(c)$ is the form of the appropriate isotherm, then—

$$m = wf(c_0) = \text{constant} \quad \ldots \quad (1)$$

Column chromatography is usually carried out in dilute solutions and, as for the five sugars that have been studied, adsorption can frequently be expressed by the Freundlich form of the adsorption isotherm. In this instance equation (1) may be written—

$$m = wkc_0^{\frac{1}{n}} \quad \ldots \quad (2)$$

where k and n are the parameters of the Freundlich equation.

For a uniform band of adsorbate, the equation may be written—

$$M = wxkc_0^{\frac{1}{n}} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

where M is the total solute in a volume V of solution passed through the column and x the length of the band.

Since $M = Vc_0$, equation (3) may be written—

$$x = \frac{Vc_0^{\left(\frac{n-1}{n}\right)}}{wk} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

This provides a method for calculating x, the band length, from a knowledge of the isotherm. Sharp boundary conditions are assumed, whereas, in fact, a diffuse zone (or "beak") occurs at the lower end of the band (see Fig. 1).

So that equation (4) may be used, it is necessary to define a datum line on the diffuse "beak" from which to measure. In all the examples studied, the profile of adsorption distribution was nearly symmetrical, and the effective length of the band was measured from the top of the column to the point in the "beak" where the level of adsorption fell to half that in the main band (see Fig. 1). The measured band lengths are compared in Table I with those calculated over a range of concentration for which the Freundlich equation was found to apply.

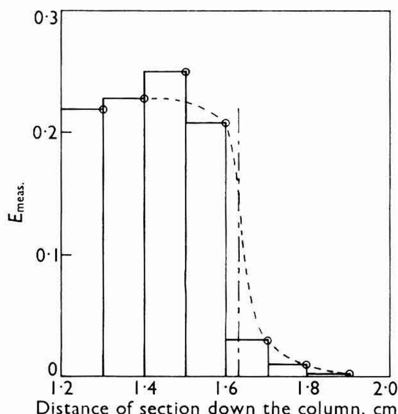


Fig. 1. A typical "beak" profile obtained with 30 ml of a solution containing 1 mg of lactose per ml of solution. The sugar content of each section is given in terms of the measured optical density of the coloured solution produced by the procedure of Somogyi and Nelson and is plotted against the distance of the section down the column. The broken line indicates the position chosen to define the end of the band

Consider the situation in which x, the band length, is equal to the length of the column V is then the volume of solution required to produce this band. The passage of further solution through the column would then have the effect of extending the band beyond the limits of the column, solute would appear in the effluent and the column would "break down."

Again, equation (1) does not specify boundary conditions, but only implies that solution will leave the column at concentration c_0. However, as the level of adsorption in the "beak" changes from a maximum given by equation (1) to zero over a finite length of column, then under equilibrium conditions we may expect the concentration of effluent to follow a related pattern in which concentration varies from 0 to c_0.

If the same criterion is taken for the measurement of band width as before, then the volume that has to be passed through the column would be given by some value for which

the effluent concentration lies between 0 and c_0. As with the measurement of band width, equation (4) would only be true if this change of effluent concentration took place over an infinitely small change of V. As before, a datum concentration would have to be chosen to compare measured and calculated "breakthrough" volumes. Since it was found that for a given value of c_0, the profile of effluent concentration appeared to be the same for different lengths of column (see Figs. 2 and 3), a more satisfactory approach was to compare the

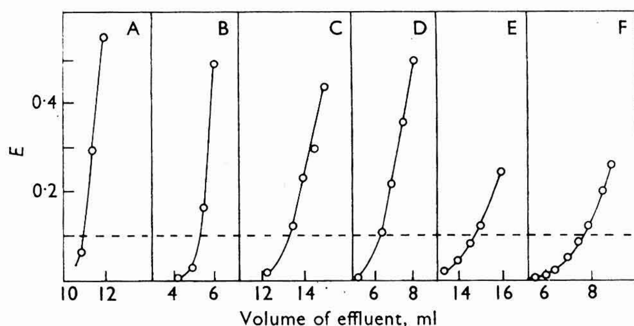


Fig. 2. Graph of optical density against "breakthrough" volume for a solution of dextrose.

Curve	A	B	C	D	E	F
c_0, mg per ml ..	**2·143**	2·143	**0·5**	0·5	**0·25**	0·25
W, g	**3·5**	2·0	**3·5**	2·0	**3·5**	2·0

where c_0 is the initial concentration of the solution and W is the weight of absorbent in the column

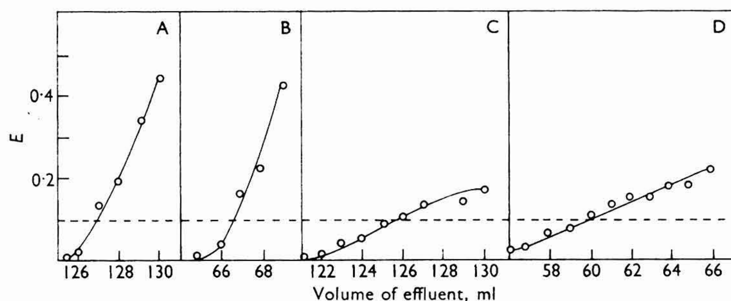


Fig. 3. Graph of optical density against "breakthrough" volume for a solution of lactose.

Curve	A	B	C	D
c_0, mg per ml	1·21	1·21	0·1	0·1
W, g	3·5	2·0	1·0	0·5

where c_0 is the initial concentration of the solution and W is the weight of adsorbent on the column

difference in "breakthrough" volumes (ΔV) for the different lengths of column, when both the "beak" effect and dead-space volume in the apparatus would be eliminated. In this instance, the most convenient point of measurement may be chosen, which is the initial appearance of solute in the effluent.

Now suppose $wx = W$, where W is the weight of adsorbent in the column. Equation (4) may be written—

$$V = \frac{Wk}{c_0^{(n-1)/n}}$$

and hence—

$$\Delta V = \frac{\Delta W k}{c_0^{(n-1)/n}} \quad \ldots \quad (5)$$

where $\Delta V = V_1 - V_2$ and $\Delta W = W_1 - W_2$, and

V_1 and V_2 are the measured "breakthrough" volumes for weights W_1 and W_2 of adsorbent, respectively. Written in the logarithmic form, equation (5) becomes—

$$\log\left(\frac{\Delta V}{\Delta W}\right) = \log k - \left(\frac{n-1}{n}\right) \log c_0 \quad \ldots \quad (6)$$

This is linear in $\log\left(\frac{\Delta V}{\Delta W}\right)$ and $\log c_0$; n can then be determined from the slope of the

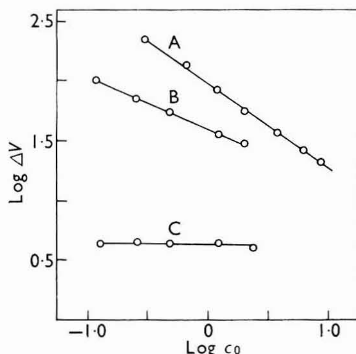


Fig. 4. Typical logarithmic graphs of the difference in "breakthrough" volume, ΔV, against the initial concentration at 25° C, c_0, for sugar solutions. Curve A, raffinose; curve B, sucrose; curve C, dextrose

graph, and $\log k$ from the values of $\log\left(\frac{\Delta V}{\Delta W}\right)$ when $\log c_0 = 0$ (see Fig. 4).

Experimental

Reagents—

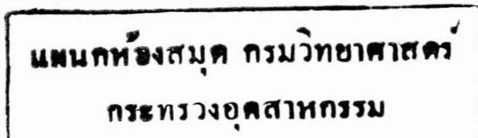
Activated charcoal—Since the adsorption properties of charcoal may vary considerably, a sufficient amount (1500 g) from a single batch of activated charcoal was obtained from B.D.H. Ltd. This was subjected to a continuous washing with water to remove, as far as possible, the phosphoric acid found to be present. The charcoal was then dried at 105° C and thoroughly mixed.

Kieselguhr—Metasil A was used.

Sugars—Analytical-reagent grades of sugars were used.

Determination of sugars—

This was carried out by using a modification of the Somogyi - Nelson method[12,13,14] over the range 0 to 250 μg per ml of solution. By gently shaking the solution after Nelson's reagent had been added, and subsequently setting the mixture aside for 30 minutes, the error of estimation was less than ± 2 per cent. when the colour formed was measured at 750 mμ with a Unicam SP600 spectrophotometer. The non-reducing sugars, raffinose and sucrose, must be inverted before estimation, and for this purpose an adaptation of the Clerget procedure was used. One millilitre of sugar solution was heated with 1 ml of a suitable strength sulphuric acid in a stoppered tube immersed to the neck in a thermostatically controlled heating-bath. The tube was then removed, cooled and the solution neutralised



with the calculated volume of a solution containing 3 per cent. w/v sodium carbonate and 1·5 per cent. w/v sodium hydrogen carbonate. This mixture of carbonate and hydrogen carbonate was used because pH was critical in the reduction stage.[8] Since this solution has the same pH as the Somogyi solution, the reduction was not affected by a slight excess of the neutralising solution. The reducing sugars were then estimated. For sucrose, N acid was used for 3 to 5 minutes at 100° C. Raffinose was heated for 50 minutes with 0·1 N acid at 75° C, after which treatment the products of hydrolysis were almost entirely fructose and melibiose. The method gave satisfactorily linear graphs in the range 0 to 250 μg per ml of solution for the di- and trisaccharides, and in the range 0 to 140 μg per ml of solution for the monosaccharide.

ADSORPTION ISOTHERMS—

These were obtained at 25° C in the usual way by analysing the equilibrium solution obtained after a known volume of sugar solution had been shaken with a weighed amount of charcoal and the mixture had been set aside in a thermostat for 30 minutes with occasional shaking. Samples of the equilibrium solution were obtained by means of a filter-stick fitted with a No. 3 sintered-glass disc. In this way the sample was obtained free from charcoal at the temperature of the bath. Portions (1 ml) were then taken, and the sugar determined after suitable dilution.

MEASUREMENT OF ADSORPTION-BAND LENGTH—

The apparatus and technique used in the preparation of the column was that of Patterson and Savage,[15] except that the reservoir at the top was replaced by a burette, so that the amount of solution entering the column could be measured. This burette was enclosed in a water jacket to ensure that the solution entered the column at the required temperature. The temperature was maintained at 25° C by circulating water from a thermostat through both jackets (see Fig. 5).

With the reservoir disconnected, solution was allowed to enter the column slowly by opening the taps T_2, T_3 and T_4; at all times a small layer of solution was maintained above the column. The rate of flow from the column was kept at 1 drop every 2 to 3 seconds. Slower rates of flow produced no significant difference in band length, whereas increasingly long "beaks" were obtained when the rate was faster. This suggests a failure to establish equilibrium under which conditions the theory would no longer apply. After the solution had passed through the column, stronger suction was applied for about 15 seconds, which had the effect of removing some of the surplus liquid and leaving the column sufficiently dry to be extruded. It was now possible to slice sections from the column down to 1 mm in thickness with a thin blade as the column was extruded from the end of the tube. Each section was then washed on a filter-paper with 70 per cent. alcohol to remove the sugar, which was then estimated. Graphs were made of the distribution of sugar with the length of the column, and the band lengths were determined (see Fig. 1).

MEASUREMENT OF "BREAKTHROUGH" VOLUME—

The wet column of Patterson and Savage[15] was used in the measurement of band widths. For general ease in manipulation and measurement of "breakthrough" volume, it was found more convenient to start with a dry column. A dry, intimate mixture of equal parts of kieselguhr and charcoal was introduced into the tube, B (see Fig. 5), and suction was applied to compact the column. After the column had been set aside to allow it to attain the required temperature, solution was allowed to enter the column, with the taps T_1, T_2, T_3 and T_4 open. When the space above the column was completely filled, tap T_1 was closed and the solution was allowed to pass down the column without suction, until liquid began to emerge from the lower end. The rate of flow was then controlled by adjustment of either tap T_3 or tap T_4. In order to maintain equilibrium, particular care was taken to avoid disturbance in the flow of solution. Attempts to control the rate of flow before the column had been completely wetted tended to trap air in the column and disturb the flow. For this reason particular care was taken to ensure that tap T_3 was open during the wetting process; desorbed gases otherwise became trapped in the column.

With the reservoir, A, connected, a measured volume of liquid (less than the calculated "breakthrough" volume) was collected in the calibrated flask, taps T_2 and T_3 were closed

and the reservoir was disconnected. The flask was then replaced by a test-tube, taps T_2 and T_3 were opened, a volume of effluent, measured by means of the burette, was collected and the sugar was determined. A series of convenient portions (usually 1 ml) was taken and the volume of solution that had passed through the column was plotted against the

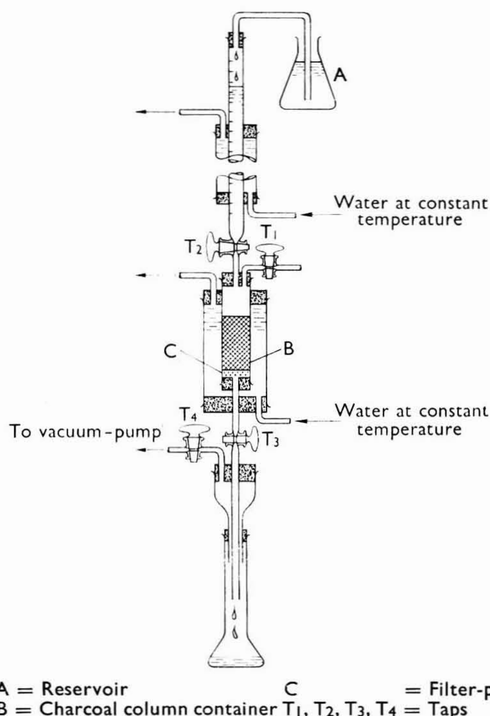


Fig. 5. Diagram of apparatus

optical density of the coloured solution obtained in the Somogyi - Nelson determination on the corresponding portion of effluent. The "breakthrough" volume was then determined from the graph as the volume corresponding to an optical density of 0·100 for the mono- and disaccharides, and of 0·200 for the trisaccharide. In each instance, the point of reference on the curve was chosen as near as possible to the initial "breakdown" and where a small change of volume gave a large change in the optical density (see Figs. 2 and 3). W_1 and W_2 were chosen in each instance to give a reasonable difference in V_1 and V_2, and to avoid the tedium of collecting large volumes. Generally, for values of c_0 of 0·5 mg per ml of solution, and above, W_1 and W_2 were made 3·5 and 2·0 g, respectively, whereas 1·0 and 0·5 g were used for lower concentrations. For the larger pair of columns, a tube of 18-mm diameter was used, and for the smaller columns this was replaced by a smaller tube about 9 mm in diameter. This maintained a reasonable ratio of length to diameter, which was thought to be desirable. Optimum rates of flow were found to be 1 drop every 2 to 3 seconds for the wide column, and 1 drop every 5 to 6 seconds for the narrow one. Faster rates of flow had the same effect of extending the "beak" as in the measurement of band width. Reproducibility was found to be within ± 2 per cent. of the determined value for "breakthrough" volume.

Results

The adsorption parameters for sugar on charcoal fell into the pattern expected by analogy with the chromatography of sugars, where the difficulty of elution was in the order

mono- < di < trisaccharides.[13,14] Thus values of k and n increase in the same order, as is shown below—

Sugar	Raffinose	Maltose	Lactose	Sucrose	Dextrose
k	98·9	43·2	42·3	39·3	4·5
n	3·20	1·99	1·91	1·69	1·06

The failure of the separation of individual disaccharides on a charcoal column is explained by their similarity in adsorption characteristics.

Metasil was found to be virtually non-adsorbing, and therefore only charcoal needs to be considered in the calculation of band width or "breakthrough" volume.

The measured and calculated adsorption-band lengths are compared in Table I. The measured band widths are reproducible within ± 1 mm.

TABLE I

ADSORPTION-BAND LENGTHS

Sugar	Concentration, mg per ml	Volume of solution applied, ml	Column length, cm	Band length measured, cm	Band length calculated, cm
Raffinose.. ..	3	20	5·0	1·1	1·1
	1	20	4·9	0·6	0·5
	1	40	4·6	1·0	0·9
	1	80	4·8	2·0	2·0
	0·5	70	4·8	1·1	1·1
	0·1	125	4·4	0·6	0·6
Maltose	3	10	4·5	0·9	0·9
	1	10	4·8	0·6	0·6
	1	20	4·6	1·0	1·1
	1	30	4·7	1·6	1·6
	0·5	20	4·6	0·8	0·8
	0·1	30	4·5	0·6	0·5
Lactose	3	10	4·7	0·9	0·9
	1	10	4·6	0·6	0·6
	1	20	4·5	1·1	1·1
	1	30	4·8	1·7	1·8
	0·5	20	4·6	0·8	0·9
	0·1	30	4·7	0·6	0·7
Sucrose	3	10	4·6	0·9	0·9
	1	10	4·6	0·7	0·6
	1	20	4·5	1·2	1·1
	1	30	4·7	1·8	1·8
	0·5	20	4·6	0·9	0·9
	0·1	30	4·6	0·8	0·7
Dextrose.. ..	3	5	4·7	2·0	2·8
	1	10	4·6	3·0	5·1
	1	4	4·5	1·5	2·2
	1	2	4·8	0·8	1·1
	0·5	5	4·8	1·7	2·6
	0·1	5	4·7	1·6	2·3

If the adsorption is of a significant order, as for the di- and trisaccharides, the results support equation (4). The values for dextrose were lower than those predicted. For the purpose of the theory, all the solution is assumed to pass through the band, whereas in practice a small volume is retained by the column. If $\frac{V}{x}$ is relatively small this becomes significant. When an approximate correction was made for the retained volume, close agreement with the calculated values was found.

Fig. 2 shows several typical examples of effluent-concentration graphs used to determine "breakthrough" volumes as defined. In these it may be seen that although the profile of effluent concentration varies with c_0, there is no apparent difference when the length of column is varied and c_0 is kept constant. This satisfies the conditions assumed in the derivation of equation (4).

The values of k and n found by using both this method and the static one are in sufficiently close agreement to justify the assumptions made (see Table II), and the linearity of the graphs

shows that, within limits, the "breakthrough" volume is dependent on the weight of adsorbing material and is independent of the diameter of the column. This contradicts previous views that a long narrow column made of a given weight of adsorbing material was more efficient than a short wider one of the same weight.[16] The probable explanation lies in the fact that the longer column presents a greater resistance to the flow of solution than the short wide one. In this instance the slower rate of flow, which was of the order of that required for equilibrium conditions, would reduce the tendency of separate bands to overlap by keeping both the "beaks" and the "tails" to a minimum.

TABLE II

COMPARISONS OF FREUNDLICH PARAMETERS FOUND BY USING (*a*) A STATIC METHOD AND (*b*) THE COLUMN

Sugar	Method (a)		Method (b)	
	k	n	k	n
Dextrose	4·5	1·06	4·5	1·06
Sucrose	39·3	1·69	38·7	1·71
Lactose	42·3	1·91	42·4	1·93
Maltose	43·2	1·99	44·9	1·97
Raffinose	98·9	3·20	97·2	3·55

A technique of frontal analysis thus provides a method for determining the parameters k and n of the Freundlich adsorption isotherm. Conversely, from a knowledge of k and n, concentrations may in principle be determined by measurements of "breakthrough" volume.

So far the investigation has been restricted to one solute. The problems involved in multi-component solutions are much greater, but it is here that the greatest analytical value of such a technique lies. Some preliminary experiments have been carried out in this direction with the intention of developing frontal techniques of analysis in the field of solid - liquid chromatography.

We thank Mr. J. L. Buchan both for suggesting the topic and his helpful advice throughout, and the Government Chemist, Department of Scientific and Industrial Research for permission to publish this Paper.

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First received *September* 19*th*, 1963
Amended *March* 26*th*, 1964

The Colorimetric Determination of *N*-Hydroxyurethane and Related Compounds

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Urethane, a carcinogen used in the treatment of myelomatosis, is metabolised to the active *N*-hydroxy derivative. *O*-Alkyl-*N*-hydroxycarbamates differ from true hydroxamic acids in that they give unstable ferric complexes, and from true esters in that they cannot be determined colorimetrically by the hydroxylamine - ferric chloride reaction. *O*-Alkyl-*N*-hydroxycarbamates give coloured complexes with pentacyano-ammine ferroates in the presence of magnesium ions, and this reaction has been developed for the determination of *N*-hydroxycarbamates. The effects of variation of time, pH, reagent concentration and magnesium ions on complex formation have been determined. The pentacyano-ammine ferroate complexes of related substances have also been investigated.

