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Dioxymes of Large Ring 1,2-Diketones and their Applications to the Determination of Bismuth, Nickel and Palladium

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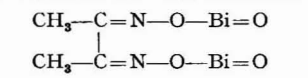
The preparation of alicyclic *vic*-dioximes containing 5 to 12 carbon atom rings from the corresponding 1,2-diketones is reported, and their application to the gravimetric determination of bismuth in the presence of several complexing agents is described. The dependence of complete precipitation of bismuth upon pH, and the effect of foreign ions has been studied. A polymeric structure is suggested for the bismuth - dioxime compounds. The application of large ring (8 to 12 carbon atoms) *vic*-dioximes to the gravimetric determination of nickel and palladium has been studied; the infrared and ultraviolet spectra of the nickel(II) and palladium(II) complexes are presented.

BISMUTH

EARLY attempts[1,2] to use dimethylglyoxime for the gravimetric determination of bismuth were largely unsuccessful owing to the formation of basic bismuth salts at the high pH required for the complete precipitation. Lott and Vitek[3] overcame this difficulty by carrying out the precipitation in the presence of ethylenediaminetetra-acetic acid and of nitrilotriacetic acid; these complexing agents possess the dual function of preventing the precipitation of basic bismuth salts and of masking certain interfering ions. The results were found to be consistent with the formula Bi_2O_2DMG (where H_2DMG is dimethylglyoxime). Lott and Vitek proposed the following structural formula for the bismuth - dimethylglyoxime compound—



The work described here was undertaken so as to study the application of a wider range of *vic*-dioximes to the gravimetric determination of bismuth and, as far as possible, to ascertain the nature of the bismuth compounds formed.

EXPERIMENTAL

PREPARATION OF *vic*-DIOXIMES—

The *vic*-dioximes (melting-points are given in parentheses) of the following 1,2 diketones were used: cyclopentane-1,2-dione (216° to 217° C), cyclohexane-1,2-dione (188° to 189° C), cycloheptane-1,2-dione (178° to 179° C), cyclo-octane-1,2-dione (169° to 170° C), cyclononane-1,2-dione (177° to 178° C), cyclodecane-1,2-dione (189° to 190° C), cyclo-undecane-1,2-dione (206° to 207° C), cyclododecane-1,2-dione (213° to 214° C), 3-methylcyclohexane-1,2-dione (164° C), 4-methylcyclohexane-1,2-dione (181° to 182° C), 4-isopropylcyclohexane-1,2-dione (182° to 183° C) and 4-t-butylcyclohexane-1,2-dione (202° C). The preparation of the diketones, with the exception of 4-isopropylcyclohexane-1,2-dione and 4-t-butylcyclohexane-1,2-dione, has been reported elsewhere.[4] The latter compounds were prepared from the corresponding pure ketones.[5]

The *vic*-dioximes were obtained from the diketones by addition of the latter to an ice-cold alkaline solution of hydroxylammonium chloride in the presence of sufficient ethanol to dissolve the diketone. The resulting dioximes were re-crystallised from water or dilute ethanol and finally from benzene.

The purity of the dioximes was established by thin-layer chromatography. About 5 μg of the dioxime in 5 μl of the eluting solvent (benzene - tetrahydrofuran - chloroform, 80 + 15 + 5 v/v) were applied to a silica-gel plate. Each dioxime was found to give only one spot on the resulting chromatogram.

The reagent used for precipitating the metal ions was either a saturated aqueous solution of the dioxime or, for the less soluble dioximes, a solution in ethanol or in acetone.

EFFECT OF pH—

The influence of pH upon the precipitation of bismuth by *vic*-dioximes was studied by using procedures (*a*) and (*b*) as described below. The bismuth nitrate solutions used were prepared by dissolving appropriate amounts of pure bismuth oxide (Johnson, Matthey Specpure) in 1 + 1 nitric acid and diluting to 1 litre. The solutions were standardised by titration with standard EDTA solution, with xylenol orange as indicator,[6] and checked gravimetrically by precipitation of bismuth phosphate from homogeneous solution.[7]

The results in Table I were obtained by using 4-methylcyclohexane-1,2-dione dioxime (4-methylnioxime). With procedure (*a*), precipitation is virtually quantitative in the pH range 11·5 to 12·0. The presence of calcium ions, procedure (*b*), however, enables bismuth to be precipitated quantitatively at the lower pH range of 9·0 to 10·0. Similar experiments with other alicyclic *vic*-dioximes indicated that the pH required for quantitative precipitation of bismuth does not vary appreciably with the dioxime used.

TABLE I

EFFECT OF pH ON THE PRECIPITATION OF BISMUTH

Procedure (*a*)—

pH	11·0	11·5	11·8	12·0	12·5	
Bismuth taken, mg	100·4	100·4	100·4	100·4	100·4	
Bismuth found, mg	92·2	100·3	100·2	100·7	102·7	

Procedure (*b*)—

pH	8·7	9·0	9·3	9·5	9·8	10·0	10·5	11·0	11·5
Bismuth taken, mg	102·4	99·5	102·4	99·5	102·4	102·4	102·4	102·4	102·4
Bismuth found, mg	94·8	99·2	102·4	99·8	102·1	102·5	104·1	110·1	116·6

MASKING AGENTS—

The use of the following complexing agents was studied with procedures (*a*) and (*b*): cyclohexanediaminetetra-acetic acid (CDTA), ethylenediaminetetra-acetic acid (EDTA), hydroxyethylethylenediaminetetra-acetic acid (HEEDTA), iminodiacetic acid (IDA), nitrilotriacetic acid (NTA) and propylenediaminetetra-acetic acid (PDTA). The pH required for complete precipitation of bismuth in the presence of these masking agents appears to be related to the stability of the bismuth complex formed.[8,9] Thus in the presence of IDA, bismuth was precipitated at a pH greater than 4·0; CDTA was found to complex bismuth so strongly that precipitation did not occur, even at pH 12·0; and HEEDTA interfered with the complete precipitation of bismuth. The most satisfactory masking agents appeared to be EDTA, NTA and PDTA; the optimum values of pH for quantitative precipitation in the presence of these complexing agents are given in Table II.

TABLE II

OPTIMUM pH FOR QUANTITATIVE PRECIPITATION OF BISMUTH

Procedure	Masking agent	pH	Bismuth taken, mg	Bismuth found, mg
(*a*)	EDTA	11·5 to 12·0	100·2	100·2
(*b*)		9·5 to 10·0	99·6	99·7
(*a*)	NTA	10·5 to 11·0	99·5	99·3
(*b*)		8·5 to 9·0	99·6	99·8
(*a*)	PDTA	11·5 to 12·0	100·4	100·3
(*b*)		9·0 to 9·5	100·4	100·3

Method

Reagents—

vic-*Dioxime solution*—Prepare as described under Experimental.

Disodium ethylenediaminetetra-acetate (EDTA) solution, 0·05 M—Dissolve 18·6 g of disodium ethylenediaminetetra-acetate dihydrate in water and dilute to 1 litre with water.

Calcium chloride solution, 0·5 M—Dissolve 50 g of analytical-reagent grade calcium carbonate in a minimum volume of dilute hydrochloric acid and dilute to 1 litre with water.

All other reagents were of analytical-reagent grade.

Procedure (*a*)—

To the bismuth solution containing 50 to 500 mg of bismuth in 100 ml, add sufficient EDTA solution to mask bismuth and the interfering ions present, and adjust the pH to between 7 and 8 with dilute ammonia solution. Add 0·5 to 1 g of potassium cyanide (required only if nickel, cobalt, copper, palladium, zinc, silver, cadmium or mercury are present), followed by sufficient dioxime solution to provide at least a 30 per cent. excess of the reagent. Adjust the pH to 11·5 with 2·5 N sodium hydroxide solution and digest the resulting yellow precipitate, with occasional stirring, for 45 minutes at 60° to 70° C. Filter through a sintered-glass crucible (No. 4) and wash thoroughly with water; wash the precipitates formed with water-insoluble dioximes with hot water followed by ethanol. Dry the precipitate to constant weight at 105° to 110° C and calculate the bismuth content; base the conversion factor on the general formula $Bi_2O_4N_2D$ where $D(NOH)_2$ is the formula of the dioxime.

Procedure (*b*)—

Carry out the initial stages of the determination as for procedure (*a*) but, after adding the dioxime reagent, add sufficient calcium chloride solution (1 to 2 ml excess) to displace the bismuth completely from its EDTA complex. Precipitate the bismuth by adjusting the pH to 9·5 with 2·5 N sodium hydroxide solution and complete the determination as for procedure (*a*).

Results

The results for the determination of bismuth by procedure (*a*) with nioxime, 4-methylnioxime and 4-isopropylnioxime as precipitating reagents are given in Table III; similar results were obtained with procedure (*b*). These three dioximes formed clean, yellow bismuth precipitates which, unlike the bismuth dimethylglyoximate precipitate, did not "creep" or form films on the walls of the beaker. It is seen that results were satisfactory when the method was applied to solutions containing from 50 to 500 mg of bismuth.

Table III

Determination of bismuth by procedure (*a*)

Cyclohexane-1,2-*dione dioxime* (*nioxime*)—

Weight of precipitate, mg ..	70·1	140·2	280·5	351·2	700·9
Bismuth taken, mg	49·7	99·4	198·7	248·4	496·8
Bismuth found, mg	49·7	99·3	198·7	248·8	496·5

4-*Methylcyclohexane*-1,2-*dione dioxime* (4-*methylnioxime*)—

Weight of precipitate, mg ..	71·7	143·7	287·4	359·6	724·8
Bismuth taken, mg	49·7	99·4	198·7	248·4	501·9
Bismuth found, mg	49·6	99·4	198·8	248·8	501·4

4-*Isopropylcyclohexane*-1,2-*dione dioxime* (4-*isopropylnioxime*)—

Weight of precipitate, mg ..	78·9	157·8	238·2	316·6
Bismuth taken, mg	52·3	104·7	157·0	209·3
Bismuth found, mg	52·2	104·3	157·5	209·3

The water-soluble dioximes of cycloheptane-1,2-dione (heptoxime) and cyclo-octane-1,2-dione (octoxime) gave satisfactory results for bismuth (less than 100 mg) by procedure (*b*) only. Some typical results are given in Table IV.

TABLE IV

DETERMINATION OF BISMUTH WITH CYCLOHEPTANE-1,2-DIONE DIOXIME

Weight of precipitate, mg ..	71·4	142·7	285·4
Bismuth taken, mg	49·4	98·8	197·6
Bismuth found, mg	49·4	98·7	197·5

The 9 to 12-membered alicyclic *vic*-dioximes gave easily filterable precipitates with bismuth, but the conditions for quantitative precipitation were not discovered during the course of the present work.

The effects of foreign ions, added as solutions of their chlorides, nitrates or sulphates, on the precipitation of bismuth by 4-methylnioxime are shown in Table V. The results by procedure (*a*) are similar to those found by Lott and Vitek[3] for dimethylglyoxime; lead, however, interfered even in the presence of tartrate or acetate added as auxiliary complexing agents. With procedure (*b*) only those foreign ions that form stable cyano complexes did not interfere. Similar trends were observed with nioxime and 4-isopropylnioxime as the precipitating agents.

TABLE V

INFLUENCE OF FOREIGN IONS ON THE PRECIPITATION OF BISMUTH BY 4-METHYLNIOXIME

Foreign ions added	Amount of each foreign ion, mg	Bismuth taken, mg	Bismuth found, mg
Procedure (a)—			
Pd, Ni, Cu, Al	25	49·7	49·7
Ba, Sr, Ca, As	25	49·7	49·8
Ba, Mg, Hg, Ag	25	99·4	99·4
Zn, Cd, Pd, Co	25	99·4	99·4
Ca, Sr, Ni, Cu	50	94·5	94·2
Pd, Ca, Zn, Co	25	198·7	198·9
Ca, Sr, Ni, Cu	100	189·6	188·4
Procedure (b)—			
Zn, Ag, Cd, Hg	25	100·4	100·3
Ba, Al, Mg, Sr	25	100·4	133·6
Ni, Co, Cu, Pd	25	100·4	100·5
As	25	100·4	102·5

The reproducibility of results obtainable by procedures (*a*) and (*b*) was studied by using aliquots of bismuth nitrate solution containing 104·5 mg of bismuth. The results obtained with 4-methylnioxime as precipitating agent are shown in Table VI. Similar values of standard deviation were obtained with nioxime and 4-isopropylnioxime as precipitating reagents.

TABLE VI

REPRODUCIBILITY OF THE METHODS

	Bismuth found by procedure (*a*), mg	Bismuth found by procedure (*b*), mg
Individual values	104·5, 104·5, 104·5, 104·7, 104·9, 104·6, 104·1, 104·5, 104·5, 104·5, 104·7	104·5, 104·3, 104·5, 104·5, 104·3, 104·5, 104·8, 104·4, 104·2, 104·9, 104·3
	Mean = 104·5	Mean = 104·5
	Standard deviation = 0·20	Standard deviation = 0·22

Samples of the various bismuth precipitates were analysed for carbon, hydrogen and nitrogen; the bismuth content of each precipitate was determined by EDTA titration, with xylenol orange as indicator. The results of these analyses are presented in Table VII. The theoretical values given have been calculated on the basis of the general formula $Bi_2O_4N_2D$ where $D(NOH)_2$ represents the formula of the *vic*-dioxime.

TABLE VII

RESULTS OF MICRO ANALYSES

Dioxime	Carbon, per cent.		Hydrogen, per cent.		Nitrogen, per cent.		Bismuth, per cent.	
	Theory	Found	Theory	Found	Theory	Found	Theory	Found
Pentoxime	10·4	10·6	1·05	1·1	4·9	4·95	72·5	72·0
Nioxime	12·2	12·5	1·4	1·5	4·75	4·2	70·8	70·8
4-Methylnioxime	13·9	14·5	1·7	1·7	4·6	4·3	69·2	68·8
4-Isopropylnioxime ..	17·1	17·6	2·2	2·3	4·4	4·8	66·1	65·7
4-t-Butylnioxime ..	18·6	18·8	2·5	2·45	4·3	4·9	64·7	64·6
Heptoxime	13·9	14·3	1·7	1·8	4·6	5·1	69·2	69·3
Octoxime	15·5	15·9	2·0	2·0	4·5	5·1	67·6	67·6
Nonoxime	17·1	17·5	2·3	2·35	4·4	5·0	66·1	66·4
Decoxime	18·6	18·05	2·5	2·4	4·3	5·0	64·7	64·6

The discrepancies between the theoretical and practical results are in some instances somewhat larger than usual; it is suggested that this may be connected with the fact that many of these bismuth compounds decompose violently when heated.

DISCUSSION

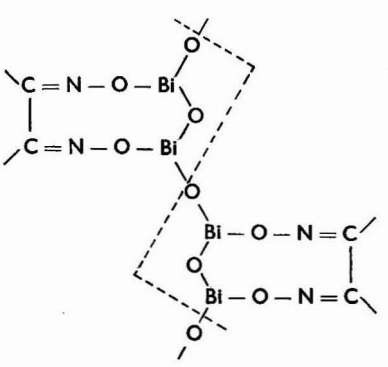
We conclude from the above results that bismuth may be quantitatively determined in the presence of a wide range of foreign ions by using procedure (*a*) and that the reagents nioxime, 4-methylnioxime and 4-isopropylnioxime are superior to dimethylglyoxime in respect of the properties of the precipitate formed.

The modified procedure (*b*) was suggested by the work of Přibil and Čuta[10] on the precipitation of pure bismuth hydroxide with ammonia solution in the presence of EDTA and an equivalent amount of calcium nitrate. This procedure enables the quantitative precipitation of bismuth at a lower pH than is required for procedure (*a*), but interference from foreign ions is more serious owing to the removal of the masking agent (EDTA) by the added calcium ions.

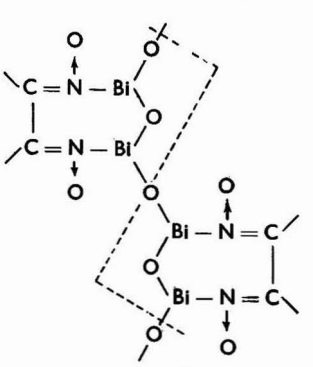
A limitation to the determination of bismuth with *vic*-dioximes in the presence of a complexing agent is the relatively narrow range of pH (about 1 unit) in which the pure compound is quantitatively precipitated (see Table I).

The conversion factors used to calculate the results (bismuth found) in the gravimetric determinations were based on the formula[3] $Bi_2O_4N_2D$ where $D(NOH)_2$ is the dioxime. This formula was also used in calculating the "Theory" values in Table VII. The insoluble nature of the bismuth precipitates both in water and in common organic solvents may, however, indicate a polymeric structure. The observations during preliminary experimental work that cyclohexane-1,3-dione dioxime failed to precipitate bismuth quantitatively and that cyclohexane-1,4-dione dioxime did not give a precipitate (in contrast to cyclohexane-1,2-dione dioxime) are in accord with a polymeric structure in which the bismuth atoms are joined by oxygen bridges. The strain in such a system (1,3- and 1,4-dione dioximes) would increase as the distance between the two oxime groups became greater.

Structures **I** and **II** given below, based on the oximino and nitrone forms[11] of the oxime group, respectively, are possible polymeric structures that have the composition $(Bi_2O_4N_2D)_n$.



I



II

Polymeric structures involving bismuth - oxygen bonds are a well established feature of bismuth chemistry.[12,13]

The infrared spectra of the various dioximes and the corresponding bismuth compounds have been determined as Nujol mulls with a Perkin-Elmer Infracord spectrophotometer (model 137), and as potassium bromide discs (concentration about 0·5 per cent.) with a Unicam SP200 infrared spectrophotometer. Some typical spectra obtained with the latter instrument are presented in Figs. 1 and 2.

The infrared spectra of the other dioximes and bismuth compounds are included in the Ph.D. thesis of Leton.[5]

Comparison of the various infrared spectra shows, for each pair, that the O–H band of the dioxime at about 3350 cm^{-1} is absent from the spectrum of the bismuth compound. In the spectra of the nickel(II) - *vic*-dioxime complexes the appearance of the O–H band at about 1775 cm^{-1} has been attributed to hydrogen bonding[14]; the absence of such a band in the spectra of the bismuth compounds suggests that they are not structurally analogous to the nickel complexes. The effect of deuteration of the bismuth compounds was not studied in view of the absence of the O–H band from their infrared spectra.

The bands at about 1540 and 1150 cm^{-1} are also striking features of the infrared spectra of the series of bismuth - *vic*-dioxime compounds. The band found between 1500 and 1600 cm^{-1} may probably be attributed to the C=N stretching vibrations.[15] A possible value for an N → O stretching frequency would seem to be between that obtained in pyridine *N*-oxides[16] (about 1250 cm^{-1}) and tertiary amine *N*-oxides[17] (about 950 cm^{-1}). It is therefore suggested that the band found at about 1150 cm^{-1} may be assigned to N → O vibrations, in accord with structure **II**.

The infrared spectra of certain of the dioximes and the corresponding bismuth compounds have also been examined, over the range 1300 to 400 cm^{-1}, as Nujol mulls on a Perkin-Elmer infrared grating spectrophotometer, model 337. These studies do not provide any evidence for the assignment of Bi–O bands in the spectra of the bismuth compounds.

NICKEL AND PALLADIUM

The study of the application of alicyclic *vic*-dioximes to the gravimetric determination of nickel and palladium was confined to those possessing 8 to 12-membered carbon rings. These *vic*-dioximes have not previously been used for the determination of these elements.

Experimental

Standard nickel solutions—

Nickel solutions were prepared by dissolving about 1 g of pure nickel (Mond Nickel Co.) in hot concentrated hydrochloric acid and diluting to 1 litre with water. These solutions were standardised electrolytically by using platinum electrodes and gravimetrically with 4-methylcyclohexane-1,2-dione dioxime as the precipitant.

Standard palladium solutions—Palladium solutions were prepared by dissolving about 2 g of anhydrous palladium chloride (Johnson, Matthey & Co. Ltd.,) in hot concentrated hydrochloric acid and diluting to 1 litre with water. The solution was standardised gravimetrically with 4-methylcyclohexane-1,2-dione dioxime.

Procedures—

The procedure used by Banks and Hooker[18] for the gravimetric determination of nickel with 4-methylcyclohexane-1,2-dione dioxime was found to be unsuitable with the 8 to 12-membered alicyclic *vic*-dioximes. The latter form yellow nickel(II) complexes, and it was found difficult to observe the appearance of a persistent yellow colour analogous to the pink colour utilised by Banks and Hooker. The following procedure was therefore adopted.

Dilute an aliquot of the nickel solution to about 250 ml with water and adjust the pH to between 5 and 6 with dilute ammonia solution. Heat the solution to 60° C, add excess of dioxime (not less than 30 per cent.), dropwise with stirring, and digest the resulting precipitate for 45 minutes at 60° to 70° C. Collect the precipitate in a sintered-glass filter-crucible, wash with hot water and with a little ethanol to remove excess dioxime; dry to constant weight at 105° to 110° C.

The procedure used for the gravimetric determination of palladium was similar to that described by Banks and Hooker,[18] except that the precipitate was washed with hot water followed by a little ethanol.

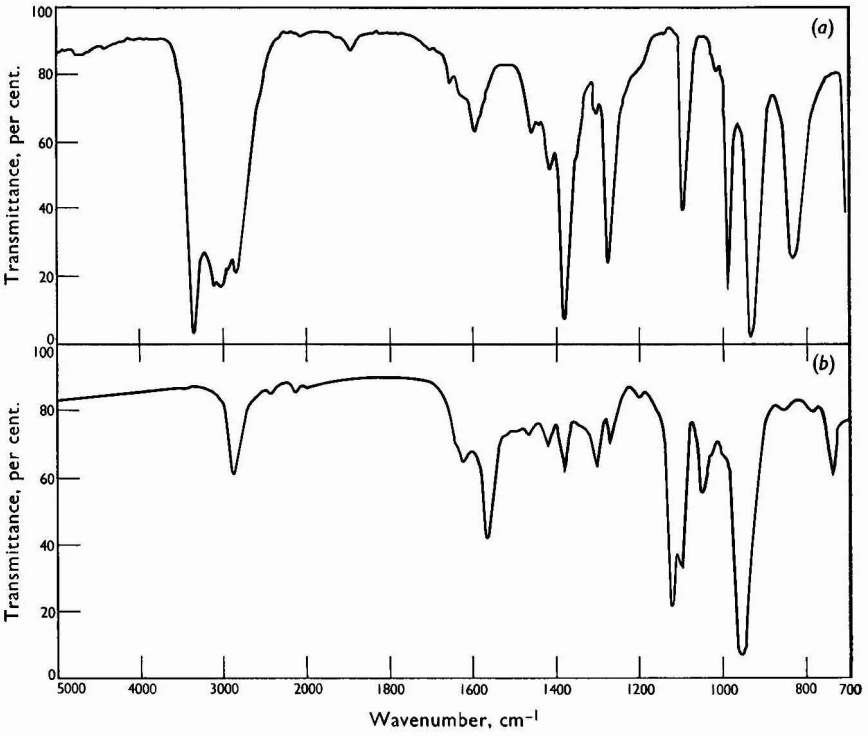


Fig. 1. Infrared spectra of (*a*) cyclopentane-1,2-dione dioxime, and (*b*) the corresponding bismuth compound

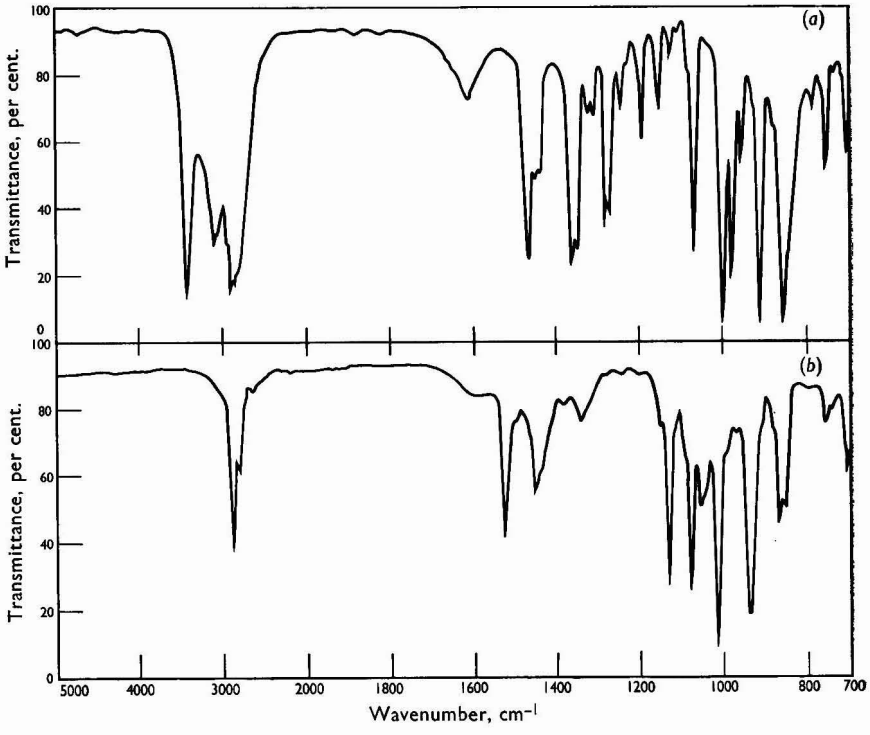


Fig. 2. Infrared spectra of (*a*) cyclo-octane-1,2-dione dioxime, and (*b*) the corresponding bismuth compound

MEASUREMENT OF ABSORPTION SPECTRA IN CHLOROFORM—

Solutions of known concentration were prepared by accurately weighing samples of the solid complex on a microbalance and dissolving them in analytical-reagent grade chloroform. The solutions were transferred into 100-ml calibrated flasks and diluted to the mark with chloroform immediately before making the absorption measurements. The solutions were rapidly transferred into 0·5-cm cells and the absorption spectra scanned on an automatic recording Unicam SP800 spectrophotometer.

INFRARED SPECTROPHOTOMETRIC STUDY—

The infrared spectra of the complexes were recorded as Nujol mulls on a Perkin-Elmer Infracord spectrophotometer (model 137) and as potassium bromide discs (concentration about 0·5 per cent.) on a Unicam SP200 infrared spectrophotometer.

