THE ANALYST

Recommended Methods of Assay of Crude Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS OF ASSAY OF CRUDE DRUGS

The Assay of Pyrethrum

THE Panel of the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry responsible for the preparation of this Report held its first meeting in February, 1960. Its membership was: Dr. W. Mitchell (Chairman), Dr. E. A. Baum (co-opted May, 1962), Mr. H. E. Coomber, Mr. L. Donegan, Dr. M. Elliott, Dr. J. R. Furlong (co-opted December, 1960; resigned April, 1962), Mr. A. D. Harford, Mr. S. C. Jolly, Mr. W. S. Manson, Mr. R. A. Rabnott, Mr. F. H. Tresadern and Dr. T. F. West.

Professor M. E. Alessandrini was appointed as a corresponding member in January, 1963. Dr. E. A. Parkin attended the first meeting as an observer, and promised to collaborate in biological aspects of the Panel's work at a later date, if necessary.

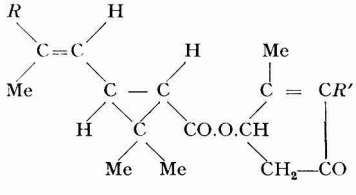
Miss A. M. Parry acted as Secretary until October, 1961, when she was succeeded first by Dr. C. H. Tinker, in November, 1962, by Miss V. Lewis and in November, 1963, by Mr. P. W. Shallis.

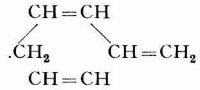
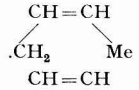
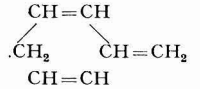
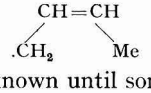
The Panel's terms of reference were: "To investigate methods of assay of pyrethrum flowers and pyrethrum extract with a view to recommending a standard chemical or physical method of assay."

REPORT

Pyrethrum consists of the dried flower heads of *Chrysanthemum cinerariaefolium* Vis (Fam. Compositae). The flowers themselves were mainly used in the powdered form in early days, but concentrated extracts have latterly been prepared from them, and it is in this form that pyrethrum is mainly used to-day. Consequently, most of the Panel's attention has been given to the assay of extracts; this has been a convenience in helping to eliminated sampling error to a large extent. Nevertheless, some attention has been given to the assay of flowers.

The insecticidal activity of pyrethrum is thought to reside in its four constituents, pyrethrin I, pyrethrin II, cinerin I and cinerin II. The formulae of these are shown below.



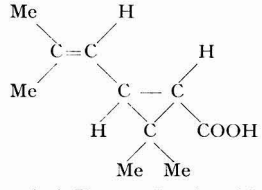
	R	*R'*
Pyrethrin I	Me	
Cinerin I	Me	
Pyrethrin II	MeO.CO	
Cinerin II	MeO.CO	

The existence of the cinerins was not known until some 15 years ago; before that it had become customary to estimate "pyrethrin I" (comprising true pyrethrin I and cinerin I) and "pyrethrin II" (comprising true pyrethrin II and cinerin II). This practice has been

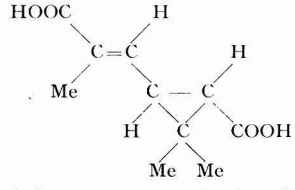
continued, and throughout this report the terms "pyrethrin I," "pyrethrin II" and total "pyrethrins" will be used as having that significance.

EVALUATION OF PYRETHRUM—

The active principles named above are neutral esters that do not lend themselves to direct determination, though methods of doing so have been proposed. One of the earlier methods used in practice, the Gnadinger - Corl method,[1] depended on determination of the keto-alcoholic components of the esters. It was soon superseded by methods involving the determination of the acids liberated on alkaline hydrolysis of the active principles. The earlier methods effected a separation of the two acids by utilising the fact that (+)-*trans*-chrysanthemic acid (derived from true pyrethrin I and from cinerin I) was volatile in steam, whereas (+)-*trans*-chrysanthemum dicarboxylic acid (derived from true pyrethrin II and from cinerin II) was not. The formulae of these two acids are shown below.



(+)-Chrysanthemic acid



(+)-Chrysanthemum dicarboxylic acid

TABLE I

RESULTS OF COLLABORATIVE TESTS ON KENYA PYRETHRUM EXTRACT BY MODIFIED P.B.K. METHOD

Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins" %	Mean, %
A	I	14·5	12·2	26·7	26·65
		14·3	12·3	26·6	
B	I	14·6	12·3	26·9	
		14·8	11·9	26·7	26·73
		14·6	12·0	26·6	
C	I	14·3	10·4	24·7	24·90
		14·6	10·5	25·1	
D	I	14·3	12·6	26·9	26·75
		14·0	12·6	26·6	
E	I	13·8	12·0	25·8	25·95
		14·0	12·1	26·1	
	II	13·8	12·3	26·1	25·90
		13·8	11·9	25·7	
F	I	14·1	12·5	26·6	
		13·7	12·5	26·2	26·60
		14·1	12·9	27·0	
	II	14·3	13·0	27·3	
		14·0	12·8	26·8	26·87
		14·0	12·5	26·5	
G	I	13·8	11·7	25·5	
		13·9	11·7	25·6	25·60
		13·8	11·9	25·7	
	II	13·5	11·9	25·4	25·55
		13·8	11·9	25·7	
H	I	14·5	11·1	25·6	
		14·7	11·4	26·1	25·87
		14·3	11·6	25·9	
	II	14·2	11·1	25·3	25·15
		14·1	10·9	25·0	
J	I	13·9	12·4	26·3	26·15
		14·0	12·0	26·0	

The best known and most widely used of these methods was that described by Seil.[2] Because the acids were determined separately, it was possible to calculate the amounts of "pyrethrin I" and of "pyrethrin II" present. It was subsequently shown[3,4,5] that some of the chrysanthemic acid became hydrated during the steam-distillation, the product not being volatile in steam, so that the results for "pyrethrin I" were low by some 15 per cent. This caused the method to be abandoned in favour of the "mercury-reduction" method.