URETHANE is carcinogenic to mice,[1] rats[2] and hamsters,[3] retards growth of the Walker rat carcinoma 256[4] and is used in the treatment of multiple myeloma.[5] In the rat, rabbit and man, it is partly metabolised by *N*-hydroxylation giving rise to the excretion of *N*-hydroxyurethane and the *N*- and *O*-acetyl derivatives in urine.[6] *N*-Hydroxyurethane has similar carcinogenic effects to those of urethane.[7] Although *N*-hydroxyurethane and other *N*-hydroxycarbamates give purple colours with ferric chloride, this colour reaction is unsuitable for determining *N*-hydroxycarbamates because of its low optical density and the instability of the ferric complex. This Paper describes a sensitive method for determining *N*-hydroxycarbamates in water or urine.

METHOD

APPARATUS—

Spectrophotometers—Visible and ultraviolet spectra were plotted on a Perkin-Elmer model 137 recording ultraviolet spectrophotometer, optical densities on a Unicam SP500 instrument and infrared spectra on a Perkin-Elmer model 137 Infracord spectrophotometer fitted with sodium chloride discs.

REAGENTS—

Light petroleum—Boiling-range 60° to 80° C.

Acetate buffer, 0·2 M—A mixture of 0·2 M aqueous solutions of sodium acetate and acetic acid.

Phosphate buffer, 0·2 M, *pH* 7·5—A mixture of 0·2 M aqueous solutions of sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate.

Borate buffer, 0·2 M—A mixture of 0·2 M aqueous solutions of boric acid and sodium hydroxide.

Sodium pentacyano-ammine ferroate—8 and 0·8 per cent. w/v solutions, dissolved in phosphate buffer.

Magnesium chloride hexahydrate solution, 0·5 *per cent. w/v, aqueous.*

MATERIALS—

Hydroxylammonium chloride (analytical-reagent grade) and ethyl carbamate were obtained from Hopkin & Williams Ltd.; magnesium chloride (analytical-reagent grade), sodium pentacyano-ammine ferroate, methyl carbamate, and methyl, ethyl and n-butyl chloroformates from The British Drug Houses Ltd.; and *O*-methylhydroxylammonium chloride and n-butyl carbamate from Eastman Kodak Ltd. Sodium hadacidin (sodium *N*-formyl hydroxyamino-acetate) dihydrate was the gift of Dr. G. Boxer of Merck, Sharp & Dohme Research Laboratories, Rahway, N.J., and *N*-hydroxyurea was a gift of E. R. Squibb & Sons, New York. *N*-Methylhydroxylamine,[8] m.p. 42° C, was prepared by reducing nitromethane with zinc dust and ammonium chloride.

PREPARATION OF *N*-HYDROXYCARBAMATES—

Methyl-N-hydroxycarbamate—Methyl chloroformate (18·8 g) was added dropwise over a period of 40 minutes to a stirred solution of 13·6 g of hydroxylammonium chloride and 35 g of sodium carbonate in 200 ml of water. After 5 hours, the mixture was acidified to pH 4·0 with 4 N hydrochloric acid and extracted continuously with 200 ml of ether for 16 hours. The ethereal extract was washed with 20 ml of water, dried over sodium sulphate and evaporated under reduced pressure to yield a colourless oil that solidified after several days at 0° C. The solid was recrystallised from an ether - light petroleum mixture to yield 14·8 g of colourless prisms of methyl-*N*-hydroxycarbamate, m.p. 50° to 51° C. The composition of the prisms is given below—

Element	C	H	N
Found, per cent.	26·6	5·7	15·3
$C_2H_5NO_3$ requires, per cent.	26·4	5·5	15·4

N-Hydroxyurethane—This was prepared by the method of Fuller and King,[9] and yielded an oil contaminated with about 5 to 15 per cent. (on chromatographic evidence) of *O*-carbethoxy-*N*-hydroxyurethane. The oil was further purified through the sodium derivative by the method given below: 50 ml of 2 M sodium ethoxide in ethanol were added to a solution of 11 g of the oil in 50 ml of ethanol. The precipitate was filtered off, washed with 50 ml of ice-cold ethanol and recrystallised from aqueous ethanol to yield 8·4 g of the sodio-derivative as colourless rhombs that decomposed without melting at temperatures above 120° C. The composition of the rhombs is given below—

Element	C	H	N
Found, per cent.	28·3	5·0	10·7
Calculated for $C_3H_6NO_3Na$, per cent.	28·3	4·6	11·0

Solutions of this compound in ethanolic sodium ethoxide undergo solvolysis within 16 hours at room temperature to yield ethyl carbonate and then sodium carbonate, which is deposited. A solution of 5 g of sodium *N*-hydroxyurethane in 20 ml of water was adjusted to pH 4 with 2 N hydrochloric acid and extracted continuously for 16 hours with 50 ml of ether. The ethereal extract was washed with 5 ml of water, dried over sodium sulphate, evaporated under reduced pressure and the residue dried over phosphorus pentoxide at 60° C and 15 mm pressure for 2 hours to yield 3·2 g of *N*-hydroxyurethane as a colourless oil (b.p. 86° to 88° C at 0·6 mm pressure); $n_D^{22} = 1·4478$, $d_4^{22} = 1·225$. The composition of the oil is given below—

Element	C	H	N
Found, per cent.	34·5	7·1	12·9
Calculated for $C_3H_7NO_3$, per cent.	34·3	6·7	13·1

O-Carbethoxy-N-hydroxyurethane—Ethyl chloroformate (60 g) was added slowly to a stirred mixture of 20·7 g of hydroxylammonium chloride and 84 g of sodium hydrogen carbonate in 600 ml of ether and 25 ml of water. After 16 hours the ethereal fraction was separated, washed twice with 50-ml portions of water, dried over sodium sulphate, evaporated under reduced pressure, and the residual oil fractionally distilled to yield 28 g of *O*-carbethoxy-*N*-hydroxyurethane as a colourless oil (b.p. 96° to 98° C at 0·7 mm pressure); $n_D^{22} = 1·4258$, $d_4^{22} = 1·170$. The composition of the oil is given below—

Element	C	H	N
Found, per cent.	40·8	6·6	8·1
$C_6H_{11}NO_5$ requires, per cent.	40·7	6·3	7·9

The infrared spectrum showed strong N–H and carbonyl bands, indicating that the compound was an *O*-substituted derivative.

O-Acetyl-N-hydroxyurethane—Acetylation of 3 g of *N*-hydroxyurethane with 5 ml of acetic anhydride in 20 ml of ether or 10 g of sodium *N*-hydroxyurethane with 8 ml of acetyl chloride in a stirred suspension of 10 g of sodium hydrogen carbonate in 60 ml of ether yielded 2·1 g and 9·8 g, respectively, of *O*-acetyl-*N*-hydroxyurethane as a colourless oil (b.p. 72° C at 0·8 mm pressure); $n_D^{22} = 1·4182$, $d_4^{22} = 1·155$. The composition of the oil is given below—

Element	C	H	N
Found, per cent.	40·8	6·3	9·3
$C_5H_9NO_4$ requires, per cent.	40·8	6·2	9·5

The infrared spectrum showed strong N–H and carbonyl absorption bands.

N,O-*Diacetyl*-N-*hydroxyurethane*—Acetylation of 5 g of *N*-hydroxyurethane or 7 g of *O*-acetyl-*N*-hydroxyurethane with acetic anhydride in pyridine yielded 6 g and 7·5 g, respectively, of *N*,*O*-diacetyl-*N*-hydroxyurethane as a colourless oil (b.p. 72° C at 0·6 mm pressure); $n_D^{22} = 1{\cdot}4334$, $d_4^{22} = 1{\cdot}154$. The composition of the oil is given below—

Element	C	H	N
Found, per cent.	44·7	5·8	7·3
$C_7H_{11}NO_5$ requires, per cent.	44·4	5·9	7·4

The infrared spectrum showed no N–H, but strong carbonyl, absorption bands. If set aside with equal volumes of aqueous N sodium hydroxide, N hydrochloric acid or 2N ammonium hydroxide, solutions of this compound in methanol gave mixtures of *N*-hydroxyurethane, *O*-acetyl-*N*-hydroxyurethane and small amounts of *N*-acetyl-*N*-hydroxyurethane.

n-Propyl-N-*hydroxycarbamate*—This was prepared as described for the methyl ester from 12·2 g of n-propyl chloroformate, 6·9 g of hydroxylammonium chloride and 15 g of sodium carbonate in 100 ml of water. Evaporation of the ethereal extracts and distillation of the residual oil under reduced pressure yielded 10·8 g of a colourless oil (b.p. 90° to 92° C at 0·6 mm pressure); $n_D^{22} = 1{\cdot}4484$, $d_4^{22} = 1{\cdot}162$. The composition of the oil is given below—

Element	C	H	N
Found, per cent.	40·5	7·6	11·6
$C_4H_9NO_3$ requires, per cent.	40·3	7·6	11·8

n-Butyl-N-*hydroxycarbamate*—This was prepared as described for the n-propyl ester from 34 g of n-butyl chloroformate, 17·3 g of hydroxylammonium chloride and 35 g of sodium carbonate in 200 ml of water. Evaporation of the ethereal extracts under reduced pressure yielded 24 g of a colourless oil (b.p. 100° to 102° C at 0·8 mm pressure); $n_D^{22} = 1{\cdot}4473$, $d_4^{22} = 1{\cdot}099$. The composition of the oil is given below—

Element	C	H	N
Found, per cent.	45·4	8·3	10·6
$C_5H_{11}NO_3$ requires, per cent.	45·1	8·3	10·5

Like the methyl-, ethyl- and n-propyl-*N*-hydroxycarbamates, it gave a purple colour with 1 per cent. w/v aqueous ferric chloride solution and reduced ammoniacal silver nitrate.

PROCEDURES—

(*i*) *Determination in water*—The alkyl-*N*-hydroxycarbamate was dissolved in 3·5 ml of water in stoppered tubes, treated with 1 ml of 0·8 per cent. sodium pentacyano-ammine ferroate solution and 0·5 ml of magnesium chloride solution, mixed, set aside at room temperature for 1·5 hours, and the optical density measured at the appropriate wavelength in a 1-cm cell against a blank solution containing the reagents only.

(*ii*) *Determination in urine*—The alkyl-*N*-hydroxycarbamate was dissolved in urine adjusted to pH 7·5 with N sodium hydroxide, and 3·5-ml samples were treated with 1 ml of 8 per cent. sodium pentacyano-ammine ferroate solution and 0·5 ml of magnesium chloride solution. The resulting solutions were mixed, spun in a centrifuge if necessary, and the optical density measured after 1·5 hours against the appropriate blank solutions for urine.

RESULTS

EFFECT OF VARIATION OF REAGENT CONCENTRATION—

Solutions of *N*-hydroxyurethane in water or urine (3 ml, adjusted to pH 7·5 with N sodium hydroxide) were treated with 0·5 ml of magnesium chloride solution and 1 ml of phosphate buffer containing varying amounts of sodium pentacyano-ammine ferroate, so that the ratio of the molar concentrations of sodium pentacyano-ammine ferroate to *N*-hydroxyurethane varied between 1 and 80. All the final solutions were 0·00022 M in *N*-hydroxyurethane. Corresponding blank solutions containing all the ingredients except *N*-hydroxyurethane were also prepared. Optical densities were measured at 475 mμ in 1-cm cells against the appropriate blank solutions between 1·5 and 2 hours after each solution had been mixed. The results (see Fig. 1) indicate that for maximum colour development under these conditions, the minimum molar ratio of pentacyano-ammine ferroate to *N*-hydroxyurethane is 8 in water and 70 in urine.

EFFECT ON VARIATION OF THE pH OF THE REACTION MEDIUM—

Steady conditions of pH were maintained by using the buffer solutions listed below: 0·2 M acetate buffer for pH 5 to 5·8; 0·2 M phosphate for pH 6·0 to 8·0; and 0·2 M borate for pH 8·5 to 9·0. *N*-Hydroxyurethane was dissolved in urine or water previously adjusted to the required pH by addition of small volumes of N sodium hydroxide or N hydrochloric acid.

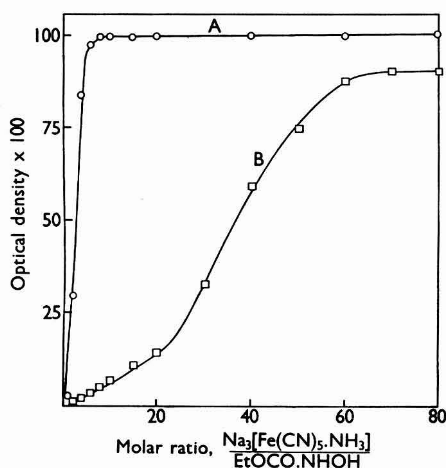


Fig. 1. Effect of variation of reagent concentration on the optical density of the *N*-hydroxyurethane - pentacyanoferroate complex: curve A, in water; curve B, in urine

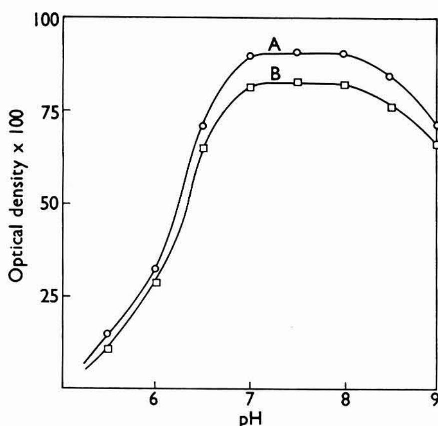


Fig. 2. Effect of variation of the pH on the optical density of the *N*-hydroxyurethane - pentacyanoferroate complex: curve A, in water; curve B, in urine

Portions (3 ml) of each water or urine solution were treated with 1·5 ml of 0·8 per cent. or 8 per cent. w/v, respectively, of sodium pentacyano-ammine ferroate solution in the appropriate buffer and 0·5 ml of magnesium chloride solution, and mixed. All final solutions were 0·0002 M in *N*-hydroxyurethane. The optical densities were measured at 475 mμ in 1-cm cells after 1·5 to 2 hours against the corresponding blank solutions, prepared similarly, but containing no *N*-hydroxyurethane. The results (see Fig. 2) indicate a maximum stability of the *N*-hydroxyurethane - pentacyanoferroate complex between pH 7 and 8.

EFFECT OF VARIATION OF TIME AND EFFECT OF MAGNESIUM IONS ON THE RATE OF COLOUR DEVELOPMENT—

Solutions of the alkyl-*N*-hydroxycarbamates in 3·5 ml of water were treated with 0·8 per cent. sodium pentacyano-ammine ferroate and magnesium chloride solutions, as in procedure

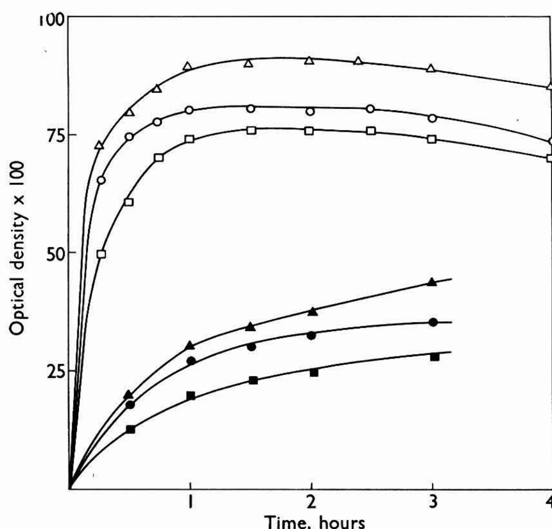


Fig. 3. Effect of variation of time on the optical density of alkyl-*N*-hydroxycarbamate - pentacyanoferroate complexes in water in the presence and absence of magnesium ions. ○ methyl-, □ ethyl-, and △ n-butyl-*N*-hydroxycarbamate in the presence of magnesium ions: ● methyl-, ■ ethyl-, and ▲ n-butyl-*N*-hydroxycarbamate in the absence of magnesium ions

(*i*), so that final solutions were 0·0002 M in the hydroxycarbamate. Similar solutions containing 0·5 ml of water instead of magnesium chloride solution were also prepared. Optical densities were measured against the appropriate blank solutions at varying time intervals after mixing. The results (see Fig. 3) indicate that maximum colour development in solutions

TABLE I

COLOUR, OPTICAL-DENSITY MAXIMA AND MOLECULAR EXTINCTION COEFFICIENTS OF THE PENTACYANOFERROATE COMPLEXES OF ALKYL-*N*-HYDROXYCARBAMATES AND RELATED COMPOUNDS

Pentacyanoferroate complex of	Colour	$\lambda_{max.}$, mμ	Molar extinction coefficient (ϵ) $\times 10^{-3}$, in— water	urine
MeOCO.NHOH	reddish pink	480	4·0	3·8
EtO.CO.NHOH	reddish pink	475	4·5	4·2
nPrO.CO.NHOH	reddish pink	465	4·2	3·9
nBuO.CO.NHOH	reddish pink	460	3·8	3·6
EtO.CO.NHOAc	mauve	540	0·24	0·23
EtO.CO.N(Ac)OAc	mauve	545	0·13	0·13
EtO.CO.NHOCO$_2$Et	mauve	540	0·25	0·20
MeO.CO.NH$_2$	none	—	—	—
EtO.CO.NH$_2$	none	—	—	—
nPrO.CO.NH$_2$	none	—	—	—
nBuO.CO.NH$_2$	none	—	—	—
NH$_2$CO.NHOH	reddish pink	465	1·1	—
Me.NHOH	reddish pink	485	0·16	—
NH$_2$OMe	none	—	—	—
HO.N(CHO)CH$_2$CO$_2$Na	none	—	—	—

containing magnesium ions is obtained after 1·5 hours, that the colours are stable for at least 1 hour thereafter and that magnesium ions caused a two- to threefold increase in the rate of colour development. Variation of the molar ratio of magnesium chloride to alkyl-*N*-hydroxycarbamate from 1 to 10 did not affect the rate of colour development.

DETERMINATION IN WATER AND URINE—

The alkyl-*N*-hydroxycarbamate was dissolved in water or urine and 3·5-ml portions were treated as described in procedures (*i*) or (*ii*). The results (see Figs. 4 and 5 for the determination in water and urine, respectively) indicate that for the three alkyl-*N*-hydroxycarbamates, the optical density is proportional to the concentration.

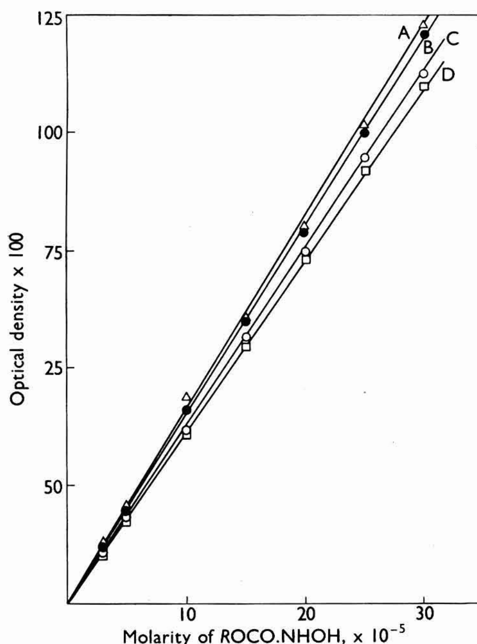


Fig. 4. The optical density of alkyl-*N*-hydroxycarbamate - pentacyanoferroate complexes at varying concentrations in water; procedure (*i*): curve A, ethyl-*N*-hydroxycarbamate; curve B, n-propyl-*N*-hydroxycarbamate; curve C, methyl-*N*-hydroxycarbamate; curve D, n-butyl-*N*-hydroxycarbamate

MOLECULAR EXTINCTION COEFFICIENTS (ϵ) AND OPTICAL-DENSITY MAXIMA ($\lambda_{max.}$) OF THE PENTACYANOFERROATE - ALKYL-*N*-HYDROXYCARBAMATE COMPLEXES AND RELATED COMPOUNDS—

Each compound listed in Table I was dissolved in water or urine and their optical densities measured as in procedures (*i*) or (*ii*). Methyl-, ethyl- and n-butyl-*N*-hydroxycarbamates were determined at concentrations of 0·0002 M; others were 0·0002 M in the final solutions. The ϵ values given for *O*-acetyl-, *N*,*O*-diacetyl- and *O*-carbethoxy-*N*-hydroxyurethane are reliable only when the final solutions of hydroxycarbamates are greater than 0·001 M; at lower concentrations, lower and erratic ϵ values are obtained. This is probably owing to varying decomposition of the corresponding substituted alkyl-*N*-hydroxycarbamate - pentacyanoferroate complexes in dilute solutions.