RESULTS AND DISCUSSION

The results obtained for the gravimetric determination of nickel and palladium with the 8 and 9-membered alicyclic *vic*-dioximes are given in Tables VIII and IX, respectively.

TABLE VIII

DETERMINATION OF NICKEL WITH ALICYCLIC *vic*-DIOXIMES

Cyclo-octane-1,2-*dione dioxime*—			
Weight of precipitate, mg ..	73·5	148·0	167·9
Nickel taken, mg	11·0	21·9	24·8
Nickel found, mg	10·9	21·9	24·8
Cyclononane-1,2-*dione dioxime*—			
Weight of precipitate, mg ..	76·4	155·0	
Nickel taken, mg	11·0	21·9	
Nickel found, mg	10·6	21·4	

TABLE IX

DETERMINATION OF PALLADIUM WITH ALICYCLIC *vic*-DIOXIMES

Cyclo-octane-1,2-*dione dioxime*—				
Weight of precipitate, mg ..	25·0	50·6	100·9	125·2
Palladium taken, mg ..	6·0	11·9	24·1	30·0
Palladium found, mg ..	6·0	12·1	24·1	30·0
Cyclononane-1,2-*dione dioxime*—				
Weight of precipitate, mg ..	26·9	55·2	110·0	
Palladium taken, mg ..	6·2	12·4	24·7	
Palladium found, mg ..	6·1	12·4	24·8	

The analyses for nickel and palladium with the 10, 11 and 12-membered alicyclic *vic*-dioximes were, in general, less satisfactory.

The nickel and palladium precipitates were analysed for carbon, hydrogen and nitrogen and some of the results are included in Table X.

TABLE X

MICRO ANALYSES

Dioxime used	Formula of complex	Carbon, per cent. Theory	Carbon, per cent. Found	Hydrogen, per cent. Theory	Hydrogen, per cent. Found	Nitrogen, per cent. Theory	Nitrogen, per cent. Found
Cyclo-octane-1,2-dione dioxime ..	$(C_8H_{13}N_2O_2)_2Ni$	48·4	48·5	6·6	6·85	14·1	14·7
	$(C_8H_{13}N_2O_2)_2Pd$	43·2	43·1	5·9	5·9	12·6	12·9
Cyclononane-1,2-dione dioxime ..	$(C_9H_{15}N_2O_2)_2Ni$	50·85	50·6	7·1	7·0	13·2	13·65
	$(C_9H_{15}N_2O_2)_2Pd$	45·7	45·5	6·4	6·6	11·85	11·4
Cyclodecane-1,2-dione dioxime ..	$(C_{10}H_{17}N_2O_2)_2Ni$	53·0	53·4	7·6	7·7	12·4	12·7
	$(C_{10}H_{17}N_2O_2)_2Pd$	47·95	48·1	6·8	6·8	11·2	10·9
Cyclo-undecane-1,2-dione dioxime	$(C_{11}H_{19}N_2O_2)_2Ni$	54·9	54·6	8·0	7·72	—	—
Cyclododecane-1,2-dione dioxime	$(C_{12}H_{21}N_2O_2)_2Ni$	56·6	56·6	8·3	8·22	—	—

These results indicate that the precipitates are bis-complexes of nickel(II) and palladium(II), and the conversion factors were calculated on this basis.

The most satisfactory of the *vic*-dioximes studied was cyclo-octane-1,2-dione dioxime which precipitates relatively small amounts of nickel and palladium quantitatively. The yellow precipitates filter easily and can be dried to constant weight at 105° to 110° C for about $1\frac{1}{2}$ hours.

The ultraviolet absorption spectra of chloroform solutions of nickel(II) and palladium(II) bis-(cyclo-octane-1,2-dione dioxime) are shown in Fig. 3. The values of $\lambda_{max.}$ and $\epsilon_{max.}$

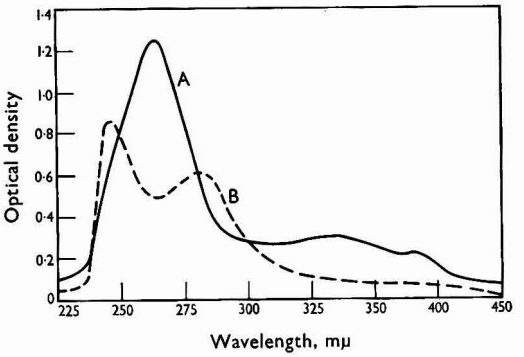


Fig. 3. Ultraviolet absorption spectra of A, chloroform solution of nickel(II) bis-(cyclo-octane-1,2-dione dioxime); and B, chloroform solution of palladium(II) bis-(cyclo-octane-1,2-dione dioxime)

(Table XI) indicate the marked similarity exhibited by the series of nickel(II) - *vic*-dioxime complexes. Owing to the limited amounts of certain of the dioximes available, the absorption spectra of only two of the palladium complexes were recorded. These values are in accord with the observation of Banks and Barnum,[19] that the absorption spectra of chloroform solutions of nickel(II) and palladium(II) complexes of *vic*-dioximes that do not contain aromatic substituents are practically identical.

TABLE XI

ABSORPTION PROPERTIES OF NICKEL(II) AND PALLADIUM(II) - *vic*-DIOXIME COMPLEXES IN CHLOROFORM SOLUTION

Complex	Colour	$\lambda_{max.}$, mμ	$\epsilon_{max.}$, 1 mole-cm
Nickel(II) bis-octoxime	Orange - yellow	264	$2{\cdot}43 \times 10^4$
		332	$5{\cdot}78 \times 10^3$
		380	$4{\cdot}43 \times 10^3$
Nickel(II) bis-nonoxime	Orange - yellow	263	$2{\cdot}21 \times 10^4$
		333	$4{\cdot}90 \times 10^3$
		380	$3{\cdot}68 \times 10^3$
Nickel(II) bis-decoxime	Orange - yellow	264	$2{\cdot}11 \times 10^4$
		335	$4{\cdot}69 \times 10^3$
		385	$3{\cdot}71 \times 10^3$
Nickel(II) bis-undecoxime ..	Orange - yellow	248	$1{\cdot}82 \times 10^4$
		263	$2{\cdot}17 \times 10^4$
		333	$4{\cdot}86 \times 10^3$
		385	$3{\cdot}65 \times 10^3$
Nickel(II) bis-dodecoxime ..	Orange - yellow	245	$1{\cdot}09 \times 10^4$
		264	$1{\cdot}97 \times 10^4$
		334	$4{\cdot}20 \times 10^3$
		384	$3{\cdot}36 \times 10^3$
Palladium(II) bis-octoxime ..	Yellow	246	$1{\cdot}74 \times 10^4$
		280	$1{\cdot}24 \times 10^4$
Palladium(II) bis-decoxime ..	Yellow	246	$1{\cdot}82 \times 10^4$
		282	$1{\cdot}27 \times 10^4$

The infrared spectra of nickel(II) and palladium(II) bis-(cyclo-octane-1,2-dione dioximes) are shown in Figs. 4 and 5, respectively. The spectra of the series of nickel(II) complexes with 8 to 12-membered alicyclic *vic*-dioximes were found to show a marked similarity to each other and to the spectra of the palladium(II) complexes with the 8 and 10-membered dioximes.

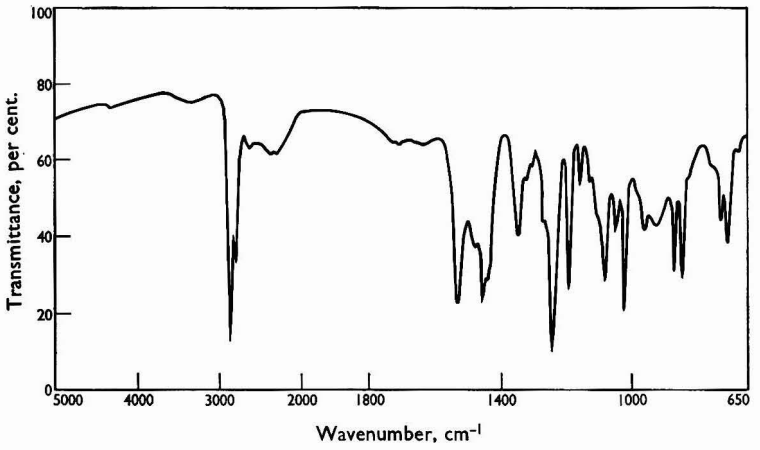


Fig. 4. Infrared spectrum of nickel(II) bis-(cyclo-octane-1,2-dione dioxime)

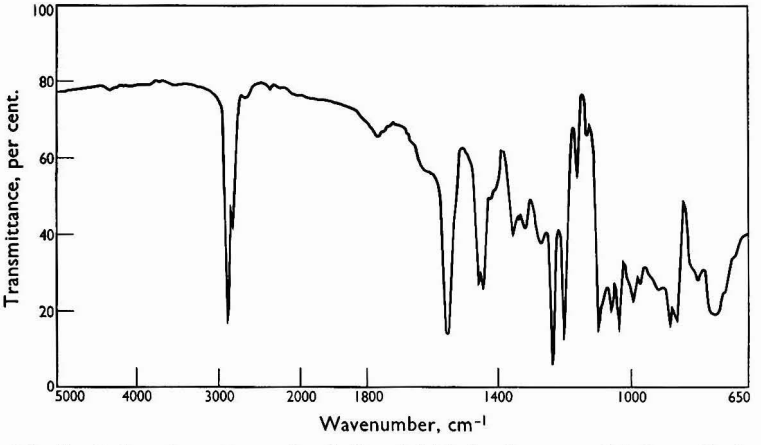


Fig. 5. Infrared spectrum of palladium(II) bis-(cyclo-octane-1,2-dione dioxime)

The observed bands were found to be in general accord with the assignments made by Blinc and Hadži[15] on the basis of their study of the infrared spectra of nickel dimethylglyoxime and related complexes. The weak, broad band near 2340 cm^{-1} reported by Blinc and Hadži, and attributed by them to the OH stretching frequency was, however, apparent only in the infrared spectra of our palladium complexes and was not observable in the spectra of the nickel complexes.

The authors' thanks are due to Imperial Chemical Industries for a grant, to Mr. J. W. Betts for the ultraviolet and infrared spectra of the nickel and palladium compounds, and to the United Kingdom - Nigerian Technical Assistance Scheme for a grant to G.B.L.

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A Limit Test for 4-Chloroacetanilide in Phenacetin and its Preparations*

A thin-layer chromatographic method is presented that is suitable for use as a limit test for 4-chloroacetanilide in phenacetin. 4-Chloroacetanilide is detectable at 0·01 per cent., but the test may be made more stringent by suitable adjustment of sample size. The method has been applied on a collaborative basis to samples of phenacetin and to several commonly used tablets containing phenacetin.

It has been suggested that the nephrotoxic action of phenacetin may be caused by impurities, and 4-chloroacetanilide has been particularly referred to in this respect, although the case against it has not been proved conclusively. The monograph in the British Pharmacopoeia 1963, p. 580, includes a test, based upon the determination of chloride after reduction, which limits chlorine-containing impurities, expressed as 4-chloroacetanilide, to about 0·11 per cent. It was considered desirable to provide a more specific test, together with a more stringent limit.

The British Pharmaceopoeia Commission appointed an *ad hoc* group to study the problem, consisting of: Dr. D. C. Garratt (Chairman), Dr. L. E. Coles, Mr. J. Deavin, Dr. G. E. Foster, Dr. A. R. Moss, Mr. R. A. Savidge and Mr. J. S. Wragg, together with members of the Commission's staff. Published methods available for study were the paper-chromatographic method of the U.S.P. XVII,[1] a spectrophotometric method[2] and two thin-layer chromatographic methods.[3,4]

During the course of the work a method based on gas chromatography was published,[5] but this was not investigated by the group.

The paper-chromatographic method was rejected as, in the opinion of several members, it was extended to the limit of its sensitivity if a standard of not greater than 0·03 per cent. is required. The spectrophotometric method involves total hydrolysis with hydrobromic acid; 4-chloroaniline produced from any 4-chloroacetanilide present is separated by extraction into cyclohexane from an alkaline medium and determined by measurement of the light absorption at 298 mμ with suitable base-line correction. 4-Hydroxyaniline produced from phenacetin remains in the alkaline aqueous phase. This was found to be a sensitive and precise method when used for phenacetin, but it was abandoned because other ingredients of dosage forms and compound preparations are likely to interfere with the procedure. Further, the conditions of hydrolysis did not seem to be sufficiently precisely defined.

It appeared that thin-layer chromatography offered the most promising approach and the published method of Savidge and Wragg,[3] together with procedures developed in members' laboratories, were examined. For a method designed as a pharmacopoeial limit test for 4-chloracetanilide, rather than as a more general test to reveal all possible impurities, it was agreed that the spot caused by this specific contaminant should be compact and well separated from any other spots. It was also agreed that, for such a purpose, a method of treatment that produced a stable visible colour capable of being examined and compared in daylight was to be preferred to one dependent on the use of two ultraviolet light sources that would have to be of the correct type and intensity to give satisfactory results. The method of Savidge and Wragg provides adequate separation of a compact spot but involves the use of ultraviolet irradiation for viewing purposes.

A method that appeared to meet the required objectives was developed in the laboratory of Dr. Moss from an original proposal by Fresen,[4] and was examined in its application to phenacetin and to combinations of phenacetin with other drugs. The method involves a short acid hydrolysis which converts the 4-chloroacetanilide to 4-chloroaniline and part of the phenacetin to *p*-phenetidine. After making the hydrolysate ammoniacal, it is extracted

* Enquiries relating to this publication should be addressed to The Secretary, British Pharmacopoeia Commission, General Medical Council Office, 44 Hallam Street, London, W.1.

with cyclohexane and the extract is subjected to thin-layer chromatography on silica gel with dichloromethane as the mobile phase. 4-Chloroaniline is made visible by diazotisation followed by coupling with *N*-(1-naphthyl)ethylenediamine; some *p*-phenetidine is also extracted and similarly made visible.

Members applied the method to three samples of phenacetin, one of which was believed to contain very little 4-chloroacetanilide, another, an amount of 4-chloroacetanilide approximating to the limit of 0·03 per cent. that is applied in the United States Pharmacopoeia, and the third, an amount of 4-chloroacetanilide in excess of this limit. Although it was intended that the method should be used as a limit test in which the 4-chloroaniline derived from the sample under test would be compared with a single standard representing the limiting amount, for the purpose of this collaborative examination a series of standards was applied so that estimates of the amounts of 4-chloroacetanilide present could be obtained. It was found that a suitable gradation of colour between successive standards is obtained if amounts equivalent to between 0·02 and 0·08 μg are used. Above this loading the spots are too intense in colour for a clear gradation to be observed. For an accurate assessment of samples that contain amounts of 4-chloroacetanilide outside the normally encountered range, it is necessary simply to adjust the amount of the sample taken for hydrolysis. Results reported on the three samples examined are given in Table I.

TABLE I

PERCENTAGE OF 4-CHLOROACETANILIDE FOUND IN SAMPLES OF PHENACETIN

Sample	Laboratory A	B	C	D
Phenacetin 1	<0·01	<0·01	<0·01	0·005
Phenacetin 2	0·02	0·045	0·02	0·03
	0·03	0·03	—	—
Phenacetin 3	0·04	0·08	0·07	0·06
	0·05	—	—	—

Two further samples of phenacetin were then obtained and batches of Compound Acetylsalicylic Acid Tablets B.P.C., Compound Codeine Tablets B.P. and Soluble Compound Codeine Tablets B.P. were prepared from them. The results obtained are shown in Table II.

TABLE II

PERCENTAGE OF 4-CHLOROACETANILIDE FOUND IN TWO FURTHER SAMPLES OF PHENACETIN AND IN TABLETS* PREPARED FROM THEM

Sample	Laboratory A	B	C	D	E
Phenacetin 4	0·01	<0·03†	0·01	<0·03†	0·02
Phenacetin 5	0·07	0·05 to 0·06	0·07	0·07	0·06
Compound acetylsalicylic acid tablets ..	0·07	0·05 to 0·06	0·07	0·05	0·05
(prepared from 5)				0·04	
Compound codeine tablets	0·07	0·05 to 0·06	0·06	0·06	0·05
(prepared from 5)				0·05	
Soluble compound codeine tablets ..	0·07	0·05 to 0·06	0·06	0·04	0·05
(prepared from 5)				0·04	
Soluble compound codeine tablets ..	0·01	<0·03†	0·01	<0·03†	0·01
(prepared from 4)					

* For tablets, the results are expressed in terms of the amount of phenacetin present.
† A more precise figure was not determined as these samples readily met the proposed limit of 0·03 per cent.

Results, which agreed well with those given above, were also obtained by one laboratory with the method of Savidge and Wragg.[3]

When applied as a limit test, and assuming 0·03 per cent. to be an acceptable value, all of the laboratories would have accepted phenacetin 4, and the tablets prepared from it, and would have rejected phenacetin 5 and its preparations.

In addition to these collaboratively examined samples, many samples of commercially available tablets containing phenacetin were successfully examined in the laboratory of one

member. These samples represented a wide range of formulations in which phenacetin was present, together with acetylsalicylic acid, caffeine, codeine, phosphate, guaiphenesin, prepared ipecacuanha, phenylephrine hydrochloride, phenylpropanolamine hydrochloride and quinine sulphate. The method was also used for paracetamol and paracetamol tablets, and found to be equally applicable. (It is possible for paracetamol to be manufactured by a route involving 4-chloroacetanilide, although this does not appear to be used in practice, at least for material of British origin.)

In view of this work, the group recommended that the following method (which sets a limit for 4-chloroacetanilide at a level of 0·03 per cent.) should be used for phenacetin and its preparations.

Method

Reagents—

Ethanol—As described in the British Pharmacopoeia **1963**, p. 26, Alcohol (95 per cent.).

Dilute hydrochloric acid—As described in the British Pharmacopoeia **1963**, p. 367.

Strong ammonia solution—As described in the British Pharmacopoeia **1963**, Appendix I, p. 898.

Phenacetin—The British Chemical Reference Substance (Note 1).

Other reagents and solvents are of analytical-reagent grade.

Procedure—

To 0·20 g of the sample, or a quantity of powdered tablets equivalent to this amount, add 6 ml of ethanol and 10 ml of dilute hydrochloric acid and boil the mixture under a reflux condenser for 15 minutes. Allow the solution to cool, transfer it to a stoppered cylinder, add 4 ml of strong ammonia solution, mix, and again allow to cool (Note 2). Add 5·0 ml of cyclohexane, shake the mixture for 2 minutes, allow the phases to separate, and use the cyclohexane layer (solution *A*) for the test. In a similar way prepare another solution (solution *B*) by using 0·20 g of phenacetin that is free from 4-chloroacetanilide and 0·06 mg of 4-chloroacetanilide instead of the substance being examined.

Spread a layer of a suitable silica-gel adsorbent [Kieselgel G (Merck) has been found to be satisfactory] about 0·25-mm thick on a glass plate, heat at 105° C for 1 hour and allow to cool. Apply separately to the plate, 0·005 ml of solutions *A* and *B*. Place the plate with one end in a shallow layer of dichloromethane contained in a closed tank, the atmosphere of which is saturated with dichloromethane. Allow to stand until the solvent front has travelled 10 cm beyond the points of application of the solutions, dry the plate in a current of warm air, transfer immediately to a closed glass tank and expose to nitrous fumes for 15 minutes. The nitrous fumes may be generated by the dropwise addition of sulphuric acid (50 per cent. w/w) to a solution containing 10 per cent. w/v of sodium nitrite and 3 per cent. w/v of potassium iodide in a beaker inside the tank. Place the plate in a current of warm air to remove nitrous fumes from the surface and spray evenly with a 0·5 per cent. w/v solution of *N*-(1-naphthyl)ethylenediamine hydrochloride in ethanol. Allow it to dry and repeat the spraying, if necessary, until maximum colour development is obtained. The purplish blue spot (R_F about 0·4) derived from the 4-chloroacetanilide in the chromatogram obtained with the solution *B* is more intense than any spot of the same R_F in the chromatogram obtained with solution *A*. Any *p*-phenetidine derived from the phenacetin has an R_F of about 0·1 (Note 3).

Notes—

1. It is expected that British Chemical Reference Substance, phenacetin, will be available in the near future.

2. If the test is applied on a more stringent basis and a larger amount (say 0·5 g) of phenacetin is examined, crystallisation may occur at this point. Difficulties arising from this crystallisation may be overcome by shaking the solution with cyclohexane, then spinning it in a centrifuge.

3. The R_F values given are approximate and may vary according to the source of the silica-gel adsorbent and the degree of activation.

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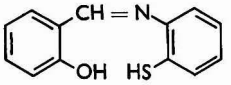
Salicylideneamino-2-thiophenol—A New Reagent for the Photometric Determination of Tin: Application to the Analysis of Ores, Rocks and Minerals

By G. R. E. C. Gregory and P. G. Jeffery
(*Warren Spring Laboratory, Stevenage, Herts.*)

Salicylideneamino-2-thiophenol can easily be prepared by the reaction of 2-aminobenzenethiol with salicylaldehyde. This reagent reacts with silver, copper, molybdenum, tin and several other metals to give coloured complexes, but by a suitable choice of conditions it can be made selective for tin. The tin complex is readily extracted into organic solvents and can be used for the photometric determination of this metal in ores, rocks and minerals.

Aromatic amines condense readily with certain aldehydes to form crystalline compounds known collectively as anils. By suitable choice of aldehyde and amine this reaction can be used to produce compounds with interesting analytical properties. Dagnall, Smith and West,[1] for example, describe the use of salicylidene-2-aminophenol for the fluorimetric determination of aluminium. The reagent was prepared by the condensation of salicylaldehyde with 2-aminophenol. A sulphur analogue of this reagent can be prepared in a similar way from salicylaldehyde and 2-aminothiophenol, which react together to give the compound salicylideneamino-2-thiophenol, **I**, referred to in this paper by the abbreviation SATP.



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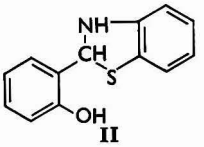

II

This compound **I** is obtained as colourless needles melting at 133° to 136° C. It is insoluble in water, slightly soluble in dilute acids to give colourless solutions and soluble in alkali hydroxide or carbonate to give yellow solutions. It is generally soluble in organic solvents.

Like most anils, SATP tends to hydrolyse in solution, particularly in the presence of acids. Continued heating of the solution results in the formation of a stable yellow phenolic compound, with a marked yellow fluorescence in ultraviolet light, which is thought to be a benzothiazoline, **II**. Alkaline solutions of the compound, **II**, show a strong blue fluorescence.

SATP reacts readily with tin in aqueous solution to give an insoluble yellow complex. The complex can be extracted into immiscible organic solvents to give a bright yellow solution that can be used for the photometric determination of tin.

Experimental

Salicylideneamino-2-thiophenol can be prepared as follows.

Dissolve 10·0 g of salicylaldehyde in 25 ml of chloroform and mix in a 100-ml flask with a solution of 10·25 g of 2-aminothiophenol also dissolved in 25 ml of chloroform. Slowly distil off part of the chloroform by immersing the flask in a water-bath at 67° C. When about half of the chloroform has distilled, a turbidity appears in the flask. Maintain the bath at 67° C for a further 5 minutes, then remove the flask and allow it to cool to room temperature. Filter the solid on to a Buchner funnel and alternately stir with small volumes of chloroform and suck dry until all of the yellow colour has been removed. After re-crystallisation from ethanol, a yield of 5·8 g is obtained. It is important that the temperature is not allowed to rise above 70° C at any stage of the preparation and that heating is not unnecessarily prolonged.

An analysis of SATP gave sulphur 13·95 per cent. and nitrogen 6·12 per cent. (theoretical, sulphur 13·98 per cent.; nitrogen 6·11 per cent.).

SATP is soluble in acetone, ethyl methyl ketone and isobutyl methyl ketone, and less soluble in aliphatic alcohols. However, the decomposition of the reagent is extremely rapid in ketonic solution and also in methanol. This decomposition is still appreciable in other alcoholic solutions, but can be considerably retarded by adding ascorbic acid and by avoiding exposure to light. Solutions in ethanol containing ascorbic acid and stored in brown glass bottles are sufficiently stable for use over a period of 8 hours.

In neutral aqueous solutions SATP reacts with many cations to give mainly yellow reaction products. Under these conditions V(V), V(IV), Cr(VI), Fe(III), Co(II), Cu(II), Zn(II), Ga(III), Ge(IV), Mo(VI), Ag(I), Cd(II), In(III), Sn(IV), Sn(II), Pt(IV), Au(III), Hg(II), Hg(I), Tl(III), Pb(II) and U(VI) all react visibly with the reagent.

In acid solution at pH 2 the reagent reacts with a reduced number of cations. These are listed in Table I. Most of these reaction products give coloured solutions in organic solvents.

TABLE I

REACTIONS OF SALICYLIDENEAMINO-2-THIOPHENOL IN ACID SOLUTION, pH 2

Ion	Reaction	Extraction of complex into xylene	Extraction of complex into isobutyl methyl ketone
V(V)	Pale yellow precipitate	None	None
V(IV)	Pale blue precipitate	Colourless	Colourless
Cr(VI)	Pale yellow precipitate	Pale yellow*	Yellow
Fe(III)	Pale yellow precipitate (fading)	Colourless	Colourless
Cu(II)	Green precipitate	Yellow†	Yellow*
Ga(III)	Pale yellow precipitate	Pale yellow	Colourless
Mo(VI)	Brown precipitate	None	Yellow
Ag(I)	Yellow colour	Faint yellow†	Yellow*
Sn(IV)	Yellow precipitate	Yellow	Yellow
Sn(II)	Yellow precipitate	Yellow	Yellow
Bi(III)	Pale yellow colour	None	None
Pt(IV)	Pale yellow precipitate	None	Pale yellow

Transient yellow colours are also given by In(III), Au(III), Hg(II), Hg(I) and Tl(III).
* Partial extraction. † Slight extraction.