This mercury-reduction method was originally devised by Wilcoxon[6] and modified by Holaday.[7] It depends on the fact that Denigès's reagent (defined on p. 702) is reduced by chrysanthemic acid. The reaction, the mechanism of which is not fully understood, is accompanied by a series of characteristic colour changes. The resulting mercurous sulphate can be determined volumetrically, but the reaction is not stoicheiometric; the factor used to calculate "pyrethrin I" was arrived at by collaborative work[8] in several laboratories, on pure chrysanthemic acid. The determination of "pyrethrin II" was virtually the same as in the Seil method.

TABLE II

RESULTS OF COLLABORATIVE TESTS ON BELGIAN CONGO PYRETHRUM EXTRACT BY MODIFIED P.B.K. METHOD

Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	Mean, %
A	I	14·8	10·2	25·0	
		14·8	10·4	25·2	25·03
		14·7	10·2	24·9	
	II	15·0	10·0	25·0	25·0
B	I	14·9	10·0	24·9	
		14·9	10·1	25·0	25·0
		15·1	10·0	25·1	
C	I	15·0	9·9	24·9	24·75
		14·8	9·8	24·6	
D	I	14·3	11·5	25·8	25·8
		14·3	11·5	25·8	
E	I	14·9	10·5	25·4	25·4
		14·9	10·5	25·4	
	II	14·5	10·4	24·9	25·0
		14·7	10·4	25·1	
G	I	14·4	10·4	24·8	24·9
		14·7	10·3	25·0	
	II	14·7	10·4	25·1	25·05
		14·8	10·2	25·0	
H	I	14·7	10·1	24·8	24·75
		14·7	10·0	24·7	
	II	13·9	9·8	23·7	23·9
		14·2	9·9	24·1	
J	I	14·6	10·8	25·4	
		15·2	11·2	26·4	
		15·8	10·6	26·4	
		15·6	11·1	26·7	25·81
		15·0	10·5	25·5	
		15·2	10·7	25·9	
		15·2	9·2	24·4	

Two variants of the mercury-reduction method have been used almost exclusively in recent years. One, the A.O.A.C. method, adopted by the Association of Official Agricultural Chemists,[9] is used in the United States of America (though it has been increasingly used in Europe recently). The other, used in most of the rest of the world, is the P.B.K. method, adopted by the Pyrethrum Board of Kenya.[10] An almost identical procedure, the E.A.E.C. method, was described by the East African Extract Corporation,[11] and a closely similar variant was described in the British Pharmaceutical Codex, 1954[12] and in the British Veterinary Codex, 1953.[13]

Having considered these methods, the Panel decided to work initially on the mercury-reduction method; because the P.B.K. variant was then in almost exclusive use in the U.K.,

TABLE III

EFFECT OF DIFFERENT SAMPLES OF LIGHT PETROLEUM ON THE DETERMINATION OF "PYRETHRINS" IN BELGIAN CONGO EXTRACT

Laboratory	Aromatic content of light petroleum, % by volume	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	Mean, %
With laboratories' own supply of light petroleum—						
A	<0·01	I	15·0	10·6	25·6	25·5
			14·8	10·6	25·4	
		II	15·0	10·5	25·5	25·45
			14·8	10·6	25·4	
B	0·01	I	14·9	10·3	25·2	25·45
			15·1	10·6	25·7	
C	0·06	I	14·8	10·6	25·4	25·4
			14·8	10·6	25·4	
D	—	I	14·5	10·2	24·7	25·0
			14·9	10·2	25·1	
			14·5	10·7	25·2	
E	0·001	I	14·7	10·6	25·3	25·35
			14·7	10·7	25·4	
		II	14·3	10·7	25·0	25·0
F	0·73	I	14·9	10·4	25·3	25·1
			14·9	10·0	24·9	
		II	14·8	10·2	25·0	24·85
			14·7	10·0	24·7	
G	0·004	I	14·7	10·4	25·1	25·15
			14·8	10·4	25·2	
		II	14·7	10·4	25·1	25·1
H	0·12	I	15·2	10·1	25·3	25·4
			15·1	10·4	25·5	
		II	14·6	10·1	24·7	24·8
			14·7	10·2	24·9	
With light petroleum containing 2 per cent. by volume of added benzene—						
A		I	14·7	10·8	25·5	25·4
			14·7	10·6	25·3	
		II	14·9	10·2	25·1	25·25
			15·1	10·3	25·4	
B		I	14·7	10·2	24·9	25·25
			15·1	10·5	25·6	
C		I	15·1	10·5	25·6	25·55
			15·0	10·5	25·5	
D		I	14·7	10·6	25·3	25·3
E		I	14·5	10·6	25·1	25·15
			14·5	10·7	25·2	
		II	14·5	10·9	25·4	25·4
F		I	14·9	10·9	25·8	25·3
			14·5	10·3	24·8	
		II	15·0	10·6	25·6	25·6
			15·0	10·6	25·6	
G		I	14·6	10·3	24·9	24·8
			14·5	10·4	24·9	
			14·6	10·1	24·7	
H		I	15·0	10·3	25·3	25·5
			15·3	10·4	25·7	
		II	14·1	10·2	24·3	24·65
			14·7	10·3	25·0	

it was selected for initial study. Further, it was known that it gave results for "pyrethrin I" some 15 per cent. higher than those obtained by the A.O.A.C. method; published work[14,15] had suggested that this was because chrysanthemic acid was lost at one stage in the A.O.A.C. method, and that the P.B.K. method therefore gave a truer assessment of the content of "pyrethrin I."