THE IRON^{III} - *N*-HYDROXYURETHANE COMPLEX—

By analogy with the sensitive iron^{III} - hydroxamate method for determining hydroxamic acids, esters, lactones and amides, the iron^{III} - *N*-hydroxyurethane complex was made to investigate the possibility of its use in an alternative method for estimating *N*-hydroxy-

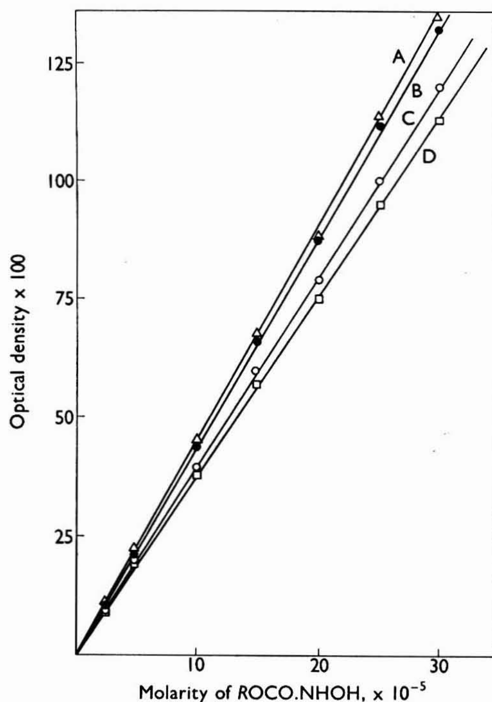
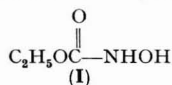


Fig. 5. The optical density of alkyl-*N*-hydroxycarbamate - pentacyanoferroate complexes at varying concentrations in urine; procedure (*ii*); curve A, ethyl-*N*-hydroxycarbamate; curve B, n-propyl-*N*-hydroxycarbamate; curve C, methyl-*N*-hydroxycarbamate; curve D, n-butyl-*N*-hydroxycarbamate

carbamates. Fig. 6 shows the optical density at 570 mμ of an aqueous solution that was 0·01 M and 0·1 M in *N*-hydroxyurethane and ferric chloride, respectively, in neutral solution. (It was less stable in acid or alkali.) A solution of *N*-hydroxyurethane in water (0·02 M, 2·5 ml) was treated with 0·5 ml of 0·2 M hydroxylammonium chloride and 0·5 ml of 0·25 M potassium hydroxide, mixed, heated at 90° C for 5 minutes, cooled and treated with 1·5 ml of a 1 per cent. w/v solution of ferric chloride in N hydrochloric acid. The optical density, measured against a blank solution prepared similarly, but without the *N*-hydroxycarbamate, at 570 mμ in a 1-cm cell was 0·21. These results show that *N*-hydroxyurethane does not behave in this respect either as an ester or a hydroxamic acid.

DISCUSSION

N-Hydroxyurethane (**I**) is bifunctional in the sense that it is an ester and a hydroxamic acid. Unlike other hydroxamic acids, it forms an unstable inner complex (see Fig. 6) with ferric chloride in neutral solution. Unlike true esters, its reaction product with hydroxylamine



in alkaline solution, probably *N,N'*-dihydroxyurea, also does not form a stable ferric hydroxamate with ferric chloride. *N*-Hydroxyurea forms an unstable ferric complex.[10] Further, because of the low optical densities of solutions of the complexes formed, these reactions are not suitable for determining *N*-hydroxyurethane.

Ferrocyanides and pentacyanoferroates form 6-co-ordinate complexes with various substances, including aromatic amines,[11] thioureas[12] and arylhydroxylamines.[13]

Taube[14] has shown that the ethylenediamine and ammine complexes of 6-co-ordinated iron are labile, but that the cyano complexes are stable. The activation energy for the breakdown of ferrocyanide to pentacyanoferroate is 19·8 kcal. per mole,[15] and the reaction is catalysed by light[16] and magnesium ions.[17]

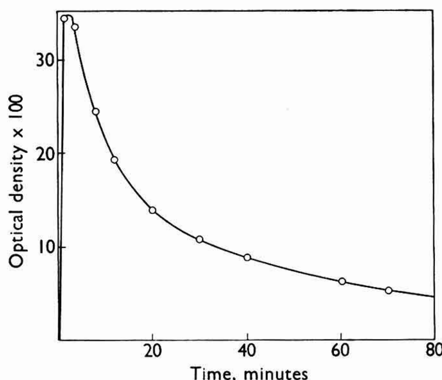
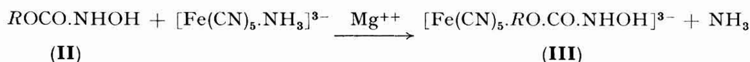


Fig. 6. The variation of optical density of a 0·01 M iron^{III} - *N*-hydroxyurethane complex in water with time

The mechanism common to these complex-forming reactions is the substitution of a cyano group (in the ferrocyanides) or of a more labile group (in the pentacyanoferroates) by an arylamino, arylhydroxylamino or other molecule. The reaction of the alkyl-*N*-hydroxycarbamates (**II**) with pentacyano-ammine ferroate may be represented as—



$$\underset{\textbf{(II)}}{R\text{OCO.NHOH}} + [\text{Fe(CN)}_5.\text{NH}_3]^{3-} \xrightarrow{\text{Mg}^{++}} \underset{\textbf{(III)}}{[\text{Fe(CN)}_5.R\text{O.CO.NHOH}]^{3-}} + \text{NH}_3$$

The magnesium ions catalyse the substitution process, probably by increasing the rate of replacement of NH_3 in the transition complex. The complex (**III**) is stable for at least 90 minutes (see Fig. 3) at pH 7·5, but decomposes with increasing acidity or alkalinity (see Fig. 2). The rapid decomposition of complex (**III**) with increasing acidity parallels that of the amminoprussides,[18] which have similar structures.

Methyl-, ethyl-, n-propyl and n-butyl-*N*-hydroxycarbamates give complexes having optical-density maxima in the 460 to 480 mμ region. The complexes of the *N*- and *O*-substituted derivatives (*O*-acetyl-, *O*-carbethoxy- and *N,O*-diacetyl-*N*-hydroxyurethane - pentacyanoferroates) absorb mainly in the 540 mμ region and have lower extinction coefficients. *N*-Hydroxyurethane may therefore be estimated in the presence of its *O*-acetyl-, *N,O*-diacetyl- and *O*-carbethoxy derivatives.

We thank Dr. G. Boxer of Merck, Sharp & Dohme and Dr. C. C. Stock of the Sloan - Kettering Institute for Cancer Research for gifts of sodium hadacidin dihydrate and *N*-hydroxyurea, respectively, and Mr. P. Detchon for technical assistance. This investigation has been supported by grants to the Chester Beatty Research (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Received *March 6th*, 1964

The Determination of Vitamin D in Pharmaceutical Preparations by Thin-layer Chromatography

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A two-stage thin-layer chromatographic procedure is used for separating and determining vitamin D in pharmaceutical preparations. In the first stage, vitamin D is separated from oil, vitamin A and some sterols. The determination is achieved in the second stage by comparing the separated spot with a series of standards. Saponification and extraction previously used in determinations of vitamin D are avoided.

THE quantitative determination of vitamin D has always been a matter of some difficulty. Commonly used biological methods are costly and take considerable time to complete. Alternative colorimetric methods[1,2,3] can only be used when there is a relatively large amount of vitamin D, and when the effect of other substances on the reagents used is either negligible or can be taken into account.[4] The presence of appreciable amounts of sterols or vitamin A necessitates some separation of the vitamin D. Various methods for this have been suggested,[5 to 10] in which saponification and extraction and then chromatography are used. Such a method was published in the U.S. Pharmacopoeia XVI.[11] In the same year it was shown by Janecke and Maass-Goebels[12] that saponification, and chromatography through Superfitrol, produce changes in the vitamin D molecule, and they concluded that such procedures should be avoided in determinations of vitamin D.

It was therefore necessary to find other means of separating vitamin D from the lipid material and vitamin A. Janecke and Maass-Goebels showed that vitamin D could be separated from other sterols by thin-layer chromatography, and Davidek and Blattna,[13] using thin layers of dry alumina, were able to separate various pure vitamins. It seemed therefore that some method of thin-layer chromatography might provide a means of separating vitamin D from oil and other substances in pharmaceutical preparations. The Davidek and Blattna method was found to give a good separation of vitamin D from vitamin A ester, but not from oil, and the method of detection with 98 per cent. sulphuric acid was somewhat inconvenient. Chromatography on thin layers of silica gel gave a better separation of the constituents of pharmaceutical preparations. Various solvent systems were tried and it was found that a 4 + 1 cyclohexane - ether mixture gave the best separation. The oil travelled to a point near the solvent front with the vitamin A, leaving an area of separated bands, among which the vitamin D could be located when the plate was viewed in ultraviolet light.

If the sample is of suitable viscosity it is possible to apply it in an even line across the the thin-layer plate, so that subsequent development will produce a separation in a series of lines, from which the area containing the vitamin D can be removed. We have found, however, that it is often extremely difficult to apply the sample evenly and have therefore made use of plates shaped as shown in Fig. 1. The sample is applied in a large elongated spot at the base of the narrow portion, and the result of the development by normal thin-layer ascending-solvent chromatography is to produce a series of curved bands. The appearance of the chromatogram viewed in ultraviolet light is shown in Fig. 2.

The position of the vitamin D is found by a preliminary qualitative experiment. It is not possible to use the R_F value of vitamin D for this purpose, since it is altered by the oil passing up the silica-gel layer. It is therefore necessary to add a spot of vitamin D solution to the original sample spot. The position of the vitamin D spot after development can be found by spraying the chromatogram with 50 per cent. sulphuric acid, and if a portion of the chromatogram is screened, the position of the vitamin D spot can be seen in relation to the bands of fluorescence when the plate is viewed in ultraviolet light. Although vitamin D can be seen as a dark spot in light of 254 mμ wavelength, the samples that we have examined have generally shown the vitamin D to be associated with a bright, narrow fluorescent band in light of 350 mμ. The area containing this band is removed from the plate and extracted.

The extract is concentrated by evaporation under nitrogen and applied to a second silica-gel plate (200 × 200 mm) together with a series of pure vitamin D standards. After development in 4 + 1 cyclohexane - ether mixture, the spots are detected by spraying the plate with 50 per cent. sulphuric acid. After the plate has been treated for 5 minutes at 80° C, the spots are seen to be a brown-purple colour, and a comparison of colour intensity is made between the sample and the standards. Other detecting reagents were tried, *e.g.*, antimony trichloride in chloroform, phosphotungstic acid, iodine, and furfural and sulphuric acid, but

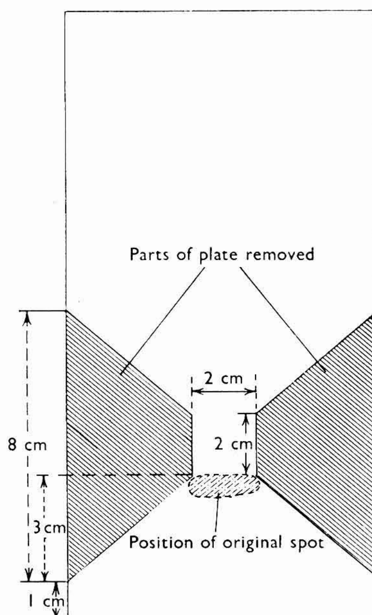

Fig. 1. Diagram showing shape of plates used for thin-layer chromatography

50 per cent. sulphuric acid was found to be the most satisfactory. The limit of detection was found to be 0·2 μg. It is possible to make an accurate comparison of the intensity of the sample spot with those of the standards if the amount of the sample is arranged to be between 0·5 and 1·5 μg, and if all the spots are applied in 5 μl of cyclohexane. It was found that development of the sample sometimes produced an irregularly shaped spot, which made comparison difficult. By a two-dimensional development, however, the sample spot was re-formed into a shape more nearly circular.

The method may be used for solid preparations, and also for liquid preparations containing at least 3200 units per gram.

METHOD

APPARATUS—

Applicator for thin-layer plates—Use apparatus manufactured by Camag, Switzerland, to the design of Mutter and Hofstetter and described by Wollish, Schmall and Hawrylyshyn.[14] Adjust it to produce an absorbent thickness of 300 microns on 100 × 200-mm plates.

Plates have also been produced by a simple method (see Fig. 3), in which they were coated by means of a frame consisting of two pieces of glass rod joined at their ends by paper clips. Two layers of Sellotape fixed along the edges of the plates held them together and at the same time kept the frame away from the glass. Adsorbent slurry was poured into the frame, which was then moved along the line of plates, to give a layer the same thickness as the layers of Sellotape. The adsorbent slurry was made by grinding silica gel with water in the proportion of 10 g of silica gel to 22 ml of water.

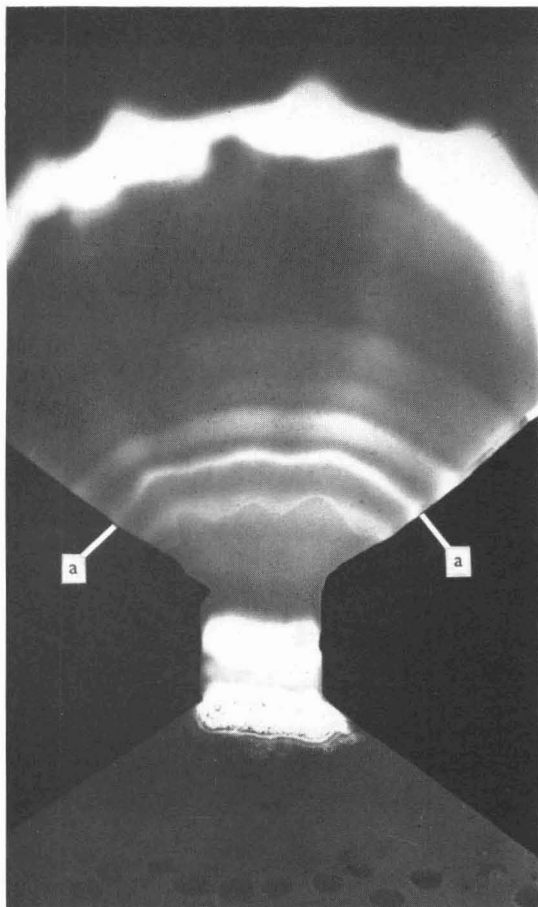

Fig. 2. Appearance of chromatogram in ultraviolet light; the band marked "a" is the zone containing vitamin D

[To face p. 530

Suction sampling apparatus—This apparatus designed by Matthews, Pereda V. and Aguilera P.[15] is a device for removing and extracting portions of thin-layer chromatograms. Silica gel is drawn in through an inlet tube by means of suction applied to the top of a long-necked 10-ml flask, and collects on a sintered-glass disc situated between the end of the tube and the point at which the suction is applied. The inlet tube is then dipped into solvent, which passes through the tube and into the flask together with the material extracted from the silica gel and collected on the sintered-glass disc.

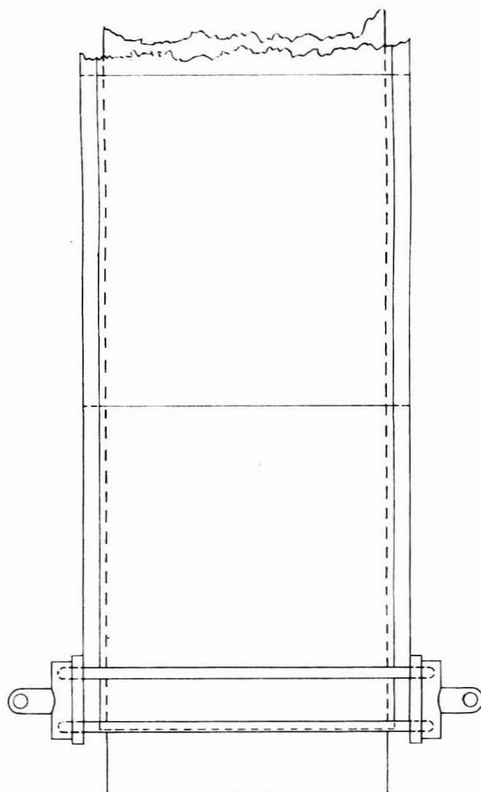

Fig. 3. Diagram of arrangement for preparing thin-layer plates

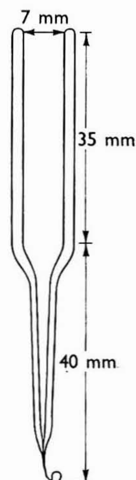

Fig. 4. Diagram of evaporation tubes

Glass plates—100 × 200 mm and 200 × 200 mm.

Ultraviolet lamp—Obtainable from Camag, Switzerland, and capable of operating at a long wavelength (350 mμ) and at a short wavelenth (254 mμ).

Chromatographic tubes—10 × 120 mm, fitted with porosity 2 sintered-glass discs.

Evaporating tubes (see Fig. 4)—These are made from glass tubing of approximately 7-mm internal diameter. One end is drawn out to a fine capillary, and sealed.

Test-tubes—6 × 50 mm.

REAGENTS—

All reagents should be of analytical-reagent grade whenever possible.

Cyclohexane.

Spectrosol—Obtainable from Hopkin & Williams Ltd.

Diethyl ether—Use peroxide-free material.

Mixed solvent—Mix 80 ml of cyclohexane with 20 ml of ether.

Silica gel—Silica gel G (obtainable from E. Merck & Co. Inc.).
Sodium sulphate, anhydrous.
Vitamin D—Use pure, crystalline vitamin D_2.

PROCEDURE—

Chromatography should be carried out in the dark. Other operations should be carried out with reasonable speed in subdued light.

Prepare two 100 × 200-mm thin-layer plates and shape them as shown in Fig. 1 by removing the shaded portions. Activate the plates by heating them to 110° C for 20 minutes. Line a chromatographic separating chamber with filter-paper, and pour in sufficient mixed solvent to cover the bottom to a depth of 1 cm.

If the sample is in tablet form, grind it and mix sufficient of the powder to contain about 800 units of vitamin D with an equal amount of sodium sulphate. Place the mixture in a chromatographic tube and elute it with 25 ml of cyclohexane. Evaporate the eluate under nitrogen until its volume is small enough to be transferred to an evaporating tube, and continue the evaporation until the volume is reduced to about 50 μl.

If the sample is an oil, place about 50 μg in an evaporating tube.

Break off the tip of the evaporating tube and apply the contents drop by drop to the lower part of the narrow portion of the shaped 100 × 200-mm thin-layer plate, until the elongated spot so formed covers the area shown in Fig. 1. When the oil has soaked into the silica, add 5 μl of a solution containing 1 μg of vitamin D per μl of cyclohexane to the sample spot at a point near its centre. Place the plate in the separating chamber and allow development to proceed until the solvent front has reached a point 16 cm from the bottom of the plate. Remove the plate from the chamber, dry it in air and screen a portion of the plate (not containing the spot caused by the added vitamin D). Spray the plate with 50 per cent. sulphuric acid and heat it to 80° C for a few minutes until the sprayed portion of the plate becomes coloured, showing the vitamin D among a series of coloured bands. Observe the whole plate in ultraviolet light at 350 mμ, and note the fluorescent bands that, if continued into the sprayed portion, would contain the vitamin D spot.

Repeat the process with an accurately weighed amount of sample, without the addition of the vitamin D spot, and without the spraying with sulphuric acid. Observe the second chromatogram in ultraviolet light, and mark with a needle the fluorescent bands or lines noted in the preliminary experiment.

Activate a 200 × 200-mm plate at 110° C for 20 minutes. Scrape the silica gel clear of the glass in the area marked out with the needle and remove it with the suction sampling apparatus. Extract the silica gel with ether, and evaporate the ethereal solution to dryness under nitrogen; use a small (6 × 5-mm) test-tube to complete the evaporation. Dissolve the residue in 50 to 100 μl of cyclohexane so that 5 μl of the solution will contain about 1 μg of vitamin D.

Prepare standard solutions of vitamin D in cyclohexane containing 0·4, 0·6, 0·8, 1·0 and 1·2 μg of vitamin D per 5 ml of cyclohexane and apply 5 μl of each to the activated 200 × 200-mm plate, together with 5 μl of the sample solution, on a line 3 cm from the edge of the plate. Place the plate in the separating chamber and allow development to proceed by ascending-solvent chromatography. After 30 minutes, remove the plate, dry it in air, then continue the development in a direction at right angles to the previous development. After a further 30 minutes remove the plate, spray it with 50 per cent. sulphuric acid and heat it at 80° C until the spots can be clearly seen. Compare the intensity of the sample spot with those of the standards to obtain a measure of the amount of vitamin D in the sample.