No reaction has been observed in either neutral or acid solution with the following cations: Li(I), Na(I), Mg(II), Al(III), K(I), Ca(II), Ti(IV), Cr(III), Mn(II), Fe(II), Ni(II), As(V), As(III), Sr(II), Y(III), Zr(IV), Sb(V), Sb(III), Ba(II), the lanthanons, W(VI), Tl(I) and Th(IV).

Formation of the tin complex at as low a pH as possible increases the selectivity to tin and also avoids a tendency for tin to hydrolyse from solution. Below pH 2 where there is still a tendency for tin to hydrolyse, both the reagent and the tin complex decompose fairly rapidly. Attempts to keep the tin in solution by complex formation at pH 2 with citric, tartaric or oxalic acids were unsatisfactory, as the presence of these acids also prevented the formation of the tin - SATP complex. However, the addition of lactic acid not only prevented hydrolysis but also permitted the reaction of tin with the reagent. The inclusion of lactic acid also prevented formation of the molybdenum complex.

The tin complex, together with excess of reagent, extracts readily into all the common immiscible organic solvents, although the extracted tin complex is not very stable in ketones and alcohols—the yellow colour fading fairly rapidly with time. The use of hydrocarbon solvents, in which the copper, silver and molybdenum - SATP complexes are only slightly soluble, confers greater selectivity towards tin. It was observed, however, that in aliphatic hydrocarbons such as hexane or iso-octane the tin complex gives first a clear yellow solution, then precipitates slowly as the extract ages. This did not occur in benzene, toluene or xylene solution. Because of its lower toxicity and higher flash-point, xylene was the preferred extractant.

The use of ascorbic acid to stabilise the reagent solution also reduces iron(III) to the non-reacting iron(II) form. Both copper and silver still interfere to some extent. The effect of small amounts of these cations can be further reduced by masking with sodium thiosulphate. The thiosulphate ion does not interfere with tin complex formation, but its decomposition in acid solution to give finely divided sulphur limits the amount that can be added.

From the absorption curves of the reagent and of the tin complex in the xylene extract, shown in Fig. 1, it can be seen that the maximum optical density occurs at 415 mμ. There is a relatively small residual absorption due to the extracted reagent. The molar absorptivity, calculated from a calibration, is 16,100. Straight-line calibration graphs indicate that, provided a considerable excess of reagent is present, the Beer - Lambert law is valid up to 50 μg of tin in 10 ml of xylene.

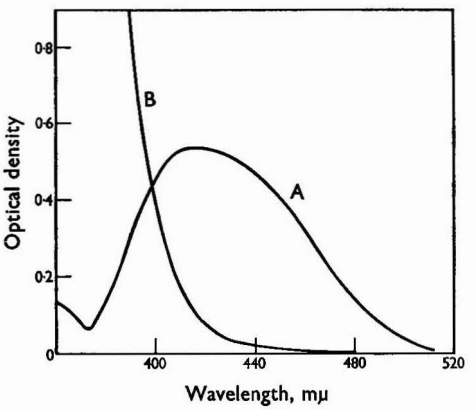

Fig. 1. Absorption spectrum for A, tin complex measured against reagent blank (50 μg of tin in 10 ml of solution, with 1-cm cells); and B, reagent blank measured against xylene

Method

Reagents—

Salicylideneamino-2-thiophenol solution—Dissolve 1 g of ascorbic acid in 100 ml of warm ethanol. Add 0·1 g of salicylideneamino-2-thiophenol and stir until dissolved. Store in a brown glass bottle and prepare freshly each day.

2,4-*Dinitrophenol indicator solution*—Dissolve 0·25 g of 2,4-dinitrophenol in a mixture of 50 ml of ethanol and 50 ml of water.

Sodium hydroxide, about 2·5 N.

Lactic acid solution—Mix 20 ml of lactic acid and 80 ml of water.

Sodium thiosulphate solution—Dissolve 1 g of sodium thiosulphate pentahydrate in 100 ml of water.

Xylene, AnalaR grade.

Tin(IV) standard stock solution—Weigh accurately about 0·5 g of high purity tin metal into a conical flask. Add 200 ml of concentrated hydrochloric acid, cover with a clock-glass and allow to stand until dissolved (up to 2 days may be necessary). Add 1 ml of 100-volume hydrogen peroxide and dilute to 1 litre with water in a calibrated flask.

Tin(IV) dilute standard solution—Dilute accurately 25 ml of the stock standard solution to 250 ml with diluted (1 + 9) hydrochloric acid. This solution contains 50 μg of tin per ml.

Procedure for tin ores—

Mix intimately a weighed sample of about 0·25 g with 2·5 g of sodium peroxide in a nickel crucible. Heat until just sintered and then bring up to the fusion-point for a few minutes. Allow the melt to cool and dissolve it in 100 ml of 6 N hydrochloric acid. Dilute to 500 ml with water in a calibrated flask.

Transfer an aliquot containing not more than 50 μg of tin to a 75-ml, all-glass, stoppered boiling-tube. Add 2 drops of the 2,4-dinitrophenol indicator solution and neutralise carefully to a yellow end-point, or to just permanent precipitation of metal hydroxides, if these are present, by adding 2·5 N sodium hydroxide solution. Dilute to about 20 ml with water and add 2 ml of lactic acid solution. Mix and allow the solution to stand until any precipitated metal hydroxides have re-dissolved. Add 1 ml of the sodium thiosulphate solution and mix. Add by pipette 5 ml of the salicylideneamino-2-thiophenol

solution, shake the tube immediately and allow it to stand for 20 minutes. Extract with exactly 10 ml of xylene, shake the mixture vigorously for 20 seconds and allow the phases to separate. After allowing the solution to stand for 5 minutes, measure the optical density of the organic extract with a spectrophotometer set at a wavelength of 415 mμ with a reagent blank solution, prepared at the same time as the sample solution, as reference.

CALIBRATION—

With a 1-ml semi-micro burette, measure aliquots of the dilute standard tin solution containing 0, 10, 20, 30, 40 and 50 μg of tin into stoppered boiling-tubes. Follow the procedure as described above from the point at which the sample aliquot is transferred to the stoppered boiling-tube. Draw a calibration graph; this should be linear and pass through the origin.

DISCUSSION

CONSTITUTION OF THE TIN COMPLEX—

The mole ratio, the Job and the Harvey and Manning methods all fail when used to investigate the constitution of the complex. This is because no appreciable reaction occurs until the reagent is present in at least a 10-molar excess. The effect of increasing reagent concentration is shown in Fig. 2.

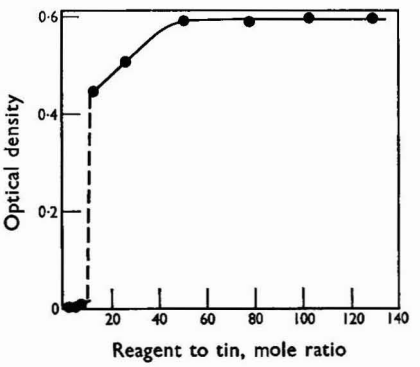

Fig. 2. Effect of reagent concentration on the formation of the tin complex

The tin complex can be almost quantitatively precipitated by the addition of ethanolic reagent to an aqueous tin solution in the presence of lactic acid at pH 2. The final ethanol concentration should be at least 40 per cent. to avoid co-precipitation of excess of reagent but not above 45 per cent., when the tin complex becomes appreciably soluble. The complex is stable to drying at 110° C. Precipitation of 50 mg of tin by this method gave 241·7 mg of dried complex, corresponding to a reagent-to-tin ratio of 2·00. A titrimetric determination of tin in the dried complex after a wet oxidation gave 19·46 per cent. of tin, corresponding to a reagent-to-tin ratio of 2·16.

The complex formed with tin(II) has an identical absorption curve in xylene solution and the same molar absorptivity to that formed with tin(IV). It is possible that oxidation of the tin(II) occurs, although this seems unlikely in the presence of ascorbic acid.

From Fig. 2 it can be seen that at least a 50-molar excess of reagent must be used to ensure complete formation of the complex. Too great an excess must, however, be avoided or the absorption of the blank becomes excessively high.

EFFECT OF TIME AND TEMPERATURE ON THE FORMATION OF THE COMPLEX—

Sufficient time must be allowed after addition of the reagent for the insoluble complex to form, but low recoveries are obtained if this period is too protracted. This effect is shown in Fig. 3, from which it can be seen that 20 minutes appears to be the optimum time for measurement to be made.

No temperature effect has been found between 5° and 40° C, but above 50° C the complex tends to decompose.

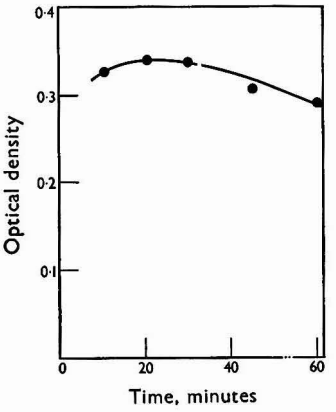

Fig. 3. Effect of time of standing at 20° C on the formation of the tin complex

STABILITY OF THE EXTRACTED COMPLEX—

When measured against the extractant, the optical densities of both the extracted tin complex and the blank first fall slightly and then increase slowly with time. The effect, shown in Fig. 4, is small but, for the most consistent results, it is advisable to take the measurements 5 minutes after separation of the phases. A reagent blank prepared at the same time as the sample must be used as the reference.

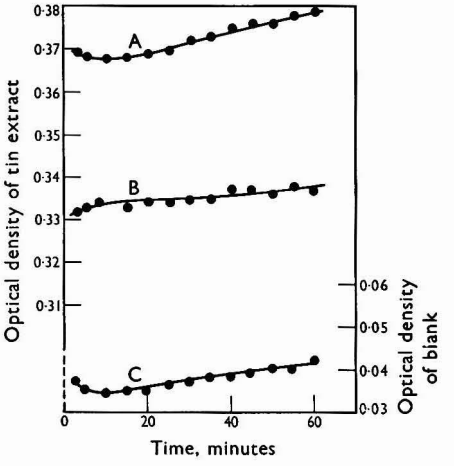

Fig. 4. Effect of time of standing on the extracted complex: A, blank; B, 25μg of tin sample; and C, after correction for the blank

INTERFERING IONS—

The effect of several possible interferences on the determination of 25 μg of tin is given in Table II. The most serious interference is that from copper, of which not more than 5 μg can be tolerated. Large amounts of chloride and of sulphate do not interfere.

One of the advantages of the proposed method is that, for most materials, a preliminary separation of the tin is not necessary. If a separation is required, the bromide distillation method of Onishi and Sandell[2] or the iodide extraction method of Tanaka[3] as modified by Newman and Jones[4] should prove satisfactory.

TABLE II

INVESTIGATION OF INTERFERENCES

Tests were made by adding the interfering ion to a solution containing 25 μg of tin

Ion added	Amount added, μg	Amount of tin recovered, μg	Recovery, per cent.
V(V)	1000	26·9	107·5
	100	25·5	102·0
V(IV)	1000	26·5	106·0
	100	25·0	100·0
Cr(VI)	100	26·4	105·5
	25	25·4	101·5
Cr(III)	1000	21·0	84·0
	100	24·0	96·0
Fe(III)	10,000	21·8	87·5
	1000	25·3	101·0
Co(II)	1000	25·0	100·0
Cu(II)	100	30·9	123·5
	25	27·5	110·0
	5	25·4	101·5
Zn(II)	1000	25·0	100·0
As(III)	1000	27·5	110·0
	100	25·0	100·0
Mo(VI)	1000	25·8	103·5
	100	25·0	100·0
Ag(I)	1000	Extract turbid	—
	100	24·3	97·0
Cd(II)	1000	25·4	102·0
Sb(III)	1000	32·0	128·0
	100	25·5	102·0
Hg(II)	1000	25·0	100·0
Pb(II)	1000	26·2	104·5
	100	25·4	102·0
Bi(III)	1000	25·3	101·0
Phosphate	1000	17·2	68·0
	500	23·5	94·0
	100	25·0	100·0
Fluoride	1000	24·6	98·0
	100	24·8	99·0

RESULTS

Several samples have been analysed by the proposed method. The results obtained are given in Table III.

TABLE III

COMPARISON OF RESULTS WITH THOSE OBTAINED BY A TITRIMETRIC METHOD

Laboratory reference number	Sample	Tin, per cent. colorimetric	Tin, per cent. titrimetric
S 453	Canadian tin ore, tin concentrate	45·4	45·5
S 454	Canadian tin ore, mill sample	0·62	0·37 (0·58)*
S 455	Canadian tin ore, tail sample	0·80	0·69
S 456	Geevor mine, mill sample "A"	0·66	0·66
S 457	Geevor mine, mill sample "B"	1·02	1·02
S 451	Cornish beach sand, composite sample	0·14	0·14
S 460	Cornish beach sand, No. 1 concentrate	21·1	20·4
S 461	Cornish beach sand, No. 2 concentrate	6·0	5·97
S 462	Cornish beach sand, table concentrate	2·25	2·06

* This result was obtained by X-ray fluorescence analysis.

REPRODUCIBILITY OF THE METHOD—

Eight replicate aliquots were taken from one sample solution of a tin concentrate (S 453) and the tin complex extracted into xylene as described above. The optical densities recorded were 0·622, 0·624, 0·622, 0·622, 0·615, 0·618, 0·620 and 0·624 (standard deviation 0·003, and the coefficient of variation 0·5 per cent.). Four completely separate determinations of tin on this concentrate gave values of 45·7, 45·2, 45·4 and 45·0 per cent.

APPLICATION OF THE REAGENT TO SILICATE ROCKS AND MINERALS—

As an extension of this work, attempts were made to determine tin in several silicate materials. Silica was removed by evaporation with hydrofluoric and sulphuric acids and the residue fused with potassium pyrosulphate. After solution of the melt in dilute hydrochloric acid, the tin present was determined by the procedure described above, except that additional ascorbic acid was added to reduce iron(III). The results obtained are given in Table IV.

TABLE IV

COMPARISON OF RESULTS FOR SOME SILICATES

Sample material	Tin, p.p.m. with SATP	Tin, p.p.m. other methods
G–1, granite	2·0, 2·8, 3·6	4*
W–1, diabase	2·9, 3·4	3*
T–1, tonalite	1·2, 1·5	50, 29, 50†

* "Recommended values," Fleischer.[5]
† Quoted by Thomas.[6]

The reproducibility of these results is far from satisfactory and further work is clearly indicated before this method can be recommended.

CONCLUSIONS

Salicylideneamino-2-thiophenol is proposed as a new photometric reagent for determining tin. It is sufficiently specific to permit the direct determination of the metal in most ores, rocks and minerals without the necessity of a preliminary separation.

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Determination of Trace Amounts of Magnesium, Strontium and Nickel in Lake-water Samples by Neutron-activation Analysis

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Neutron-activation analysis has been used to determine magnesium, strontium and nickel in water from eleven Greek lakes. The elements were separated by an isolation procedure (taking up to 30 minutes); the magnesium and strontium were determined by γ-spectrometry, and the nickel by β-coincidence counting. The ranges found were 11 to 31 p.p.m. of magnesium, 0·02 to 1·12 p.p.m. of nickel and 0·07 to 0·48 p.p.m. of strontium.

Neutron-activation analysis is a rapid modern analytical method that is available for most precise analytical work for determining trace elements in different matrices.[1,2,3] Because of its high sensitivity and accuracy, neutron-activation analysis has become a useful tool for analytical chemists.[4 to 11] In the past years spectrographic methods had been applied for determining minute amounts of elements in lake waters,[12] but these methods or other conventional ones were sometimes restricted for some elements because of insufficient sensitivity, the impurity of the reagent or the large reagent blanks. A promising method for determining microgram or submicrogram amounts of elements present in lake water is neutron-activation analysis, which will supply complementary or new information for most trace elements in lake waters.

Radioactivation analysis has also been used by several investigators for determining trace elements in water.[13,14,15,16] A review of the chemical composition of the river and lake waters of the world has been made.[17] Recently 14 trace elements were determined in the Greek lakes by activation analysis.[18] Magnesium and strontium (simultaneously) and also nickel were determined in the same lakes by neutron-activation analysis.

The half-lives of the nuclides are 9·45 minutes for magnesium-27, 2·8 hours for strontium-87*m* and 2·56 hours for nickel-65. Various competing nuclear reactions[19,20,21] have been considered, but are found to be insignificant when the levels of the interfering elements, present in the lake waters, have been taken into account.

Experimental

Reagents—

Materials of analytical-reagent grade were used.

Magnesium nitrate carrier solution—Prepare an aqueous solution of magnesium nitrate containing 10 mg of magnesium per ml.

Standard magnesium nitrate solution—Prepare an aqueous solution of magnesium nitrate containing 0·2 mg of magnesium per ml.

Manganese chloride hold-back carrier solution—Prepare an aqueous solution of manganese chloride containing 10 mg of manganese per ml.

Sodium chlorate, solid.

Nitric acid, fuming, 6 N, concentrated, 20 and 50 per cent., aqueous.

Sodium nitrate hold-back carrier solution—Prepare an aqueous solution of sodium nitrate containing 10 mg of sodium per ml.

Ethanol, absolute.

Phenolphthalein indicator, 0·1 per cent. alcoholic solution.

Ammonia solution, concentrated, 2·5 per cent., aqueous.

Diammonium hydrogen orthophosphate solution, 25 per cent., aqueous, freshly prepared.

Hydrochloric acid, 2 N, 3 N and 4 N.

Strontium nitrate carrier solution—Prepare an aqueous solution of strontium nitrate containing 10 mg of strontium per ml.

Standard strontium nitrate solution—Prepare an aqueous solution of strontium nitrate containing 5 mg of strontium per ml.

Barium chloride hold-back carrier solution—Prepare an aqueous solution of barium chloride containing 10 mg of barium per ml.

Copper nitrate hold-back carrier solution—Prepare an aqueous solution of copper nitrate containing 10 mg of copper per ml.

Iron(III) nitrate hold-back carrier solution—Prepare an aqueous solution of iron(III) nitrate containing 10 mg of iron per ml.

Ammonium acetate, 6 M, *aqueous.*

Acetic acid, 6 M.

Sodium chromate solution, 1·5 M, *aqueous.*

Sodium carbonate solution, 10 *per cent., aqueous.*

Nickel metal—Supplied by Johnson, Matthey & Co. Ltd., spectrographically pure.

Nickel nitrate carrier solution—Prepare an aqueous solution of nickel nitrate containing 10 mg of nickel per ml.

Benzene.

Sodium chloride hold-back carrier solution—Prepare an aqueous solution of sodium chloride containing 10 mg of sodium per ml.

Ammonium arsenate hold-back carrier solution—Prepare an aqueous solution of ammonium arsenate containing 10 mg of arsenic per ml.

Potassium sodium tartrate solution, 10 *per cent., aqueous.*

Dimethylglyoxime, 1 *per cent. alcoholic solution.*

Chloroform.

Irradiation

Lake-water samples were stored in polythene bottles. Before irradiation, samples were filtered through a Pyrex funnel with Whatman filter-paper and transferred with a Pyrex pipette into small polythene tubes. In all irradiations the targets were sent to the core of the reactor by a pneumatic transfer system (rabbit). The flux to which targets were irradiated was 2×10^{12}n per cm^2 per second supplied by the "Democritos" swimming-pool nuclear reactor operating at 1 MW.

With magnesium and strontium, a volume of more than 10 ml of lake-water sample was placed in the external tube of the specially adjusted polythene vial.[22] An amount of more than 1 ml of a mixture consisting of equal volumes of the magnesium and strontium standard solutions was put in the central tube. The irradiation time was 25 minutes.

For nickel the target was adjusted in the same manner as above. A volume of more than 10 ml of lake water was transferred by pipette into the external tube. Nickel turnings, 10 mg, were weighed into two small snap-closure polythene vials, which were then inserted in the central tube.[23] The irradiation time was 45 minutes.

Radiochemical separations[24,25,26]

Magnesium and strontium—

After irradiation of the target, 5 ml of the lake-water sample were transferred by pipette (sampling in duplicate) into a 50-ml centrifuge tube containing 1 ml of magnesium and 2 ml of strontium carrier solutions, and 1 ml each of hold-back carrier solutions of manganese, barium and sodium. The solution was cooled in an ice-bath, and 25 ml of cold fuming nitric acid were added. The precipitate was spun in a centrifuge. The supernatant liquid was collected in another 50-ml centrifuge tube for isolating the magnesium, while the precipitate was subjected to the strontium isolation procedure.

Supernatant liquid—The solution was heated in a water-bath, and a few crystals of sodium chlorate were added. The manganese dioxide precipitate was separated by spinning the solution in a centrifuge. The supernatant liquid was decanted into another 250-ml centrifuge tube, cooled in an ice-bath, and 1 ml of sodium hold-back carrier solution was added. Careful and complete neutralisation of the nitric acid was carried out with concentrated ammonia solution (about 35 ml) in the presence of phenolphthalein indicator, and 5 ml

of diammonium hydrogen orthophosphate solution were added while stirring and cooling the mixture. The ammonium magnesium orthophosphate precipitate was separated by spinning the solution in a centrifuge for 1 minute at 3000 r.p.m. The ammonium magnesium orthophosphate precipitate was dissolved in a few drops of 3 N hydrochloric acid and then transferred into a 50-ml centrifuge tube and cooled in an ice-bath The hydrochloric acid solution was completely neutralised in the presence of phenolphthalein indicator with a few drops of concentrated ammonia solution. To the solution 1 ml of diammonium hydrogen orthophosphate solution was added and the ammonium magnesium orthophosphate precipitate was filtered, rinsed with 2·5 per cent. ammonia solution and finally with ethanol. The filter-paper and precipitate were transferred into a culture tube for counting. After counting, the filter-paper and precipitate were transferred into a pre-weighed porcelain crucible and then ignited at 1100° C to magnesium pyrophosphate. (The chemical yield was, on the average, about 65 per cent.)

Precipitate—The precipitates of barium nitrate and strontium nitrate were dissolved in 2 to 3 ml of water. Aliquots of copper, sodium and iron hold-back carrier solutions, 1 ml of each, were added to the solution together with 1 drop of phenolphthalein indicator. Concentrated ammonia solution was added dropwise to precipitate iron as iron(III) hydroxide scavenger. The precipitate was separated by spinning the solution in a centrifuge. The supernatant liquid was decanted into another 50-ml centrifuge tube. The ammoniacal solution was completely neutralised with 6 N nitric acid; 2 ml of 6 M ammonium acetate and 1 ml of 6 M acetic acid were added to the solution, which was heated to boiling, and 1 ml of 1·5 M sodium chromate was then added dropwise. The solution was stirred and cooled in an ice-bath. The barium chromate precipitate was separated by spinning in a centrifuge. The supernatant liquid was decanted into another 50-ml centrifuge tube, neutralised with concentrated ammonia solution (pH 7) and then 10 ml of 10 per cent. sodium carbonate solution were added. The solution was heated to coagulate the strontium carbonate precipitate, which was then filtered with a pre-weighed filter-paper, rinsed with water and then with ethanol. The filter-paper with the precipitate were transferred into a culture tube for counting. After counting they were heated at 110° C. (The chemical yield, on the average, was about 60 per cent.)

For the standards a 0·5-ml aliquot was used (sampling in duplicate) and the same analytical steps were followed exactly.

NICKEL—

After irradiation of the target, 5 ml of lake-water sample were transferred by pipette (sampling in duplicate) into a 100-ml beaker containing 1-ml portions of hold-back carriers of sodium chloride and ammonium arsenate and 5 ml of 20 per cent. potassium sodium tartrate. The solution was adjusted to pH 9 with ammonia solution. A standard precipitation of nickel as nickel - dimethylglyoxime was carried out and the nickel - dimethylglyoxime was then extracted into chloroform. The same step was once again repeated with the aqueous phase. Nickel was back-extracted from chloroform with 2 N hydrochloric acid. The same step was once again repeated in the chloroform layer. The aqueous solution was adjusted to pH 9 with ammonia solution, and a standard precipitation of nickel as nickel - dimethylglyoxime was carried out in the presence of the hold-back carriers and tartrate. The nickel - dimethylglyoxime precipitate was rinsed with hold-back carriers (Na^+, Cl^-, As^{5+}) and hot water. The nickel - dimethylglyoxime precipitate was dissolved in hot 4 N hydrochloric acid. After dissolution, the filtered solution was neutralised with ammonia solution and brought to pH 9; a standard precipitation of nickel - dimethylglyoxime was then carried out in the presence of the same hold-back carriers and tartrate.

The precipitate was filtered with a pre-weighed filter-paper, rinsed in the same way as before, air dried by suction and counted. After counting it was heated at 110° C. (The chemical yield, on the average, was about 90 per cent.)

For the standards, the nickel turnings were transferred into two beakers, 1-ml volumes of 50 per cent. nitric acid were added and then heated until dissolution occurred. The two nickel nitrate solutions were transferred into two 1-litre calibrated flasks and were then diluted to volume. Aliquots of 100 μl, taken from solutions in each calibrated flask (sampling in duplicate), were subjected to the same analytical steps as before.

Determination of radioactivity

The radioactivity measurements for magnesium-27 and strontium-87*m* were made by using a well-type crystal connected to a single channel analyser to count γ-rays at the areas 0·83 MeV and 0·39 MeV, respectively.

Because of the presence of nickel-65 a β-count was made with a Geiger counter connected anti-coincidentally.

Results

Identification and control of radiochemical purity of magnesium-27, strontium-87*m* and nickel-65—

The isolated precipitates of ammonium magnesium phosphate, strontium carbonate and nickel - dimethylglyoxime were subjected to a γ-ray spectrometric examination,[27,28] which confirmed the absence of any γ-emitting radionuclide as contaminant. Half-life measurements of the isolated radio-elements were also performed by plotting the decay curve on semi-log paper, for each isolated precipitate.

The half-life was obtained from the slope of the straight line which was calculated by the method of least squares. Values were found corresponding to those reported in the literature.[19,20]

Concentration of elements determined—

Quantitative results for the magnesium, strontium and nickel content of lake-water samples taken from the surface and depth of the most important Greek lakes have been obtained by radiochemical methods. The results, being the average of at least duplicate analyses, are given in Table I. Samples analysed were collected with a Ruttner water sampler (made of Plexiglass) from the surface and 5 or 15 metres' depth, depending on the maximum depth of each lake. The collection of samples was made at the deepest point, often the centre of the lakes.