Experimental

Because experience had established that analysts in East Africa consistently got higher results for "pyrethrin I" than did U.K. analysts, it was thought that this discrepancy might arise because of some difference in procedure or in interpretation. Accordingly, it was decided to redraft the P.B.K. method in considerable detail and to carry out collaborative tests by it on a shared sample of Kenya pyrethrum extract (ordinary grade), light petroleum supplied by A. D. Harford being used. The results are shown in Table I.

With one exception, the agreement between these results was surprisingly good, though the differences, relatively small as they were, would in commercial practice probably have been submitted to arbitration, because of the high value of the material.

Further trials of the method, on a sample of Belgian Congo extract (ordinary grade), gave even better agreement between results; see Tables II and III. The results in Table III also appeared to establish that the purity of the light petroleum used for the preliminary treatment was less important than had been thought, and that commercial grade "aromatics-free light petroleum" was suitable. Later, F. H. Tresadern established that the addition of as much as 6 per cent. v/v of cyclohexane to light petroleum increased the "pyrethrin II" results by about 5 per cent.; thus a small content of alicyclic hydrocarbons would also appear admissible.

Further collaborative tests were carried out on the same sample of Belgian Congo pyrethrum extract, and also on samples of Kenya and English extracts. Agreement continued to be satisfactory (much better than normally obtained in ordinary commercial practice) and it was decided that nothing would be gained by carrying out further tests on extracts. These results are recorded in Tables IV, V and VI. Further small modifications to the method were adopted; the modified method is given in Appendix I.

Table IV

Results of collaborative tests on Belgian Congo extract by a further modification of the P.B.K. method

Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	Mean, %
A	I	14·6	10·6	25·2	25·2
B	I	14·3	9·8	24·1	24·1
		14·3	9·8	24·1	
C	I	14·8	10·6	25·4	25·45
		14·8	10·7	25·5	
D	I	14·6	10·4	25·0	25·1
		14·8	10·4	25·2	
E	I	14·8	10·3	25·1	25·2
		14·9	10·4	25·3	
	II	14·6	10·3	24·9	24·9
F	I	14·2	10·3	24·5	24·75
		14·7	10·3	25·0	
	II	15·0	10·4	25·4	25·45
		15·0	10·5	25·5	
G	I	14·9	10·5	25·4	25·35
		14·7	10·6	25·3	
	II	14·7	10·4	25·1	25·1

At this stage, the E.A.E.C. laboratory, in Nairobi, Kenya, and the laboratories of the Cooper Technical Bureau and of Stafford Allen & Sons Ltd., in the U.K., adopted the Panel's method for commercial analyses. Unfortunately, inter-laboratory agreement was no better than formerly, the U.K. laboratories regularly returning significantly lower results for "pyrethrin I." On the assumption that higher ambient laboratory temperatures would

TABLE V

RESULTS OF COLLABORATIVE TESTS ON KENYA EXTRACT BY A FURTHER MODIFICATION OF THE P.B.K. METHOD

Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	Mean, %
A	I	13·5	11·0	24·5	24·6
		13·6	11·1	24·7	
	II	13·6	11·1	24·7	24·7
		13·6	11·1	24·7	
B	I	12·7	10·3	23·0	23·0
		12·8	10·2	23·0	
C	I	15·0	11·0	26·0	25·95
		14·7	11·2	25·9	
D	I	13·4	11·4	24·8	24·8
		13·2	11·6	24·8	
E	I	13·5	10·9	24·4	24·45
		13·6	10·9	24·5	
	II	13·7	10·7	24·4	24·4
F	I	13·6	11·0	24·6	24·45
		13·4	10·9	24·3	
	II	13·4	11·5	24·9	24·65
		13·2	11·2	24·4	
G	I	13·3	11·1	24·4	24·5
		13·4	11·2	24·6	
H	I	14·5	11·1	25·6	25·55
		14·3	11·2	25·5	
	II	13·3	10·4	23·7	23·85
		13·3	10·7	24·0	

TABLE VI

RESULTS OF COLLABORATIVE TESTS ON ENGLISH EXTRACT BY A FURTHER MODIFICATION OF THE P.B.K. METHOD

Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	Mean, %
A	I	14·8	12·0	26·8	26·85
		14·7	12·2	26·9	
	II	14·8	12·2	27·0	27·0
		14·7	12·3	27·0	
B	I	14·8	11·6	26·4	26·25
		14·6	11·5	26·1	
C	I	15·9	12·3	28·2	28·1
		16·0	12·0	28·0	
D	I	14·2	12·4	26·8	26·75
		14·3	12·4	26·7	
E	I	14·7	11·7	26·4	26·45
		14·7	11·8	26·5	
	II	15·0	11·6	26·6	26·6
F	I	14·5	12·3	26·8	26·7
		14·4	12·2	26·6	
	II	14·3	12·4	26·7	26·75
		14·4	12·4	26·8	
G	I	14·1	12·4	26·5	26·6
		14·1	12·6	26·7	
H	I	14·7	12·3	27·0	26·95
		14·7	12·2	26·9	
	II	14·4	11·6	26·0	25·95
		14·3	11·6	25·9	

apply in Kenya, the two U.K. laboratories simulated these conditions, and found that higher results for "pyrethrin I" were in fact obtained. However, a collaborative trial by the Panel failed to confirm this finding.

It was then decided to carry out collaborative tests on a sample of recently ground pyrethrum flowers (the method described in Appendix III was used, the pyrethrins "I" and "II" being subsequently determined as described in Appendix I). Comparison was made between the results obtained by warm extraction (Soxhlet) and by continuous cold extraction in an apparatus described by Barker.[16] In both methods extraction was followed by a chilling procedure. Concordance of results was very good, as was agreement between the two sets of results (see Table VII). It was decided to carry out similar tests on a sample of one-year-old pyrethrum flowers, to see whether older flowers might behave differently. In fact, concordance of results, and agreement between the two sets of results, was just as good as for recently harvested flowers (see Table VIII). Since warm extraction is probably more convenient in practice, it was decided to adopt it. The recommended procedure for pyrethrum flowers is given in Appendix III.