RESULTS

The results of examination of several tablet samples of nominal vitamin D content of 800 units per 0·5-g tablet are shown below—

Vitamin D found—								
in μg ..	20·7	21·0	17·5	23·0	19·2	21·7	22·5	20·0
in i.u. ..	828	840	700	920	768	868	900	800

Although one sample shows a difference of 15 per cent. from the nominal amount, the majority show a difference of less than 10 per cent., and the average figure of 828 units is only a difference of 3·5 per cent.

It is possible to carry out a colorimetric determination on the extract from the first plate with a solution of antimony trichloride in chloroform. There are, however, objections to the use of this reagent. Not only is it not specific for vitamin D, but there is also the possibility of corrosion of the mirrors when it is used in a spectrophotometer. The extract from the first plate does not contain vitamin D only. The second stage of chromatography shows a separation of the extract into vitamin D and at least two other substances, both of which give a colour with antimony chloride reagent. In the comparison with the standards, only the spot having the same R_F value as the standards is compared. It may therefore be claimed that the method is specific for vitamin D. As the method applies equally well to both vitamin D_2 and vitamin D_3, no attempt has been made to differentiate between the two.

With regard to oils containing less than 3200 units of vitamin D per g of oil, it is necessary to find some way of increasing the proportion of vitamin D before the method can be applied. Work on this problem is in progress, and it is hoped to publish a procedure for such products in the near future.

We thank the Government Chemist, Department of Scientific and Industrial Research, for permission to publish this Paper.

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Received *January 10th*, 1964

Spectrophotometric Determination of Vitamin D in Presence of Vitamin A

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The determination of vitamin D in the presence of vitamin A is a formidable task, since vitamin A masks vitamin D both in the ultraviolet-absorption and colour tests.

It is proposed to remove vitamin A by converting it to anhydrovitamin A by treatment of the unsaponifiable material with 0·033 N ethanolic hydrogen chloride, and then by chromatography of a light-petroleum extract of the resulting product on a column of weakened alumina containing 8 per cent. v/w of water. The chromatogram is developed with light petroleum. Anhydrovitamin A flows quickly through the column. Three distinct yellow bands develop on the column; the colourless portion, between the second and third yellow bands on the column, contains all the vitamin D. This colourless portion is extruded and then eluted with diethyl ether, the eluate is evaporated to dryness under reduced pressure and the colour developed with an antimony trichloride - acetyl chloride reagent is measured within 30 seconds of the addition of the reagent.

About 94 per cent. of the vitamin D is recovered.

THE quantitative determination of vitamin D in fish-liver oils is beset with great difficulties, mainly owing to the presence of vitamin A, which remains with vitamin D in the non-saponifiable fraction and masks vitamin D both in the ultraviolet-absorption and colour tests. The presence of provitamins D is another obstacle, since these also accumulate and they are difficult to eliminate.

An antimony trichloride reagent, first used by Brockmann and Chen,[1] has been found to give highly erratic results by several workers. Morton[2] has found that cholesterol, ergosterol and calciferol all gave colour tests with antimony trichloride reagent, but the colour, developed at different rates, showed different intensities that were insufficiently reproducible. The introduction of the modified antimony trichloride reagent of Zimmerli, Nield and Russell[3] is a distinct advance, since the colour produced reaches maximum intensity in 30 seconds, is stable for up to 5 minutes, has a maximum absorption at 500 mμ and has a value for $E^{1\%}_{1\,cm}$ of 1800. The common congeners of vitamin D (cholesterol, etc.) develop similar bands, but more slowly.[3,4,5]

Various methods have been tried for eliminating vitamin A, *viz.*, treatment of concentrates with maleic anhydride in dioxan,[6] and the chromatographic methods of Ewing, Kingsley, Brown and Emmett,[7] Ewing, Powell, Brown and Emmett[8] and Green.[9] These methods, however, are not found to be entirely satisfactory, especially when the ratio of vitamin A to vitamin D is high.

In this Paper a different method of eliminating vitamin A is proposed, *viz.*, converting vitamin A to anhydrovitamin A by treatment of the non-saponifiable fraction with dry ethanolic hydrogen chloride. The separation of vitamin D from anhydrovitamin A can be achieved by chromatography on weakened alumina that contains 8 per cent. v/w of water.[10]

METHOD

APPARATUS—

The spectrophotometric determinations were carried out with a Beckman DK-2 spectrophotometer and a Hilger & Watts Uvispek photo-electric spectrophotometer. All the absorption spectra of colours produced by adding antimony trichloride reagent to the substances that separated on the chromatogram were recorded on a Beckman DK-2 spectrophotometer.

REAGENTS—

Use chemically pure material except where otherwise stated.

Alcohol—Heat a solution of 20 g of potassium hydroxide and 10 g of silver nitrate per litre of absolute alcohol for 8 hours under reflux. Distil the alcohol and collect the distillate, discarding the first and the last 20-ml portions.

Light petroleum—Boiling-range, 40° to 60° C. Purify light petroleum by shaking it with concentrated sulphuric acid for 24 hours, and separate and discard the acid. Wash the light petroleum twice with 10 per cent. sodium carbonate solution, then with 5 per cent. potassium permanganate solution and finally 15 times with water. Dry it over sodium and distil it under reduced pressure. Collect the distillate, discarding the first 5 per cent. and the last 10 per cent.

Diethyl ether—Wash B.P. ethyl ether 10 times with water to remove alcohol, and dry it over phosphorus pentoxide. Filter the mixture and distil the ether from reduced iron as needed.

Chloroform—Wash chloroform 7 times with equal volumes of water and dry it over calcium chloride. Distil the mixture and collect the distillate, discarding the first, turbid portion and the last 10 per cent.

Antimony trichloride reagent—Dissolve 20 g of antimony trichloride in 100 ml of purified chloroform and 2 ml of freshly distilled acetyl chloride. Mix the solution and add a few pieces of zinc to it.

Calciferol—B.P. grade.

Alumina—Use aluminium oxide that has been standardised for chromatographic analysis to Brockmann grading I.

Potassium hydroxide solution, 12 *per cent. w/v, alcoholic.*

Sodium sulphate, anhydrous.

Hydrogen chloride, 0·033 N *in ethanol.*

SAPONIFICATION—

Take a convenient weight of oil that contains not less than 300 i.u. of vitamin D and saponify it with freshly prepared alcoholic potassium hydroxide solution under a slow stream of nitrogen for 5 to 15 minutes, depending on the nature of the substance to be analysed. Maintain a ratio of 2·5 g of potassium hydroxide to 1 g of oil. Dilute the alcoholic soap solution obtained with an equal volume of water and extract it four times with suitable volumes of light petroleum. Combine the extracts and wash them with water until the washings are neutral to phenolphthalein. Dry the solution over sodium sulphate and quantitatively transfer it to a calibrated flask. Determine the vitamin A content from the ultraviolet-absorption spectrum of the solution and apply a Morton and Stubb's correction.

Evaporate the remainder of the solution (a known volume) to dryness, and treat the residue with a convenient volume, 10 to 40 ml, of ethanolic hydrogen chloride for 40 minutes under an inert atmosphere. Neutralise the excess of acid with the least amount of solid sodium hydrogen carbonate. Extract the solution with light petroleum as described in the previous paragraph. Wash the extract free from alkali and dry it over sodium sulphate.

CHROMATOGRAPHY—

Evaporate the light-petroleum solution to a small volume, 1 to 2 ml, and carefully transfer it to a chromatographic column, 1 × 9 cm, packed with weakened alumina that contains 8 per cent. v/w of water. Develop the chromatogram with light petroleum. Maintain the rate of elution at approximately 1·5 ml per minute by applying a slight positive pressure to the column. Anhydrovitamin A will be contained in the first 25 ml of eluate. Further development with light petroleum will give three yellow bands and a colourless portion between the second and third yellow bands. Described from the top of the column they will be—

(*i*) A thin, well defined yellow band, whose value for $\lambda_{max.}$ is 280 to 290 mμ in the ultraviolet, in the first centimetre of the column; the colour with antimony trichloride reagent has a value of $\lambda_{max.}$ of 580 to 585 mμ.

(*ii*) A well defined, relatively wider yellow band about 5 mm below the first. This substance has values for $\lambda_{max.}$ of 330, 350 and 370 mμ in the ultraviolet; the colour with antimony trichloride reagent has a value for $\lambda_{max.}$ of 615 mμ.

(*iii*) A colourless portion, 4 to 5 cm long, containing vitamin D.

(*iv*) A wide, pale yellow band at the bottom of the column. This substance has a low absorption in the ultraviolet and no selective absorption.

From several preliminary experiments it was found that vitamin D was contained in the colourless portion, (*iii*), of the column.

EXTRACTION AND DETERMINATION OF VITAMIN D—

Extrude the column, cut a 2-cm length of the colourless portion from 0·5 cm below the second yellow band and elute the cut piece with two 15-ml portions of diethyl ether. Evaporate the eluate to dryness and dissolve the residue in a convenient volume of light petroleum. Evaporate a portion of this solution to dryness under nitrogen in a small flask. Add 3 ml of antimony trichloride reagent to the residue and swirl the flask gently for 30 seconds. Transfer the solution quickly to an absorptiometer cell and measure the optical density at 500 mμ against antimony trichloride reagent as the blank solution.

RESULTS

Recovery experiments were carried out with pure calciferol mixed with an oil that contained vitamin A, in this instance, Prepalin (obtainable from Glaxo). Measured volumes of an alcoholic solution of calciferol of known strength were added to different weights of the oil before it was saponified. Prepalin contains no vitamin D and has a value of $E_{1cm}^{1\%}$ at 326·5 mμ of 39·6, equivalent to 75,240 i.u. of vitamin A. The procedure described above was used to separate vitamin D from vitamin A, with the results shown in Table I.

TABLE I

RECOVERY OF VITAMIN D IN THE PRESENCE OF VITAMIN A

Weight of oil, g	Calciferol added, μg	Calciferol recovered, μg, calculated— from optical density at 500 mμ	from ultraviolet optical density at 265 mμ	Recovery of calciferol, per cent. at 500 mμ	at 265 mμ	Ratio of vitamin A (i.u.) to vitamin D (i.u.)
0·2095	96·7	91·4	103·4	94·5	107	4
0·2182	55·2	52·65	66·6	95·5	120·7	7·8
0·2765	31·12	29·59	—	94·8	—	16·7
0·2164	17·2	15·9	—	92·8	—	23·6
0·3163	15·79	14·9	18·57	94·8	117·5	37·7

Table I shows that vitamin D, determined by measuring the optical density at 265 mμ, gave results with an error ranging from 7 to 20 per cent., depending on the ratio of vitamin A (i.u.) to vitamin D (i.u.). The results obtained by measuring the optical density at 500 mμ gave better values with an error ranging from 5 to 7 per cent. In all these recovery experiments it has been possible to get a sharp maximum at 265 mμ, which shows the efficiency of elimination of vitamin A.

TABLE II

DETERMINATION OF VITAMIN D IN VARIOUS PREPARATIONS

Preparation	Weight of oil, g	Potencies of vitamins as given, i.u. per g: Vitamin A	Vitamin D	Vitamin D found, i.u. per g
Shark-liver oil	4·0	1500	100	82
	4·0	1500	100	78
Shark-liver oil, medicinal preparation ..	10·0	190	35	32
	10·0	190	35	31
Multivitamin tablets—				
Becadex (Glaxo, India)	1 tablet	5000	1000	600
	1 tablet	5000	1000	593
Multivite (The British Drug Houses Ltd.)	5 tablets	12,500	1250	1080
	5 tablets	12,500	1250	1027

By using the same procedure, vitamin D was estimated in two fish-liver oils and two proprietary preparations containing vitamins A and D. The analysis was carried out in duplicate, and the results are shown in Table II.

Table II shows the values for vitamin D determined by the proposed method against the potencies as stated by the manufacturers. In the absence of any bioassy being carried out in this laboratory, it is not possible to say how far the determinations carried out will compare with actual bioassay values. Nevertheless, the duplicate determinations carried out agree well, and as such are reproducible. Further work is in progress.

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Received *December 30th*, 1963.

Studies of the Separation of Trace Metals by the Manganese Dioxide "Collection" Method

Part I. The Behaviour of Antimony, Bismuth and Tin: Separation of Traces of Antimony and Tin from Bismuth

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Studies of the "collection" of antimony, bismuth and tin, both alone and in admixture, by means of manganese dioxide are described. The concentration ranges over which the precipitations are quantitative have been determined, and the effect of nitric acid concentration on the inhibition of the precipitation of bismuth has been investigated. Procedures for determining each element in the absence and presence of one, or both, of the other two are given, with particular reference to the separation of trace amounts of antimony and tin from bismuth. Certain aspects of the results are discussed.

THE use of manganese dioxide, produced by the reaction of manganese^{II} ions with potassium permanganate in nitric acid solution, as a "collector" for the co-precipitation of certain metals was first described by Blumenthal.[1] He showed that the method could be applied to antimony and indicated that tin and bismuth were also quantitatively co-precipitated. Further studies by Kallman and Prestira[2] showed that if the acidity of the solution was greater than 0·07 N, the precipitation of bismuth was incomplete. Previous work by one of us (G.F.R.) has shown that, for antimony and tin, quantitative co-precipitation is obtained over a wide range of acid concentration. Similar results have been obtained for antimony by Babko and Shtokalo[3] who used radioactive antimony-124, which was also used by one of us (G.F.R.) in earlier work.

Several analytical applications have been reported in the literature, *e.g.*, the determination of antimony with Rhodamine B as indicator by MacNulty and Woollard,[4] the determination of antimony in copper by Park and Lewis,[5] antimony and bismuth in anode copper by Yamazaki,[6] antimony, arsenic and tin in lead by Luke[7] and antimony in cast iron by Rooney.[8] No comprehensive study of the elements to which it is, or is not, applicable has, however, been published, and no investigation of the concentration ranges over which it is effective has been reported.

This Paper describes a study of some factors that affect the quantitative "collection" of antimony, bismuth and tin and the effective concentration ranges for the co-precipitation of these elements. Since most published procedures deal with separation of trace amounts and the efficiency at these levels is well established, our major interest was in the upper limits of concentration from which the metal could be effectively "collected." A wide concentration range has, however, been covered.

In view of the important application of the procedure for determining traces of antimony and tin in bismuth, special attention has been given to establishing conditions for inhibiting the bismuth precipitation, while allowing the quantative removal from solution of the other two elements.

EXPERIMENTAL

The sample solution, in a 400-ml beaker, was treated with 1·2 N nitric acid to bring the volume up to 200 ml. Five millilitres of a 5 per cent. solution of manganese sulphate was then added and the solution heated to boiling. A portion (2·5 ml) of 1·25 N potassium permanganate was added dropwise and the solution heated for a further 2 minutes. The solution was kept at 70° C for 30 minutes and then the manganese dioxide precipitate was filtered off on a Whatman No. 40 filter-paper. The precipitate was washed 3 times with 10-ml portions of hot 1·2 N nitric acid; the first two portions were used to transfer all the residual precipitate from the beaker to the filter-paper. The precipitate was dissolved in a suitable

acid and the "collected" element determined by methods appropriate to the species present. The foregoing procedure was devised in the course of earlier work by one of us (G.F.R.).

Unless otherwise stated all polarography was carried out on a Tinsley Mark 19 Polarograph at 25° C *versus* the mercury pool anode. A Spekker absorptiometer was used for colorimetric determinations of antimony in conjunction with a tungsten lamp, Ilford spectrum yellow-green filters and 1-cm cells.

TIN—

A series of standard solutions of tin was prepared and treated as described above. The tin solution used was obtained by dissolving 0·600 g of pure tin in 10 ml of 10 M hydrochloric acid and diluting the solution to 500 ml in a calibrated flask with 1·2 N nitric acid. The filter-paper containing the precipitate was wet oxidised by repeated evaporation with sulphuric and nitric acids, and the resulting solutions diluted to 500 ml in calibrated flasks after addition of sufficient sulphuric acid to make the final concentration 2 M.

The tin contents of these solutions were determined polarographically. Three millilitres of each solution were placed in a polarographic cell and treated with 2 ml of 10 N hydrochloric acid. The solution was de-oxygenated by passing nitrogen through the solution for 10 minutes. A typical step is shown in Fig. 1. Concentrations were found by reference to a calibration curve. Recoveries are listed in Table I.

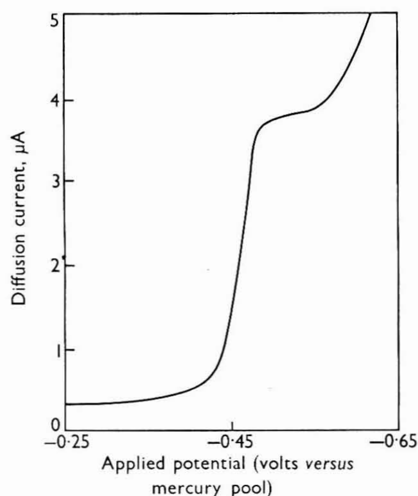

Fig. 1. Polarogram showing step for tin in sulphuric acid - hydrochloric acid solution mixture after manganese dioxide "collection"

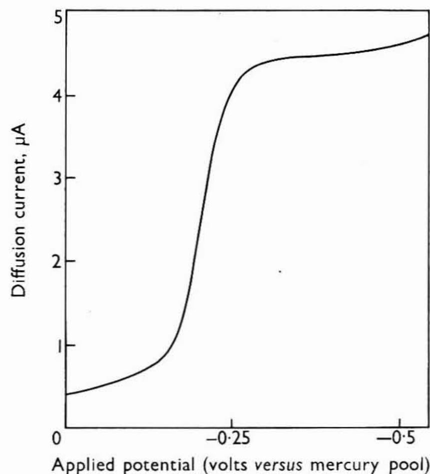

Fig. 2. Polarogram showing step for bismuth in sulphuric acid - hydrochloric acid solution mixture after manganese dioxide "collection"

Alternative procedures involving sulphide precipitation and oxidation with hydrogen peroxide were tried. Although easier to carry out than the wet oxidation method, these were abandoned because the resulting solutions were unsuitable for direct polarography.

The effect of varying the amount of potassium permanganate used to produce the manganese dioxide was next studied by using a constant amount of tin (24·0 mg) in 1·2 N nitric acid. The results are given below—

Volume of 1·25 per cent. potassium permanganate used, ml	1·25	2·5	5·0	10·0
Tin found, mg	6·0	14·0	19·9	20·0

BISMUTH—

A series of experiments was made by using standard solutions of bismuth in 1·2 N nitric acid. The procedure and polarographic determination were the same as those used for tin.

Bismuth recoveries are shown in Table II, and a typical bismuth step is shown in Fig. 2.

In view of the previous results[2] the effect of varying the nitric acid concentration on the "collection" of bismuth was investigated. For this work 10-ml portions of a standard solution containing 1·00 g of bismuth per litre of solution were used and the initial nitric acid concentration was varied from 0·06 to 1·2 N. The results are shown in Table III.

TABLE I

RECOVERY OF TIN

Tin added, mg	Tin concentration in sample solution, µg per ml	Tin found, mg
0·3	1·5	0·35*
0·3	1·5	0·4*
1·5	7·5	1·3
1·5	7·5	1·4
3·0	15	3·0
3·0	15	2·8
6·0	30	6·0
6·0	30	5·9
12·0	60	11·7
12·0	60	11·5
18·0	90	17·9
18·0	90	18·4
24·0	120	12·4
24·0	120	14·7

* A linear-sweep cathode-ray polarograph[9,10] was used for these determinations.

TABLE II

RECOVERY OF BISMUTH

Bismuth added, mg	Bismuth found, mg	Bismuth added, mg	Bismuth found, mg
10	0·97	100	1·95
10	0·95	200	2·21
20	1·91	500	2·08
20	1·94	500	2·11
50	2·52	1000	1·95
50	2·36	1000	2·00
100	2·20	5000	2·28

TABLE III

EFFECT OF NITRIC ACID CONCENTRATION ON THE RECOVERY OF BISMUTH

Bismuth added, mg	Nitric acid concentration, N	Bismuth found, mg
10	1·2	2·0
10	0·6	7·4
10	0·12	8·6
10	0·06	9·7
20	0·06	19·1

ANTIMONY—

For these studies a standard solution, prepared by dissolving 0·5 g of antimony powder in 25 ml of 18 M sulphuric acid and diluting the solution to 250 ml with 25 per cent. sulphuric acid, was used. The usual precipitation procedure was employed. In view of the difficulties in obtaining good polarographic steps for antimony under the conditions of the experiment, determinations were made colorimetrically by using the reaction of Rhodamine B with the element in the quinquivalent state. This has been fully described by MacNulty and Woollard[4] and antimony recoveries obtained by using their method are given in Table IV.