Table I

Concentration of elements determined in lake-water samples*

Lake	Magnesium		Strontium		Nickel	
	Surface	Depth	Surface	Depth	Surface	Depth
Aghios Vassilios†	28·3	30·6	0·07	0·09	0·02	0·09
Doirani†	19·7	19·8	0·26	0·44	0·66	0·27
Ioannina†	12·7	10·5	0·17	0·17	1·22	0·52
Kastoria‡	14·5	14·4	0·10	0·16	0·64	0·76
Marathon‡	13·7	16·9	0·20	0·22	0·09	0·10
Ostrovon‡	25·9	27·1	0·12	0·09	0·46	0·21
Paralimni ‡	21·5	25·3	0·16	0·17	0·44	0·74
Prespa mikri†	11·2	11·7	0·07	0·09	0·93	0·29
Stymphalis§	13·0	—	0·48	—	0·23	—
Trichonis‡	10·9	16·2	0·29	0·40	0·54	0·58
Volvi‡	26·7	23·6	0·15	0·08	0·50	0·57

* Concentrations expressed in parts per million.
Each lake-water sample analysis was performed at least in duplicate.
† Depth of sample collection, 5 metres.
‡ Depth of sample collection, 15 metres.
§ Maximum depth less than 4 metres.

Discussion

The analytical methods proposed involve the use of radiochemical separations, and result in a remarkable radiochemical purity of the isolated precipitates of ammonium magnesium orthophosphate, strontium carbonate and nickel - dimethylglyoxime. Experimental values are reproducible, with a relative error of less than ± 2 per cent. From the results given it is apparent that activation analysis with its high sensitivity should play an important rôle in supplying complementary or new information on elements present in microgram or submicrogram amounts in lakes. The information given by our results in this paper may have, for the elements determined, not only geological but also biological interest. The presence

or absence of some trace elements can affect the eutrophic, oligotrophic or atrophic behaviour of the lake. Determination of the trace elements in a given lake should, therefore, be performed several times a year.

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A Rapid Method for the Determination of Iron in Plant Material with Application of Automatic Analysis to the Colorimetric Procedure

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A colorimetric procedure is described for the determination of iron in plant material by using sulphonated 4,7-diphenyl-1,10-phenanthroline. The method may be applied directly to the AutoAnalyzer, and gives good and reproducible recoveries over a wide range of added iron. The possibility of low recoveries of iron resulting from the wet digestion of material in the presence of sulphuric acid is discussed.

THE accurate determination of iron in biological materials has often proved difficult, even though many colorimetric methods have been described.[1] This is borne out in a report of the Agricultural Research Council,[2] in which identical samples of several plant materials were sent to ten centres for analysis by their own methods. Solution preparation involved wet or dry ashing, and was followed by the use of 1,10-phenanthroline, dipyridyl, potassium thiocyanate or salicylic acid in colorimetric determinations. One centre used direct spectrographic arcing of the powdered samples. The results showed large inter-laboratory variations, ranging from 20 to 60 per cent. of the mean, according to the material analysed. Replicate determinations at the same centre of sub-samples of a given material also gave variable results, but were of a smaller range than those from different centres. Subsequent examination showed that plant material is particularly susceptible to surface contamination by iron-bearing dust and soil particles; moreover, the actual distribution of iron within the tissues may vary locally, thus making representative sub-sampling difficult. Differences in the amount of sample taken and in methods of preparation for analysis were also suspected of contributing significantly to the variability of the results.

The method described involves the wet digestion of the plant material, with colorimetric determination of the iron by a fully automatic or relatively rapid manual method. It has been shown to give good recovery of a wide range of added iron, and with replicate samples of oak leaf litter gave values varying by less than 2 per cent. of the mean.

THE MANUAL METHOD

Case[3] introduced 4,7-diphenyl-1,10-phenanthroline, generally referred to as bathophenanthroline,[4] as an improvement on the well known 1,10-phenanthroline. However, as this substituted compound is barely soluble in water, it has to be dissolved in ethanol or aqueous ethanol, and the iron(II) complex is generally extracted into an organic solvent before measurement. This increases the sensitivity of the determination, but the method is tedious when dealing with large numbers of samples. The present method is simpler, and makes use of a sulphonated form of bathophenanthroline as described by Riley and Williams.[5]

REAGENTS—

All reagents should be of analytical grade.
Nitric acid, concentrated, sp.gr. 1·42.
Sulphuric acid, concentrated, sp.gr. 1·84.
Perchloric acid, 60 *per cent., sp.gr.* 1·54.

Sulphonated bathophenanthroline—Treat 0·4 g of 4,7-diphenyl-1,10-phenanthroline with 4·0 ml of fuming sulphuric acid (20 per cent. sulphur trioxide). Stir until dissolved, leave to stand for 30 minutes and pour into 400 ml of water. Neutralise the excess of acid with ammonium hydroxide to pH 4 to 5 and make up to 1 litre.

Hydroxylammonium chloride, 2·5 per cent. w/v, aqueous.

Sodium acetate, 33 per cent. $NaOOC.CH_3.3H_2O$ w/v, aqueous.

Standard iron solutions—Prepare a stock solution by dissolving 0·1000 g of clean iron wire in about 10 ml of 10 per cent. sulphuric acid and making the volume up to 1 litre. Dilute 100-fold to give a standard solution containing 1 μg of iron per ml.

Colorimetric reagent—Just before the determination mix sodium acetate, sulphonated bathophenanthroline and hydroxylammonium chloride solutions in the proportions 4 + 3 + 1.

INITIAL TREATMENT OF SAMPLE—

Plant material should be dried at room temperature, or at 40° C in an air-circulated oven, until sufficiently brittle to be ground. Any suitable knife or hammer mill may be used, taking care that the entire sample passes through the sieve. In this laboratory, a mesh of 0·4 to 0·7 mm has been found suitable for sample weights of 50 to 500 mg. Dry the ground sample in an oven at 105° C for 3 hours before weighing for digestion.

PREPARATION OF SAMPLE SOLUTION—

Digest up to 0·5 g of the ground vegetation with 1 ml of 60 per cent. perchloric acid, 6 ml of concentrated nitric acid and 1 ml of concentrated sulphuric acid in a 200-ml Kjeldahl flask. Blank digestions are required as a check on possible contamination of the digest acids. Bring to the white fume stage and heat for a further 15 minutes. Normally, on cooling, the remaining liquid should be colourless. Dilute the digest with about 15 ml of water, boil for 10 minutes, filter into a 100-ml calibrated flask and dilute to the mark when cool.

PROCEDURE—

Transfer by pipette 20 ml of the sample solution containing up to 0·03 mg of iron into a 50-ml calibrated flask. If a smaller aliquot is taken, the acid content should be adjusted to give a final concentration equivalent to 20 ml of 1 per cent. sulphuric acid. Add 16 ml of the colorimetric reagent, dilute to volume, mix thoroughly and read the optical density at 536 mμ. Beer's law is obeyed up to at least 0·4 mg of iron per 50 ml, and the colour is stable for at least 24 hours. The standards (0 to 0·03 mg of iron) are transferred by pipette into 50-ml calibrated flasks and the acidity made equivalent to 20 ml of 1 per cent. sulphuric acid. They are then treated as described above.

RESULTS AND DISCUSSION

Riley and Williams applied the bathophenanthroline method to rocks and minerals that are normally very rich in iron, and they required only 1 ml of sample digest for colour development. The iron(II) - bathophenanthroline complex is stable over the pH range 2 to 9, and they found that a sodium acetate buffer at a final concentration of 0·04 M was sufficient to maintain the final pH within the required range. Plant materials, however, usually have a relatively low iron content and larger aliquots of the digests are required. Furthermore, the amount of acid remaining after wet digestion may vary slightly. In most of the methods described, the excess acid is neutralised with dilute ammonia solution by using pH papers or a pH meter, and sodium acetate buffer is then added to stabilise the final pH. To improve the speed of the manual method however, and to enable it to be adapted to a fully automatic procedure, it was found possible to prepare a buffer of sufficient strength both to dispense with the neutralisation and to control adequately any variations in the final acidity of the digests. It was found that 8 ml of 33 per cent. w/v hydrated sodium acetate in a final volume of 50 ml was sufficient to buffer 20 ml of 1 per cent. sulphuric acid (the theoretical final acid concentration in the diluted digests) to pH 4·8. This value was chosen as being in the region of maximum buffering capacity of sodium acetate - acetic acid. It was shown that, under these conditions, a final acid concentration in the diluted digest solution of 0·5 to 1·5 per cent. sulphuric acid can be tolerated. These values are considered to be the extremes likely to be met in practice.

COLOUR STABILITY—

It was shown that under the conditions described, the colour develops fully within 20 seconds and is stable for at least 24 hours.

RECOVERY OF IRON FROM DIGESTION FLASKS—

Standards prepared directly from stock solution and treated as above gave reproducible results, and good agreement was also found between duplicate determinations on a single digest. However, determinations made on replicate digests often gave widely varying results.

To investigate this, appropriate amounts of the iron stock solution were digested as described above. The digest was diluted and immediately filtered into 100-ml calibrated flasks without further heating. After dilution to volume, 20-ml aliquots were taken for the determination of iron. A series of undigested standards was prepared to cover the same range for comparison.

TABLE I

EFFECT OF DILUTING DIGESTS AND BOILING THEM FOR 10 MINUTES ON THE RECOVERY OF DIGESTED IRON

A. Sample digests diluted and filtered directly into flasks			B. Sample digests diluted and boiled before filtering into flasks		
Iron digested, mg	Iron found, mg	Recovery, %	Iron digested, mg	Iron found, mg	Recovery, %
0·025	0·024	96	0·050	0·049	98
0·050	0·047	94	0·100	0·096	96
0·075	0·055	73	0·150	0·152	101
0·100	0·046	46	0·200	0·200	100
0·125	0·024	19	0·300	0·304	101
0·150	0·039	26	0·400	0·407	102
0·200	0·033	17	0·500	0·506	101
0·250	0·098	39	1·00	0·99	99
—	—	—	2·00	1·98	99
—	—	—	3·00	2·98	99
—	—	—	4·00	3·98	100

The results (Table I A) show that the recovery of iron becomes progressively poorer when more than 0·05 mg of iron is digested per flask. Subsequent tests with bathophenanthroline as a spot reagent revealed the presence of iron particles, both in the Kjeldahl flask and on the filter-paper. Further, if more than 0·05 mg of iron was digested and washed directly into a calibrated flask without filtering, the aliquot taken for iron determination gave a colour developing gradually with time. Over a period of about 20 hours the optical density approached that given by an undigested iron standard of the same iron content. These results are in agreement with those of Smith and Sullivan,[6] who stated that digestion of 100 mg of potassium ferricyanide with perchloric acid - sulphuric acid mixtures resulted in the precipitation of anhydrous iron(III) sulphate. The present work indicates the low solubility of iron(III) sulphate in anhydrous sulphuric acid.

It was found that dilution of the acid in the Kjeldahl flask with about 15 ml of water, followed by boiling for 10 minutes was sufficient to bring all the precipitated iron into solution. There was no advantage in adding a reducing agent such as hydroxylammonium chloride in an attempt to convert the iron(III) sulphate to the more soluble iron(II) salt.

Table I B shows that boiling the digest with water before filtering allows full recovery of up to at least 4 mg of iron per flask.

Comparison with other methods showed that elimination of the neutralisation stage produced a nearly 2-fold increase in the rate at which samples could be analysed. When using semi-automatic equipment for transferring sample solutions and reagents by pipette, colorimetric determinations could be made at the rate of 30 samples per hour.

THE AUTOMATIC METHOD

APPARATUS—

The instrument used for the automatic determination was the Technicon AutoAnalyzer, which is fully described elsewhere.[7]

As the sample solution is filtered before use, and the colour develops fully within 20 seconds of adding the buffer at room temperature, the dialyzer and heating-bath modules are not required. The flow diagram is shown in Fig. 1.

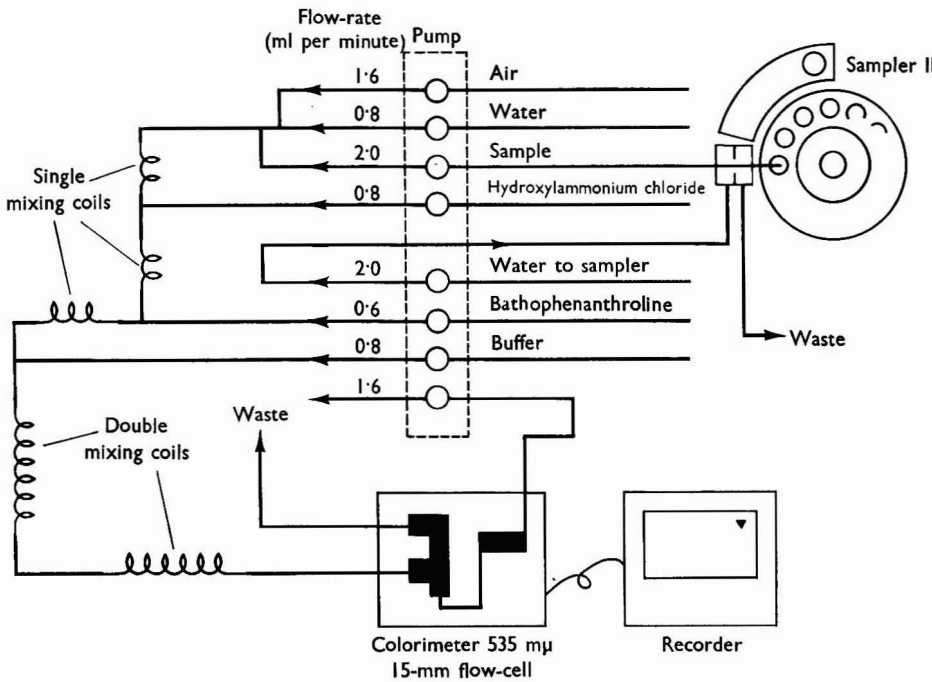

Fig. 1. Flow diagram of the AutoAnalyzer as used in the automatic method for determining iron. All pump tubing is in Tygon

REAGENTS—

These are prepared as described for the manual method with the following exceptions—

Hydroxylammonium chloride, 0·5 per cent. w/v, aqueous.

Colorimetric reagent—This is not required as the components are added as separate reagents.

Standard iron solutions—Prepare from the stock solution a series of standards containing from 0 to 5 p.p.m. of iron, each made up in 1 per cent. v/v sulphuric acid.

PROCEDURE—

The large 3-ml cups are used. Sampler II is fitted with a standard cam to give a sampling rate of 40 samples per hour, with a sample-to-wash time-ratio of 2 to 1 to allow for adequate washing. As a further precaution, a cup containing water is inserted after every third sample. It is preferable to use water for this purpose rather than 1 per cent. sulphuric acid, as the former gives more well defined minima for the construction of the base-line (Fig. 2). Standards, samples and digestion blanks are always run together, and although the blanks give a small peak this should not be greater than that given by the zero standard. In the present work, the reagent blank was found to be caused by iron impurities in the bathophenanthroline, but was not great enough to warrant further purification of the reagent.

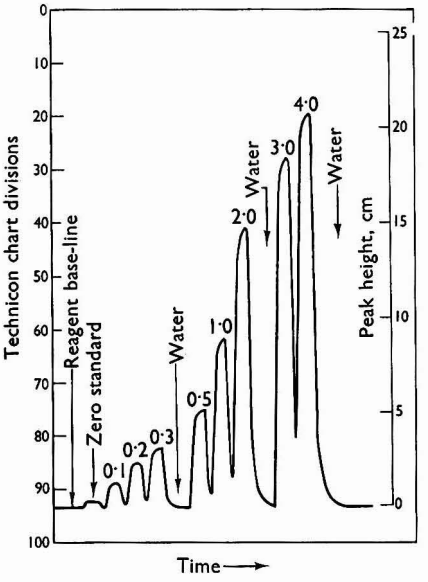

Fig. 2. A typical recording of the peaks obtained by using calibration standards. The figures above each peak refer to the concentration of iron in the standard in p.p.m. The time interval between successive peaks is 90 seconds

Results and discussion

Interferences—

The interferences in the determination of iron by bathophenanthroline have been reviewed.[8] It has been shown that chloride, nitrate, sulphate, perchlorate and acetate do not interfere. The metal ions listed that may cause interference are unlikely to be present in plant material in amounts sufficient to have any significant effect. On the other hand, citrate, oxalate, lactate, tartrate, cyanide, sulphide, fluoride, phosphate and pyrophosphate all gave a less intense iron colour. Of these however, only phosphate is expected to remain after the wet digestion described above, and is therefore the only ion in plant material likely to cause interference. It was found with the automatic method that a full recovery of iron was obtained on digesting 0·1 mg of iron with at least 10 mg of phosphate phosphorus, equivalent to 1 p.p.m. of iron and 100 p.p.m. of phosphate phosphorus in the final diluted digest, and that 4·0 p.p.m. of iron could be recovered fully in the presence of up to 50 p.p.m. of phosphate phosphorus. Phosphate phosphorus rarely exceeds 0·3 per cent. in unfertilised plant material, and therefore this ion is unlikely to interfere in the method described, provided the recommended sample weights are not exceeded.

Tests confirmed that perchloric acid is without effect at concentrations up to 100 p.p.m. in the final digest. Full recovery of up to 4·0 mg of iron was also obtained in the presence of 10 mg of dehydrated silica.

Table II shows the recovery of iron from six plant materials to which known amounts of iron had been added. The weight of iron digested varies from 0·06 to 0·37 mg per flask, and the final percentage figures for iron are in good agreement. To determine the reproducibility of the method, ten sub-samples of oven-dried oak leaf litter were digested as described above, a portion being taken from each digest solution for analysis on the Auto-Analyzer. The sample weights taken were in the range of 225 to 275 mg. At a level of 0·044 per cent. of iron, the standard deviation is 0·001 per cent. and indicates the accuracy to be expected of the method under conditions of routine analysis.

We are indebted to Mr. S. E. Allen for his help and advice during the course of this work.

Table II

Recovery of iron added to various plant materials

Type of material and weight digested	Iron added, mg	Total iron found, mg	Iron in plant material, percentage dry weight	Percentage recovery of added iron
Heather (*Calluna vulgaris*) shoots, 500 mg	—	0·210	0·042	—
	—	0·216	0·043	—
	0·050	0·261	0·042	96
	0·100	0·315	0·043	102
	0·150	0·366	0·043	102
Mat grass (*Nardus stricta*), 250 mg	—	0·062	0·025	—
	—	0·062	0·025	—
	0·050	0·113	0·025	102
	0·100	0·166	0·026	104
	0·150	0·224	0·030	108
Pine (*Pinus sylvestris*) fresh needles, 500 mg	—	0·108	0·022	—
	—	0·119	0·024	—
	0·050	0·164	0·023	101
	0·100	0·210	0·022	97
	0·150	0·260	0·022	98
Oak (*Quercus petraea*) fresh leaves, 500 mg	—	0·082	0·016	—
	—	0·080	0·016	—
	0·050	0·137	0·017	112
	0·100	0·181	0·016	100
	0·150	0·230	0·016	99
Oak (*Quercus petraea*) leaf litter, 250 mg	—	0·112	0·045	—
	—	0·112	0·045	—
	0·050	0·164	0·046	104
	0·100	0·214	0·046	102
	0·150	0·264	0·046	101
Acid peat, 50 mg	—	0·184	0·368	—
	—	0·184	0·368	—
	0·050	0·235	0·370	102
	0·100	0·283	0·366	99
	0·150	0·335	0·370	101

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The Determination of Nitrate in Soil Solutions by Ultraviolet Spectrophotometry

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Ultraviolet spectrophotometric analysis has been applied to the determination of nitrate nitrogen over the 0·5 to 10 p.p.m. range in soil solutions and perfusates in the absence of organic matter, and over the 5 to 50 p.p.m. range in its presence. If necessary, a 4-fold increase in ultimate sensitivity can easily be obtained. Sulphamic acid can be used for the destruction of nitrite which would otherwise interfere, and a short procedure has been suggested for the reduction of severe interference present in extracts from highly organic samples such as peat. The more important nitrification inhibitors used in perfusion experiments are well tolerated. Nitrate contents of soil solutions were measured by the method and gave results in good agreement with the nitrate reduction and ammonia distillation method.

Various methods have been proposed for the determination of nitrate in soil solutions, extracts and perfusates. They are mainly dependent on either the reduction of nitrate followed by the determination of nitrite[1] and ammonia,[2] or the colorimetric measurement of a nitration product formed with reagents such as brucine,[3] chromotropic acid,[4] 4-methylumbelliferone,[5] phenoldisulphonic acid[6] and 2,4-xylenol.[7] A polarographic method[8] and the interference of nitrate in the determination of rhenium by α-furildioxime[9] have also been suggested.

Some of these techniques have the disadvantage of being fairly tedious, while others show non-linear calibration graphs, poor colour stability, low sensitivity and severe interference from chlorides, nitrites and organic matter. The use of strong sulphuric acid in nitration procedures promotes interference from organic compounds in soils.

A more simple, reliable and rapid technique was required for the analysis of soil solutions and perfusates. According to Bastian, Weberling and Palilla,[10] the absorption spectra of the nitrate ion in 5 per cent. v/v perchloric acid shows a double peak in the 210 and 300-mμ regions, and readings at the shorter wavelength are about a thousand times more sensitive. These authors investigated the interference from many anions and cations, and applied the method to the measurement of nitrate in alkaline earth carbonates that had been dissolved in dilute perchloric acid. Although Hoather and Rackham[11] carried out the direct determination of nitrate in waters at 210 mμ, there appears to be no parallel investigation dealing with the reliability of the method for soils.

The work described here was designed to test the suitability of ultraviolet spectrophotometry for the measurement of nitrate, firstly in soil solutions extracted by a centrifugation method,[12] where the most serious interferences would be expected to come from iron and organic matter, and secondly in soil perfusates where the presence of nitrite or various nitrification inhibitors could cause further errors; in addition, the interference of boron has not been previously studied.

Experimental

A good absorption by nitrate can be obtained by the measurement of soil solutions diluted with water, but dilution of aliquots with 5 per cent. v/v perchloric acid was retained as a final step before ultraviolet spectrophotometry; it was found that the presence of acid reduced nitrite interference (from sodium nitrite) and iron interference (from ammonium iron(III) sulphate) by 73 and 23 per cent., respectively, at 210 mμ, compared with water

alone. The acid also prevented any bacterial action in the samples that could be accumulated for at least 1 week at room temperature without variation in optical density.

INTERFERENCE AND REMOVAL OF NITRITE—

Sulphamic acid has been used for the destruction of nitrite in soil extracts in the microdiffusion analysis of nitrate.[13] The effectiveness of this removal procedure and its influence on nitrate recovery was tested by adding 1 ml of 2 per cent. sulphamic acid to solutions that contained known amounts of nitrate, and 50 μg of nitrite nitrogen. Solutions were shaken and allowed to stand for 2 minutes before making up the volumes to 10 ml with 5 per cent. perchloric acid. Absorbance was measured at 210 mμ in a Unicam SP500 spectrophotometer with 1-cm silica cells. The results were compared with a calibration graph obtained from known amounts of nitrate in perchloric acid, and the recoveries were satisfactory—

	with 50 μg of nitrite nitrogen added				
Nitrate nitrogen taken, μg	2	4	6	8	10
Nitrate nitrogen found, μg	1·96	3·91	5·97	7·98	9·95
Recovery, per cent.	98·0	97·8	99·5	99·8	99·5

The absorption spectra of the same concentration of sulphamic acid in perchloric acid was measured in a Unicam SP700 recording spectrophotometer, and showed a large peak at 192 mμ. Although readings taken at 210 mμ were free from interference because of the sharp decline in the sulphamic acid absorption peak, it would not be advisable to use lower wavelengths.

The interference of nitrite was examined by preparing a fresh sodium nitrite standard in quartz distilled water, and measuring the absorption arising from 25 μg of nitrite nitrogen in 10 ml of 5 per cent. perchloric acid. A correction for traces of nitrate present in the nitrite standard was obtained by deducting the reading of a sulphamic acid blank from a nitrite *plus* sulphamic acid treatment. At a wavelength of 210 mμ, 0·55 μg of nitrite nitrogen gave the same optical density as 0·1 μg of nitrate nitrogen, and it would not be advisable to exceed this amount of nitrite in test samples. With some of the soils used in experimental work, whose properties are shown in Table I, there is no interference because only 0·1 or 0·2-ml aliquots of soil solution are required for nitrate analysis, and these particular soils are not prone to nitrite accumulation.

TABLE I

DESCRIPTION OF ARABLE SOILS USED FOR ANALYSIS

Soil reference	Soil origin and description	pH	Total carbon dry soil, per cent.	Soil moisture at extraction, per cent.	Soil solution, p.p.m. nitrite	Soil solution, p.p.m. iron
1	Black Series, Sonning organic loam ..	7·0	3·5	50	0·06	0·27
2	Broad Series, Sonning organic clay loam	7·7	6·6	62	0·05	0·19
3	Faringdon light loam	6·3	2·2	25	0·05	0·13
4	Grove heavy loam	7·0	3·5	45	0·05	0·25

INTERFERENCE OF ORGANIC MATTER—

One-ml aliquots of soil solutions from the soils described in Table I, and a coloured water extract from moss peat, were decolorised by mixing with 4 ml of alumina cream suspension and spinning in a centrifuge.

Faringdon soil gave the most intensely coloured extract, but colour removal had no effect on the ultraviolet absorption spectra; analysis of nitrate by the distillation method of Piper[14] gave good agreement with ultraviolet spectrophotometry without any decolorising procedure. The peat extract was an exception, and results consistently exceeded the distillation figures by as much as 120 per cent., probably because of organic matter absorption in the lower ultraviolet region. The interfering substances were completely removed by alumina cream treatment, and results from both methods were almost identical; interference could be conveniently estimated by comparing absorption readings with and without alumina cream reagent.

INTERFERENCE OF IRON(III)—

The absorption of iron(III) ion in perchloric acid solution at 240 mμ has been used for quantitative analysis.[15] Interference in nitrate determination was measured at 210 mμ with a solution of ammonium iron(III) sulphate. Aliquots were fumed with 0·5 ml of perchloric acid to expel nitrate, and made up to 10 ml with water; 0·83 μg of iron(III) gave an absorption equivalent to 0·1 μg of nitrate nitrogen.