TABLE VII

RESULTS OF COLLABORATIVE TESTS ON PYRETHRUM FLOWERS

		Warm extraction			Cold extraction		
Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %
A	I	0·89	0·66	1·55	0·89	0·66	1·55
		0·88	0·65	1·53	0·88	0·65	1·53
	II	0·85	0·66	1·51	0·84	0·67	1·51
		0·86	0·66	1·52	0·84	0·67	1·51
B	I	0·86	0·65	1·51	0·84	0·63	1·47
		0·87	0·64	1·51	0·84	0·63	1·47
		0·88	0·64	1·52	0·85	0·63	1·48
C	I	0·60	0·90	1·50	0·88	0·70	1·58
		0·63	0·90	1·53	0·81	0·71	1·52
D	I	0·83	0·63	1·46	0·79	1·18	1·97
		0·84	0·64	1·48	0·79	1·07	1·86
E	I	0·86	0·64	1·50	0·81	0·61	1·42
		0·86	0·64	1·50	0·81	0·63	1·44
	II	0·85	0·65	1·50	0·81	0·63	1·44
		0·85	0·64	1·49			
F	I	0·87	0·67	1·54	0·89	0·63	1·52
		0·90	0·67	1·57	0·88	0·61	1·49
	II	0·86	0·66	1·52	0·89	0·64	1·53
		0·90	0·66	1·56	0·90	0·65	1·55
G	I	0·88	0·68	1·56	0·84	0·68	1·52
		0·87	0·67	1·54	0·86	0·64	1·50
		0·88	0·70	1·58	0·85	0·68	1·53
H	I	0·84	0·62	1·46			
		0·84	0·62	1·46			
	II	0·84	0·61	1·45			
		0·84	0·61	1·45			

At about this time, the Panel was informed by Mr. D. McClellan (Associate Referee for Pyrethrins to the Association of Official Agricultural Chemists, in the U.S.A.) that the A.O.A.C. was examining the modified "pyrethrin II" determination recommended by Mitchell and Tresadern.[17] A collaborative trial of this modification by the Panel showed, rather surprisingly, that most members found it not to give lower results for "pyrethrin II." This was at variance with the results duly recorded by the U.S. Committee, results that fully supported the original claim to the extent that the modification has now been incorporated into the A.O.A.C. method.[18]

Independent work had in the meantime been under way in the laboratories of the Cooper Technical Bureau and of Stafford Allen & Sons Ltd. A publication from the former[19] showed that the amount of chrysanthemic acid (free and combined) present in pyrethrum

TABLE VIII

RESULTS OF COLLABORATIVE TESTS ON OLD PYRETHRUM FLOWERS

		Warm extraction			Cold extraction		
Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %
B	I	0·69	0·53	1·22	0·68	0·54	1·22
		0·70	0·53	1·23	0·68	0·54	1·22
D	I	0·67	0·57	1·24	0·64	0·49	1·13
		0·69	0·57	1·26	0·63	0·53	1·16
E	I	0·68	0·57	1·25	0·65	0·53	1·18
		0·67	0·58	1·25	0·63	0·51	1·14
	II	0·69	0·56	1·25			
		0·67	0·60	1·27			
F	I	0·70	0·56	1·26	0·70	0·55	1·25
		0·69	0·58	1·27	0·67	0·54	1·21
	II	0·69	0·57	1·26	0·64	0·55	1·19
		0·70	0·56	1·26			
G	I	0·66	0·58	1·24	0·66	0·57	1·23
		0·65	0·57	1·22	0·66	0·58	1·24
	II	0·66	0·57	1·23	0·65	0·56	1·21
H	I	0·68	0·56	1·24	0·64	0·50	1·14
		0·69	0·57	1·26	0·65	0·50	1·15
	II	0·71	0·58	1·29	0·63	0·49	1·12
		0·71	0·58	1·29	0·63	0·49	1·12

TABLE IX

RESULTS OF COLLABORATIVE TESTS BY THE A.O.A.C. METHOD ON TWO SAMPLES OF KENYA PYRETHRUM EXTRACT

		Sample (1)			Sample (2)		
Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %
A	I	11·9	11·3	23·2	11·2	11·1	22·3
		12·0	11·4	23·4	11·5	11·3	22·8
	II	12·2	11·0	23·2			
		11·9	11·6	23·5			
B	I	10·9	10·2	21·1	11·2	10·4	21·6
		10·7	10·3	21·0	11·0	10·4	21·4
D	I	11·3	11·0	22·3	10·9	11·0	21·9
		11·1	11·0	22·1	10·7	10·8	21·5
E	I	11·7	10·3	22·0	11·2	10·2	21·4
		11·8	10·2	22·0	11·2	10·1	21·3
	II	11·5	10·6	22·1	10·8	10·6	21·4
		11·6	10·5	22·1	10·8	10·6	21·4
F	I	11·9	11·3	23·2	11·4	11·4	22·8
		12·0	11·4	23·4	11·4	11·3	22·7
	II	11·5	11·6	23·1	11·3	11·4	22·7
		11·6	11·5	23·1	11·3	11·3	22·6
G	I	11·9	10·8	22·7	11·2	10·7	21·9
		12·1	10·9	23·0	11·4	10·7	22·1
					11·3	10·6	21·9
H	I	11·5	11·1	22·6	10·9	10·5	21·4
		11·5	10·8	22·3	10·9	10·6	21·5
	II	11·2	10·4	21·6	10·7	10·8	21·5
		11·4	10·4	21·8	10·4	10·8	21·2

extracts is less than is indicated by the P.B.K. method. Substantially similar results were obtained by each of three procedures—

(1) steam-distillation, under conditions not resulting in hydration of part of the chrysanthemic acid,[3]
(2) infrared measurement,
(3) a chromatographic separation on silica gel.