TIN AND ANTIMONY—

This part of the work necessitated a change in the procedure for dissolving the precipitate. The wet oxidation method used for tin alone was found to introduce sufficient organic material to cause interference with the colorimetric determination of antimony. Similarly, the

hydrogen peroxide - nitric acid method used by MacNulty and Woollard[5] resulted in interference with the polarography of tin, owing to the presence of traces of un-decomposed peroxide in the final solution.

TABLE IV

RECOVERY OF ANTIMONY

Antimony added, mg	Antimony found, mg	Antimony added, mg	Antimony found, mg
0·5	0·45	20	18·2
1·0	0·9	20	18·7
1·0	0·8	30	28·0
5·0	4·8	40	9·8
10	9·0	50	2·5
		60	0

After some experiments, however, the hydrogen peroxide - nitric acid method was adopted, and a portion of the final solution was used directly for the determination of antimony. A further portion of this solution was then treated with sulphur dioxide to decompose the residual peroxide.

After the solution had been boiled to remove excess of sulphur dioxide and diluted to give a solution approximately 2 M in sulphuric acid, a 3-ml portion was treated with 2 ml of 10 M hydrochloric acid and polarographed as before.

The results obtained with mixtures of the standard antimony and tin solutions are given in Table V.

TABLE V

RECOVERY OF ANTIMONY AND TIN FROM AN ADMIXTURE OF THE TWO

Antimony		Tin	
added, mg	found, mg	added, mg	found, mg
1	0·9	1·5	1·6
1	0·85	3	3·0
5	4·5	3	2·8
10	9·5	6	5·8
20	18·8	12	11·6
20	19·0	15	14·4
25	21·2	15	14·0
30	5·5*	18	4·3
30	5·5*	18	7·1

* Incomplete precipitation of the manganese dioxide occurred.

TIN AND BISMUTH—

As the polarographic steps of tin and bismuth are close together (half-wave potentials less than 0·2 V apart) in the solutions used, separation was necessary. The procedure given below, based on earlier work by one of us (G.F.R.), was used.

Solutions containing standard amounts of tin and bismuth were precipitated and wet oxidised as before. The resulting solutions were diluted to 100 ml and treated with 10 ml of 10 per cent. tartaric acid solution and 0·1 ml of phenolphthalein. Ammonium hydroxide (18 M) was then added dropwise until the indicator was just pink. The solutions were then heated to boiling and treated with 5 ml of 3 per cent. oxine solution with continuous stirring. After the solution had been set aside for 30 minutes it was filtered, and the residue was washed with two 10-ml portions of hot 2 per cent. tartaric acid solution that had been made just alkaline to phenolphthalein by adding ammonium hydroxide. The residue was rejected. The filtrate was extracted with successive 25-ml portions of a 3 per cent. dithizone solution in chloroform until the chloroform layer remained green. The aqueous layer was then washed twice with 25 ml of chloroform and diluted to the mark in 250-ml calibrated flasks with 2 M sulphuric acid.

Portions (3 ml) of these solutions were treated with 2 ml of 10 M hydrochloric acid, deoxygenated and polarographed as before. Good steps were again obtained, but they were smaller than those with standard solutions of equivalent concentration. There was, however, a linear relationship between step height and tin concentration, as is shown in Table VI.

ANTIMONY AND BISMUTH—

Preliminary experiments confirmed that small amounts of bismuth had no effect on the determination of antimony with Rhodamine B. The method for antimony was therefore used without modification for the solutions obtained from the manganese dioxide precipitate. Results obtained with mixtures of standard antimony and bismuth solutions are given in Table VII.

TABLE VI

STEP HEIGHT OF TIN OBTAINED FROM AN ADMIXTURE OF TIN AND BISMUTH

Tin added, mg	Bismuth added, mg	Step height of tin, μA	Tin added, mg	Bismuth added, mg	Step height of tin, μA
1·0	10·0	0·13	1·0	100·0	0·14
1·0	10·0	0·15	1·0	100·0	0·14
3·0	10·0	0·45	3·0	100·0	0·46
3·0	10·0	0·44	3·0	100·0	0·44
6·0	10·0	0·90	6·0	100·0	0·95
6·0	10·0	0·90	6·0	100·0	0·90
12·0	10·0	1·79	12·0	100·0	1·78
12·0	10·0	1·81	12·0	100·0	1·82
18·0	30·0	2·65	6·0	500·0	1·00
18·0	30·0	2·64	6·0	500·0	0·92
24·0	30·0	2·10	12·0	500·0	1·80
24·0	3·0	2·00	12·0	500·0	1·81

TABLE VII

RECOVERY OF ANTIMONY FROM AN ADMIXTURE OF ANTIMONY AND BISMUTH

Antimony added, mg	Bismuth added, mg	Antimony found, mg	Antimony added, mg	Bismuth added, mg	Antimony found, mg
0·5	10	0·4	10	50	10·00
0·5	20	0·45	20	20	18·6
1	10	0·96	20	30	19·1
1	20	0·91	20	100	19·3
5	10	4·45	30	50	29·4
5	20	5·05	30	50	29·0
10	10	9·40	40	100	13·0*
10	20	9·00	40	100	9·2*

* Incomplete precipitation of the manganese dioxide occurred.

ANTIMONY, BISMUTH AND TIN—

For studies of all three metals present, the oxidation procedure used for antimony and tin and the extraction procedure for tin and bismuth were applied. The antimony was determined absorptiometrically and the tin polarographically. The results are presented in Table VIII.

TABLE VIII

RECOVERY OF ANTIMONY FROM AN ADMIXTURE OF ANTIMONY, TIN AND BISMUTH

Bismuth added, mg	Antimony added, mg	Tin added, mg	Antimony found, mg	Step height of tin, μA
100	0	1·0	0·96	0·13
100	1·0	3	0·95	0·45
100	5	3	4·8	0·44
100	5	6	4·85	0·95
200	10	3	9·50	0·5
200	10	6	9·60	0·9
200	20	6	18·3	0·94
500	20	12	18·1	1·75
500	25	15	21·0	2·00
500	30	18	0	0

DISCUSSION

The results given above show that the maximum amounts of antimony and tin that may be "collected" by manganese dioxide from bismuth solutions under the conditions used are approximately 30 mg and 18 mg, when they are present singly. When present together these amounts become 20 mg and 15 mg, respectively. These amounts can be increased by

increasing the amount of potassium permanganate solution used, but quantitative results were not readily obtained and this study was not pursued as the present upper limit was considered sufficient for determining "trace" amounts of tin and antimony. The results in Table II show that, in agreement with other workers, the affinity of manganese dioxide for bismuth in 1·2 N nitric acid is small. It can be seen from Tables II, VI and VIII that high bismuth concentrations can be tolerated without significant increase in the amount of bismuth "collected." The greatest affinity for manganese dioxide is shown by antimony under these conditions and the relative affinities may be correlated with the position of these elements in the electrochemical series.

The results in Table III agree with the findings of Blumenthal[1] and Kallmann and Prestira[2] of the effect of acidity on the co-precipitation of bismuth.

The decrease in the amount of tin and antimony "collected" when the concentration was increased above a specific value was quite unexpected. A rise to a limiting value would have been more easily explained. In the presence of antimony in excess of the maximum amount that gave satisfactory recovery, there was visual evidence of a decrease in the amount of the manganese dioxide precipitate. It seems probable that the behaviour is similar in the presence of tin. A suggestion is that the decrease is due to a preferential reaction between potassium permanganate and antimony during which the latter is oxidised to the quinquivalent state. Since the tin standard was prepared in hydrochloric acid solution it is possible that this acid interfered with the precipitation and "collection" in the solutions to which larger amounts of the tin standard had been added. No evidence for this was obtained, however, and it is considered unlikely.

The use of two successive extraction procedures for separating tin from residual bismuth proved to be necessary because the oxine precipitation did not remove the last traces of bismuth, and the dithizone procedure required many extractions to cope with the 1 to 2 mg of bismuth "collected." This was too time consuming and costly in terms of dithizone used, but the method involving the two procedures was found to be effective and comparatively rapid.

The height of the tin step obtained with solutions that had been subjected to the manganese dioxide "collection" was less than those of standard solutions of similar concentration. This was not unexpected as contents of the two series of solutions were dissimilar. For analytical purposes it is unimportant, since the tin concentration - step height relationship after "collection" is linear. It is only necessary to treat a standard tin solution by the same procedure as the sample for accurate quantitative results to be obtained. The amount of bismuth in the sample appeared to have no appreciable effect on the recovery of tin As shown in Table VI, high concentrations have no depressive effect, and reduction of the bismuth content to only 3 mg gave no improvement. The apparent recovery of tin under these conditions, obtained by comparisons of the step heights in Tables VI and VIII with that from the standard solution of tin, was in the range 80 to 83 per cent.

This work shows that the manganese dioxide "collection" provides a satisfactory method for separating antimony and tin from other materials, especially bismuth; it is also shown that if antimony and tin are present in amounts exceeding the upper limit for quantitative recovery, the amount apparently present falls below this value. This should be borne in mind when the separation is used. The details given under "Experimental" form the basis for a satisfactory method for determining these two elements when present as trace impurities in bismuth and its compounds.

It is evident that this method of "collection" and concentration is of considerable value. Further studies are necessary to ascertain its applicability to other species and to the separation of other metal combinations. These are in progress and will be reported in later Papers in this series.

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Received *August 28th*, 1963

The Determination of Radiostrontium in Milk and Herbage

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A method has been developed for determining strontium-90 and strontium-89 in milk and herbage. No attempt has been made to separate strontium from calcium during the analysis although standard radiochemical purifications are carried out. Strontium-90 is determined via its yttrium-90 daughter, and strontium-89 is determined by counting a mixed strontium - calcium source. Strontium-85 is added as a tracer at the beginning of the analysis to permit a correction for yield to be made by subsequent γ-ray counting.

Experiments indicate that this analysis is substantially quicker, safer and cheaper than earlier procedures, whilst retaining satisfactory precision and accuracy.

THE determination of radiostrontium in environmental samples has been an analytical requirement since the advent of nuclear weapons and reactors. The potential dangers to man from radiostrontium, together with other radiation hazards, have been reviewed nationally[1] and internationally.[2]

Apart from radiochemical considerations, the main analytical problem encountered in determining radiostrontium is the separation of small amounts of strontium (as added carrier) from large amounts of calcium salts, which are present in most biological and agricultural samples.

In the U.S.A., the Atomic Energy Commission[3] and in this country, the Atomic Energy Authority[4] have favoured the use of fuming nitric acid in separating strontium from calcium, strontium nitrate being preferentially precipitated in strong solutions of nitric acid[5]. Since these reports were issued most workers in this field have used this technique.

Although methods involving this separation yield accurate and precise results, they are time consuming and expensive, and the use of fuming nitric acid, potentially an extremely dangerous substance, presents a significant hazard to the analyst. In view of this it was felt worthwhile to explore the possibility of conducting the analysis without separating the strontium from the calcium. This would obviate the use of fuming nitric acid and should reduce the time taken for analysis.

Calcium is usually separated gravimetrically as its oxalate under controlled pH conditions, and re-precipitation yields a product containing virtually only alkaline and rare-earth cations. It was decided to use this technique with subsequent standard radiochemical decontamination processes.[4] It appeared unnecessary to add strontium carrier in these circumstances, but rather to rely on strontium naturally present in biological materials to carry the radiostrontium activities. It was then proposed to add yttrium carrier to the purified calcium (and strontium) solution and to "milk" off yttrium-90 after equilibration with the strontium-90 present, in order to determine the strontium-90 content of the sample. The calcium (and strontium) would then be precipitated and weighed as carbonate and a portion taken for counting strontium-89.

The first problem apparent was the large amount of material obtained at the end of the analysis, particularly from milk samples. This would present difficulties with respect to counting strontium-89 since a normal portion of milk would yield gram amounts of calcium carbonate. Low-background β-particle counters used at this Laboratory are at present calibrated for sources weighing up to 100 mg, and to take only this amount from the final precipitate would mean counting a small fraction of the available activity with consequent loss of accuracy and sensitivity. It was decided, therefore, to attempt the preparation and counting of much larger sources, bearing in mind the attendant reduction of counting efficiency owing to increased self-absorption.

The second difficulty was that of determining the chemical yield of strontium in the presence of large amounts of calcium. Alternative approaches were to determine the calcium yield and the strontium - calcium ratio before and after processing by flame photometry, or to use strontium-85 as an isotopic tracer.

Experimental

β-particle counting of large sources—

The aluminium counting trays normally used are approximately 23 mm in diameter and 1·6 mm deep, and are only suitable for sources up to about 100 mg in weight. Some stainless-steel trays were obtained, of similar diameter, but 6·5 mm deep, and it was found that 1 g of calcium carbonate could conveniently be slurried into these. In order to ascertain the efficiency of the β-particle counters for strontium-90 - yttrium-90 with these larger sources, and to check the reproducibility of counting them, 8 were prepared and counted. Each one contained 1 g of calcium carbonate, which was precipitated in the presence of 216 pC of strontium-90. The sources, when slurried into the deep trays with methanol, and dried, were approximately 3 mm thick, and the tops of the sources, when placed in the counter, were approximately 6 mm from the window of the counting tube (MX152 with guard tube).

The results are given in Table I.

Table I

Counting reproducibility of large sources

Source No.	Weight, g	Total count	Net counts per minute
1	1·0083	4200	63·8
2	1·0245	4300	64·2
3	1·0151	3900	63·8
4	1·0195	7300	64·9
5	1·0158	4800	63·9
6	1·0248	3500	64·2
7	1·0140	3900	64·1
8	0·9515	3400	63·2

Mean count rate .. 64·0 counts per minute
Standard deviation .. 0·5 counts per minute

The results give an efficiency figure, relative to disintegrations per minute of strontium-90 - yttrium-90, of 6·7 per cent., compared with 22 per cent. obtained with normal thin sources (approximately 50 mg). The reproducibility was clearly satisfactory.

Preliminary trials of the proposed method—

Preliminary work showed that there were no obvious disadvantages in the proposed method, and so 12 milk samples were analysed by using both the proposed and the current method, to permit a comparison to be made. An assessment of the two methods of determining chemical yields was incorporated in the experiment.

Table II

Comparison of current and proposed methods

	Strontium-90 per g of ash, pC		
Milk sample	Current method	Proposed method, yield via strontium - calcium ratio	Proposed method, yield via strontium-85
M. 1	5·0	4·1	—
M. 2	6·3	7·0	—
M. 3	10·5	11·5	—
M. 4	6·1	*	—
M. 5	2·5	2·3	—
M. 6	3·6	3·6	—
M. 7	5·4	6·8	5·4
M. 8	6·5	6·3	4·5
M. 9	3·1	4·0	2·3
M.10	2·6	*	2·5
M.11	3·3	3·7	3·4
M.12	4·5	5·4	4·7

* Suspect analyses owing to difficulties with the flame photometer.

The samples used for this trial were milk samples collected during the first Quarter of 1963, and the analyses listed below were made—

(*i*) All 12 samples were processed by the current method.[4]

(*ii*) Nos. 1 to 6 were processed by the proposed method and the yield determined via the strontium - calcium ratio by using an SP900 flame photometer.

(*iii*) Nos. 7 to 12 were processed by the proposed method and the yield determined via the strontium - calcium ratio and by using strontium-85 as a tracer.

The results of these analyses are given in Table II.

It can be deduced from the results in Table II that—

(*a*) There is no bias between the current and the proposed method.

(*b*) The precision of the proposed method is similar to that of the conventional method.[4,6] (The coefficient of variation is approximately 5 per cent.)

(*c*) The results show that both methods of determining the yield are equally satisfactory.

In practice the determination of yield with strontium-85 as a tracer was quicker and more convenient than the alternative, and hence was used for subsequent determinations.

The strontium-89 content of these samples was too low to permit a comparison to be made for this isotope.

COMPLETION OF ANALYTICAL TRIALS—

Having established that the proposed method was adequate for determining strontium-90 in milk, the analysis was extended to herbage samples. This also allowed an appraisal of the proposed method with respect to removal of unwanted activities and the accuracy of the determination of strontium-89, since the herbage samples used contained approximately 7-months old fission products from nuclear debris.

The results are given in Table III.

TABLE III

COMPARISON OF RESULTS ON SAMPLES OF HERBAGE

Herbage sample	Strontium-90, pC per sq. metre		Strontium-89 - strontium-90 ratio	
	Proposed method	Current method	Proposed method	Current method
H. 1	1700	1600	6·0	6·2
H. 2	3300	3200	5·5	6·0
H. 3	3700	3600	5·5	6·2
H. 4	2200	2200	5·9	6·8
H. 5	880	870	5·7	6·0
H. 6	3800	3700	5·0	5·6
H. 7	1850	2000	5·4	5·5
H. 8	590	590	5·0	5·9
H. 9	620	650	4·1	5·5
H.10	810	710	4·2	4·9
H.11	360	340	4·7	6·2
H.12	970	1050	6·2	4·8

TABLE IV

REPRODUCIBILITY OF RESULTS

Portion	Strontium recovered, per cent.	Strontium-90, pC per g of ash	Strontium-89, pC per g of ash
1	82	5·4	+1·3
2	76	5·8	+1·3
3	83	5·8	+0·8
4	83	6·0	+0·3
5	88	5·9	+0·4
6	82	6·0	−0·1
7	85	5·9	+0·3
8	83	5·9	+0·5
9	76	5·9	−0·1
10	81	6·0	+0·5
11	72	6·5	−1·4
12	74	5·6	+0·1
Mean	80	5·9	+0·3
Standard deviation ..	4·8	0·26	0·71

It can be seen from Table III that the agreement for strontium-90 is again excellent, but that the proposed method produced, in general, slightly lower figures for strontium-89.

These results also show that decontamination from fresh fission products is as good as that obtained by the standard method, and, to illustrate the order of decontamination, one sample of ash was counted before analysis, giving 28,000 pC gross beta; it was subsequently shown to contain 435 pC of strontium-90.

A final check on reproducibility was carried out with 12 portions of bulked-milk ash. Each portion (approximately 5 g) was processed by using the proposed method, and the results obtained for strontium-90 and strontium-89 are given in Table IV.

The conclusions to be drawn from the results in Table IV are—

(*a*) The proposed procedure gives consistently high recoveries for strontium.

(*b*) For strontium-90, the coefficient of variation is less than 5 per cent., which compares favourably with the standard method.[6]

(*c*) The limit of detection obtained for strontium-90, by using the commonly accepted criterion of three times the standard deviation, is better than 1 pC per g of ash.

(*d*) For strontium-89 the limit of detection depends largely on the amount of strontium-90 present (since a difference method is used) and therefore the limit is best expressed as the ratio of strontium-89 to strontium-90. By using three times the standard deviation as the criterion, as before, we find on this basis, the limit of detection for the strontium-89 - strontium-90 ratio to be 0·4. This is similar to the limits found for the standard method.[6]

(*e*) Since undetectable amounts of strontium-89 were present in the milk at this time (January, 1964: strontium-89 - strontium-90 ratio was less than 0·2), it is not possible to derive a coefficient of variation for strontium-89. However, as indicated above, this would depend on the strontium-89 - strontium-90 ratio in any instance, and in these circumstances a standard deviation of 0·7 pC of strontium-89 per g of ash must be regarded as satisfactory.

The "spread" obtained covers all types of errors in the analytical procedure (including counting) and also the personal error, since 3 analysts processed 4 samples each.

METHOD

REAGENTS—

Hydrochloric acid, 2 M.
Nitric acid, 6 M.
Ammonium hydroxide, 6 M.
Acetic acid, 6 M.
Hydrogen peroxide, 100-*volume*.
Oxalic acid solution, 8 *per cent. w/v, aqueous*.
Ammonium acetate solution, 25 *per cent. w/v, aqueous*.
Sodium chromate solution, 30 *per cent. w/v, aqueous*.
Barium carrier solution—Prepare this solution such that—
1 ml of solution ≡ 50 mg of barium approximately.
Iron carrier solution—Prepare this solution such that—
1 ml of solution ≡ 5 mg of iron approximately.
Yttrium carrier solution—Prepare this solution such that—
1 ml of solution ≡ 20 mg of yttrium approximately, and standardise it.

PROCEDURE—

Ash the sample of dried milk (equivalent to 1 litre) or herbage (100 g) at 700° C and add a known amount of strontium-85 (approximately 3000 disintegrations per minute). Add the same amount of strontium-85 to a solution containing the equivalent of 1 g of calcium carbonate and reserve this solution for the preparation of a strontium-85 standard. Prepare a new standard for each batch of samples.