When 10 μg of iron(III) was subjected to alumina cream treatment, analysis of the supernatant solution showed that 90 per cent. had been removed, and therefore interference from this element in soil solutions is unlikely.

INTERFERENCE OF BORON—

A solution of Specpure boric acid in quartz distilled water showed that 16·7 μg of boron gave the same absorption as 0·1 μg of nitrate nitrogen at 210 mμ. Vinogradov[16] states that the average boron content of soils is 1×10^{-3} per cent. Water-soluble boron comprises about 10 per cent. of this, so that it is unlikely to interfere.

INTERFERENCE OF NITRIFICATION INHIBITORS—

Seven inhibitors were dissolved in water at their effective strengths, and 0·2-ml portions were diluted to 10 ml with 5 per cent. perchloric acid for test. Aliquots from 0·006 M potassium chlorate, 0·001 M ethyl urethane, 0·015 M 2-chloro-6-(trichloromethyl)pyridine and 0·005 M guanidine carbonate did not affect the nitrate absorption spectra. Serious interference was found with aliquots from 0·002 M allylthiourea, 0·005 M DL-methionine and 0·001 M nitrourea.

SULPHAMIC ACID AND ALUMINA CREAM TREATMENT OF SOIL SOLUTIONS—

The effect of sulphamic acid and alumina cream treatments on nitrate recovery was tested with 0·1 ml of soil solution and 15 μg of nitrate nitrogen, and finally, the effect of 250 μg of nitrite nitrogen and 10 μg of iron(III) on the analysis of soil solutions was examined. After spinning the alumina cream in a centrifuge, 1 ml of the supernatant solutions was taken for sulphamic acid treatment, then diluted with 5 per cent. perchloric acid for absorption measurement. The results showed that both nitrate recovery and removal of nitrite and iron(III) were satisfactory.

	Nitrate nitrogen, μg			
Soil	Soil solution alone	Soil solution + 15 μg of nitrate nitrogen	Recovery of nitrate, per cent.	Soil solution + 250 μg of nitrite nitrogen and 10 μg of iron(III)
1	12·55	27·20	97·7	12·00
2	25·45	40·45	100	25·70
3	7·70	22·50	98·7	7·40
4	10·25	24·75	96·7	9·75

METHOD

REAGENTS—

All reagents are of analytical-reagent grade.

Ammonia solution, 15 N.

Perchloric acid, 70 *per cent. w/w.*

Sulphamic acid solution, 2 *per cent. w/v.*

Alumina cream—Prepare by dissolving 30 g of potassium aluminium sulphate in 1 litre of water. Filter, and add the filtrate to a mixture of 225 ml of distilled water and 25 ml of ammonia solution. Free the aluminium hydroxide precipitate from sulphate by decanting with water, and finally dilute a suspension of the precipitate to 1 litre. The preparation has a final pH of about 6·8, and should be well agitated during use.

Nitrate stock solution—Prepare by dissolving 3·6090 g of potassium nitrate in water and diluting to 1 litre.

Nitrate standard solution—Dilute 10 ml of stock solution to 100 ml with water.

1 ml ≡ 50 μg of nitrate nitrogen.

PROCEDURE—

If organic matter interference is present, take a 1-ml portion of the sample, which should contain between 5 and 50 μg of nitrate nitrogen, and portions of nitrate standard solution to cover this range. Mix with 4 ml of alumina cream and spin in a centrifuge. Take 1 ml of the supernatant fraction in a 10-ml calibrated flask, add 1 ml of sulphamic acid solution, and allow the mixture to stand for 2 minutes. Dilute to 10 ml with 5 per cent. v/v perchloric acid, and measure the absorbance at 210 mμ with 1-cm silica cells. A blank correction is required for small amounts of nitrate in reagents.

A second procedure can be used in the absence of organic matter interference, whereby the alumina cream treatment is omitted. In this event, samples should contain 0·5 to 10 μg of nitrate nitrogen.

RESULTS

The method was compared with a nitrate reduction and ammonia distillation technique without decolorisation of soil solutions. Three replicate determinations were made for each method and good agreement was found for the mineral soils, but decolorisation was essential for a moss-peat extract. The results are shown in Table II.

TABLE II

NITRATE CONTENT OF SOIL SOLUTIONS

Soil		Nitrate nitrogen, p.p.m. found by— ultraviolet technique	distillation
1	not decolorised ..	102·5	103·6
2	not decolorised ..	148·5	151·2
3	not decolorised ..	81·4	80·1
4	not decolorised ..	100·0	100·8
Moss peat	not decolorised ..	13·9	6·2
1	decolorised	101·6	—
2	decolorised	148·0	—
3	decolorised	80·5	—
4	decolorised	99·4	—
Moss peat	decolorised	6·3	6·0

DISCUSSION

SENSITIVITY AND ACCURACY—

An optical density of 0·1 can be obtained in 1-cm silica cells at 210 mμ, with 2 μg of nitrate nitrogen diluted to 10 ml with 5 per cent. v/v perchloric acid. The calibration graph is linear over the range specified in the procedure, and a concentration of 0·5 p.p.m. of nitrate nitrogen in the soil solution is a practical lower limit. If necessary, greater sensitivity can be achieved by increasing the optical path of the spectrophotometer cells or by reducing the dilution with perchloric acid.

By using the full procedure one operator analysed 40 samples in 2¼ hours, with a standard deviation of $\pm$0·0480 μg of nitrate nitrogen on a single sample. With the shorter procedure the standard deviation was $\pm$0·0023 μg of nitrate nitrogen.

The soils examined gave no interference from organic or inorganic compounds, unlike the peat extract, and it should be noted that a strongly coloured solution is not a reliable indication of the degree of interference to be expected.

Soil nitrate is frequently extracted by water at neutral pH, or by saturated calcium sulphate, and according to the calcium interference levels quoted by Bastian[10] there would be no problem with the use of 0·2-ml aliquots. Chlorides are also well tolerated, but the use of extractants that increase the solubility of organic matter and iron should be avoided. If nitrite determination is not required on the same extract, it can be more conveniently destroyed by the use of sulphamic acid in the extractant.

CONCLUSIONS

Ultraviolet absorption can be successfully applied to the determination of nitrate in soil solutions and perfusates; the sensitivity of the method to nitrate is sufficiently predominant to make the effect of interfering elements unimportant. It is a simple, rapid technique, which is suitable for the daily control of perfusion experiments in which large numbers of samples can be involved.

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Determination of Terminal Hydroxyl Groups in Polyethyleneoxy Compounds

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The 3,5-dinitrobenzoyl chloride method has been adapted for the semi-micro analysis of hydroxyl groups in polyethylene glycol and non-ionic surfactants of the polyethylene oxide type. The 3,5-dinitrobenzoates are determined colorimetrically after removal of the excess of acid chloride by column chromatography.

By this method 100 μg of hydroxyl can be determined with a relative accuracy of 4·5 per cent. Products containing fewer than ten ethylene oxide units per molecule do not respond adequately.

Results of the analyses of some technical non-ionic detergents and polyethylene glycol are reported.

Several methods for the determination of hydroxyl groups on a macro or micro scale are known. In the macro method acetic anhydride or phthalic anhydride are used as acylating agent, whereas in a semi-micro method, based on the two-phase titration of anionic surfactants,[1] chlorosulphonic acid is used. No accurate micro or semi-micro methods are available for determining polyethylene glycols and their mono-ethers. Johnson and Critchfield's method[2] for determining the hydroxyl group in fatty alcohols on the micro scale has been examined for its applicability to these polyethyleneoxy compounds.

In Johnson and Critchfield's method, dinitrobenzoyl chloride is used to esterify the higher alcohol. The excess of reagent is eliminated by acid hydrolysis and the ester is extracted with hexane. The hexane extract is coloured by a strong base, propylene diamine, and the absorbance of the solution measured at 525 nm. As the dinitrobenzoates of polyethylene glycols and non-ionics do not dissolve in hexane, the solvent was replaced with chloroform. This, however, produced high blanks, which were caused by traces of co-extracted dinitrobenzoyl chloride and dinitrobenzoic anhydride. The interference has been reduced by chromatographic separation of the ester from all other reaction products after hydrolysis.

Separation appeared to be possible by thin-layer chromatography on silica gel G (obtained from Merck), with 0·3-mm layers activated at 120° C for 2 hours. The plates were developed in chloroform, which completely removed the hydrolysis products, leaving a stationary spot of the polyethyleneoxy dinitrobenzoate esters at the start. For column chromatography the silica gel G was replaced by Kieselgel 0·05 to 0·2 mm (obtained from Merck), which was completely de-activated with water. This was necessary to allow the dissolution of the purified ester from the column by dimethylformamide.

Instead of pyridine, dimethylformamide was used as solvent in the acylation. It has the same catalytic properties,[3] can be dried more easily and is almost odourless.

The ester was coloured by anhydrous ethylene diamine, instead of propylene diamine. This did not significantly change the wavelength of maximum absorbance.

Experimental

Reagents—

All reagents are of analytical-reagent quality.

3,5-Dinitrobenzoyl chloride—This is prepared by reacting 3,5-dinitrobenzoic acid with thionyl chloride.[4] The 3,5-dinitrobenzoyl chloride formed is then dissolved in dimethylformamide, and this freshly prepared solution is used for the esterification of the terminal hydroxyl group of the polyethyleneoxy compound.

Hydrochloric acid, 2N.

Chloroform.

Ethylene diamine—Dehydrate commercial ethylene diamine by adding to it an equal weight of sodium hydroxide pellets and heating the mixture for half an hour at 100° C, occasionally swirling it. After cooling it to room temperature, pour off the liquid and distil the solution at atmospheric pressure. The pure ethylene diamine is collected between 117° and 118° C.

Dimethyl formamide.

APPARATUS—

A Beckman DB spectrophotometer was used for the absorbance measurements.

The chromatographic column (200 mm long and 14 mm in diameter) was packed with Kieselgel 0·05 to 0·2 mm, which is prepared as follows.

For each column, 5 g of Kieselgel is dried at 150° C for 2 hours. The material is slurried with distilled water into the column. The column is then flushed with 25 ml of dimethylformamide to remove free water and the dimethylformamide is finally removed by washing with 50 ml of chloroform.

When using the column for the first time, the Kieselgel should be thoroughly purified by passing 5 ml of a 10 per cent. w/v solution of 3,5-dinitrobenzoyl chloride in dimethylformamide through it and then washing it with pure dimethylformamide. The absence of the acid chloride in the effluent is ascertained by a spot test with ethylene diamine. After this preliminary purification, the column is de-activated again with distilled water, then flushed with 25 ml of dimethylformamide and 50 ml of chloroform, as described above.

An amount of sample, equivalent to 100 μg of hydroxyl, is dissolved in 2 ml of dimethylformamide in a 100-ml stoppered conical flask. One millilitre of a freshly prepared solution of 1 g of 3,5-dinitrobenzoyl chloride in 10 ml of dimethylformamide is added to the sample. The reaction mixture is left at room temperature for 1 hour, then 25 ml of 2 N hydrochloric acid is added, followed by 20·00 ml of chloroform (from a pipette). The flask is stoppered and shaken vigorously for half a minute. After de-gassing, shaking is resumed for half a minute. The layers are allowed to separate and 2·00 ml of the chloroform are transferred (by pipette) to the chromatographic column. The column is eluted with 50 ml of chloroform, and the effluent containing the by-products is discarded. The purified ester is dissolved by passing dimethylformamide over the column. The movement of the ester band is observed carefully. The band is collected in a 10-ml calibrated flask. When the effluent fills the flask to the mark, 1 ml of anhydrous ethylene diamine is added, the flask is stoppered and finally shaken. The absorbance of the dimethylformamide solution is measured at 528 nm, against dimethylformamide containing 10 per cent. of ethylene diamine as reference. Correction for impure reagents is made by running a blank.

RESULTS AND DISCUSSION

Table I gives the results of hydroxyl determinations in some polyethylene glycols and non-ionics. The hydroxyl figures in the column "Hydroxyl added" are based on the

TABLE I

DETERMINATION OF HYDROXYL GROUPS IN POLYETHYLENEOXY COMPOUNDS*

	Hydroxyl added, μg	Hydroxyl found, μg	Difference, μg	Difference, per cent.
PEG-40EO	68·0	70·3 (±3·2)†	2·3	3·4
PEG-11EO	95·0	92·12 (±4·2)	−2·8	−3·0
PEG-5EO	96·0	30·0 (±1·4)	−66·0	−69·0
NP-32EO	143·0	146·5 (±6·6)	3·5	2·5
NP-28EO	105·0	103·0 (±4·7)	−2·0	−1·9
NP-14EO	90·0	58·0 (±2·6)	−32·0	−36·0
NP-8·5EO	170·0	54·0 (±2·5)	−116·0	−68·0
TA-35EO	141·0	142·0 (±6·4)	1·0	0·7
TA-34EO‡	160·0	158·0 (±7·1)	−2·0	−1·3
TA-8·6EO	110·0	48·0 (±2·2)	−62·0	−57·0

* PEG Polyethylene glycol.
NP Nonylphenol.
TA Tallow alcohol.
† Experimental (2s) error.
‡ PEG-free.

conventional macro method in which acetic anhydride - pyridine solution is used. The spectrometric results are obtained by using one calibration line determined with nonylphenol-28EO. The 2*s* error of the results calculated from 20 determinations of known samples is 4·5 per cent. relative. The absolute blank absorbances range from 0·060 to 0·080 (92 observations over a period of a few months).

As Table I indicates, the method does not give satisfactory results for polyethylene glycol-5EO. PEG–11EO and higher appear to be determined satisfactorily. With polyethylene glycols, the results depend on the size of the reacting molecules. These products are mixtures of adducts of different molecular weight. PEG-5EO is a mixture for which an average of 5 ethylene oxide units per molecule are found. It may be composed, however, of compounds ranging in ethylene oxide number from 1 to about 15. A negative response of the lower members of the range means a partial loss of hydroxyl groups and causes a low result.

As for non-ionics, it must be noted that the technical products invariably contain polyethylene glycol as contamination. The low results found for NP-14EO and NP-8·5EO may, therefore, be caused by an inadequate response of their low molecular polyethylene glycol fractions or by the lower nonylphenol ethoxylates themselves.

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Precipitation from Homogeneous Solution by Cation Release at Constant pH

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Precipitation from homogeneous solution by cation release at constant pH from an iron - EDTA complex has been described by MacNevin and Dunton for the precipitation of hydrated iron oxide.[1] They concluded that the method should be applicable to the precipitation of a large number of metal ions. The technique has subsequently been studied in greater detail,[2,3] and it is now possible to form a clearer picture of the scope of the method. Some of the more important aspects are briefly discussed below.

Choice of oxidising agent—MacNevin and Dunton considered several oxidising agents before deciding that hydrogen peroxide was to be preferred. Oxidising agents, such as ammonium persulphate and sodium hypochlorite, were found to be too vigorous, while the use of sodium bromate was restricted to acid solution. The complex formed by iron with EDTA is, however, extremely easy to break up by oxidation, despite its relatively high stability constant ($K_{abs.} = 25$). Other metals, and in particular bismuth, form complexes that are extremely resistant to attack over certain ranges of pH. Hydrogen peroxide has the advantage that the products of its breakdown are not themselves precipitating anions, but it suffers in that it may be catalytically destroyed by traces of ions in solution, or by first-formed precipitate particles, and its use is frequently restricted to solutions containing stabilising anions, notably the phosphate ion; this imposes a limitation on the method.

Selectivity in separation—Hydrogen peroxide is not selective in its attack upon metal - EDTA complexes. All cations present in complexed form are released into solution as their complexes are destroyed. The method is therefore in itself non-selective, and only serves to release all cations at a slow rate into an initially homogeneous solution. A certain degree of selectivity can be achieved by choosing a pH range at which only one cation forms a precipitate, or by including an anion that will precipitate only one cation. There are few occasions, however, on which these techniques can be applied successfully.

Masking efficiency—It is essential in carrying out precipitation from homogeneous solution that precipitation should not occur immediately on addition of the precipitating anion to a solution of the metal - EDTA complex. Immediate precipitation will occur with all cations over pH ranges in which the metal - EDTA complexes have low stability. This can occur in both acidic and alkaline conditions according to the nature of the cation, and imposes a limitation on the choice of pH at which precipitation can be carried out from homogeneous solution. It is possible that other complexing agents that form stronger complexes than EDTA may offer some assistance, but little use appears to have been made of these agents.

Nature of the precipitate—The method frequently results in the formation of denser precipitates that are more readily filtered than those obtained by the direct addition of reagents. Errors caused by absorption and occlusion are also reduced. The method is not always successful, however, and gives gelatinous precipitates, for example, with thorium and zirconium phosphates. Gordon, Salutsky and Willard have similarly reported voluminous precipitates with hydrated thorium oxide.[4]

Conclusions

The technique of precipitation from homogeneous solution by cation release from metal - EDTA complexes, by oxidation with hydrogen peroxide, is capable of giving good results in certain cases. There are severe limitations to its general application to the precipitation of a wide range of cations, and the selectivity of the method is poor.

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Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Tin in Organic Matter

Part I: Amounts of Tin up to 30 μg

THE Analytical Methods Committee has received the following Report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. W. C. Johnson (Chairman), Dr. J. C. Gage, Dr. T. T. Gorsuch (resigned November, 1966), Dr. R. A. Hoodless, Miss E. M. Johnson, Dr. H. Liebmann, Dr. R. F. Milton, Dr. E. J. Newman and Mr. G. B. Thackray, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

Tin may be present in organic materials, such as foodstuffs, in concentrations ranging from less than one to several hundred parts per million. The Sub-Committee has considered several colorimetric, titrimetric and polarographic methods for the determination of small amounts of tin, and has concluded that no single method could conveniently be adapted to the determination of such a wide range of tin concentrations in organic matter. The Sub-Committee's investigation has therefore been divided into the examination of methods for determining tin in two separate ranges of concentration. For amounts of tin up to 30 μg a colorimetric method based on the reaction of tin(IV) with catechol violet is recommended; the other method under consideration is the colorimetric method involving the use of the zinc complex of toluene-3,4-dithiol for amounts in the range 30 to 150 μg. For amounts above 150 μg a method involving titration of tin(II) with iodine would undoubtedly prove suitable.

METHOD A: FOR AMOUNTS OF TIN NOT GREATER THAN 30 μg

The method recommended is that of Newman and Jones,[1] which is based on, but contains modifications of, the procedures of Tanaka[2] and Tanaka and Yamayoshi.[3] This method was chosen because it contains a solvent-extraction stage designed for the selective extraction of tin from sulphuric acid solutions such as would be produced from wet oxidations of organic matter.

EXPERIMENTAL—

In view of the work that had been carried out on behalf of the Analytical Methods Committee by Newman and Jones,[1] the Sub-Committee decided that the usefulness or otherwise of the method could be demonstrated adequately by having its members carry out a simple collaborative test.

A sample of orange squash was divided between the collaborating laboratories. Each laboratory determined the tin content of the sample by the recommended method after wet oxidation with nitric and sulphuric acids, or with 50 per cent. hydrogen peroxide and sulphuric acid. Recovery tests were also conducted in which amounts of tin equivalent to 1·0, 2·0 and 10·0 p.p.m. were added to the sample before wet oxidation. The tin was added

as portions of the dilute standard tin solution (tin(IV) sulphate) prepared as described under "Method" in the Appendix to this Report. In one laboratory, tin was also added as tin(IV) chloride to find out whether volatilisation losses of tin(IV) chloride could occur during the early stages of the digestion.

The results obtained are shown in Table I. The figures for the tin content of the sample are in good agreement and the recoveries of added tin were all satisfactory.

It was found by two of the collaborating laboratories during preliminary experiments on the orange squash that reproducible results were obtained only when completely colourless digestates were produced.

TABLE I

DETERMINATION OF TIN IN ORANGE SQUASH

Laboratory	Tin added, p.p.m.	Tin found, p.p.m.	Tin recovered, p.p.m.
A	0	2·0	—
	1·0	3·0	1·0
	5·0	6·7	4·7
	10·0	10·9	8·9
	0	2·2	—
	1·0	3·2	1·0
	5·0	6·8	4·6
	10·0	11·9	9·7
B	0	2·1	—
	0	2·4	—
	1·0	3·3	1·1
	1·0	3·4	1·2
	5·0	7·8	5·6
	5·0	7·8	5·6
	10·0	12·3	10·1
	10·0	12·2	10·0
C	0	2·3	—
	0	2·2	—
	0	2·1	—
	1·0*	3·3	1·1
	1·0*	3·1	0·9
	1·0†	3·3	1·1
	5·0*	7·0	4·8
	5·0*	7·2	5·0
	5·0†	7·3	5·1
	10·0*	12·5	10·3
	10·0*	11·8	9·6
	10·0†	12·1	9·9
D‡	0	2·7	—
	0	2·7	—
	1·0	3·6	0·9
	1·0	3·6	0·9
	5·0	8·1	5·4
	5·0	7·7	5·0
	10·0	13·6	10·9
	10·0	13·1	10·4
	0	2·5	—
	0	2·3	—
	1·0	3·3	0·9
	1·0	3·5	1·1
	5·0	7·0	4·6
	5·0	7·3	4·9
	10·0	11·8	9·4
	10·0	12·6	10·2

* Tin added as the chloride.

† Tin added as the sulphate.

‡ The two sets of results quoted were obtained with different supplies of catechol violet. A separate calibration graph was prepared for each.

Appendix

Recommended method for the determination in organic matter of amounts of tin not greater than 30 μg

Principle of method—

After destruction of the organic matter by wet oxidation with nitric and sulphuric acids,[4] nitric, perchloric and sulphuric acids[4] or 50 per cent. hydrogen peroxide in the presence of sulphuric acid,[5] the residual sulphuric acid is diluted to four times its volume with water to give an approximately 9 N concentration of the acid.

Tin is selectively separated from this solution by treating it with potassium iodide and extracting tin(IV) iodide into toluene. Tin(IV) is then returned to aqueous solution by shaking the toluene extract with a solution of sodium hydroxide. After acidification, and removal of free iodine from the solution, the tin(IV) is determined spectrophotometrically as its coloured complex with catechol violet, the solution being buffered to pH 3·8 with acetate.

Range—

For tin contents in the range 1 to 30 μg in the sample taken.

Applicability—

The colour reaction between tin(IV) and catechol violet is far from selective. However, Newman and Jones[1] have shown that the solvent-extraction step is highly selective or even specific. For the present application, therefore, the recommended method can be regarded as specific.

Reagents—

All reagents should be of analytical grade.

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

Sulphuric acid, approximately 9 N—Cautiously mix 250 ml of sulphuric acid, sp.gr. 1·84, with 500 ml of water, cool to room temperature, and dilute to 1 litre with water.

Potassium iodide, approximately 5 M—Dissolve 83 g of potassium iodide in water to produce 100 ml. Prepare freshly each day.

Toluene (*low in benzene*).

Sodium hydroxide, approximately 5 N *and approximately* 0·1 N.

Hydrochloric acid, approximately 5 N.

Ascorbic acid solution—A freshly prepared 5 per cent. w/v aqueous solution.

Catechol violet solution—A 0·05 per cent. w/v aqueous solution. Prepare freshly each week.

Sodium acetate trihydrate solution—A 20 per cent. w/v aqueous solution.

Ammonia solution, approximately 5 N.

Tin(IV) stock solution—Dissolve 0·1000 g of pure granulated tin in 20 ml of sulphuric acid, sp.gr. 1·84, by heating until fumes appear. Cool, cautiously dilute with 150 ml of water, and cool again. Add 65 ml of sulphuric acid, sp.gr. 1·84, cool, and transfer to a 500-ml calibrated flask. Dilute to the mark with water.

1 ml of solution ≡ 200 μg of tin.

Tin(IV) dilute standard solution—Dilute 5·0 ml of tin(IV) stock solution to 100 ml with water in a calibrated flask. Prepare freshly each day.

1 ml of solution ≡ 10 μg of tin.

Preparation of calibration graph—

Transfer by pipette, or small-capacity burette, suitable volumes of dilute standard tin solution, to cover the range 0 to 30 μg of tin, to a series of 50-ml beakers and treat each as follows: dilute to 7 ml with water, add 1 ml of 5 N sodium hydroxide, and mix. Add 2·5 ml of 5 N hydrochloric acid, mix, add 2·0 ml of catechol violet solution, mix again, and add 5 ml of sodium acetate solution (see Note 1). Adjust the pH of the solution with 5 N ammonia solution to 3·8 ± 0·1 units, with the aid of a pH meter. Transfer to a 25-ml calibrated flask, dilute to the mark with water, mix thoroughly, and set aside for 30 minutes. Measure

the optical density of the solution in a 1-cm cell at a wavelength of 552 mμ, with the solution containing no added tin in the reference cell. Construct a graph relating the amount of tin to the optical density (see Note 2). The graph should be rectilinear and pass through the origin.

PROCEDURE—

Dilute the sulphuric acid solution containing not more than 30 μg of tin to approximately 9 N, cool, and transfer it to a separating funnel. For each 25 ml of solution add 2·5 ml of 5 M potassium iodide, mix, and add 10 ml of toluene. Insert the stopper, shake the funnel vigorously for 2 minutes, allow the layers to separate, and discard the aqueous phase. Wash the toluene layer, without shaking it, with 5 ml of a solution prepared by mixing 25 ml of 9 N sulphuric acid and 2·5 ml of 5 M potassium iodide, and discard the washings. The toluene layer will be coloured pink with extracted iodine.

Add 5 ml of water to the toluene extract and then 5 N sodium hydroxide dropwise, with shaking, until the toluene layer is colourless. Add 2 drops of 5 N sodium hydroxide in excess (usually a total of 8 to 10 drops is required). Insert the stopper, shake the funnel for 30 seconds, allow the phases to separate, and transfer the aqueous layer into a 50-ml beaker. Shake the toluene layer with 3 ml of 0·1 N sodium hydroxide for 30 seconds, allow the layers to separate, and add the aqueous layer to the contents of the 50-ml beaker. Retain the organic (toluene) phase.