A publication[20] from the second laboratory concentrated on infrared measurements, and results agreed closely with those obtained by the first laboratory. Details of a proposed infrared method were given. Results for "pyrethrin I" were some 12 to 14 per cent. lower than those by the P.B.K. method. Further, the results were closely similar to those by the A.O.A.C. method.

These important findings caused the Panel to re-assess the whole matter. Because it had been shown[14] that chrysanthemic acid was retained on the barium sulphate residue discarded during the A.O.A.C. assay, it had been assumed that this explained the lower results, and the P.B.K. method had therefore been regarded (outside the U.S.A.) as more accurate. It now seemed likely that much of the extraneous acidic matter, responsible for the higher P.B.K. results, was retained by the barium sulphate residue from the A.O.A.C. procedure, but that enough passed through to compensate approximately for the lost chrysanthemic acid—in other words, it seemed that the A.O.A.C. method might give the "correct" result by a summation of positive and negative errors.

TABLE X

RESULTS OF COLLABORATIVE TESTS ON SIX SAMPLES OF PYRETHRUM EXTRACT BY THE MODIFIED A.O.A.C. METHOD

Sample	Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %
(*a*)*	A	I	24·1	22·6	46·7
			24·3	22·6	46·9
	B	I	23·3	23·7	47·0
	D	I	22·7	22·7	45·4
	E	I	23·7	22·8	46·5
	F	I	23·8	22·7	46·5
			23·6	22·7	46·3
	G	I	23·6	22·2	45·8
	H	I	22·9	21·6	44·5
			24·0	21·8	45·8
			24·9	22·5	47·4
		II	23·8	22·5	46·3
			24·0	22·5	46·5
(*b*)	A	I	11·8	11·3	23·1
			11·5	10·7	22·2
	B	I	11·2	10·7	21·9
	D	I	11·2	11·9	23·1
	E	I	11·4	11·0	22·4
	G	I	11·7	11·5	23·2
	H	I	11·6	11·1	22·7
			11·9	10·9	22·8
		II	11·5	10·6	22·1
			11·5	10·8	22·3
(*c*)	A	I	11·2	11·4	22·6
	B	I	10·7	11·3	22·0
	D	I	10·8	11·9	22·7
	E	I	11·1	11·3	22·4
	G	I	11·3	11·2	22·5
	H	II	11·0	11·1	22·1
			11·0	11·0	22·0

* A purified extract containing approximately twice the total "pyrethrins" content of the other samples

TABLE X—*continued*

Sample	Laboratory	Analyst	"Pyrethrin I" found, %	"Pyrethrin II" found, %	Total "pyrethrins," %
(*d*)	A	I	12·9	10·9	23·8
			12·9	10·9	23·8
	B	I	12·3	10·9	23·2
	D	I	12·4	11·4	23·8
	E	I	12·7	10·3	23·0
	F	I	13·0	10·9	23·9
	G	I	12·8	11·2	24·0
			12·9	11·1	24·0
	H	I	13·1	10·1	23·2
			13·1	10·4	23·5
		II	12·8	10·9	23·7
			13·0	10·8	23·8
(*e*)	A	I	12·1	12·7	24·8
	B	I	11·6	12·4	24·0
	D	I	11·8	13·3	25·1
	E	I	11·9	12·7	24·6
	G	I	11·9	12·9	24·8
	H	I	12·3	12·7	25·0
			12·3	12·6	24·9
		II	11·8	12·3	24·1
			12·2	12·3	24·5
(*f*)	A	I	11·9	12·1	24·0
	B	I	11·5	11·9	23·4
	D	I	11·4	12·8	24·2
	E	I	11·5	12·1	23·6
	G	I	11·3	11·7	23·0
	H	I	11·6	12·0	23·6
			11·6	11·6	23·2
			11·6	12·4	24·0
		II	11·5	12·0	23·5
			11·5	11·9	23·4

These results, coupled with the knowledge that increasingly buyers, *outside* the U.S.A., were calling for sales to be effected on the basis of A.O.A.C. tests, and the fact that it was therefore troublesome to have two assays currently in use, led the Panel to decide not to pursue work on the P.B.K. method, but simply to record what had been done. Instead, it was decided to re-examine the A.O.A.C. method. With this in view, Mr. McClellan was asked to provide details of the latest revision of the A.O.A.C. method, incorporating the revised determination of "pyrethrin II." This information was duly received. As a result of Mr. McClellan's answers to various questions about details of the method, a version revised in minor details, and relevant to concentrated extracts, was prepared by the Panel. In particular, provision was made for "blank" determinations; it seems unfortunate that the A.O.A.C. method does not include this requirement.

A collaborative test by this method on two ordinary-grade Kenya pyrethrum extracts was undertaken. The results are shown in Table IX. These were not entirely satisfactory; although four of the participants got results in close agreement, one laboratory in E. Africa and one in the U.K. got results, themselves in close agreement, but some 4 per cent. higher than those of the rest of the participants—this applied to both samples.

Accordingly, it was decided to test four further samples of ordinary-grade pyrethrum extracts and two of decolorised extracts. Because W. S. Manson had shown that Filtercel caused a slight loss (at the purification stage) from ordinary-grade extracts, but a serious loss from decolorised extracts, it was decided that Filtercel should not be used when the solution of a decolorised pyrethrum extract in light petroleum (at the purification stage) was bright, or not more than slightly opalescent—as is also recommended in the W.H.O. method.[21]

A further revision of certain details of the A.O.A.C. method was made; its final form is given in Appendix II.