For herbage samples, add 2 g of calcium carbonate (to permit a 1-g source to be taken for counting) and 10 ml of water to the solution. Heat the solution to incipient dryness

with 40 ml of 40 per cent. hydrofluoric acid and 30 ml of 60 per cent. perchloric acid. Heat the residue to fuming twice with 30-ml portions of perchloric acid.

The remainder of the analysis is common to both milk and herbage samples. Dissolve the ash (or residue) in 250 ml of 2 M hydrochloric acid and boil the solution. Cool it, and remove any unwanted insoluble matter by filtration. Add 250 ml of water and 500 ml of oxalic acid solution and heat the solution to approximately 80° C. Adjust the pH of the solution to 4 by using concentrated ammonium hydroxide with bromo-cresol green as indicator, and set the solution aside until the precipitate settles. Remove the supernatant liquid by suction or by decantation, and discard it. Re-dissolve the precipitate in 50 ml of concentrated hydrochloric acid and re-precipitate the oxalates under the conditions described above. Separate the precipitate by filtration on a Buchner funnel. Wash the precipitate well with water and then methanol, and dry it by suction.

Ignite the re-precipitated oxalates at red heat in a platinum crucible, cool them, dissolve the residue in the minimum amount of 6 M nitric acid and transfer the solution to a 40-ml centrifuge tube. Dilute the solution to 10 to 15 ml with water and add 1 ml of barium carrier solution and 1 drop of methyl red indicator. Neutralise the excess of acid with 6 M ammonium hydroxide and buffer the solution with 1 ml of 6 M acetic acid and 2 ml of ammonium acetate solution. Dilute the solution to 30 ml and heat it in a boiling-water bath. Add 1 ml of sodium chromate solution and continue to heat the tube for 5 minutes. Spin the tube in a centrifuge and remove the supernatant liquid. Re-heat the removed liquid and add a further 1 ml of barium carrier solution, stirring the solution well. Remove the second barium chromate precipitate by centrifugation, and filter the supernatant liquid into another centrifuge tube. Precipitate the strontium and calcium by adding solid ammonium carbonate to the tube. Heat the tube and separate the precipitates by centrifugation.

Dissolve the carbonates in dilute nitric acid, and add 1 drop of 100-volume hydrogen peroxide, to permit removal of the occluded chromate, and 2 ml of iron carrier solution. Heat the solution and stir it to remove carbon dioxide. Dilute the solution to 30 ml with water and precipitate the iron and chromium with an excess of ammonium hydroxide solution. Reject the precipitate and repeat the iron scavenge. Acidify the supernatant liquid with 6 M nitric acid.

Add standardised yttrium carrier solution to the solution and store it for 16 days to allow equilibration of strontium-90 with its yttrium-90 daughter. Add carbonate-free ammonium hydroxide to precipitate the yttrium carrier and activity. Re-precipitate the yttrium three times to ensure complete removal of calcium and strontium. Finally mount the yttrium as its oxalate, and count the source twice to check its decay. When the counting is complete, ignite the yttrium oxalate and weigh the residue to determine the yttrium recovery.

Precipitate the calcium and strontium carbonate from the combined supernatant liquids with solid ammonium carbonate and heat the mixture. Filter off the precipitate on a tared sintered-glass crucible and wash it with water and then methanol. Dry the precipitate in an oven at 110° C for 1 hour. Cool the crucible and the precipitate in a desiccator and weigh the precipitate. Transfer a slurry of 1·00 g of the precipitate in methanol into a deep counting tray and dry it under an infrared lamp. Prepare a similar source from the strontium-85 reserved at the beginnging of the analysis.

Count the β-radiation after a further 16 days in order to assess their strontium-89 content. Carry out the γ-ray counting of the samples and standard on the same day to avoid decay corrections for strontium-85.

Calibrations and calculations—

The low-level anti-coincidence β-particle counters used for this work were calibrated for yttrium-90, strontium-89 and strontium-90 - yttrium-90 against standards prepared from active solutions that have been calibrated absolutely by the Radiochemical Centre, Amersham. For strontium-85, we used a reference solution from Amersham.

The γ-ray counting of stronium-85 was carried out on a 100-channel pulse-height analyser, although a simpler instrument would suffice.

Calculations for strontium-90—

Correct the counting rate on the yttrium-90 source for background and dead-time and compute the corrected counting rate from the mid-time of the count to the time the yttrium

was separated (for yttrium-90, $t_{\frac{1}{2}} = 64$ hours). The strontium-90 content of the sample is then given by—

$$\text{Strontium-90, disintegrations per minute} = \frac{100\,C}{Y} \times \frac{S_1}{S_2} \times \frac{1}{W} \times \frac{100}{E}$$

where C = corrected mean β-particle count rate (counts per minute from yttrium-90) at the time of separation,

Y = percentage yttrium yield,

S_1 and S_2 = γ-ray count rates, corrected for background and dead-time on the 1-g standard and sample sources, respectively,

W = the weight of the final calcium (and strontium) carbonate precipitate,

and E = the percentage counting efficiency for yttrium-90 (approximately 27 per cent.).

CALCULATIONS FOR STRONTIUM-89—

Correct the β-particle counting rate on the 1-g calcium (strontium) sample source for background and dead-time. The strontium-89 content of the sample is then given by the formula—

$$\text{Strontium-89, disintegrations per minute} = \left[\left(C - a\,E_1 \times \frac{S_2}{S_1}\right)\frac{S_1}{S_2} - 2bE_2\right]\frac{100}{E_3}$$

where C = corrected β-particle count rate of the sample,

S_1 and S_2 = γ-ray count rates, corrected for background and dead-time on the 1-g standard and sample sources, respectively,

a = disintegrations per minute of strontium-85 in the standard,

b = disintegrations per minute of strontium-90 in the sample (see calculations for strontium-90),

and E_1, E_2 and E_3 = the percentage β-particle counting efficiencies for strontium-85 (approximately 0·1 per cent. from γ-radiation), strontium - yttrium (50 disintegrations per minute each of strontium-90 and yttrium-90; approximately 7 per cent.) and strontium-89 (approximately 8·5 per cent.), respectively.

DISCUSSION

It has been demonstrated that the proposed method gives results for strontium-90 in milk and herbage that compare favourably with those obtained by using other techniques.

For strontium-89, the precision and the limit of detection are similar to those found by using existing methods, although increased counting times are necessary owing to the lower counting efficiencies for the thick sources. The apparent slight bias in the strontium-89 - strontium-90 ratios presented in Table III is not highly significant, but, if real, may indicate better decontamination from extraneous fission products.

One of the main advantages of the method is that the use of fuming nitric acid is eliminated. Two potentially dangerous accidents involving the use of this acid have occurred in this Laboratory during the last three years, and other analytical laboratories carrying out this separation have experienced similar incidents.

Experience on the experimental samples has shown that the analysis is somewhat shorter than the standard method because of the fewer separation stages involved.

The experiments have been conducted on milk and herbage samples, since these represent analytical commitments of this Laboratory; we see no reason why the technique should not be extended to other biological and agricultural materials. If samples are known to have low calcium contents, either calcium should be added at the beginning of the analysis, leading to some dilution of the available calcium activity, or the method adopted to count smaller calcium sources. The method would not be suitable for materials containing gross amounts of calcium, such as some soil samples, since the sensitivity would be much reduced.

We thank the Regional Director, South Eastern Region, C.E.G.B., for permission to publish this Paper. In addition, we thank Messrs. M. B. Lovett and K. McGrath for their experimental assistance and Messrs. E. S. Brown and G. J. Seal for their respective γ-ray spectrometric and flame-photometric determinations.

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Received *February* **11***th*, **1964**

SHORT PAPERS

Determination of Zinc in Sewage and Sewage Sludge

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RELATIVELY low concentrations of zinc or other metals may interfere with the biological purification of sewage or with the treatment and disposal of sewage sludge. In preparation for work in this field, methods alternative to the dithizone procedure described in official publications[1,2] have been examined in the hope that they might be less time consuming in the repetitive work involved. There is enough zinc present in sludge from even domestic sewage to permit titration, for example with ferrocyanide,[3] but to determine zinc in settled sewage of domestic origin a colorimetric method would be more appropriate. The colour reaction at pH 9 with zincon[4] requires a small volume of material containing about 1 p.p.m. zinc, but the zinc must be isolated before this reagent can be applied. Separation of zinc from the alkaline-earth elements and from such metals as iron, nickel, copper, chromium and manganese, but not cadmium, is easily effected by passing the mixed metals in N hydrochloric acid through a column of Dowex 1X4 resin, washing the column with N hydrochloric acid, and eluting it with dilute nitric acid (1 + 1000). Described as a necessary step for determining zinc in mixtures before the use of the zincon reagent,[4] this procedure isolates zinc from interfering metals other than cadmium, and could be applied to industrial sewages and sludges; the zinc could then be determined in any manner that was convenient, as for example, by titration with EDTA.[5] Zinc has been determined on a single sample of domestic sewage sludge by titration with ferrocyanide and by the dithizone reagent without first separating interfering metals, and, after the use of resin columns to separate the zinc, by titration with EDTA and by the zincon reagent.

PROCEDURE

PRELIMINARY OXIDATION—

It is necessary first to destroy organic matter, and for this purpose wet oxidation with nitric and perchloric acids is effective. Instead of the procedure described in "Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents,"[1] it was found better to add 5 ml of nitric acid, sp.gr. 1·40, and 2 ml of 60 per cent. perchloric acid to 10 ml of wet sludge in a 50-ml Kjeldahl flask, and to heat the flask until it was clean and the residue nearly colourless; extra precautions were taken when the mixture began to char. Unused perchloric acid was removed by transferring the digest to a 50-ml beaker, adding 2 drops of 50 per cent. v/v sulphuric acid to the mixture, evaporating it on a hot plate and baking it for a short time at 250° C; this removed not only the unused perchloric acid but residual perchlorates as well, and is necessary if ion-exchange columns are to be used.

Wet oxidation with nitric and perchloric acids is dangerous. An alternative procedure is to add 5 ml of nitric acid, sp.gr. 1·40, and 2 ml of sulphuric acid, sp.gr. 1·84, to 10 ml of wet sludge in a 50-ml Kjeldahl flask and to heat the flask until the residue is nearly colourless. The flask is then heated for a further 30 minutes and then the digest is transferred to a 50-ml beaker, evaporated on a hot plate and baked for a short time at 250° C. The dry residue should show no signs of charring.

EXTRACTION OF METALS—

For the dithizone procedure, warm the residues from the wet oxidation with 2 ml of 5 N hydrochloric acid and 8 ml of water, filter the solution through a pad of glass wool covered with asbestos (suitable for Gooch crucibles, thoroughly washed with hydrochloric acid before use). Dilute the filtrate to a known volume. For the ferrocyanide titration, boil the residues from the wet oxidation with 10 ml of water and 5 drops of 25 per cent. v/v sulphuric acid and filter the solution through a pad of glass wool covered with asbestos. For the zincon procedure and for titration with EDTA, warm the residues from the wet oxidation with 2 ml of 5 N hydrochloric acid and 5 ml of water, filter the solution through a pad of glass wool covered with asbestos, and then wash the pad with N hydrochloric acid.

TITRATION WITH FERROCYANIDE—

Add 1 ml of 25 per cent. w/v ammonium sulphate to the filtered extract of the metals, add 2 ml of saturated sodium pyrophosphate solution to complex iron^{III}, and adjust the pH value of the solution to 1·5 with N ammonium hydroxide. Transfer the solution to a 100-ml wide-necked stoppered flask, add 4 drops of freshly prepared 0·1 per cent. w/v potassium ferricyanide and 2 drops of a 1 per cent. w/v solution of 3,3'-dimethylnaphthidine in glacial acetic acid. Run in 0·001 M potassium ferrocyanide rapidly with shaking until the pink colour is discharged. Add a further known volume (less than 1 ml will suffice) of ferrocyanide to the solution, shake the mixture for 2 minutes and then back-titrate the excess of ferrocyanide with a standard solution of zinc sulphate containing 100 p.p.m. of zinc, 0·1 ml at a time, with shaking, until the pink colour re-appears.[3] Standardise the ferrocyanide against a standard solution of zinc sulphate.

Preparation of standard solution of zinc sulphate—Dissolve 0·1000 g of analytical-reagent grade zinc in 20 ml of water and 1 ml of 20 per cent. v/v analytical-reagent grade sulphuric acid in a beaker. Cover the beaker with a watch glass and warm it until the zinc is dissolved. Dilute the solution to 1 litre with water to obtain a solution containing 100 p.p.m. of zinc.

TITRATION WITH EDTA—

Pass the filtrate and washings from the extract of the metals through a column of Dowex 1X4 resin (75 to 150 mesh) 8 mm in diameter and 5 cm deep, and wash the column with 10 ml of N hydrochloric acid. Discard the filtrate and washings. Elute the column with 20 ml of dilute nitric acid (1 + 1000). Neutralise the eluate to pH 7·5 with N ammonium hydroxide, add Eriochrome black T in water as indicator, and titrate the solution with a solution that contains 0·635 g of the dihydrate of the tetra-sodium salt of ethylenediaminetetra-acetic acid per litre of solution till the red colour becomes blue. Standardise the EDTA solution against the standard solution of zinc sulphate.

ZINCON—

Pass the filtrate and washings from the extract of the metals through a column of Dowex 1X4 resin, and wash the column with 10 ml of N hydrochloric acid. Elute the column with 20 ml of dilute nitric acid (1 + 1000) as above. Adjust the pH of the eluate to 8 with N ammonium hydroxide. Dilute the eluate to a known volume (50 ml). Take a suitable portion, adjust the pH to 9 with Clark and Lubs's buffer, add 3 ml of zincon reagent (prepared by dissolving 0·13 g of ground zincon in 2 ml of N sodium hydroxide, and diluting the solution to 100 ml with water), and dilute the mixture to 50 ml. Measure the optical density of the solution against a reagent blank solution in a Spekker absorptiometer fitted with orange (Ilford No. 607) filters, which transmit at a wavelength of 620 mμ.

DITHIZONE—

Use the procedure set out in "Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents."[1]

RESULTS

The results of replicate determinations of zinc in a single sample of sewage sludge of domestic origin by the four methods given above are given in Table I.

TABLE I

COMPARISON OF RESULTS OF DETERMINATIONS OF ZINC IN SEWAGE SLUDGE

	Zinc found, mg per 10 ml of wet sludge, by using—			
	Dithizone method	Zincon method	EDTA method	Ferrocyanide method
Individual values	0·29	0·28	0·33	0·43
	0·27	0·27	0·32	0·42
	0·25	0·30	0·33	0·45
	0·25	0·29	0·32	0·42
	0·28	0·28	0·33	0·43
	0·28	0·27	0·33	—
Mean	0·27	0·28	0·33	0·43
Standard deviation	0·02	0·01	0·01	0·01

Conclusions

The results show that zinc may be determined in sewage sludge equally well by dithizone, by zincon or by titration with EDTA, but that in the specimen of sludge analysed, titration with ferrocyanide gave high results indicating interference from some source other than iron^{III}. For repetitive work the dithizone procedure is unduly time consuming but the time required for isolating zinc by passage through the resin columns is of little consequence. After isolating the zinc on resin columns, choice of method of determination would depend on the weight of zinc available for analysis. Titration with EDTA is the best method if the sample contains 0·2 mg of zinc or more; it may therefore be most suitable for analysis of sludges. When sewage is examined, 500 ml might have to be evaporated to obtain this concentration; it would therefore be better to evaporate 100 ml and to determine the zinc with zincon. For determination by zincon, the sample should contain less than 0·15 mg of zinc. For the standard dithizone procedure the sample should contain not more than 0·02 mg of zinc.

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Received *January 6th*, 1964

An Improved Method for the Identification of Furfuraldehyde in Hydrocarbon Oil

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In accordance with Statutory Instrument No. 861 of 1961 made under the Hydrocarbon Oils (Marking of Gas Oil) Regulations, 1961, furfuraldehyde is added to gas oil as a marker in amounts of approximately 10 p.p.m. In an earlier Paper,[1] a method was described for the detection, determination and identification of furfuraldehyde in gas oil in the range 1 to 10 p.p.m. Our experience in this Laboratory over the last 2 years has shown the necessity of being able to identify furfuraldehyde in concentrations as low as 0·2 p.p.m. The column previously recommended,[1] *i.e.*, 20 per cent. w/w of squalane on Celite, proved to be unsuitable at low concentrations of furfuraldehyde owing to interference by certain components of the gas oil that had not been removed by the extraction with sodium hydrogen sulphite ($NaHSO_3$) solution. Several stationary phases were tried, the most successful being 3,3′-iminodipropionitrile. The polarity of this compound is such that paraffins up to eicosane emerge before furfuraldehyde and do not interfere with the identification. It also proved necessary to reduce the analysis time to cope with the large number of samples. Of the methods used for this purpose, increasing the temperature of the column was unsatisfactory owing to the high rate of stationary-phase "bleed" at temperatures above 50° C. Both high detector voltages and high amplifier sensitivities were required when such small amounts of furfuraldehyde were to be detected. The shortening of retention times was achieved by reducing the amount of stationary phase from the 5 per cent. normally used. The minimum permissible stationary-phase content is determined by (*a*) the level at which active centres in the support cause peak-tailing by adsorption; this was reduced by the use of acid-washed glass beads (100 to 120 mesh) instead of Celite; (*b*) the point at which the furfuraldehyde peak is masked by the broad ether peak. The optimum amount of stationary phase was found to be 0·4 per cent., at which concentration the furfuraldehyde peak appears in about 40 minutes.

The amount of sample injected is normally in the range 0·25 to 0·5 ml. This large amount of sample, together with the use of a high detector voltage (usually 1500 V), causes initial gross overloading of the system, resulting in a broad-based ether peak; the base-line is, however, regained before the furfuraldehyde peak emerges. To minimise the overloading, the smallest amount of sample that will produce a furfuraldehyde peak large enough for identification (at least an inch high) must be injected. The size of the sample is therefore related to the furfuraldehyde content of the oil as determined by the aniline acetate spectrophotometric method,[1,2] and varies from 0·5 ml for an oil containing 0·2 p.p.m. of furfuraldehyde down to 0·25 ml for an oil containing 1 to 3 p.p.m.

Chromatograms were recorded for n-butyraldehyde, n-hexaldehyde and 5-methylfurfuraldehyde, none of which gave a peak capable of interfering with the identification of furfuraldehyde.

Several gas oils, shown to be free from furfuraldehyde by the aniline acetate method, were distilled and then extracted with sodium hydrogen sulphite solution and chromatographed. None of the chromatograms showed a peak where the furfuraldehyde peak would be expected to appear. Known amounts of furfuraldehyde ranging from 0·1 to 0·2 p.p.m. were added to these oils and the procedure was repeated. At concentrations of 0·2 p.p.m. and above, the furfuraldehyde peak was easily identified; below 0·2 p.p.m. extraneous peaks were relatively more prominent and made identification less positive. The effect is shown in Figs. 1 (*a*) and (*b*). The lower limit of identi-

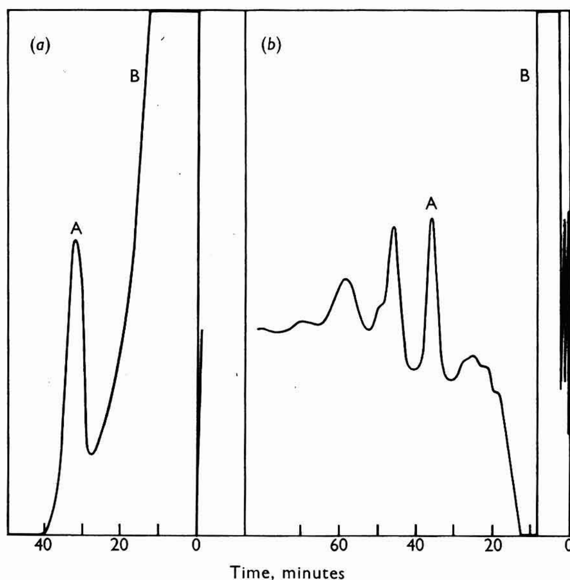

Fig. 1. Chromatograms showing results for (*a*), 1·0 p.p.m. of furfuraldehyde in gas oil and (*b*), 0·12 p.p.m. of furfuraldehyde in gas oil. Peaks A, furfuraldehyde; peaks B, ether

fication of furfuraldehyde for the purpose of legal proceedings is therefore taken to be 0·2 p.p.m. in the original gas oil. (It is important to note that the distillation and extraction processes concentrate the aldehyde content by a factor of about × 60; therefore, at the lower limit of detection the amount of furfuraldehyde in the ethereal extract will be about 12 p.p.m.) The extraneous peaks referred to above are believed to arise from the mechanical carry-over of small amounts of gas oil through the extraction procedure. To reduce this, a slight modification has been made to the extraction method; the method is described below. To minimise the risk of contamination from other sources, grease-free taps and stoppers are used throughout, and analytical-reagent grade peroxide-free ether is used for the final extraction.