Acidify the aqueous solution in the beaker with 2·5 ml of 5 N hydrochloric acid, and decolorise the liberated iodine by the dropwise addition of ascorbic acid solution. Add 2·0 ml of catechol violet solution, and mix. Wash the toluene retained from above, without shaking, with 5 ml of sodium acetate solution. Add the washings to the contents of the beaker, mix, and adjust the pH of the solution to 3·8 ± 0·1 units with 5 N ammonia solution by means of a pH meter. Transfer the solution to a 25-ml calibrated flask, and complete the determination of tin as described above under "Preparation of Calibration Graph." Calculate the amount of tin present by reference to the calibration graph.

NOTES—

1. The order of addition of reagents is important, and the stated order should be strictly adhered to.

2. When a new bottle or batch of catechol violet is used a fresh calibration graph should be prepared.

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Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

The Determination of Small Amounts of Zinc in Organic Matter

THE Analytical Methods Committee has received the following Report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee of the Analytical Methods Committee recommended, in 1963, a titrimetric dithizone procedure that was generally applicable to the determination in fertilisers and feeding stuffs of up to 100-μg amounts of zinc in the final solution.[1] An alternative spectrophotometric procedure suitable for the determination of concentrations of less than 10 p.p.m. of zinc was also given.[1]

As part of its work in investigating methods for determining the more important metals that can appear as impurities in organic matter, this Sub-Committee had also to consider the determination of small amounts of zinc. The method recommended by the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee was therefore examined to ascertain whether or not it could be recommended as being directly applicable to the determination of zinc in organic matter generally. This investigation indicated that, with a few comparatively minor modifications, this method could be recomended by this Sub-Committee, and members were of the opinion that these modifications could also, with advantage, be incorporated in the method as published in 1963.

The modifications proposed, with reasons for their adoption, are—

Method A (*titrimetric procedure*)—

1. The pH of the ammonium citrate solution is sufficiently high to allow a little of the dithizone to be transferred to the aqueous phase. It is therefore proposed that after the ammonium citrate solution has been extracted with dithizone, the aqueous phase should be washed with carbon tetrachloride, 2 ml being sufficient to remove any dithizone present.

2. If a little of the ammonium citrate phase is inadvertently transferred to funnel (B) with the dithizone extracts, then the 5 ml of 0·02 N hydrochloric acid may be insufficient to neutralise it and produce a distinctly acid phase, with the consequent danger that all the zinc may not be reverted to the aqueous phase. Care must therefore be taken to ensure that the aqueous solution is distinctly acid when the dithizone extracts are being washed.

Method B (*spectrophotometric procedure*)—

1. The dithizone extract is obtained from an aqueous solution of pH 4·0 to 4·5, and if any of this aqueous solution has been entrained with the dithizone, treatment with the sodium sulphide solution without first washing the dithizone extract with water will cause decomposition of the sulphide. It is therefore proposed that the dithizone extract should be washed with two 15-ml portions of water before being washed with the sodium sulphide solution.

Recommendation

The Sub-Committee recommends that, after destruction of organic matter by some suitable method,[2,3] the titrimetric dithizone procedure for up to 100 μg of zinc in the final solution and the alternative spectrophotometric finish for up to 10 μg of zinc in the final solution previously recommended by the Trace Elements in Fertilisers and Feeding Stuffs Sub-Committee[1] are, with the modifications given below, applicable to the determination of zinc in organic matter generally. It is also recommended that these modifications should be incorporated in the method as previously published.[1]

Modifications—

Page 35, *line* 15—After the sentence ending ". . . carbon tetrachloride layer" insert, as a new sentence, "Shake the solution with 2 ml of carbon tetrachloride, and discard the carbon tetrachloride layer."

Page 35, *line* 22—After the words "0·02 N hydrochloric acid" insert "(ensure that the aqueous layer is acid)."

Page 36, *line* 5—To read "Wash the combined dithizone extracts run off during the titration with two 15-ml portions of distilled water; shake the washed solution for 10 seconds. . . ."

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Analyst, May, 1967, Vol. 92, *pp.* 326–327

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factor for Tongue

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

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this Report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. J. Cooper (appointed August, 1965), Mr. P. O. Dennis, Mr. J. R. Fraser (resigned June, 1965), Mr. H. C. Hornsey, Dr. A. J. Kidney, Mr. T. McLachlan, Dr. R. A. Lawrie, Dr. A. McM. Taylor and Mr. E. F. Williams, with Mr. P. W. Shallis as Secretary.

In continuing its investigations on the nitrogen content of various types of meat,[1 to 7] the Sub-Committee has now collected results on the composition of tongue, which are summarised in Fig. 1. The figures quoted were all obtained from uncured tongues, and it is worth noting that there is no significant difference between the figures found for the roots and the blades of tongues, or between ox and pig tongues. The over-all weighted mean of all determinations was 2·99 per cent.

RECOMMENDATION

The Sub-Committee recommends an average nitrogen factor of 3·0 for use in the analysis of comminuted tongue products.

ACKNOWLEDGMENT

The Sub-Committee thanks those listed below for their assistance—

British Food Manufacturing Industries Research Association.
Brown & Knight Ltd.
Harvey's Belgravia Foods Ltd.
J. Sainsbury Ltd.

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		Laboratory	No. of samples	Range	Mean
OX TONGUE	Fresh	A*	1	—	2·72
	Fresh	B	6	2·25 – 3·59	3·15
	Frozen	B	13	2·24 – 3·17	2·90
	Blade	C	6	2·88 – 3·18	3·00
	Root	C	6	2·85 – 3·13	2·98
	Tip	D	1	—	3·00
	Blade	D	1	—	2·93
	Root	D	1	—	2·99
PIG TONGUE	Fresh	E	3	2·92 – 3·29	3·10

* *N.Z. J. Sci. Technol.*, 1926, **8**, 163

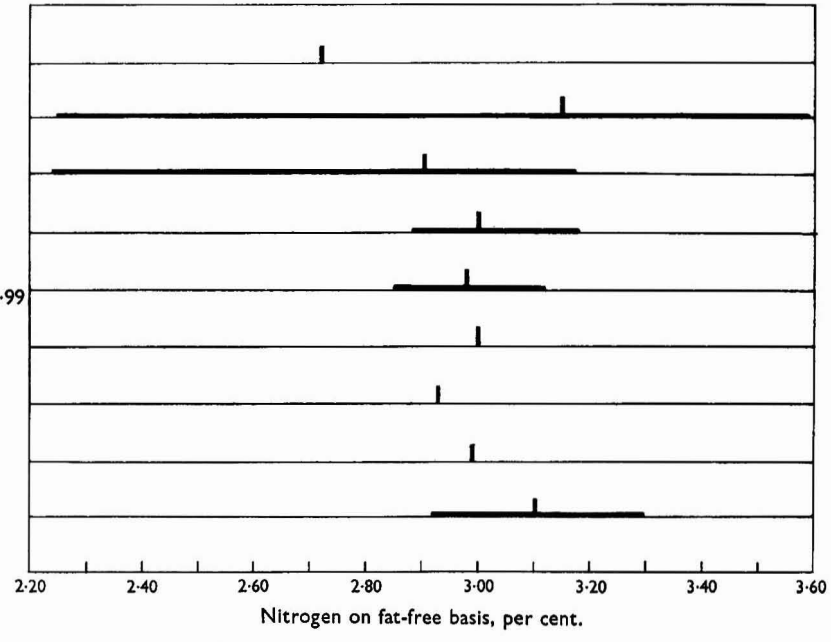

Fig. 1. Nitrogen on fat-free contents of various tongues. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values

Analyst, May, 1967, Vol. 92, *pp.* 328–342

Recommended Methods for the Evaluation of Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON RECOMMENDED METHODS FOR THE EVALUATION OF DRUGS

Evaluation of Thyroid

EARLY in 1962, the Joint Committee of the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry investigating methods for the evaluation of drugs appointed a working Panel to examine methods of assay of thyroid. The constitution of the Panel was: Mr. C. A. Johnson (Chairman), Mr. R. E. A. Drey, Miss S. J. Patterson, Mr. N. A. Terry and Mr. C. Vickers, with Mr. P. W. Shallis as Secretary and Miss B. Gartside as Assistant Secretary. Mr. R. L. Clements joined the Panel in 1963 as an additional member. During the course of the Panel's work, samples of thyroid that had been biologically tested by Mr. K. L. Smith were made available for the Panel's work and Mr. Smith attended later meetings.

INTRODUCTION

The Panel's original terms of reference were—

> "To investigate the possibility of devising a chemical method for determining the principal pharmacologically-active constituents of thyroid."

As work proceeded this objective was modified, and the Panel has confined itself to attempting to provide a method that would serve as a sorting test to indicate whether or not a sample could be expected to possess acceptable biological activity. It was felt that this could best be done by developing a procedure that could be applied without elaborate precautions by routine control laboratories, and that would allow conclusions to be drawn that would parallel those obtained by a biological evaluation.

The activity of thyroid is considered to be a summation of that of its contents of L-3,5,3′-tri-iodothyronine (referred to throughout this report as tri-iodothyronine or T_3) and of the less active L-3,5,3′,5′-tetra-iodothyronine (referred to as thyroxine or T_4); samples containing the same amount of total thyronine iodine as determined by chemical assay may have widely different biological activities, depending on the ratio in which these two active constituents are present.

These differences in activity from sample to sample are reflected in the anti-thiouracil goitre test in rats, when comparison is made between the thyroid under test and a thyroxine standard. It has been shown that, by making an assessment of tri-iodothyronine only, a sample may be evaluated and graded in a way that parallels biological assessments made by the anti-thiouracil goitre test. This is because the anti-thiouracil goitre test is about twenty-five times as responsive to tri-iodothyronine as it is to thyroxine, so that small differences in the proportion of the former constituent readily outweigh large differences in proportion of the latter. With the mouse-anoxia method of test, however, the difference in response between tri-iodothyronine and thyroxine is much less, so that comparison in this case would require assessment of both active constituents. The thin-layer chromatographic procedure described in this Report is readily adaptable to the evaluation of tri-iodothyronine only, or of both tri-iodothyronine and thyroxine.

During the course of the work a number of papers appeared that seemed to have achieved the Panel's objective, notably those by Devlin and Stephenson,[52]* Williams, Meister and Florsheim,[123] Backer and van de Langerijt[112] and Lemieux and Talmage.[113] For various reasons, detailed in the body of this Report, these methods were not considered acceptable for occasional use by routine control departments of pharmaceutical organisations although, without doubt, they are valuable methods for use in the context in which they were developed.

* Reference numbers in the text of this Report refer to the classified bibliography in Appendix III, p. 338.

Experimental

The work of the Panel was initiated after it had been reported that some batches of thyroid tablets, although meeting the requirements of the British Pharmacopoeia 1958, had a negligible thyroid activity. At an early stage of the work it was agreed that a promising approach might be to hydrolyse the thyroid, extract the iodoamino-acids, separate them by paper chromatography and then determine separately the thyroxine and the tri-iodothyronine. It was considered that the very sensitive cerium(IV) - arsenite method for determining small amounts of iodine might be suitable as an end determination in this series of steps, and the determination of iodoamino-acids by this method was first examined by the Panel. After much work, including consultation with biochemists who made regular use of the method, it was concluded that it could not be recommended for use by workers who would require to carry out determinations occasionally, as it is susceptible to minor variations in experimental conditions and to interference from small amounts of impurities. This conclusion in no way reflects on the value of this extremely sensitive method of determining iodide in specialised laboratories using it regularly.

The Panel next examined a somewhat less sensitive but more robust method for determining iodoamino-acids based on flask combustion, formation of a tri-iodide complex and spectrophotometric measurement at 352 mμ. This gave all members recoveries of between 94 and 111 per cent. in the range 10 to 40 μg of iodide, and could be used successfully by analysts applying the method for the first time. Recoveries of thyroxine after paper chromatography and oxygen-flask combustion were between 82 and 96 per cent., but application to thyroid hydrolysates was disappointing in that, when sufficient hydrolysate was loaded to permit estimation of the tri-iodothyronine, alignment of spots was poor. It was felt that this method might be of interest and value to those requiring to estimate small amounts of organic iodine-containing substances, and the procedure is therefore detailed in Appendix II to this Report.

In 1962, Devlin and Stephenson[52] published a method that was of considerable significance, as it contained a detailed study of an enzymic hydrolysis of thyroid that was subsequently adopted by the Panel. In 1964, two members of the Panel, Patterson and Clements,[84] published a thin-layer chromatographic method for the determination of thyroxine in a feeding stuffs additive and they later adapted the principles they had described to the estimation of tri-iodothyronine in thyroid.[95] At a later stage modifications were suggested by Vickers, another Panel member, that enabled both tri-iodothyrone and thyroxine to be assessed. Thus the method that forms the basis of the Panel's recommendation was developed from this work and comprises enzymic hydrolysis of the thyroid and extraction into butanol, after Devlin and Stephenson, with subsequent thin-layer chromatography and assessments of the tri-iodothyronine and thyroxine contents by comparing the sizes and intensities of the spots obtained with those from similarly chromatographed standards.

Each stage of the recommended method (given in Appendix I to this Report) has been closely studied and the method incorporates such detail as has been thought to be essential. Ten samples of commercial thyroid have been examined, and estimates obtained for tri-iodothyronine and for thyroxine are given in Table I, which also includes biological assessments of potency as determined by the anti-thiouracil goitre method and expressed as equivalents of thyroxine sodium iodine. It is considered that the inter-laboratory agreement obtained is acceptable for a method of this type. When it is considered that the response of the anti-thiouracil goitre test is approximately twenty-five times greater to tri-iodothyronine than to thyroxine, it will be seen that the chromatographic assessment gives a grading of samples that parallels that given by the biological method. It has not been possible to obtain direct numerical equivalence of thyronine iodine content between the two methods, but this is hardly surprising in view of the totally different principles involved and the wide limits of error that necessarily apply to both procedures. When tri-iodothyronine was examined by the complete procedure, recoveries in the various collaborating laboratories ranged from 74 to 90 per cent. The possible effect of lactose, commonly used as a diluent of thyroid powder, on the course of the determination has been assessed and found to be negligible, and the results are incorporated in Table I. It has been confirmed that pig, sheep and ox thyroid give similar chromatographic patterns although, as is to be expected, the content of active constituents varies, pig thyroid being the most potent and ox thyroid the least. It has also been established that, should the need arise to examine weak or diluted

TABLE I

ASSESSMENT OF SAMPLES OF THYROID BY THE THIN-LAYER CHROMATOGRAPHIC PROCEDURES

Sample No.	Laboratory	10-cm run T_3, per cent.	15-cm run T_3, per cent.	15-cm run T_4, per cent.	Thyroxine iodine by bio-assay, per cent. (limits of error P = 0·95)
1	A	0·018	0·009	0·013	0·45 (0·37 to 0·54)
	B	0·013	—	—	
	C	0·013	0·013	0·013	
			0·013	0·013	
			0·013	0·015	
			0·012*	0·013*	
			0·010†	0·013†	
			0·013	0·030	
	D	0·013	0·025	0·019	
			0·006	0·013	
	E	0·018	0·012	0·013	
2	A	0·01	0·012	0·027	0·40 (0·30 to 0·53)
	B	0·019	—	—	
	C	<0·023	0·012	0·030	
			0·012*	0·033*	
	D	<0·025	0·019	0·038	
			0·006	0·013	
	E	0·019	0·012	0·031	
3	A	0·019	0·010	0·037	0·49 (0·40 to 0·60)
	C	0·02‡; 0·02	0·020	0·035	
			0·019	0·038	
	D	0·02	0·031	0·025	
	E	0·019	0·012	0·035	
4	A	0·025	—	—	0·56 (0·46 to 0·68)
	C	—	0·013	0·044	
	E	—	0·015	0·045	
5	A	0·013	0·013	0·032	0·58 (0·45 to 0·74)
	C	0·025	0·013	0·050	
			0·010†	0·035†	
	D	0·02	0·025	0·025	
			0·013	0·031	
	E	0·019	0·015	0·031	
6	A	0·019; 0·025	—	—	0·81 (0·65 to 1·0)
	B	0·026; 0·024	—	—	
	C	0·035; 0·025	0·019	0·032	
			0·019	0·038	
	D	0·02; 0·02	0·031	0·025	
			0·019	0·031	
	E	0·025; 0·021	0·019	0·035	
7	A	0·02	—	—	0·87 (0·70 to 1·08)
	B	0·031	—	—	
	C	0·045	0·019	0·032	
			0·020	0·030	
	D	0·02	0·031	0·031	
			0·025	0·031	
	E	0·019; 0·019	0·019	0·031	
			0·019		
8	A	0·05	—	—	1·27 (1·01 to 1·60)
	B	0·044	—	—	
	C	0·068	0·038	0·050	
	D	0·05	0·044	0·038	
			0·038	0·050	
	E	0·038; 0·038	0·031	0·038	
			0·031	0·043	
			0·031		

TABLE I—*continued*

Sample No.	Laboratory	10-cm run T_3, per cent.	15-cm run T_3, per cent.	15-cm run T_4, per cent.	Thyroxine iodine by bio-assay, per cent. (limits of error P = 0·95)
9	A	0·063; 0·063	—	—	
	C	0·050‡; 0·060‡	0·056	0·050	
		0·056; 0·056	0·050	0·050	1·7
	D	0·05‡; 0·05‡	0·05	0·044	(1·4 to 2·11)
	E	0·050‡	0·050	0·050	
		0·050; 0·056			
9 *plus* equal amount of lactose	A	0·063	—	—	
	C	0·060; 0·060§	—	—	
	D	0·045	—	—	
	E	0·050; 0·056§	—	—	
10	A	0·07	—	—	
	B	0·067	—	—	
	C	0·09	0·056	0·063	
			0·056	0·050	1·78
	D	>0·075	0·05	0·05	(1·24 to 2·54)
	E	0·050; 0·050	0·056	0·031	
			0·063	0·043	
			0·050		
Tablet A	A	—	0·019	0·014	
	B	0·017; 0·016	—	—	Manufactured
	C	0·025	0·025	0·019	from
	D	0·03	0·038	0·019	satisfactory
			0·038	0·038	thyroid
	E	0·025	0·019	0·019	
Tablet B	A	—	0·009	0·021	
	B	0·006	—	—	
	C	0·006	0·006	0·019	0·229
	D	0·013	0·009§	0·013§	
			0·009	0·013	
			0·013	0·019	
	E	0·006	0·006	0·013	
Tablet C	A	—	0	0·007	
	B	0·004	—	—	
	C	Negligible	0	0·012	<0·036
	D	0·006	0·006	0·013	
			0·006§	0·013§	
	E	Negligible	0	0·006	
Tablet D	A	—	0·020	0·036	
	B	0·013	—	—	Manufactured
	C	0·013	0·025	0·038	from
	D	0·013	0·025	0·031	satisfactory
			0·025	0·038	thyroid
	E	0·019	0·019	0·038	
Tablet E	A	—	0·013	0·035	
	C	—	0·013	0·040	Manufactured
	D	—	0·019	0·025	from
			0·013	0·044	satisfactory
	E	—	0·015	0·040	thyroid

* 18 μl of extract spotted.
† 0·5 g of sample hydrolysed.
‡ 3 μl of extract spotted.
§ 12 μl of extract spotted.

thyroid powders, it is possible to increase the amount of material hydrolysed (the recommended method stipulates 0·125 g but amounts up to 0·5 g have been digested successfully) or to increase the volume of extracted hydrolysate applied to the plate (up to a 3-fold increase over the recommended volume has been found practicable). A sample of iodinated casein,

when examined by the recommended method, gave a recognisably different chromatographic pattern and appeared to contain a high proportion of thyroxine but no tri-iodothyronine.

For thyroid tablets no pre-treatment or extraction is necessary. Five samples of thyroid tablets were examined; three of these samples had been prepared from thyroid that had proved acceptable when assayed by the anti-thiouracil goitre method; the other two came from batches of tablets that had been rejected as defective. Although they did not know which samples were unacceptable at the time the assessments were made, all members of the Panel rejected the defective samples and accepted the satisfactory ones.

Other approaches to the problem of evaluation of thyroid have been considered by the Panel and, in particular, those that have appeared in the literature during recent years. In 1963, Williams, Meister and Florsheim published a procedure for the chemical identification of defective thyroid preparations. Their method was based on alkaline hydrolysis of the thyroid, extraction with butanol of the iodoamino-acids released, digestion of the butanol extract with perchloric acid and sodium chromate and subsequent measurement of the liberated iodine by the cerium(IV) - arsenite procedure as a basis for determination of the "thyronine" iodine content of the sample. The content of this "thyronine" iodine, calculated as a percentage of the total iodine content of the sample, was said to provide a guide as to the clinical effectiveness of the sample. This procedure was tested, but with the modification that, for the reasons already given, instead of the cerium(IV) - arsenite method for determining the iodine, the tri-iodide complex was formed and measured spectrophotometrically at 352 mμ. Two samples of thyroid were examined, one of which, on biological assay by the anti-thiouracil goitre method in rats, gave a result equivalent to 0·56 per cent. of thyroxine iodine (limits of error, P = 0·95, 0·43 to 0·73) and the other a result equivalent to 1·7 per cent. (limits of error P = 0·95, 1·4 to 2·11). The modified Williams, Meister and Florsheim method, in the hands of all members of the Panel, showed only a marginal difference between the two samples and the approach was therefore abandoned.

Again because of its dependence on the cerium(IV) - arsenite determination of iodine, the method of Backer and van de Langerijt[112] did not commend itself as suitable for use as a routine control method. The more recently published procedure of Lemieux and Talmage,[113] however, appeared at first sight to have a number of points in its favour. It was specifically designed for routine quality control of thyroid products and is based on hydrolysis with barium hydroxide, extraction with butanol, paper chromatography, oxygen-flask combustion of the iodoamino-acid regions of the chromatograms and determination of the liberated iodine by measurement of its absorbance in benzene at 295 mμ. When this method was considered by the Panel, however, a number of points of doubt emerged. The authors rejected enzymic hydrolysis for routine work because of the time required; this is, however, waiting time during incubation and does not add to the manipulative work required, whereas any method of chemical hydrolysis certainly does. Moreover, the work of Backer and van de Langerijt indicated that recovery of thyroxine was lower with barium hydroxide hydrolysis than with enzymatic. Considerable difficulty was experienced by Panel members in applying the necessary volume of sample solution to Whatman No. 1 chromatographic-paper and, in the final absorption measurement, the necessity of using 10-cm silica cells would be very inconvenient for routine work. Formation of the tri-iodide complex, as detailed in Appendix II of this report, would probably result in a considerable improvement as the oxidation stage yields a 6-fold increase in sensitivity. When applied to tablets the method requires a preliminary washing to remove other material and, as much thyroid available in this country is diluted with lactose, a similar pre-treatment would probably be necessary at all times.