The results obtained on these extracts are shown in Table X. The concordance of results was considered as satisfactory as could reasonably be expected in any collaborative trial. In this respect, the results were not in general superior to those obtained by the modified P.B.K. method. As expected, the figures for "pyrethrin I" were lower than when the P.B.K. method was used.

Recommendation

The Panel considers that methods involving determination of "pyrethrin I" by the mercury-reduction procedure are inherently unsatisfactory, and also believes that results for "pyrethrin II" may be falsely high (even with application of the modified procedure[17] now adopted by the A.O.A.C.). Nevertheless, the Panel, bearing in mind the results it has obtained, and taking into account commercial considerations now prevailing, feels justified in recommending that the A.O.A.C. method,[9,18] as slightly modified by the Panel and described in Appendix II, be recommended for general use as an interim measure.

Other methods for determining "pyrethrins"

Several members of the Panel are currently carrying out a collaborative trial of the ethylenediamine method (E.D.A.), originally devised by Hogsett, Kacy and Johnson[22] for the determination of allethrin, and now suitably modified for application to pyrethrum extracts. Pending the outcome of this trial, discussion on alternative methods has been deferred. However, besides the E.D.A. method, the dinitrophenylhydrazine method (D.N.P.) in the latest form described by Head,[23] the infrared method described by Mitchell, Byrne and Tresadern,[20] and both the steam-distillation method and the chromatographic method described by Brierley and Brown[19] deserve consideration. The Panel is also aware that two of its members are studying the determination of "pyrethrin II"; that they have evidence that, just as for "pyrethrin I," some false material is being included by present methods; and that they may in due course be able to offer suggested alternative procedures. Methods have also to be considered for the analysis of formulated products containing, besides pyrethrum, synergists and other insecticides.

A pyrethrum assay method giving absolute results need not necessarily give a better correlation with biological activity, unless it measures the amounts of all active principles—and this is unlikely to be a practicable outcome for routine application. Further, it is unlikely that a more accurate method would give better agreement between results than the Panel has achieved by the modified A.O.A.C. method, and previously by the modified P.B.K. method.

Appendix I

Modified P.B.K. method for the assay of pyrethrum extracts

Apparatus—

The special apparatus required is described in Appendix II.

Reagents—

Unless otherwise indicated, all reagents should be of analytical grade. Water must be distilled or de-ionised. Details of most of the reagents are given in Appendix II; the others required are described below.

Ethanolic potassium hydroxide solution—A 2·8 per cent. w/v solution of potassium hydroxide in ethanol.

Acetone—B.P. or analytical-reagent grade.

Carbon dioxide-free water—B.P. grade.

Method

Sampling—

Keep the extract at 20° to 25° C for at least 2 hours, mix thoroughly, and weigh into a 150-ml flask sufficient of the extract to contain 0·12 to 0·13 g of total "pyrethrins."

PRELIMINARY TREATMENT OF SAMPLE—

To the sample in the flask, add 50 ml of light petroleum, swirl the liquid, add 1 g of Filtercel, swirl the liquid again to mix completely, stopper the flask, and set it aside at 20° to 22° C for 16 hours.

Thoroughly mix the contents of the flask, filter them under gentle suction through a previously prepared Gooch crucible, the asbestos pad of which has been washed first with ethanol and then with light petroleum, and wash the residue with 50 ml of light petroleum, in successive small portions. Distil off the light petroleum from the combined filtrate and washings on a water-bath, until the final volume of the liquid is reduced to 1 to 2 ml, add 20 ml of ethanolic potassium hydroxide solution, and boil the mixture for 45 minutes under reflux; use an air condenser. Transfer the solution to a 600-ml beaker, and rinse out the flask into the beaker with sufficient hot water to give a volume of 200 ml in the beaker. Concentrate the solution to 150 ml on a hot-plate, cool rapidly to room temperature, and transfer to a 250-ml calibrated flask. Rinse out the beaker into the flask with three successive 20-ml portions of water, transferring any gummy material from the beaker to the flask. Add 1 g of Filtercel and 10 ml of barium chloride solution, swirl the liquid gently, dilute to the mark with water, shake the flask vigorously until the supernatant liquid is "bright," and filter the suspension through a fluted 15-cm Whatman No. 1 filter-paper.

DETERMINATION OF "PYRETHRIN I"—

Transfer 200 ml of the filtrate to a 500-ml separating funnel, rinse in with two successive 5-ml portions of water, and add 1 drop of phenolphthalein indicator solution. Neutralise the solution by the dropwise addition of concentrated hydrochloric acid, and add 1 ml of the acid in excess. Add 5 ml of sodium chloride solution and 50 ml of light petroleum, shake the funnel vigorously for 1 minute, allow the layers to separate, and run off the lower aqueous layer as completely as possible. Filter the light-petroleum extract through a plug of cotton-wool, and collect the filtrate in a 250-ml separating funnel containing 10 ml of water. Return the aqueous layer to the 500-ml separating funnel, and repeat the extraction with a 50-ml and then a 25-ml portion of light petroleum, reserving the aqueous layer for the determination of "pyrethrin II," and filter the light-petroleum extracts through the same plug of cotton-wool into the same 250-ml separating funnel. Shake the combined light-petroleum extracts for about 30 seconds with the 10 ml of water, allow the layers to separate, and run off the lower aqueous layer and add it to the aqueous liquid reserved for the determination of "pyrethrin II." Repeat the washing of the light-petroleum extracts with a further 10 ml of water, and again add the washings to the reserved aqueous liquid.