In the original Paper,[1] toluene was used as an external standard; pure furfuraldehyde is now used for this purpose and standard chromatograms are run occasionally during the day as a check on the constancy of the operating conditions of the column. It has been found convenient to compare the sample and standard chromatograms by superimposing them on a viewing box, *i.e.*, a box with a top of diffusing glass, 19 × 13 inches, illuminated by five 40-watt bulbs.

METHOD

APPARATUS—

Distillation assembly and Pye Argon Chromatograph—These should be the same as those used in the previous method.[1]

Column—Prepare a column containing 0·4 per cent. w/w 3,3'-iminodipropionitrile on acid-washed glass beads, 100 to 120 mesh.

PROCEDURE—

Extract the distillate with sodium hydrogen sulphite solution as described previously.[1] Wash the complex of sodium hydrogen sulphite and furfuraldehyde with 25 ml of light petroleum (boiling-range 40° to 60° C) before liberating the furfuraldehyde. Extract the liberated furfuraldehyde into 3 ml of diethyl ether.

For the chromatographic analysis, inject 0·25 to 0·5 ml of the anhydrous ethereal extract on to the top of the column and record a chromatogram. Immediately after the chromatogram is complete, repeat the process with pure furfuraldehyde.

NOTE—Suitable operating conditions for the Pye Argon Chromatograph are—

Column temperature	50° C
Detector voltage	1500 V for sample, 1000 V for standard
Amplifier sensitivity	× 3
Argon input pressure	12 to 15 lb per sq. in.
Chart speed	3 or 6 inches per hour

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Received *November 29th* 1963

Dispersing Agents for the Tin-Dithiol Complex

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WE have recently encountered a difficulty in estimating dissolved tin in canned foods by the dithiol method.

Williams and Whitehead[1] investigated the effect of several dispersing agents on the nature of the colloidal red complex formed by adding 4-methyl-1,2-dimercaptobenzene (dithiol) to tin ions. They found that Teepol X, a product containing a sodium secondary-alkyl sulphate, gave a clear pink solution suitable for absorptiometric measurements. We found that Teepol 710/5 from Shell Chemicals (Aust.) Pty. Ltd., also containing a sodium secondary-alkyl sulphate, may be used instead of Teepol X with satisfactory results.

Some time ago the active ingredient of Teepol supplied in Australia was changed to a mixture of alkylaryl sulphonates. We have not been able to use this successfully as a dispersing agent in the estimation of tin. At all concentrations of the new Teepol formulation that we investigated, the tin - dithiol complex quickly precipitated, leaving a white cloudy suspension. This cloudy material formed when the new Teepol was acidified, indicating that the dispersing agent changes its physical condition and is no longer available to suspend the tin - dithiol complex. The failure of the alkylaryl sulphonate formulation was unexpected since Farnsworth and Pekola[2] reported the successful use of Santomerse S for estimating tin, and this material, according to Snell and Snell,[3] is a sodium alkylaryl sulphonate. Further, Kenyon and Ovenston[4] reported that Belloid

T.D., a sulphonated condensation product of naphthylene and formaldehyde, and Dispersol L., which has a similar composition to Belloid T.D., effectively stabilise the tin - dithiol suspension.

In an endeavour to find an alternative dispersing agent, we investigated Shell's non-ionic formulation known as Nonidet P40. At low concentrations of Nonidet the dithiol formed a white cloudy suspension, and the red tin - dithiol complex formed slowly and precipitated; at higher concentrations formation of the tin - dithiol complex was inhibited.

We have investigated solutions of stannous ions with Teepol 710/5 and with Nonidet, spectroscopically and chronopotentiometrically, to obtain an explanation for the failure of Nonidet to perform satisfactorily. Ultraviolet-absorption measurements show that Teepol 710/5 and stannous ions form a colloidal suspension, whereas there is no evidence of interaction between Nonidet and stannous ions. Results in accord with this interpretation were obtained chronopotentiometrically. The addition of Teepol 710/5 in increasing concentrations to a solution of stannous ions progressively shortens the transition time and produces irreversibility in the electrode process, whereas Nonidet has no observable effect on the nature of the chronopotentiograms. It was also observed that dithiol is suspended in dilute acid solutions by Teepol 710/5, but not by Nonidet.

It appears likely that Teepol 710/5 promotes the formation of the tin - dithiol complex by its interaction with the stannous ions as well as by its dispersal of the dithiol. Teepol 710/5 is also a more effective dispersing agent for the tin - dithiol complex and itself does not form cloudy suspensions in acid solution.

Our experience again illustrates the danger inherent in using non-specific trade names for chemical reagents as has been done by Dickinson and Holt,[5] and Pearson,[6] in relation to Teepol for use in tin estimations.

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Received *February 10th*, 1964

A Rapid Method of Casting Fused Beads for Use in X-ray Fluorescence Analysis

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ONE of the major drawbacks of the fusion method of preparing samples for routine X-ray fluorescence analysis is the length of time required compared with simpler techniques, such as grinding. Its advantages in mineral analysis[1] are such that it was considered necessary to study means of reducing the time involved.

The long annealing time required to avoid shattering of the glass beads formed in a borax-fusion preparation can be reduced by grinding and pelleting the glass directly upon solidification,[2] but the preparation time is still much longer than that for a simple grinding and pressing into pellets, and additional equipment has to be cleaned. In the present work, reducing the diameter of the bead from the usual $1\frac{1}{4}$ inches to $\frac{7}{8}$ inch was found to cause little reduction in the intensity of X-rays, but the stresses caused by cooling were reduced, allowing a shorter annealing time. However, the reduction in annealing time was insufficient to make fusion methods competitive in terms of speed.

In progressively attempting to reduce the annealing time with the smaller beads it was observed that, under certain conditions, the bead cracked extensively but remained in its original form without breaking up. The conditions necessary to produce this "coherent" cracking were

then studied. The lowest frequency of spoiled discs was obtained by casting the bead on a $\frac{1}{4}$-inch aluminium sheet at room temperature. Increasing the apparent rate of cooling by using $\frac{1}{2}$-inch aluminium sheet or a water-cooled copper block sharply increased the failure frequency, as did trying to reduce the cooling time by casting the bead on a hot-plate at temperatures lower than normal.

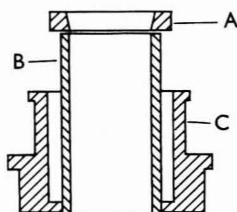

Fig. 1. Section of casting ring (A), tube (B) and sample holder (C)

The beads were accurately centred in the sample holders by keeping them in the rings of pure aluminium used as moulds. However, owing to contraction on cooling, the bead was a loose fit in the casting ring, so that it was not held together when the ring was lifted. This was overcome by tapering the inside of the ring (see Fig. 1) so that the bead was held together by the sloping walls.

PROCEDURE—

Fuse the required amount of sample with 7 g of flux in a platinum, recrystallised-alumina or other suitable crucible at a temprature of about 900° C for borate fluxes, or 500° C for pyrosulphate fluxes. When it is homogeneous, pour the melt into the pure aluminium or copper casting ring on a $\frac{1}{4}$-inch aluminium plate. After 1 to 2 minutes, when cracking has stopped, transfer the ring and bead together to the top of a metal tube placed inside a sample holder (see Fig. 1). Lift the sample holder, taking the ring and bead with it but leaving the tube behind and load it directly into the X-ray spectrograph. If, in isolated instances, cracking does not start on solidification, place a metal weight of about 50 g on top of the bead to prevent it flying apart should it subsequently crack violently.

RESULTS

The method reduces the time between the end of fusion and the start of measurement to 3 or 4 minutes. The lower surfaces of the beads produced by the method are somewhat irregular, but, when these beads were used with a rotating sample holder, no significant difference in reproducibility could be found between these and normally annealed beads. A slightly inferior performance was found if the sample was not rotated. A reproducibility of ± 1 per cent. or better has been obtained with chromium and niobium in sodium tetraborate flux, and with silicon in a lithium tetraborate flux. Concentrations as high as 10 per cent. of sample in sodium tetraborate and 50 per cent. of sample in lithium tetraborate do not affect the procedure. Metal turnings have been fused directly into potassium pyrosulphate,[3] and the melt cast under the same conditions with identical results.

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Received *February 25th*, 1964

Interference by *p*-Aminosalicylic Acid or its Sodium Salt in the Determination of Vitamin B_1 by the Thiochrome Method

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DURING the determination, by the thiochrome method given in the U.S. Pharmacopoeia XV,[1] of vitamin B_1 in urine from patients treated with *p*-aminosalicylic acid, Ostrovskiĭ[2] obtained erroneous results owing to the development of a brown colour in the assay solution. We have found similar difficulties, in that we obtained low recoveries of vitamin B_1 from tablets containing 0·5 mg of vitamin B_1 and 500 mg of sodium *p*-aminosalicylate by using the same method. Modifications have been made to the method to improve recovery of the vitamin, and an attempt has been made to characterise the interfering material.

Tablets of vitamin B_1 and sodium *p*-aminosalicylate were assayed for vitamin B_1 as described below. Ten tablets were powdered and added to 100 ml of water. Sufficient sulphuric acid to adjust the pH to 1·5 was added to the mixture and the tablets digested for 30 minutes on a steam-bath. The mixture was cooled, the final dilutions made and the vitamin determined fluorimetrically as described in the U.S. Pharmacopoeia XV. This is method (*i*) and the results are given in Table I.

Method (*i*) was then applied to a mixture of powdered standard Vitamin B_1 U.S.P. and sodium *p*-aminosalicylate in the same ratio as for the tablets, and an amount equivalent to 10 tablets was taken. The recovery results for vitamin B_1 are given in Table I.

TABLE I

RECOVERY OF VITAMIN B_1 IN ADMIXTURE WITH *p*-AMINOSALICYLIC ACID (SODIUM SALT)

Sample	Recovery of vitamin B_1, mg, obtained by using method—			
	(*i*)	(*ii*)	(*iii*)	(*iv*)
Tablet—				
Sodium *p*-aminosalicylate, 0·5 g, *plus* vitamin B_1, 0·5 g, per tablet	0·24	0·43	0·45	0·48
	0·25	0·44	0·46	0·48
	0·23	0·42	0·43	0·46
Powder—				
Mixture of sodium *p*-aminosalicylate, 0·5 g, *plus* vitamin B_1, 0·5 g	0·25	0·44	0·48	0·49
	0·26	0·45	0·48	$0{\cdot}48_5$
	0·26	0·46	0·47	0·49

Since there was only about 50 per cent. recovery of vitamin B_1 from the two samples, it was presumed that the *p*-aminosalicylic acid or its decomposition products were interfering and that the effect of the excipients was negligible. It was noticed that when the alkaline ferricyanide was added to the solution of vitamin B_1 and *p*-aminosalicylic acid, it became coloured, the colour being extracted by the isobutanol along with the thiochrome. The method was therefore modified by washing the initial solution with isobutanol several times before the addition of the alkaline ferricyanide; this is method (*ii*). The final isobutanolic extract of the thiochrome was free from extraneous colour and recoveries of vitamin B_1 were much improved (see Table I).

It is known that *p*-aminosalicylic acid decomposes readily in solution,[2] and as it appears that decomposition is enhanced by heating, method (*i*) was again modified by shaking the acidified mixture of tablets or powder and water for 15 minutes, instead of heating it for 30 minutes; this is method (*iii*). Recovery was further improved (see Table I). Hence there was less degradation of *p*-aminosalicylic acid and heat is not essential for extraction of the vitamin as it was for Ostrovskiĭ's biological material. The isobutanolic extract was practically colourless after alkaline ferricyanide oxidation.

Finally, the recently published chromatographic purification procedure[3] for determining vitamin B_1 in the presence of interfering substances was used; this is method (*iv*). Maximum recovery was obtained by using this method (see Table I).

It would not appear that vitamin B_1 is destroyed in the assay by method (*i*), but can be more completely recovered by eluting the interfering material with isobutanol.

When the amount of *p*-aminosalicylic acid was maintained at 0·5 g and the amount of vitamin B_1 increased from 0·5 mg to 1 and 1·5 mg, a higher recovery of vitamin B_1 of 82 and 92 per cent., respectively, was obtained by using method (*i*). This suggests that the interference by a fixed amount of *p*-aminosalicylic acid is almost independent of the amount of vitamin B_1 present.

It was considered possible that *p*-aminosalicylic acid is decomposed by de-carboxylation to *m*-aminophenol.[4,5] Methods (*i*), (*ii*) and (*iii*) were therefore tried on a mixture of 0·5 mg of vitamin B_1 and *m*-aminophenol; the results are given in Table II.

TABLE II

RECOVERY OF VITAMIN B_1 IN ADMIXTURE WITH *m*-AMINOPHENOL

Amount of *m*-aminophenol added to 0·5 mg of vitamin B_1, mg	Recovery of vitamin B_1, mg, obtained by using method— (*i*)	(*ii*)	(*iii*)
30	0·34	$0{\cdot}49_5$	0·35
60	$0{\cdot}27_5$	0·49	0·28
90	$0{\cdot}23_5$	0·48	0·24

m-Aminophenol reacts with alkaline ferricyanide in the same way as the decomposition products of *p*-aminosalicylic acid and may well be the interfering substance.

Further work is in progress to determine the nature of the interfering colour that appears to be associated with iron.

We gratefully acknowledge the encouragement of Mr. A. J. Judah, which helped us to investigate the problem and to publish this Paper.

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Received *August 23rd*, 1963

Book Reviews

EDUCATION IN CHEMISTRY. Vol. 1, No. 1, January, 1964. Editor: F. W. GIBBS with an editorial advisory board. Pp. 60. London: The Royal Institute of Chemistry. Annual subscription (four issues per year): 40s.; $7.

This is a new journal initiated by the British Committee on Chemical Education set up recently by the Royal Society and the Royal Institute of Chemistry. Its aims are to foster and improve the teaching of chemistry at all levels from schools to technical colleges and universities. The Editor is advised by a board of ten distinguished teachers of chemistry, including a schoolmaster, an H.M.I., and three Fellows of the Royal Society.

There is no doubt about the need for such a journal. The School Science Review has for many years held an important place among science-teaching journals and there is no intention of trespassing on its preserves, but if teachers of chemistry want any other journal they have to go outside this country to the Journal of Chemical Education, most of whose contents are not really adapted to the teaching of chemistry in this country. Now that there is a great deal of interest and activity in reviewing the content and manner of teaching chemistry, this is a most suitable time for launching a new journal dealing with the subject. It will give opportunities for the promulgation of new ideas and could be of great help to teachers anxious to know what is going on in the field of chemical education both here and overseas.

The first issue contains a good variety of articles, although the technical articles seem to concentrate on topics concerned with Chemical Structures: it may be that the Editor intends to characterise each issue by selecting articles on related topics. Thus, titles include Models of Chemical Structures, The Shapes of Molecules, Free Radicals and Ions, Chemical Bonds and

Models of Boron Hydrides. There is also an informative article on the Nuffield Foundation's Science Teaching Project, and notes on chemistry in the University of Sussex, and of courses and symposia being run for teachers of chemistry.

An interesting feature is a review of activities concerning chemical education in other countries, *viz.*, Belgium, Denmark, Holland, Eire, U.S. and U.S.S.R. Book reviews, particulars of some chemistry films and a Readers' Forum complete the list of contents.

In his introductory article, Professor R. A. Morton stresses the fact that chemistry is an experimental subject and mentions some of the problems that arise in teaching chemistry as part of education to the ordinary citizen as well as to those who will become professional scientists. "There are large and urgent problems to be faced . . . [and] discussion will be continuous. . . . If *Education in Chemistry* is destined to play a part in a great surge forward, its sponsors will be delighted. Were the new journal to need a motto it could be: 'Nobody connected with this enterprise knows all the answers'."

Readers of *The Analyst*, many of whom will be concerned with teaching, should find this new journal stimulating and informative. G. VAN PRAAGH

STANDARD METHODS OF THE OILS AND FATS DIVISION OF THE I.U.P.A.C. Fifth Edition. (Text in English and French.) Pp. xxii + 190. London: Butterworth & Co. (Publishers) Ltd. 1964. Price 50s.

The Oils and Fats Division of the International Union of Pure and Applied Chemistry is concerned with international agreement on methods of analysis and lists delegates from fifteen countries. This new edition of their standard methods covers four sections: (I) oleaginous seeds and fruits, (II) oils and fats, (III) glycerol and (IV) soaps. It differs from the fourth edition (1954) mainly in fuller and more precise description of the methods and in their publication in a loose-leaf binder to provide for incorporation of future methods and amendments. The full and exact bilingual specifications should ensure a high level of inter-laboratory agreement. It is noted that in case of dispute, the French text is regarded as official.

Although the presentation is greatly superior and this volume undoubtedly renders the preceding edition obsolete, it is a little disappointing that the number of new methods is not greater. No doubt further items are under consideration and will be issued in due course.

Section I deals with the size and preparation of the sample and determination of impurities, moisture, oil and acidity, the method for separation and determination of impurities having been greatly elaborated.

In the section on oils and fats, methods are given for the preparation of the sample and of the insoluble fatty acids, the determination of refractive index, titre, colour (spectrophotometrically), moisture and volatile matter, impurities, ash, acidity, saponification and ester values, neutral fat in highly acid fats, unsaponifiable matter, sterols, iodine and thiocyanogen values, soluble and insoluble volatile acids, A and B numbers, polybromide value, oxidised acids, peroxide value and hydroxyl value.

The principal addition to this section is a fully detailed procedure for the spectrophotometric determination of colour, which is to be expressed as a series of transmittance values at definite wavelengths or as a transmission curve between 400 and 700 mμ. No recommendations are made regarding determination of melting-point, smoke or flash points or viscosity. At least some tentative proposals regarding spectrophotometric determination of polyunsaturated acids might also have been expected. Regarding thiocyanogen values, the statement in the English text, that "it is generally considered that $(SCN)_2$ attaches itself to only one double bond of linoleic acid and to only two double bonds of linolenic acid" would be misleading if taken literally. Some definite reference to values found for pure acids by the methods specified would have been more desirable.

The newly added section on glycerol is limited simply to determination. The section on soaps gives methods for determining moisture, foreign matter insoluble in ethanol, total crude fatty acids, total alkali, total free alkali, free caustic alkali, chlorides and rosin.

The major additions here are the barium chloride method as an alternative to direct titration in ethanol for the determination of free caustic alkali, and provision for potentiometric titration in the determination of rosin.

It seems unlikely that this publication will be regarded in general other than as a supplementary work of reference in this country or the U.S.A., where the methods of the British Standards Institution and of the American Oil Chemists' Society meet the respective needs.

Nevertheless, the book offers several clearly presented methods of analysis that form a substantial basis of a collection of internationally agreed methods. Chemists concerned with international trade in oilseeds and their products will find this volume essential and will undoubtedly also look forward to the publication of further methods by the Oils and Fats Division of the International Union of Pure and Applied Chemistry. H. H. HUTT

SOLUBILITIES OF INORGANIC AND ORGANIC COMPOUNDS. Volume I. BINARY SYSTEMS. Parts 1 and 2. Edited by Prof. H. STEPHEN, O.B.E., D.Sc., F.R.I.C., and Dr. T. STEPHEN, M.Sc., Ph.D. Pp. iv + 960; viii + 963–1933. Oxford, London, New York and Paris: Pergamon Press. 1963. Price: Part 1, £10; Part 2, £12 10s.

These two parts in English provide data on the solubilities of elements, inorganic compounds, organo-metallic and organic compounds in different solvent systems, and were compiled by a panel of U.S.S.R. scientists from a survey of the international literature. As a source of such information these volumes are useful to analysts, though there are many gaps in the classes of compounds covered. This may be for the very good reason that the solubility characteristics of these compounds have not been published, and presumably subsequent volumes may make good the deficiencies.

Each volume contains a comprehensive index of both names and formulae based on the recommendations of *Chemical Abstracts*, and arranged in order of the Periodic Classification. While this is undoubtedly sound scientifically, nevertheless for a reference book, the formula index would be quicker and easier to use if it had been drawn up alphabetically. Since, however, this was originally a Russian book, there may have been real difficulties due to the differences between the Russian and Western alphabets. F. H. POLLARD

ANALYSIS OF PETROLEUM FOR TRACE ELEMENTS. By O. I. MILNER. Pp. viii + 128. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 35s.