Fig 1. Thin-layer chromatogram (10-cm run) showing tri-iodothyronine spots obtained from two samples of thyroid and four standards at different levels: A, blank *plus* T_3 standard (0·025%); B, sample; C, blank *plus* T_3 standard (0·05%); D, blank *plus* T_3 standard (0·075%); E, sample; F, blank *plus* T_3 standard (0·10%)

Fig. 2. Thin-layer chromatogram (10-cm run) showing tri-iodothyronine spots obtained from several samples of thyroid and thyroid tablets: A, blank hydrolysate; B, blank *plus* T_3 standard; C, sample No. 10; D, sample No. 8; E, sample No. 2; F, sample No. 1; G, thyroid tablet A; H, thyroid tablet C; J, iodinated casein

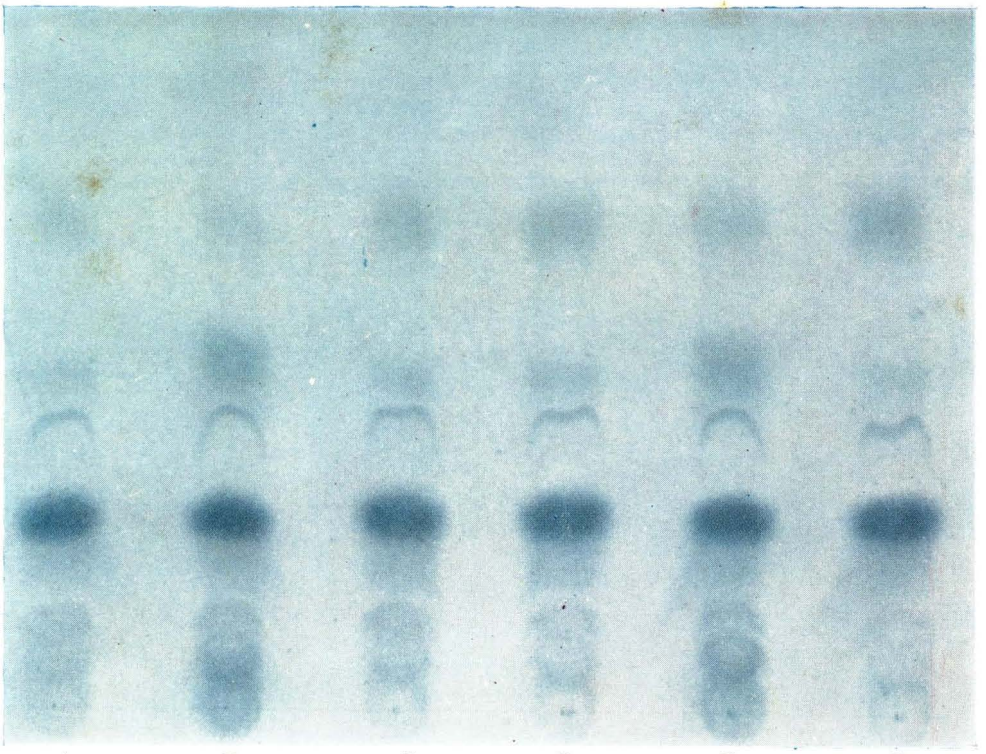

Fig. 1

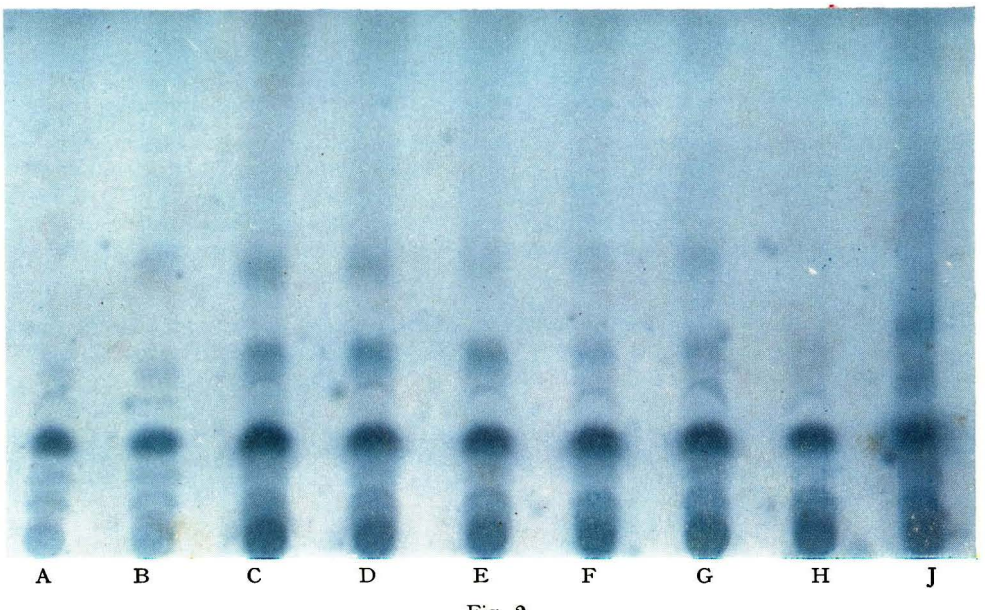

Fig. 2

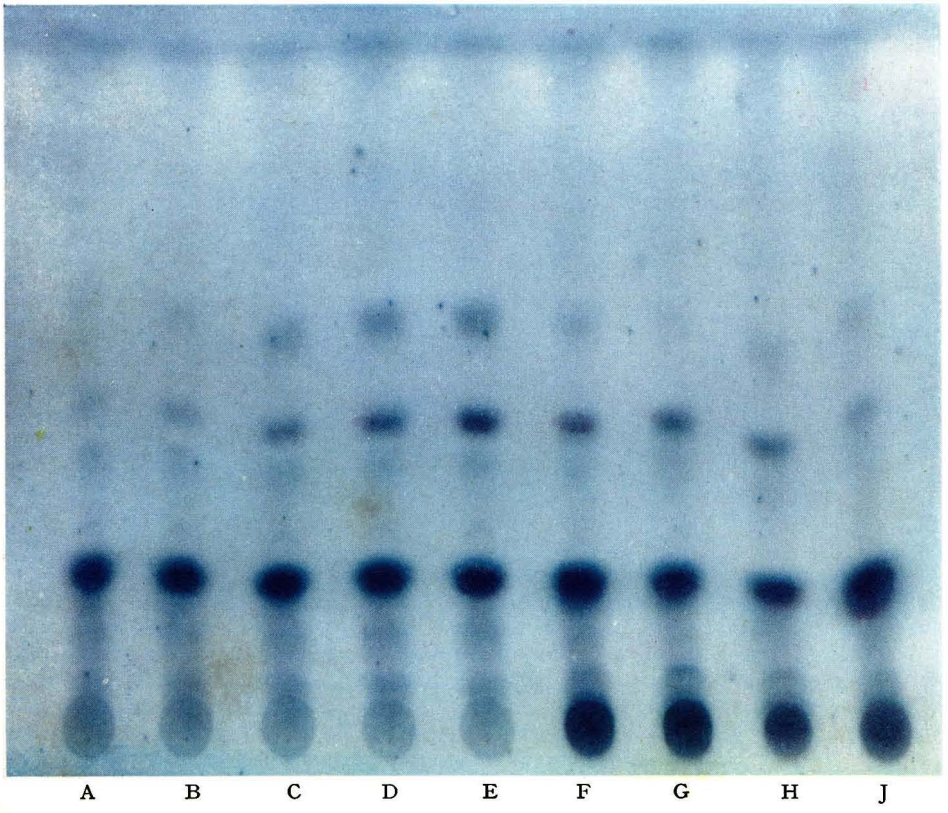

Fig. 3. Thin-layer chromatogram (15-cm run) showing tri-iodothyronine and thyroxine spots obtained from samples of thyroid and from standards at different levels: A, blank *plus* T_3 and T_4 standards (0·00625%); B, blank *plus* T_3 and T_4 standards (0·0125%); C, blank *plus* T_3 and T_4 standards (0·025%); D, blank *plus* T_3 and T_4 standards (0·0375%); E, blank *plus* T_3 and T_4 standards (0·050%); F, sample No. 6; G, sample No. 3; H, sample No. 5; J, sample No. 1

Discussion

It is suggested that the recommended test be used for sorting acceptable from defective samples of thyroid. When used in this way it may be sufficient to assess the tri-iodothyronine content only if the results are to be correlated with those obtained by the anti-thiouracil goitre method of biological assay. When correlation is to be made with other methods of biological assay that may not show such a wide difference of response between the two active constituents, it would be necessary to obtain approximate assessments of the contents of each constituent by comparison with a suitable series of standards. The results of such assessments are given in Table I, and typical chromatographic plates are shown in Figs. 1, 2 and 3. In the initial stages of the Panel's work on the thin-layer chromatographic method, some members obtained plates on which the blue background colour was so intense that assessment of spots was extremely difficult. Standardisation of the procedure overcame this difficulty, and Figs. 1, 2 and 3 show the satisfactory background colour that can be obtained.

It is not within the competence of the Panel to be definite about the limit of acceptance that should be applied to thyroid. One pharmaceutical house chose to accept samples of Thyroid B.P. offered to it only if the biologically determined activity expressed in terms of thyroxine iodine exceeded 0·3 per cent. This would exclude thyroid powders that had been the cause of clinical complaint. From the results at present obtained there are indications that, if the assessment is made with a single tri-iodothyronine standard of 2 × 2 μl of comparison solution B (see p. 335), equivalent to 0·013 per cent. of tri-iodothyronine in the thyroid, a similar acceptance level would be obtained.

During the course of the Panel's work a considerable bibliography of relevant references was compiled by Mr. R. E. A. Drey. This is presented as Appendix III to the Report.

Appendix I

RECOMMENDED METHOD FOR THE ASSESSMENT OF THYROID AND THYROID TABLETS BASED ON ESTIMATION OF THYROXINE AND TRI-IODOTHYRONINE

(See Note 1)

Enzymic hydrolysis

Apparatus—

Centrifuge tube—A tube of 50 ml total capacity, approximately 4 inches long, with parallel sides and about 1 inch i.d.

Reagents—

Borate buffer solution—Prepare a solution of 0·15 g of boric acid and 0·85 g of sodium tetraborate in 100 ml of water.

Trypsin (DIFCO 1 : 250).

Erepsin—Obtainable from Nutritional Biochemicals Corporation.

Thiomersal solution—Prepare a 0·1 per cent. solution of Thiomersal in water.

Procedure—

Into a 50-ml centrifuge tube place 0·125 g of thyroid (or a quantity of powdered thyroid tablets equivalent to 0·125 g of thyroid), 0·05 g of trypsin, 0·05 g of erepsin, 10 ml of borate buffer solution and 4 drops of Thiomersal solution. Stopper the tube, and shake the mixture into a suspension.

Incubate the tube and contents for 96 hours at 38° to 40° C, adding 0·025-g amounts of erepsin at 24, 48 and 72 hours after beginning the incubation. Agitate the tube after the addition of erepsin and each evening.

pH adjustment and butanol extraction

Apparatus—

Centrifuge—To accommodate 50-ml tubes.

Rotary-film evaporator with, preferably, a 150-ml evaporator flask.

REAGENTS—

Sulphuric acid, N.

Butanol—Analytical-reagent grade.

Ammonia - methanol solution—A 5 per cent. v/v solution of ammonia solution (35 per cent.) in methanol.

PROCEDURE—

At the end of the 96-hour incubation period, allow the tube and contents to cool to room temperature and then adjust the pH of the hydrolysed solution to 2 to 3 by the addition of N sulphuric acid (this adjustment can be followed by the use of pH indicator papers and will require about 1 ml of acid).

Extract the acidified hydrolysate four times with 15-ml amounts of water-saturated butanol. Carry out each extraction by adding the water-saturated butanol to the contents of the centrifuge tube, inserting the stopper, shaking the tube thoroughly and then separating the two phases centrifugally at 2000 r.p.m. After each extraction withdraw the upper organic phase by pipette.

Evaporate the combined butanol extracts to dryness under reduced pressure at a temperature not exceeding 45° C. To the residue add 5 ml of the ammonia - methanol solution, stopper the flask securely, and set aside for about 1 hour with occasional swirling of the solvent to ensure complete solution of the residue.

Finally, transfer this solution to a suitable centrifuge tube (*e.g.*, 10-ml graduated), insert the stopper, and spin the tube in a centrifuge to precipitate suspended material. Store the tube and contents (*Thyroid extract*) in a refrigerator.

THIN-LAYER CHROMATOGRAPHY

MATERIALS AND APPARATUS—

Chromatographic tank for thin-layer chromatography.

Glass plates—Approximately 8 × 8 inches.

Whatman 3MM *chromatographic paper.*

Starch solution, 0·8 per cent. w/v, aqueous—Triturate 0·8 g of soluble starch with a small volume of water, and add the resulting suspension, with constant stirring, to sufficient boiling water to produce 100 ml. Boil for 2 minutes, then cool, but do not filter. Prepare this solution freshly before use.

Cellulose powder—Macherey, Nagel & Co., MN-300 without gypsum, was used.

Solvent system—Shake thoroughly a mixture of 100 ml of t-pentanol, 80 ml of water and 20 ml of ammonia solution, sp.gr. 0·88, and set the mixture aside to separate into two phases.

Standard comparison solutions—Dissolve 3·75 mg of tri-iodothyronine and 3·75 mg of thyroxine (or equivalent amounts of their sodium salts) in ammonia - methanol solution and dilute with the same solvent to 200 ml (Solution A). Dilute 25 ml of Solution A to 100 ml with ammonia - methanol solution (Solution B). (See Note 1.)

Spray reagent—Dissolve 2·7 g of $FeCl_3.6H_2O$ in 100 ml of 2 N hydrochloric acid (Solution I). Dissolve 3·5 g of $K_3Fe(CN)_6$ in 100 ml of water (Solution II). Dissolve 5 g of $NaAsO_2$ in 50 ml of N sodium hydroxide at room temperature then add, with vigorous stirring, 65 ml of 2 N hydrochloric acid (Solution III).

Immediately before use mix 5 parts of I, 5 parts of II and 1 part of III. (Solutions I and III keep for some time; Solution II should be prepared daily as required.)

Blank hydrolysate solution—Carry out the procedure for enzymic hydrolysis, pH adjustment and butanol extraction, but omit the thyroid. The extract so prepared is stable for months when stored in a refrigerator, and one blank preparation would be sufficient for 100 or more determinations.

PROCEDURE—

Preparation of plates—Weigh 7·5 g of cellulose powder into a 150-ml beaker, add 20 ml of starch solution (see Note 2), and stir with a thick glass rod until homogeneous. Slowly add a further 30 ml of starch solution with continuous stirring until a homogeneous slurry is obtained, and then continue stirring for a further 3 minutes. Immediately spread the slurry

on to the plates from an applicator set at 0·25 mm, allow the plates to dry, and then heat them in an oven at 100° to 105° C for 1 hour. Wash the plates by the ascending-solvent technique overnight with the organic phase of the solvent system, and allow them to dry before use.

Chromatography—Carry out all chromatography at a temperature not lower than 20° C. Line the chromatographic tank with 3MM paper, and place a solvent trough at the bottom of the tank.

Several hours before chromatography is to be carried out ensure that the tank is saturated (see Note 3).

Apply at the starting line, drawn 2 cm above the end of the plate placed in the solvent trough during the pre-washing process, the following series of sample and comparison spots containing the stated amounts of tri-iodothyronine and thyroxine (see Note 4)—

(*i*) 3 × 2 μl of thyroid extract (equivalent to 150 μg of original thyroid);
(*ii*) 2 μl of Comparison Solution B (0·0094 μg of each of T_3 and T_4);
(*iii*) 2 × 2 μl of Comparison Solution B (0·019 μg of each of T_3 and T_4);
(*iv*) 2 μl of Comparison Solution A (0·0375 μg of each of T_3 and T_4);
(*v*) 2 μl of Comparison Solution A and 2 × 2 μl of Comparison Solution B (0·056 μg of each of T_3 and T_4);
(*vi*) 2 × 2 μl of Comparison Solution A (0·075 μg of each of T_3 and T_4); and
(*vii*) 2 × 2 μl of Comparison Solution A and 2 × 2 μl of Comparison Solution B (0·094 μg of each of T_3 and T_4).

Overspot the comparison spots with 3 × 2 μl of blank hydrolysate solution.

As soon as the spots have dried begin the chromatographic run, placing the side of the plate having the cellulose layer on it to face the lining paper (see Note 3). After the solvent front has risen about 15 cm (see Note 1) above the starting line (this is usually conveniently achieved by allowing chromatography to proceed overnight, although with some batches of cellulose rather less time may be required), remove the plate from the tank and allow it to dry (see Note 5).

Finally, spray the plate with the ferric chloride - ferricyanide - arsenite reagent until it is uniformly moist, but without making it so wet that the spray solution runs. Stand the plate horizontally away from bright light and observe the development of the tri-iodothyronine standard spot (see Note 6). When this has achieved a suitable depth of colour, wash the plate by immersing it in a layer (about ½ inch deep) of water to which 5 per cent. by volume of hydrochloric acid has been added, and then with two successive washings of water.

Assess the thyroid by comparing the sizes and intensities of the spots due to tri-iodothyronine and thyroxine obtained from the sample hydrolysate with the standard comparison spots.

NOTE 1—When it is desired to use the method simply to determine whether the sample of thyroid would be likely to be acceptable when tested by the anti-thiouracil goitre test in rats, it is sufficient to use a single application of a suitable amount of tri-iodothyronine (see p. 333). Further, it is only necessary to allow the solvent front to move 10 cm beyond the points of application, as tri-iodothyronine is readily separated in this distance from all other materials that respond to the spray reagent. It is suggested that users of the method should first establish their own criterion of acceptability and then use an appropriate comparison standard.

NOTE 2—A sample of soluble starch used by one Panel member was consistently found to give plates that were unsatisfactory, *i.e.*, the surface flaked off when excess of reagent was being washed away. Should this occur another batch of starch should be used; the majority appear to be satisfactory.

NOTE 3—Saturation of the tank before developing the plates is important and can affect the size of the separated spots. A procedure that has been found satisfactory is to place the aqueous layer, together with 2 or 3 ml of the organic layer in the bottom of the tank, which should be completely lined with paper, then place a glass trough in the bottom of the tank, close and seal the tank with adhesive tape and then leave it for several hours. To insert the plate remove the seal and slide the lid gently to one side, quickly insert the prepared plate into the trough, replace the cover and leave to stand for 30 minutes. Then introduce the organic solvent into the trough through a hole in the lid by means of a long-stemmed funnel.

NOTE 4—It is important when overspotting that the previously applied spot is completely dry.

NOTE 5—Complete drying of the chromatographed plate before spraying is essential. This can be accomplished by applying a current of warm air, or by allowing it to dry overnight at room temperature.

NOTE 6—Suitable colour development on the plate is usually achieved within 3 to 4 minutes after completion of spraying.

Appendix II

THE DETERMINATION OF SMALL AMOUNTS OF IODINE-CONTAINING ORGANIC COMPOUNDS

During collaborative investigations into the chemical assessment of thyroid the need arose to determine small amounts (of about 10 to 50 μg) of iodine-containing organic compounds. Many methods that appeared applicable have been described in the literature. These include reaction in solution with a cerium(IV) - arsenite reagent,[52,58,70,72,76,102] photodensitometric evaluation of coloured zones on chromatographic paper after treatment with cerium(IV) - arsenite reagent or with ferric - ferrocyanide - arsenious acid reagent,[100,101] ashing of chromatographic paper after spraying with ferric - ferrocyanide - arsenious acid reagent followed by photo-colorimetric determination of combined iron[106] and combustion of paper in oxygen followed by determination of iodine in the absorbing liquid with the cerium(IV) - arsenite reagent (see pp. 171 to 176 of reference 6). Some of these methods were tested, but none met the requirements of the Panel. What was required was a method that satisfied criteria of accuracy, speed and simplicity and was capable of being used for determining 10 to 50-μg amounts of iodinated amino-acids on chromatographic-paper with an over-all reproducibility of about 10 per cent. The method developed depends on combustion of the iodo-compound on filter-paper by the oxygen-flask method, followed by oxidation of the iodine to iodate with bromine, boiling to remove excess of bromine and peroxides, reaction of the iodate with excess of iodide and spectrophotometric determination of the resulting tri-iodide ion.

Experimental

During development of the method, three possible means of determining the tri-iodide ion were examined. These were all based on spectrophotometric measurements, one of the tri-iodide ion at 288 mμ, another of the tri-iodide ion at 352 mμ and the third of the tri-iodide - starch complex at about 600 mμ. The last method was very reproducible, but was rejected because of the comparatively high "threshold" value obtained. Measurements at 352 mμ were preferred to those at 288 mμ because, although a little less sensitive, they were more reproducible and less subject to the influence of extraneous absorption. The method was developed for application to iodinated amino-acids that had been separated by chromatography on Whatman 3MM paper, so the procedure detailed below makes use of a 2-litre flask for combustion, as this capacity is necessary to permit 12 × 3-cm strips of thick filter-paper to be completely burned. If previous paper chromatography is not involved, the iodine-containing organic compound, in a suitable solvent, may be spotted directly on to a 5 × 3-cm strip of Whatman No. 1 filter-paper and combustion may be carried out in a 500-ml flask.

Method

Reagents—

Sodium hydroxide, 0·1 N.
Orthophosphoric acid—A 20 per cent. w/v solution of H_3PO_4.
Potassium iodide solution, 1 per cent. w/v—Freshly prepared.
Saturated bromine solution—Freshly prepared.

Procedure—

Spot a suitable volume of a solution of the iodine-containing compound in a volatile solvent (to contain the equivalent of approximately 15 to 40 μg of iodine) on to Whatman 3MM paper (12 × 3 cm), fold the paper, and place it firmly into a platinum-gauze sample holder of a 2-litre flask for oxygen-flask combustion. (The folded paper is very bulky and it may be found convenient to bind it into the gauze with 1-mm diameter platinum wire.) Place 15 ml of 0·1 N sodium hydroxide into the flask to act as absorbing liquid, and carry out the combustion in the usual manner. After combustion shake the flask thoroughly to absorb the vapour, and then transfer the liquid to a 100-ml beaker. Wash the sample holder and flask well with water, transferring the washings to the beaker until the volume of absorbing liquid and washings is about 35 ml. Acidify the solution with 1 ml of 20 per cent. orthophosphoric acid, add 2 ml of saturated bromine solution, and boil until 5 minutes after the solution becomes colourless. Cool to 20° C, transfer the liquid to a 50-ml calibrated

flask, dilute to about 35 ml with water, add 5 ml of a 1 per cent. solution of potassium iodide in water, and dilute to volume with water. Set aside in the dark at 20° C for 30 minutes, and then measure the absorption of a 2-cm layer at the peak at about 352 mμ. Carry out a blank test on a similar piece of Whatman 3MM paper, and calculate the amount of iodine present by reference to a calibration graph.

Prepare a calibration graph by mixing in 50-ml calibrated flasks 10 ml of 0·1 N sodium hydroxide, 1 ml of 20 per cent. orthophosphoric acid, suitable volumes (equivalent to 10, 15, 20, 25, 35 and 40 μg of iodine) of a solution of potassium iodate in water and dilute each to 35 ml. Complete as described above from "add 5 ml of a 1 per cent. solution of potassium iodide in water. . . ."

Results and discussion

The procedure described has been applied in five laboratories to the determination of thyroxine sodium, both after direct application of an ethanolic solution to filter-paper and also to the same substance after paper chromatography. The results obtained are shown in Table II.

Table II

Determination of iodine in thyroxine sodium by the method given in Appendix II

Laboratory	Iodine taken, μg	Iodine found, μg	Recovery, per cent.
Without chromatographic separation—			
A	10·0	9·8	98·0
B	10·58	11·6	109·4
C	10·0	9·9	99·0
D	10·0	11·0	110·0
E	8·74	9·37	107·2
A	20·0	19·4	97·0
B	21·15	22·5	106·5
C	20·0	19·8	99·0
D	20·0	19·4	97·0
E	17·48	18·24	104·3
A	30·0	28·8	96·0
B	31·73	35·35	111·4
C	30·0	29·1	97·0
D	30·0	29·6	98·3
E	26·22	26·03	99·3
A	40·0	37·6	94·0
B	42·31	45·25	107·0
C	40·0	38·2	95·5
D	40·0	40·0	100·0
E	34·96	35·35	101·1
After chromatographic separation—			
A	20·0	18·0	90·0
B	21·15	19·6	92·6
C	20·0	18·0	90·0
D	20·0	16·4	82·0
E	17·48	16·71	96·0
A	40·0	37·2	93·0
B	42·31	38·6	91·2
C	40·0	35·6	89·0
D	40·0	33·4	83·5
E	34·96	33·72	96·4

The method of combustion and conversion of the iodine present to iodate was developed from that used for the semi-micro determination of iodine in organic compounds,[128] but for the micro-application described here it has been found necessary to boil the brominated solution thoroughly before the addition of iodide. Appreciable amounts of peroxides are formed during the combustion process and, unless these are completely removed by boiling, high results will be obtained.

The simplicity of the method, the fact that it can be applied with reasonable precision to the determination of paper-chromatographically separated iodine-containing organic compounds at levels corresponding to only 10 to 40 μg of iodine and the potential ability to be able to use organic detecting reagents on the paper without interference to the final determination, indicate that it might be of more general use than the Panel's investigations have required.

Appendix III

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(See also References 49, 51, 52, 72, 96, 100, 108, 110, 111, 112 and 113)

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Book Reviews

METHODS OF BIOCHEMICAL ANALYSIS. Volume 14. Edited by DAVID GLICK. Pp. x + 562. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons. 1966. Price 113s.

Methods for estimating magnesium in biological materials are reviewed by Nancy W. Alcock of Sydney in conjunction with I. MacIntyre of the Postgraduate Medical School, London. The article covers flame-emission spectrophotometry and atomic absorption, as well as the classical method of precipitation of magnesium as magnesium ammonium phosphate. Other methods are discussed but the above are selected for detailed treatment on the score of "accuracy and precision of techniques." Methods of preparation of samples round off an excellent article with a bibliography of 216 references.

Helen R. Skeggs of the Merck Institute at West Point, Pennsylvania, deals with the microbiological assay of vitamin B_{12} in a concise and authoritative way straight from the bench. "Tension and turmoil in the laboratory play a rôle. An analyst with personal or emotional problems is almost certain to produce erratic results although there may be no apparent problem within individual assays."

R. H. Silber of Merck, Rahway, writes on fluorimetric analysis of corticoids in plasma, urine and rat adrenals, going back to the well tried Porter - Silber procedure. For some time the clinical determination of plasma corticoid has been based upon interaction between the dihydroxyacetone side chain with phenylhydrazine in ethanolic sulphuric acid. By substituting fluorescence for the colour reaction corticosterone could then be determined. If necessary, cortisol and corticosterone can both be measured. A balance has to be struck between convenience and specificity limitations. This article is concise and the mark of the expert is obvious.

N. O. Lindh and B. L. Brantmar of Lund, Sweden, are concerned with the preparation and analysis of basic proteins. The use of Reinecke salt in a new preparative process and the technique of acrylamide disc electrophoresis are described in detail. The article includes a lucid account of the isolation of the subcellular components that contain the proteins to be studied. The basic proteins associated with the nucleic acids are relatively low in molecular weight and usually have no tryptophan, cysteine or methionine, but because they are quite rich in lysine and arginine and less rich in acid amino-acid residues their isoelectric points are between pH 10 and 11. The basic proteins tend to form complexes and to neutralise acid groups in polynucleotides. Methods are described for isolating basic proteins from non-chromatin material and for extracting whole histone from chromatin. The fractionation of histones by various procedures is discussed and the use of anionic precipitants (*e.g.*, Reinecke salt) is considered. Practical directions are given based on acidic ethanol extraction. There is a full bibliography (128 references).

H. N. Munro, formerly of Glasgow and now at M.I.T., Cambridge, Mass., and A. Fleck of Glasgow, tackle the determination of nucleic acids systematically and logically. W. C. Hutchinson and H. N. Munro (*Analyst*, 1961, **86**, 768; 1962, **87**, 303) made a study of the literature and concluded that no infallible procedure was available. Although no important new principles have emerged in this field since 1961, a good deal of clarification and consolidation has been achieved so that this new review is well worthwhile. First, the preliminary preparation of tissue and the removal of lipids and substances of "small" molecular weight are described. Then the well known Schneider, Ogur-Rosen and Schmidt-Thannhauser procedures for extracting and separating nucleic acids are critically surveyed. This is followed by an account of analytical methods based on phosphorus estimation; on sugar reactions; on ultraviolet absorption by the purine and pyrimidine moieties; on fluorimetry; on electrophoresis; and on miscellaneous reactions. The authors proceed to make general and serviceable recommendations on selecting a procedure for nucleic acid determination. The bibliography runs to 419 references.

S. Jacobs of the National Institute for Medical Research, London, discusses the determination of amino acids by ion-exchange chromatography. Sulphonated polystyrene resins (Zeo-Karb, Dowex) are more satisfactory than the starch columns used by Moore and Stein in their pioneer separations on protein hydrolysates. Jacobs describes the types of cation-exchange resins and anion-exchange resins now used, and deals with the preparation of columns and the separations of amino acids. He then points out that a reliable spectrophotometric method for the quantitative determination of the amino groups is of crucial importance and describes many approaches. However, there is ample evidence to show that indanetrione hydrate is the most sensitive reagent

for the spectrophotometric determination of amino acids "provided certain precautions are taken." The author's experience and "know how" are skilfully made available to the reader.