Add 5 ml of dilute sodium hydroxide solution to the light-petroleum extract, shake the funnel vigorously for 1 minute, allow the layers to separate, and run off the lower aqueous layer into a 150-ml conical flask, leaving any interfacial "cuff" in the funnel and draining the stem of the funnel completely. Wash the separator stem by adding 1 ml of water to the funnel and running it through into the flask, again draining the stem carefully. Repeat the extraction of the light-petroleum solution with a 2·5-ml portion and then a 1·5-ml portion of water; shake for about 30 seconds each time and add the extracts to the alkaline extract in the flask. Use 10 ml of mercuric sulphate solution to rinse down the tip of the funnel stem and neck of the conical flask and allow it to run into the alkaline solution in the flask; then stopper the flask, swirl the liquid, and set aside in complete darkness at 25° $\pm$ 0·5° C for exactly 60 minutes from the addition of the mercuric sulphate solution. Add 20 ml of acetone and 3 ml of sodium chloride solution, heat the mixture to boiling on a water-bath, allow the precipitate to settle, decant the supernatant liquid through a folded 9-cm Whatman No. 1 filter-paper, and drain carefully, retaining most of the precipitate in the flask. Wash the precipitate in the flask with 10 ml of acetone, again boil, and decant the supernatnat liquid through the same filter-paper. Repeat the washing and decanting with three successive 10-ml portions of hot chloroform. Transfer the filter-paper to the flask, add 50 ml of diluted hydrochloric acid, 1 ml of iodine monochloride solution and 6 ml of chloroform, and titrate with 0·01 M potassium iodate, running into the flask in one portion nearly all the required volume of titrant and shaking the flask vigorously for about 30 seconds after each addition, until the chloroform is colourless.

Carry out a blank determination by the entire procedure, omitting the pyrethrum extract; the blank titre should not exceed 0·1 ml. Calculate the percentage of "pyrethrin I" from the

difference between the two titres; each millilitre of 0·01 M potassium iodate is equivalent to 0·0057 g of "pyrethrin I."

DETERMINATION OF "PYRETHRIN II"—

Transfer the combined reserved aqueous liquids from the determination of "pyrethrin I" to a 600-ml beaker, cover with a clock-glass, place on an asbestos plate having a 3-inch diameter hole covered by an asbestos gauze, and evaporate over a bunsen flame to 50 ml; the evaporation should be completed in 35 to 45 minutes. Cool the contents of the beaker, rinse any droplets of liquid on the underside of the clock-glass into the beaker with not more than 5 ml of water, and filter the liquid through a plug of cotton-wool into a 500-ml separating funnel, rinsing in with a 10-ml portion and then with two successive 7·5-ml portions and two successive 5-ml portions of water, the filter being allowed to drain completely each time before the next rinsing is added.

Saturate the aqueous liquid in the separating funnel with sodium chloride (about 30 to 35 g will be required), add 10 ml of hydrochloric acid and 50 ml of ether, shake the funnel for 1 minute, allow the layers to separate completely, and run off the lower aqueous layer. Repeat the extraction of the aqueous layer with a 50-ml portion and then two successive 25-ml portions of ether. Wash the combined ether extracts with three successive 10-ml portions of sodium chloride solution, removing as much of the aqueous layer as possible, and transfer the ether layer to a 250-ml conical flask, rinsing in with 10 ml of ether. Distil off the ether on a water-bath, and remove the ether vapour in the flask with a gentle current of air. Dry the residue at 100° C for 10 minutes, removing any residual hydrochloric acid fumes with a gentle current of air. Add 2 ml of neutralised ethanol and 1 drop of phenolphthalein indicator solution, swirl the liquid to dissolve the residue, add 20 ml of freshly boiled and cooled carbon dioxide-free water, and titrate rapidly with 0·02 N sodium hydroxide to a brownish-pink end-point that persists for 30 seconds; keep the flask stoppered between additions of alkali.

Carry out a blank determination by repeating the procedure on the aqueous liquid reserved in the blank determination for "pyrethrin I"; the blank titre should not exceed 0·1 ml. Calculate the percentage of "pyrethrin II" from the difference between the two titres; each millilitre of 0·02 N sodium hydroxide is equivalent to 0·00374 g of "pyrethrin II."

Appendix II

RECOMMENDED METHOD FOR THE ASSAY OF PYRETHRUM EXTRACTS

PRINCIPLE OF METHOD—

After removal of inert polymerised material from a light-petroleum solution of the extract, the active esters are hydrolysed, and the liberated chrysanthemic acid and chrysanthemum dicarboxylic acid are converted to their water-soluble barium salts and separated from insoluble materials by filtration. After the barium salts have been converted to the free acids with sulphuric acid and the precipitated barium sulphate has been removed, the chrysanthemic acid from "pyrethrin I" is extracted with light petroleum, then taken into aqueous solution by extraction with alkali, and allowed to react with mercuric sulphate. The mercury[1] formed by the reaction is titrated with potassium iodate solution, and the amount of "pyrethrin I" present in the extract is obtained by application of an empirical factor.

The chrysanthemum dicarboxylic acid remaining in the aqueous solution after extraction of the chrysanthemic acid is extracted with ether, the ether is removed from the extract, the residue is extracted with hot water to isolate the acid and the solution is then filtered. The filtrate is titrated with standard alkali, and the amount of "pyrethrin II" present in the extract is obtained by application of another empirical factor.

APPLICABILITY—

The method is generally applicable.

APPARATUS—

Gooch crucible—Size 4 (Royal Worcester porcelain), capacity 45 ml, with perforated bottom.

Separating funnels—Of appropriate sizes, with stems shortened to 3 to 5 cm.

REAGENTS—

Unless otherwise indicated, all reagents should be of analytical grade. Water must be distilled or de-ionised.

Light petroleum—Boiling-range 40° to 60° C. Commercially available, aromatics-free.

*Filtercel**—A natural diatomaceous earth obtainable from Johns-Manville Co. Ltd., 20 Albert Embankment, London, S.E.11.

Asbestos—"For Gooch crucibles," obtainable from The British Drug Houses Ltd., Laboratory Chemicals Division, Poole, Dorset.

Ethanol, 95 per cent. v/v—B.P. grade.

Ethanol, dehydrated, 99 per cent. v/v—Aldehyde-free.