The author notes the need that has arisen in the last 20 years for more knowledge about the trace constituents of petroleum and their effect on the refining processes and the characteristics of petroleum products. He deals with those elements that are of most interest, including arsenic, copper, halogens, iron, lead, nickel, nitrogen, phosphorus, sulphur, vanadium and the alkali metals, and adds briefer notes on 11 more.

The analytical chemistry of each element is discussed, and a method of determination is recommended for each of the more important elements. These methods are supplemented by numerous references to up-to-date literature. All the methods are based on chemical procedures, even where instrumental methods would be preferable. K. A. WILLIAMS

HANDBUCH DER PAPIERCHROMATOGRAPHIE. Edited by I. M. HAIS and K. MAČEK. Part I. GRUNDLAGEN UND TECHNIK. Part II. BIBLIOGRAPHIE UND ANWENDUNGEN. Part III. BIBLIOGRAPHIE 1957–1960 UND ANWENDUNGEN. Pp. xvi + 1069; xxiv + 726; xx + 700. Jena: Veb Gustav Fischer Verlag. 1963; 1960; 1963. Price (Part I) DM 72.40; 123s. 7d.; (Part II) DM 44; (Part III) DM 40.90; 69s. 10d.

These 3 large volumes by well known Czech workers in the field undoubtedly represent a valuable contribution to the literature of paper chromatography, and though in German, must be on the shelves of all good libraries. The bibliography itself includes some 10,000 references dating from about 1850 to 1957 in Part II, and over 8,000 from 1957–60 in Part III, and must be one of the most complete compilations of paper-chromatographic literature yet published.

Although Part II appeared in 1960 with a first edition of Part I, the latter, which covers the theory and technique of paper chromatography, has been issued as a second, enlarged edition. Here again, the diligence of the two main authors, I. M. Hais and K. Maček, has to be admired, for within this one volume, almost everything one needs can be found, not merely as a collection of facts, but rather as a critical review of each aspect. The first 250 pages presents the general principles of chromatography in 8 sections: a mathematical treatment of the chromatographic processes, a discussion of the relationship between structure and chromatographic behaviour, preparation and application of sample for analysis, ordinary and modified papers, technique of developing chromatograms with references to chromatography room, detection and determination of separated substances, radioactive separations and the use of paper chromatography for preparative work. Throughout these sections one can detect the hand of the experienced chromatographer who knows what is important to mention and what can be safely omitted.

For many experienced workers, the specialised chapters that comprise the rest of the book may represent the most important contribution. How widespread paper chromatography has become is well illustrated merely by the list of classes of compounds that have been separated: hydrocarbons (olefins and polycyclics); alcohols; phenols; oxygen-containing heterocyclic compounds (flavones, cumarine); aliphatic and cyclic aldehydes and ketones; carbohydrates; organic acids; peroxides and peracids; steroids, terpenes; aliphatics and aromatic amines; nitro compounds; amino-acids; peptides; proteins; purines and pyrimidines; alkaloids; other nitrogenous heterocyclic compounds; organic sulphur compounds; organic phosphorus compounds; vitamins; antibiotics; pesticides; dyestuffs; synthetic polymers; and inorganic cations and anions. Many of these sections have been written by Czech experts in the particular field, and therefore can be regarded as authoritative, and up to date. Finally, there is an appendix of useful notes covering such items as preparation of detecting reagents, various procedures, *e.g.*, methods of extraction of dinitrophenyl amino-acids, and quantitative estimations after chromatographic separation. One can see that the authors have done all that they can to make this volume useful both for the practising, and the would-be, chromatographers.

The books are well printed, and illustrated with coloured chromatograms, clear and informative diagrams, and several tables comparing the behaviour of substances in different solvents. It is thoroughly indexed, and has no obvious faults. Part I is complete in itself and would make a good book for constant reference in the laboratory, whereas Parts II and III are more appropriate for the library for bibliographic references.

F. H. POLLARD

THE CHEMISTRY OF BERYLLIUM. By D. A. EVEREST, Ph.D., F.R.I.C., A.M.I.M.M. Pp. x + 151. Amsterdam, London and New York: Elsevier Publishing Company. **1964**. Price 45s.

To anyone wishing to acquire a basic knowledge of the chemical reactions of beryllium compounds this book will have a special appeal. The metallurgy of beryllium, and specialised topics associated with beryllium-bearing materials, for example, refractories, have been deliberately omitted, but a chapter on the Extractive Metallurgy of Beryllium, which rightly comes within the title of the book, has been included.

Other chapters include Nuclear Properties and Reactions of Beryllium, and a useful 14-page summary on The Analytical Chemistry of Beryllium that contains sub-titles dealing with, for example, Quantitative Procedures, Radiometric, Spectrometric, Polarographic and Newer Methods.

The Beryllium Health Hazard and its Control is the heading to a 4-page chapter that, like the other 10 chapters, is supported by a selected list of references. The book contains over 500 references, covering the literature up to early 1962, and is well produced and adequately indexed.

W. T. ELWELL

METHODS OF QUANTITATIVE INORGANIC ANALYSIS: AN ENCYCLOPEDIA OF GRAVIMETRIC, TITRIMETRIC AND COLORIMETRIC METHODS. By KAZUNOBU KODAMA. Pp. xiv + 507. New York and London: Interscience Publishers, a division of John Wiley & Sons Inc. **1963**. Price 160s.

In the introductory chapter the author states that originally it was intended that this book should be a compendium of all methods of inorganic quantitative analysis, but such a work would be so vast that it was necessary to limit the methods to gravimetry (including electro-gravimetry), titrimetry, and photometry. This is understandable, but his contention that many excellent textbooks are available that cover instrumental analysis whilst those that "deal with gravimetric, titrimetric, or colorimetric analysis are less satisfactory and are seldom comprehensive" is less readily acceptable.

The book is divided into three parts. Part I (pp. 3 to 41) is a brief account of the analytical methods that the book covers. Most of it consists of a chapter on titrimetry (pp. 20 to 40), which gives a good survey together with ancillary material on indicators, standards, etc. The remaining chapters in this section are largely nominal. Part II (pp. 45 to 142) is concerned with organic reagents. The longest chapter (25 pages) deals with those reagents forming inner complexes and is a masterpiece of compression. Other chapters deal with other types of complex, with ion-exchange resins, with redox indicators and with solvents for extraction.

Part III is the core of the book and treats each element or group of elements in the order adopted in Hillebrand and Lundell's "Applied Inorganic Analysis." The essential procedure of each determination is given in somewhat telegraphic form together with an indication of the amount that can be dealt with. Almost invariably, this is supplemented by notes, often copious, that

deal with interferences, modifications of the method, etc. It is here that one of the most annoying features of the book occurs, for material in the notes would often be better placed in the main sections. Thus in the chapter on tin, the iodimetric determination after reduction with lead is described; Note 3 states that reduction with nickel is preferable; again, in the section on sulphate, a barium chloride - potassium chromate procedure is described; Note 5 states that barium chloranilate can be used in a similar (?) method. The few attempts that are made to be critical are not always well chosen; thus it is doubtful whether electrolytic deposition as "PbO_2" is the best method for lead (p. 19) or that the gravimetric determination as Fe_2O_3 is one of the most satisfactory methods for iron (p. 254). There are also some surprising near-omissions, *e.g.*, Lingane and Karplus' method for manganese appears in brackets in a list of references, and the thioglycollic acid method for iron (mentioned only in a table), with all its faults, surely merits as much space as the thiocyanate method.

These are, however, minor criticisms. One cannot but admire the author for the way he has presented so much information so concisely and for the accuracy of the transcription. Owing to understandable delays in checking and, possibly, in translation, the literature is covered only up to the end of 1957; this particularly unfortunate because a number of real advances in inorganic analysis have been made since then. If one is prepared to accept this serious limitation together with the restriction to non-instrumental methods, then this book should prove useful and achieve its declared object, namely, the reduction of the labour involved in the initial search of the literature when an analytical problem arises. It is to be hoped that no one will take it as a substitute for the original paper on the method selected. J. F. HERRINGSHAW

PROGRESS IN CHEMICAL TOXICOLOGY. Volume 1. Edited by ABRAHAM STOLMAN. Pp. xiv + 436. New York and London: Academic Press. 1963. Price $14.00.

This volume is the first of a series intended to cover developments in those subjects that are mainly the concern of the forensic toxicologist. The chapters contributed by recognised authorities deal with the identification and isolation of poisons and with the interpretation of analytical results for medico-legal or similar purposes. Some attention is also given to pharmacology and pharmacognosy, in so far as these studies can assist in the diagnosis of suspected poisoning. The title is not well chosen; toxicology has several aspects and the epithet "chemical" is hardly adequate to define the scope of this volume.

The appearance of this series, so soon after the publication of the two-volume "Toxicology: Mechanisms and Analytical Methods," by Drs. Stewart and Stolman in 1960 and 1961, indicates the rapid growth of the subject. In the words of Dr. Curry, in his chapter entitled "Acidic and Neutral Poisons," the last 3 years have been dramatic years of development perhaps only paralleled by those of 1950 to 1952. Identification by spectroscopy after the use of one or more chromatographic techniques is now the accepted approach, and the detection and absolute identification of 5 to 10 μg or the detection of one part of acidic or neutral poison or drug in 50 million parts of tissue is to be considered not only possible, but routine.

Dr. Stolman has been well served by his contributors, although the chapters show some repetition and not all are of equal merit. There is a tendency towards the presentation of an uncritical review of published literature, whose value would have been enhanced by some personal comment, to be expected from the standing and experience of the authors. A few chapters show an excessive attention to experimental detail; two pages are required by Dr. Freimuth to describe an extraction procedure published in *The Analyst*, though it is to be doubted whether the method could be operated without consultation of the original paper. The chapter on the aliphatic alcohols by Professors Harger and Forney is of outstanding excellence; it provides a comprehensive account not only of analytical methods for alcohol in tissues and excreta, but also of the absorption, distribution and metabolism of alcohol in the body, and includes a discussion of the pharmacological action of alcohol and the medico-legal interpretation of blood concentrations. A useful chapter on narcotics and related bases is contributed by Drs. Farmilo and Genest; this includes a comprehensive list of identification characteristics by ultraviolet and infrared spectrometry, paper chromatography and X-ray diffraction of a range of narcotics with special reference to opium. Dr. Goldbaum and his colleagues give an interesting account of the application of gas chromatography to the analysis of poisons, though it seems hardly necessary in a chapter of this nature to include an elementary account of the principles of this technique. There are contributions on non-barbiturate sedative drugs and on anti-arthritics, antihistamines and thymoleptics, and two chapters deal with the toxicity of air pollutants and analytical methods for their quantiative determination.

One notable feature in this volume is a contribution to the limited literature on poisonous mushrooms. It is surprising to learn how little work has been done on the nature of the poisons present, and that the only certain method of recognising poisonous mushrooms, apart from eating them, is by their botanical characteristics. Tables list the toxic action and botanical description of a range of species, but the terminology is difficult; the analyst is not likely to make much of the statement that the pileus of *Rhodophyllus strictior* is umbonate and hygrophanous. The final chapter on poisonous seeds and fruits is not worthy of a place in a volume devoted to recent developments.

J. C. GAGE

CANNED FOODS: AN INTRODUCTION TO THEIR MICROBIOLOGY (BAUMGARTNER). By A. C. HERSOM, B.Sc., F.R.I.C., and E. D. HULLAND, B.Sc. Fifth Edition. Pp. viii + 291. London: J. & A. Churchill Ltd. 1963. Price 30s.

The value of "Baumgartner" is demonstrated by the fact that this is the fifth edition in 20 years, and it is only 7 years since the last edition was published. For those who do not already know it, it is an extremely practical small book that is of great interest to anyone working in the food field who is interested in microbiology—and that really means everyone in the food field.

The book starts with a short elementary discussion on bacteria, moulds and yeasts, and then are discussed methods of control of spoilage micro-organisms such as refrigeration, gas storage, moisture limitation and preservatives. This is followed by a discussion on the containers, in which is described the construction of cans with an outline of canning operations.

The authors emphasise what is so often lost sight of, namely that the prevention of bacterial contamination before sterilising, aids more efficient sterilisation without recourse to excessive heat and consequent damage to the quality of the food. There is a short discussion of what can go wrong, of factors affecting thermal destruction of micro-organisms and of food poisoning.

This edition has a new chapter on ionising radiation, an extension of the chapter on antibiotics, and the whole book is brought up to date.

Although entitled "Canned Foods," "Baumgartner" covers the subject of microbiology for the beginner. Anyone handling foods, whether in cans or not, should find this book of great value; the same knowledge and the same precautions are needed to maintain hygienic standards in food processing whatever the form of the finished product.

A. E. BENDER

GRAVIMETRIC ANALYSIS. Volume 1. By LÁSZLÓ ERDEY. Translated by GYULA SVEHLA. Edited by ILONA BUZAS. Pp. viii + 324. Oxford, London, New York and Paris: Pergamon Press. 1963. Price 50s.; $7.50.

This, the first volume of a three-part work on gravimetric methods of analysis, is devoted to the theoretical basis of gravimetry and, in particular, precipitation processes themselves. The second part of the work will be devoted to the gravimetric determination of metal ions and other cationic forms, whilst the third part will be concerned with the determination of non-metals and other anionic forms.

After a brief introductory section, the text is subdivided into chapters that deal *inter alia* with the basic theory, operations and techniques of gravimetry. The principal topics in the second chapter, which is fairly extensive, include methods of precipitation, solubility and morphology of precipitates, purification of precipitates, speed of filtration, washing, drying, ignition, thermogravimetry, differential thermal analysis and derivative thermogravimetry. A third chapter deals with methods of separation such as precipitation as sulphides, hydroxides or basic salts and with numerous organic reagents. Other separation techniques discussed include selective dissolution of solids, fractional precipitation, solvent extraction from aqueous solution, ion-exchange, adsorption and partition chromatography, and electrolysis in all its forms. A final chapter deals with the determination of moisture in solids.

Undoubtedly this is a first-class book. It is particularly strong on the practical side in relation to the details attendant on filtration and ignition of analytical precipitates. The theoretical approach is also adequate and sound. It would be extremely hard indeed to find fault with this excellent book, and it is only rarely that one would wish for a fuller treatment. For example, a minor instance occurs in dealing with adsorption phenomena; interpretation is based largely on the Langmuir isotherm and there is no real discussion of the relative merits of the Freundlich isotherm.

This text from Professor Erdey, who is happily no stranger to audiences in this country, is a timely book on a subject that has not received much attention from writers of his calibre

recently. It is well written and liberally illustrated by line diagrams, and in places it becomes so absorbing that one reaches the end of that particular section with considerable regret that the account must terminate when it does.

T. S. West

Theorie und Praxis der gravimetrischen Analyse. Part I. Theoretischer Teil. By Dr. László Erdey. Pp. 382. Budapest: Akadémiai Kiadó. 1964. Price $9.00.

This (1964) German edition of the (1963) English text is published in Budapest and is identical in almost all respects, except that it possesses the advantage of a much more detailed list of contents at the beginning. It is a pity that the English version is thus condensed, because its register really does not reveal the scope of the work. The only other difference lies in the quality of the binding and the layout of the text. In both respects, I find the Hungarian production to be superior.

T. S. West

Trace Analysis of Semiconductor Materials. Edited by J. Paul Cali. Pp. x + 282. Oxford, London, New York and Paris: Pergamon Press. 1964. Price 70s.

The need for exceptionally high purities in semiconductor materials has given a new concept to "trace analysis." Impurities at the 1 in 10^{-9} level can have a significant effect on the properties of a semiconductor material, and over the last decade or so the semiconductor analyst has been fighting a constant battle to match his sensitivity to the extremely low impurity levels achieved by such purification techniques as zone refining. This book is essentially an account of the six analytical techniques that have been most commonly applied to this problem.

Apart from a short preface and introduction, 8 pages in all, the book consists of four chapters, each by specialist authors. Chapter I on activation analysis is by the editor, J. P. Cali; this runs to 135 pages and comprises almost half the book. A brief account of the principles involved and some notes on radiochemical procedures are given, but most of this chapter is devoted to separation procedures applicable to semiconductor matrices, to radiochemical purification procedures for some 50 elements, and to a lengthy table summarising published results for impurity levels in semiconductor materials. Chapter II, by P. E. Lightly and E. W. Currier, deals with emission spectroscopy and is relatively short (28 pages). The topics discussed include excitation techniques, recording of spectra, time-selective spectroscopy and chemical pre-concentration, all viewed from the need to attain the highest possible sensitivity. Chapter III (37 pages) is by R. E. Honig on mass spectrometry. This surveys the available instrumentation and discusses the techniques of isotope dilution, complete thermal vaporization, vacuum spark and ion bombardment. Chapter IV (66 pages), by C. A. Parker and W. T. Rees, consists of three sections on absorptiometric, fluorimetric and polarographic methods; each section embraces a brief discussion of the principles involved, the sensitivities attainable and a summary of methods for determining impurities in various semiconductor materials. References are given at the end of each chapter, or at the end of each section in Chapter IV; a subject index completes the book.

This book thus is essentially a collection of monographs by specialist authors on the six particular analytical techniques that have been most widely used in semiconductor trace analysis. Judged purely on this basis, the book has much to commend it, and particularly the emphasis throughout on practical details. Some minor failings and anomalies are apparent, as is probably inevitable in a book of this type. Thus in Chapter IV due weight is given to the need for devising procedures introducing the minimum blank value, but in the discussion of the isotope-dilution method in Chapter III, the blank-value problem is barely mentioned, though it is equally important. Again, the chapter on neutron-activation analysis provides much useful information on the types of interference resulting from nuclear reactions, but the problem of self-shielding in samples, and the choice of form for irradiation standards, are not discussed. The occasional error is detectable, *e.g.*, the reference to pH 2 as the methyl red end-point on p. 63.

My main criticism of this book is that it makes virtually no attempt to link together the various techniques discussed and to consider the subject matter as a whole. There are after all numerous problems common to all methods of trace analysis, problems that become all the more acute at the extremely low impurity levels with which analysis of semiconductor materials is concerned. These include the often daunting difficulties of preparing a sample in a form suitable for analysis without introducing contamination; the problem of analysing a sample that, even if uniform in bulk, may have relatively high and variable impurity levels on the surface; and, of

course, the ever present problem of the blank value, which in one form or another affects all techniques. In regard to this last point, some information on methods available for purifying reagents would have been a welcome addition.

These omissions detract from what is otherwise a most useful compilation, and one that may be read with profit by analysts outside the field of semiconductors. H. J. CLULEY

INORGANIC ULTRAMICROANALYSIS. By I. P. ALIMARIN and M. N. PETRIKOVA. Translated by M. G. HELL. Pp. xvi + 151. Oxford, London, New York and Paris: Pergamon Press. 1964. Price 40s.

Ultramicro-analysis is a comparatively new technique in analytical chemistry and is concerned with the problem of analysing extremely small samples (10^{-6} to 10^{-12} g) of any nature and composition. This is achieved principally by virtue of an increase in the surface area of the solution and containing vessels employed in comparison to macro methods.

Ultramicro-analysis requires not only special apparatus, but also special techniques, and it is in this respect that this book excels, in spite of rather poor photographic reproductions. There is somewhat sparse application of ultramicro-analysis to specific problems, although in certain sections, for example, "Systematic Qualitative Analysis," the authors have made a considerable effort to illustrate the usefulness of this new technique. The application to the separation - concentration methods of precipitation, electrolysis, ion exchange, extraction and distillation is particularly well described.

The purpose of the book is primarily to acquaint the analyst with the necessary experimental technique of ultramicro-analysis. The authors have also suggested applications currently possible and probable future developments, *e.g.*, the use of ultraviolet light, luminescent microscopes and the study of radioactive substances, and the advantages of some physico-chemical methods.

The technique should become increasingly popular, especially as the present trend in many areas of analysis is to reduce sample size and, at the same time, strive for an even greater sensitivity. This text covers material not dealt with in other books on ultramicro-analysis and I would recommend it wholeheartedly to all who are concerned with such small-scale work. The senior author is well known to most analysts in the United Kingdom, particularly after his recent participation at symposia organised by this Society. It is a pleasure to pay tribute to his new book.

T. S. WEST

Errata

APRIL (1964) ISSUE, p. 243, reference in 1st line of 1st footnote. *For* "7" *read* "8".

IBID., p. 245, 11th equation in Table II, right-hand side. *For* "$H_2TeO_3 + 3H_2O$" *read* "$H_2TeO_3 + H_2O$".

IBID., p. 248, 3rd line from foot of page. *For* "and" *read* "but".

IBID., p. 260, reference 3. *For* "Quantitative" *read* "Qualitative".