B. Weinstein of Stanford, California, describes in a long article (120 pages) the separation and determination of amino acids and peptides by gas - liquid chromatography. "The major problem facing the newcomer in this field today is the need to prepare a suitable derivative that can be used for all amino acids . . . some fifteen of the twenty primary amino acids contain a third functional group besides the characteristic α-amino and carboxyl groups." The article discusses many derivatives and notes how they have been used. The article goes on as follows: separation of amino acids by acylation, decarboxylation, diazotisation, esterification, esterification and acylation, esterification and alkylation, oxidation, pyrolysis and reduction. Some quantitative aspects are touched on. Next comes the separation of peptides (di-, tri- up to hexa-peptides) followed by polypeptides and several well known proteins. The author decides that "in reality" the choice is quite limited. He concludes that "the outstanding derivatives at this time seem to be the methyl esters and butyl esters of *N*-trifluoroacetyl amino acids," with the amyl esters very promising, but "the fact remains that the complete analysis of a peptide or a protein of unknown composition cannot be obtained with existing methods" but the qualitative goal of analysing the amino acids by gas chromatography has been partially achieved. This is an impressive survey.

A. Kuksis of Toronto deals with newer developments in the determination of bile acids and steroids by gas chromatography. This again is a substantial chapter of 130 pages. Unlike the previous article it includes instrumentation and column technology. A long section on qualitative separations is subdivided into preparation of derivatives; relationship between steroid structure and retention time; and identification of unknowns. The quantitative aspects include the use of isotopes, and numerous applications are surveyed interestingly. The author makes it clear that gas - liquid chromatography does not at present permit the identification of many biologically important steroids in crude extracts. Standard extraction procedures followed by preliminary fractionation must first be carried out. Thin-layer chromatography, isotope dilution and mass spectrometry reinforce gas - liquid chromatography which itself "may have lost much of its independence, but by integration with other analytical systems, it has allowed the development of research techniques of unprecedented potentialities."

M. D. Poulik of Detroit offers an account of gel electrophoresis in buffers containing urea. The use of buffers containing a dissociating agent (urea) and a reducing agent (mercapto-ethanol), or both, constitutes a marked technical improvement in starch-gel electrophoresis. The gelled supporting media separate proteins according to size rather than charge. If, for example, reduced and alkylated ribonuclease is compared with native ribonuclease in starch gel, the former migrates much more slowly than the latter as a result of the unfolding of the molecule. The new methods are versatile as well as reproducible, and valuable results have been obtained on difficult problems such as genetic variation of protein and gamma globulin structure.

The whole volume maintains the high standard of its predecessors in the series.

R. A. MORTON

INDUSTRIAL CHEMISTRY—ORGANIC ADVANCED LEVEL. By D. M. SAMUEL, B.Sc., A.R.I.C. Pp. vi + 34. London: The Royal Institute of Chemistry. 1966. Price 6s.

A proper study of organic chemistry must include some consideration of its increasing importance in industry. Unfortunately, the average text-book in current use at "A" level was probably written before the recent developments got under way and unless his tutor supplies the deficiency, the student who only goes as far as "A" level can easily be left with the thought that acrylonitrile, propylene, vinyl chloride and many other organic substances are just some more carbon compounds. This booklet is designed to help teachers inject some lively reality into the subject and enable their students to realise that huge tonnages are now being produced of materials that were laboratory curiosities not so long ago, and to appreciate the significance of ease and cheapness of production. The subject matter is divided conventionally into aliphatic and aromatic compounds. In the aliphatic section there is a page or so devoted to each of the following: Paraffins, Olefins, Acetylene, Aliphatic halogen-containing compounds, Alcohols, Ethers and epoxides, Aldehydes and ketones, Carboxylic acids and derivatives, Esters and Nitrogen compounds, and in the aromatic section the subjects discussed are: Aromatic hydrocarbons, Aromatic nitro and amino compounds, Sulphonic acids, Phenols, Aromatic alcohols, aldehydes, ketones and acids, and Dyestuffs. The whole is simply and clearly written and should fulfil its declared purpose admirably. At the price it ought to find a place on many laboratory shelves, as it is not only "A" level students who need to keep up-to-date on these matters. A. G. JONES

GAS CHROMATOGRAPHY IN THE ANALYSIS OF STEROID HORMONES. By HERBERT H. WOTIZ and STANLEY J. CLARK. Pp. xvi + 288. New York: Plenum Press. 1966. Price $12.50.

Rapid advances in the techniques of gas chromatography as applied to the determination of basically labile compounds such as steroid hormones have resulted in the publication, over the last 4 or 5 years, of several hundred papers on the subject. Professor Wotiz and Dr. Clark are to be congratulated on bringing together in one volume the many and varied experiences of eminent workers in the field of steroid biochemistry and presenting such a wealth of analytical detail and methodology in a concise and assimilative fashion.

The first four chapters are devoted to an introduction to the general theory and practice of gas chromatography, and a further chapter describes ancillary techniques such as infrared spectroscopy, nuclear magnetic resonance and mass spectrometry. This is followed by an outline of the history of gas chromatography of steroids and a detailed discussion on the importance of column preparation and conditioning to achieve successful results. Further chapters on the thermal degradation of steroids, adsorption losses and purity of standards and solvents emphasise the care that must be taken, and illustrate the pitfalls awaiting the unwary analyst. Two brief chapters on derivative formation and preliminary clean-up procedures are followed by a chapter on the relationship between steroid structure and retention times. This chapter, written by a colleague, completes the first half of the book.

The remaining sections comprise detailed practical procedures for the determination of individual steroids or groups of steroids in biological fluids. These include progesterone and its metabolites, androgens such as 17-ketosteroids and testosterone, pregnanediol, adrenocortical steroids, and finally oestrogens. Each method is discussed and evaluated according to the particular needs of the analyst.

The authors justly pay tribute to the many experts who have provided much of the information contained in these later chapters. It must not be forgotten, however, that many of the procedures described have originated from or been rigidly tested in the authors' own laboratory, and this renders even more valuable the contribution of this book in the field of steroid analysis.

Finally, the authors provide a directory of suppliers of gas chromatography equipment, a bibliography and over 250 references to published literature.

This book is highly recommended not only for the biochemist but also for any analyst faced with the problem of separating and determining thermally unstable compounds by gas chromatography.

D. A. ELVIDGE

INFRARED SPECTRA OF CELLULOSE AND ITS DERIVATIVES. By ROSTISLAV GEORGIEVICH ZHBANKOV. Edited by B. I. STEPANOV. Translated by A. B. DENSHAM. Pp. xiv + 333. New York: Consultants Bureau. 1966. Price $16.00.

The ubiquity of the infrared spectrophotometric method in connection with analytical and similar methods is now generally recognised, and the work under review is an example of one of its highly specialised applications. The author is senior scientist at the Institute of Physics of the Academy of Sciences of the Belorussian S.S.R.; he is the author of more than fifty scientific papers and patents, and a co-worker of the State Prizewinner, Academician B. I. Stepanov, who is also the editor of the work. The original was published in Russian in 1964, and has been translated by A. B. Densham of the Gas Council, London, for publication in the United States. The translated edition has been corrected and up-dated by the (presumably original) "author."

The first chapter deals with the methods and technique of obtaining the infrared spectra of cellulose and related materials and, from the purely practical point of view, this is the most important part of the book although it comprises only 33 pages. A variety of methods has been used, quite apart from the investigation of the fibrous material as received. These include the suspension method, the solid immersion medium method, and the production of thin films of water-soluble compounds from their aqueous solutions. Apparatus is described, with practical directions for such operations as pressing out fibre films, the use of a vacuum press for making discs of sample material with an alkali metal halide, and a device for moving a fibre film of different thicknesses in a vertical plane. These are difficult and highly specialised techniques, evolved specifically for cellulose fibres and cellulose derivatives, but they could have obvious applications in other fields.

The following five chapters deal respectively with the application of the method to unmodified celluloses, hydrocellulose, cellulose esters and ethers, oxidation products of cellulose and so-called new types of cellulose derivative, such as xanthates, esters with phosphorus-containing acids, and graft copolymers of cellulose and carbon chain polymers. These chapters give the results

obtained, together with the appropriate curves and, where relevant, certain deductions, mainly of a theoretical nature.

Chapter 7 is concerned with the future possibilities of the method for the investigation of the properties of cellulose and its derivatives; it also is mainly theoretical in nature and is quite short. The remaining 146 pages comprise a 204-item bibliography, and tables and graphs illustrating the results obtained. These should be of use to workers making a preliminary rapid appraisal of the origin of individual absorption bands in the infrared spectra of cellulosic materials; and in assisting them to choose the spectral region for the determination of particular atomic groups.

It is evident that this book is for two types of specialist, namely, the infrared spectroscopist interested in new methods and techniques, and workers on the theory of the structure of cellulose and its derivatives. Apart from this it is of special interest as an example of the type of work that is being carried out in the U.S.S.R. at the present time, and the fact that it has been translated into the English language is a measure of its importance and reliability. JULIUS GRANT

HANDBUCH DER KOLORIMETRIE. BAND III. KOLORIMETRIE IN DER BIOLOGIE, BIOCHEMIE UND MEDIZIN. By DR. BOHUMIL KAKÁČ and ZDENĚK J. VEJDĚLEK. Pp. xiv + 857. Jena: Veb Gustav Fischer Verlag. 1966. Price 129s. 6d.

Part 1 of this book is described as the organic part, and describes the measurement by colorimetry of substances likely to be encountered in biology, biochemistry and medicine, when dealt with from an analytical point of view.

The book is divided into four main parts, the first dealing with amino-acids, proteins and peptides; the second with carbohydrates and derivatives thereof; the third with steroids and sterins; and the fourth with other interesting biological substances.

The first section, on amino-acids, etc., deals initially with the general colorimetric methods of determination, and specifically mentions ninhydrin, ninhydrin - copper complex, naphthoquinone, sulphonic acid, Schiffs reagent and copper complexing, as the chief lines of approach.

There then follows itemised specific methods for the various amino-acids. Some thirty-two amino-acids are dealt with in a very full manner. The preferential colorimetric method is given, and a full bibliography is also included.

The section on carbohydrate and its derivatives is also dealt with in a similar way. First of all the group methods for the colorimetric analysis of sugars are given, and then specific methods for the various commonly occurring sugars, *e.g.*, trioses, tetroses, pentoses and hexoses. Hexosamine, desoxy sugars, uronic acids, celluloses, starch, glycogen, inulin and dextran are fully dealt with.

There is a particularly interesting chapter on the determination of nucleic acids and glycoproteins.

Probably the most valuable part of the volume is that on the colorimetric methods for the sterins and steroids. There is a comprehensive detailed exposition and a full bibliography relating to the many steroid substances that are now recognised as being of physiological interest, including some of the synthetic hormones.

The last part of the book, on biologically interesting substances, includes colorimetric determination of substances such as acetaldehyde, formic acid, the various sugar acids, acetone and its metabolic derivatives, indole, purines, indican and many of the metabolites that have been discovered in recent biochemical investigations.

Without doubt the volume is of particular interest to those engaged in clinical biochemistry and research in physiology, but for any analyst involved in colorimetry there is material of of considerable value, and the bibliography can be recommended.

Certainly there is no book written in the English language which is nearly so comprehensive, but because of the stereotype nature of the lay-out and the format, most readers with a knowledge of technical German would find the volume comprehensible. R. F. MILTON

Errata

JANUARY (1966) ISSUE, p. 42. *For second author* "G. Catanzaro" *read* "E. W. Catanzaro."

MARCH (1967) ISSUE, p. 194, 3rd line. *For "Kieselguhr GF254" read "Kieselgel GF254."*

IBID., p. 194, 22nd line. *For* "Kieselguhr GF254" *read* "Kieselgel GF254."

Summaries of Papers in this Issue

Dioximes of Large Ring 1,2-Diketones and their Applications to the Determination of Bismuth, Nickel and Palladium

The preparation of alicyclic *vic*-dioximes containing 5 to 12 carbon atom rings from the corresponding 1,2-diketones is reported, and their application to the gravimetric determination of bismuth in the presence of several complexing agents is described. The dependence of complete precipitation of bismuth upon pH, and the effect of foreign ions has been studied. A polymeric structure is suggested for the bismuth - dioxime compounds. The application of large ring (8 to 12 carbon atoms) *vic*-dioximes to the gravimetric determination of nickel and palladium has been studied; the infrared and ultraviolet spectra of the nickel(II) and palladium(II) complexes are presented.

J. BASSETT, G. B. LETON and (the late) A. I. VOGEL
Chemistry Department, Woolwich Polytechnic, London, S.E.18.

Analyst, 1967, **92**, 279–289.

A Limit Test for 4-Chloroacetanilide in Phenacetin and its Preparations

A thin-layer chromatographic method is presented that is suitable for use as a limit test for 4-chloroacetanilide in phenacetin. 4-Chloroacetanilide is detectable at 0·01 per cent., but the test may be made more stringent by suitable adjustment of sample size. The method has been applied on a collaborative basis to samples of phenacetin and to several commonly used tablets containing phenacetin.

BRITISH PHARMACOPOEIA COMMISSION (*ad hoc* Committee)
General Medical Council Office, 44 Hallam Street, London, W.1.

Analyst, 1967, **92**, 290–292.

Salicylideneamino-2-thiophenol—A New Reagent for the Photometric Determination of Tin: Application to the Analysis of Ores, Rocks and Minerals

Salicylideneamino-2-thiophenol can easily be prepared by the reaction of 2-aminobenzenethiol with salicylaldehyde. This reagent reacts with silver, copper, molybdenum, tin and several other metals to give coloured complexes, but by a suitable choice of conditions it can be made selective for tin. The tin complex is readily extracted into organic solvents and can be used for the photometric determination of this metal in ores, rocks and minerals.

G. R. E. C. GREGORY and P. G. JEFFERY
Warren Spring Laboratory, Stevenage, Herts.

Analyst, 1967, **92**, 293–299.

Determination of Trace Amounts of Magnesium, Strontium and Nickel in Lake-water Samples by Neutron-activation Analysis

Neutron-activation analysis has been used to determine magnesium, strontium and nickel in water from eleven Greek lakes. The elements were separated by an isolation procedure (taking up to 30 minutes); the magnesium and strontium were determined by γ-spectrometry, and the nickel by β-coincidence counting. The ranges found were 11 to 31 p.p.m. of magnesium, 0·02 to 1·12 p.p.m. of nickel and 0·07 to 0·48 p.p.m. of strontium.

A. G. SOULIOTIS, E. P. BELKAS and A. P. GRIMANIS
Nuclear Research Center "Democritos," Chemistry Department, Aghia Paraskevi Attikis, Athens, Greece.

Analyst, 1967, **92**, 300–304.

A Rapid Method for the Determination of Iron in Plant Material with Application of Automatic Analysis to the Colorimetric Procedure

A colorimetric procedure is described for the determination of iron in plant material by using sulphonated 4,7-diphenyl-1,10-phenanthroline. The method may be applied directly to the AutoAnalyzer, and gives good and reproducible recoveries over a wide range of added iron. The possibility of low recoveries of iron resulting from the wet digestion of material in the presence of sulphuric acid is discussed.

C. QUARMBY and H. M. GRIMSHAW

Natural Environment Research Council, The Nature Conservancy, Merlewood Research Station, Grange-over-Sands, Lancashire.

Analyst, 1967, **92**, 305–310.

The Determination of Nitrate in Soil Solutions by Ultraviolet Spectrophotometry

Ultraviolet spectrophotometric analysis has been applied to the determination of nitrate nitrogen over the 0·5 to 10 p.p.m. range in soil solutions and perfusates in the absence of organic matter, and over the 5 to 50 p.p.m. range in its presence. If necessary, a 4-fold increase in ultimate sensitivity can easily be obtained. Sulphamic acid can be used for the destruction of nitrite which would otherwise interfere, and a short procedure has been suggested for the reduction of severe interference present in extracts from highly organic samples such as peat. The more important nitrification inhibitors used in perfusion experiments are well tolerated. Nitrate contents of soil solutions were measured by the method and gave results in good agreement with the nitrate reduction and ammonia distillation method.

P. A. CAWSE

U.K. Atomic Energy Authority, Wantage Research Laboratory, Wantage, Berks.

Analyst, 1967, **92**, 311–315.

Determination of Terminal Hydroxyl Groups in Polyethyleneoxy Compounds

The 3,5-dinitrobenzoyl chloride method has been adapted for the semi-micro analysis of hydroxyl groups in polyethylene glycol and non-ionic surfactants of the polyethylene oxide type. The 3,5-dinitrobenzoates are determined colorimetrically after removal of the excess of acid chloride by column chromatography.

By this method 100 μg of hydroxyl can be determined with a relative accuracy of 4·5 per cent. Products containing fewer than ten ethylene oxide units per molecule do not respond adequately.

Results of the analyses of some technical non-ionic detergents and polyethylene glycol are reported.

K. W. HAN

Unilever Research Laboratory, Mercatorweg 2, Vlaardingen, The Netherlands.

Analyst, 1967, **92**, 316–318.

Precipitation from Homogeneous Solution by Cation Release at Constant pH

P. F. S. CARTWRIGHT

Department of Chemistry, Sir John Cass College, London, E.C.3.

Analyst, 1967, **92**, 319.

The Determination of Small Amounts of Tin in Organic Matter. Part 1: Amounts of Tin up to 30 μg

Report prepared by the Metallic Impurities in Organic Matter Sub-Committee.

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1967, **92**, 320–323.

The Determination of Small Amounts of Zinc in Organic Matter

Report prepared by the Metallic Impurities in Organic Matter Sub-Committee.

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1967, **92**, 324–325.

Nitrogen Factor for Tongue

Report prepared by the Meat Products Sub-Committee.

ANALYTICAL METHODS COMMITTEE
14 Belgrave Square, London, S.W.1.

Analyst, 1967, **92**, 326–327.

Evaluation of Thyroid

Report prepared by the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Recommended Methods for the Evaluation of Drugs.

Joint Committee of the PHARMACEUTICAL SOCIETY and the SOCIETY FOR ANALYTICAL CHEMISTRY
14 Belgrave Square, London, S.W.1.

Analyst, 1967, **92**, 328–342.

Notice to Authors

THE Editor welcomes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Papers are submitted to the Editorial Committee, who decide on their suitability for publication.

Intending authors should consult the current Notice to Authors, last published in full in *The Analyst*, 1966, **91**, 67, reprints of which can be obtained on application to The Editor, *The Analyst*, 14, Belgrave Square, London, S.W.1. All papers submitted will be expected to conform to the recommendations there laid down, and any that do not may be returned for amendment.

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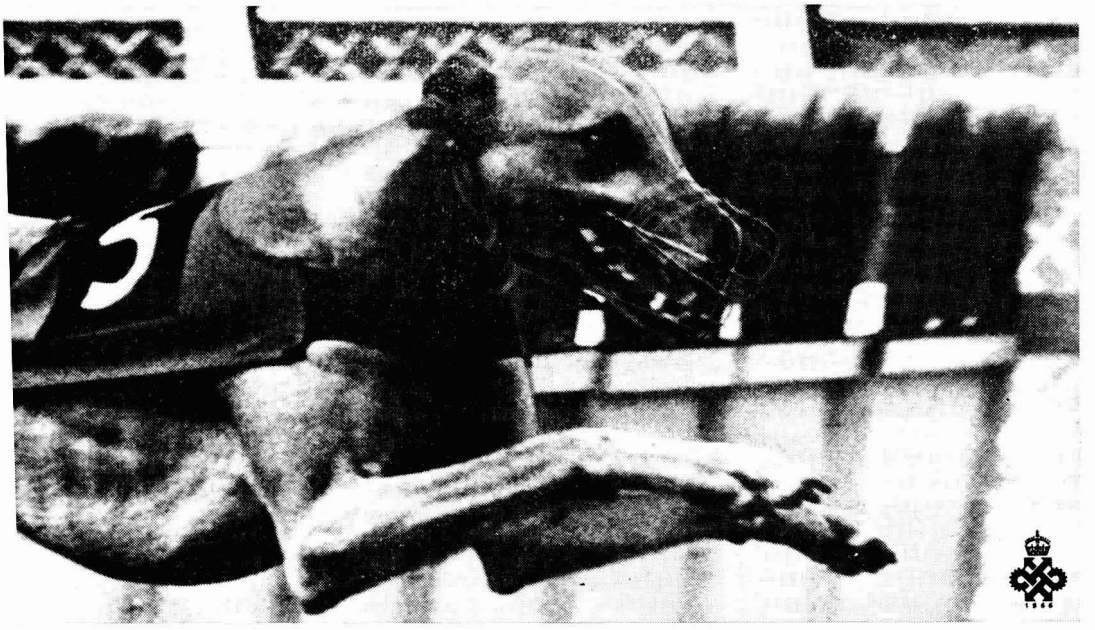

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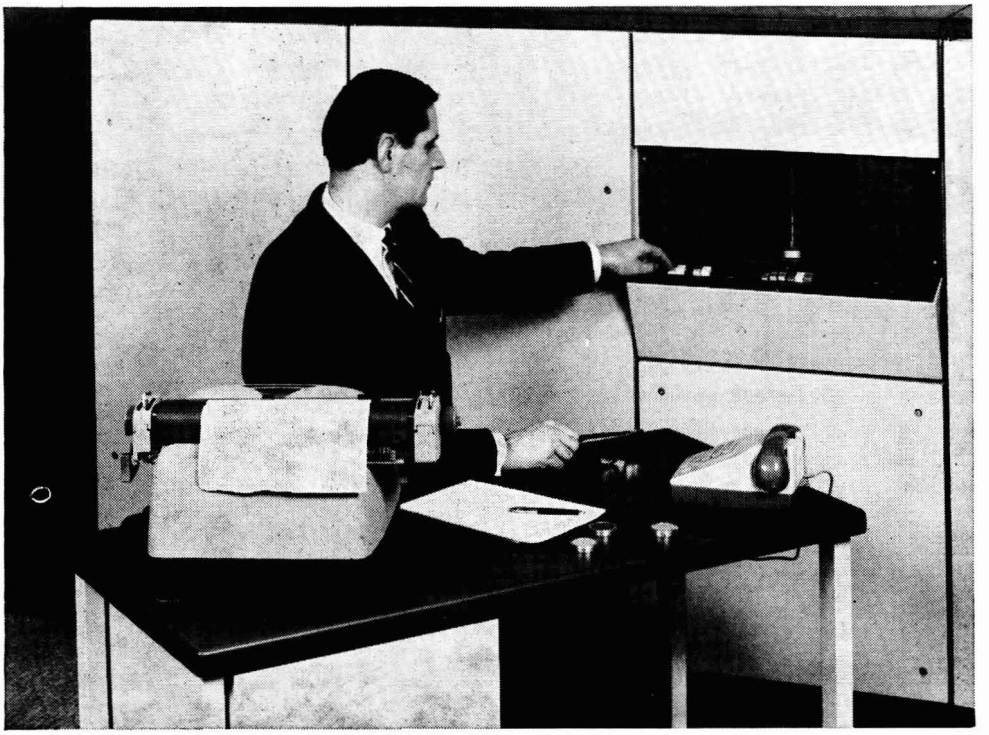

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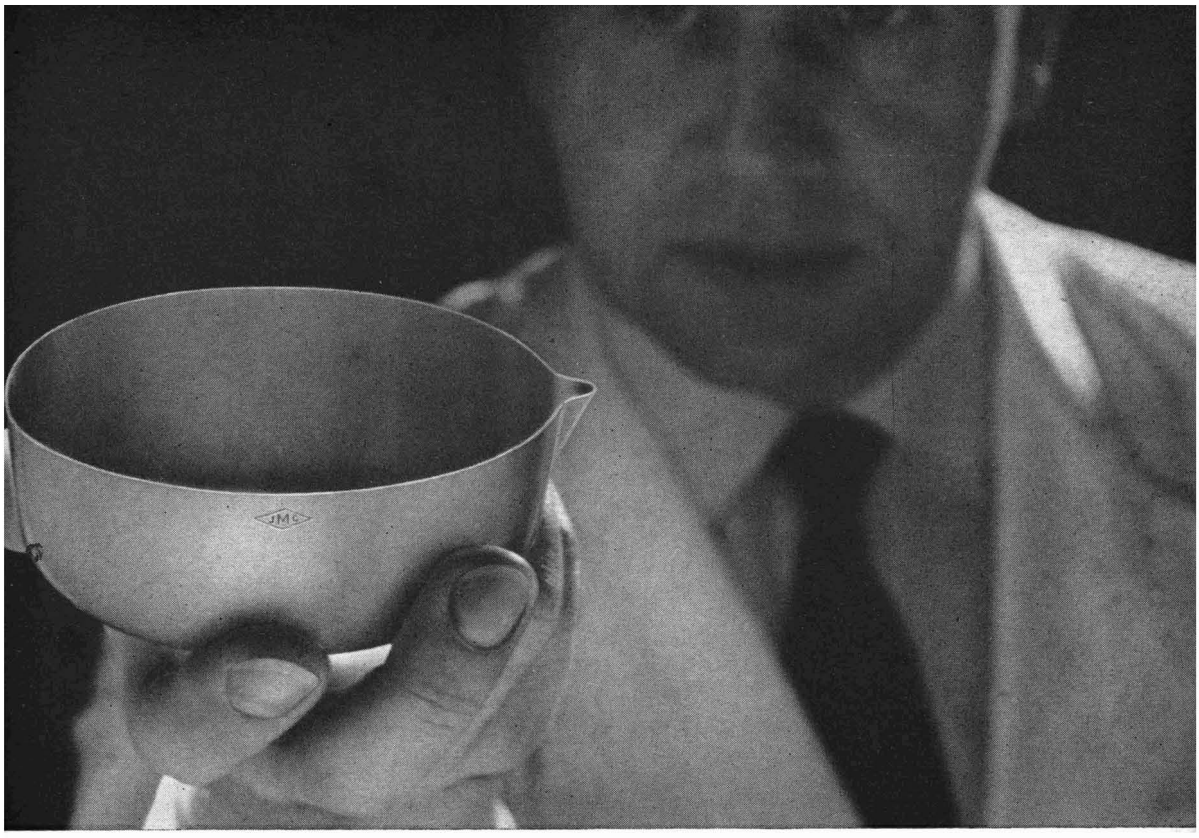

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