Ethanolic sodium hydroxide solution—A 4 per cent. w/v solution of sodium hydroxide in 95 per cent. ethanol.

Barium chloride solution—A 10 per cent. w/v solution of barium chloride, $BaCl_2.2H_2O$, in water.

Phenolphthalein indicator solution—A 1 per cent. w/v solution of phenolphthalein in 95 per cent. ethanol. If it is necessary to neutralise the solution, adjust its colour to a faint pink by adding 0·01 N sodium hydroxide or 0·01 N hydrochloric acid.

Diluted sulphuric acid—Add gradually and cautiously 1 volume of sulphuric acid, sp.gr. 1·84, to 4 volumes of water, mix, and cool.

Dilute sodium hydroxide solution—A 0·4 per cent. w/v solution of sodium hydroxide in water.

Mercuric sulphate solution (Denigès's reagent)—Mix 5·0 g of yellow mercuric oxide B.P. with 40 ml of water, and, while stirring, add slowly 20 ml of sulphuric acid, sp.gr. 1·84. Add a further 40 ml of water, and stir until the mercury salt is completely dissolved. Test for the absence of mercury[1] by adding a few drops of iodine monochloride solution to 10 ml of the reagent solution and titrating with 0·01 M potassium iodate as described below under "Determination of Pyrethrin I," beginning at "Add to the contents of the flask 50 ml of diluted hydrochloric acid, . . ." Reject the reagent if the titre exceeds 0·2 ml of 0·01 M potassium iodate solution.

Sodium chloride.

Sodium chloride solution—A saturated solution of sodium chloride in water.

Chloroform—B.P. grade.

Hydrochloric acid, sp.gr. 1·16 to 1·18.

Diluted hydrochloric acid—Mix 3 volumes of hydrochloric acid with 2 volumes of water, and cool.

Iodine monochloride solution—Dissolve 10·0 g of potassium iodide and 6·4 g of potassium iodate in 75 ml of water in a glass-stoppered bottle; add 75 ml of hydrochloric acid and 5 ml of chloroform. If necessary, add dropwise, with vigorous shaking, approximately 0·1 M potassium iodide until a faint iodine colour develops in the chloroform. Add similarly, approximately 0·01 M potassium iodate until the chloroform is just colourless.

Store the solution in a cool, dark place. Before use, re-adjust with 0·01 M potassium iodate or 0·1 M potassium iodide.

Potassium iodate, 0·01 M—Dissolve 2·14 g of potassium iodate, previously dried at 105° C for 1 hour, in water, and dilute to 1 litre.

1 ml of solution ≡ 0·0057 g of "pyrethrin I."

The solution requires no standardisation.

Diethyl ether.

Sodium hydroxide, 0·02 N—Standardise on the day of use against potassium hydrogen phthalate, previously dried at 105° C for 1 hour, with phenolphthalein as indicator.

1 ml of solution ≡ 0·00374 g of "pyrethrin II."

METHOD

SAMPLING—

Keep the extract at 20° to 25° C for at least 2 hours, mix thoroughly, and weigh into a 150-ml conical flask sufficient of the extract to contain 0·12 to 0·13 g of total "pyrethrins."

* Registered trade mark.

PRELIMINARY TREATMENT OF THE SAMPLE—

To the sample in the flask add 50 ml of light petroleum, swirl the liquid, add 1 g of Filtercel (see Note 1), swirl the liquid again to mix completely, stopper the flask, and set aside in a refrigerator at 0° ± 0·5° C overnight.

Prepare a Gooch crucible with asbestos, packing it under suction. Wash the pad, first with about 25 ml of 95 per cent. ethanol and then with about 25 ml of light petroleum.

Thoroughly mix the contents of the conical flask, and filter, under gentle suction, through the prepared Gooch crucible (see Note 2) into a 250-ml conical flask, draining the 150-ml flask as completely as possible. Rinse the 150-ml flask with 15 ml of ice-cold light petroleum, and use this rinsing to wash the residue in the crucible; collect the washings in the 250-ml flask containing the filtrate. Repeat the rinsing and washing twice more. Place one or two glass beads (1 mm in diameter) in the flask containing the combined filtrate and washings, connect the flask to a condenser, and distil off the light petroleum on a water-bath until less than 1 ml of solvent remains. Add 20 ml of ethanolic sodium hydroxide solution to the concentrated extract in the flask, connect an air condenser (100 cm long and 1 cm in internal diameter) to the flask, and boil the solution gently under reflux for 45 minutes. Transfer the solution to a 600-ml beaker, calibrated at 200, 150 and 50 ml, and rinse out the flask into the beaker with hot water; use sufficient water to produce a volume of 200 ml in the beaker. Place a few glass beads in the beaker, or preferably use a boiling-tube, and evaporate the liquid to 150 ml; play a stream of air on to the surface of the liquid, if necessary, to depress frothing. Cool the liquid rapidly to room temperature, and transfer it to a 250-ml calibrated flask. Rinse out the beaker into the flask with three successive 20-ml portions of water, transferring any gummy material from the beaker to the flask. Add 1 g of Filtercel and 10 ml or more of barium chloride solution to the contents of the flask, swirl the liquid gently, dilute to the mark with water, shake the flask vigorously until the supernatant liquid is "bright," and filter the suspension through a fully fluted (32 folds) 15-cm Whatman No. 1 filter-paper (see Note 3).

DETERMINATION OF "PYRETHRIN I"—

To 200 ml of the clear filtrate add 1 drop of phenolphthalein indicator solution, neutralise the solution by the dropwise addition of diluted sulphuric acid, then add 1 ml of the acid in excess, and mix. Filter the suspension through a 7-cm Whatman No. 1 filter-paper, previously coated lightly with Filtercel, in a Buchner funnel, and wash the residue with three successive 15-ml portions of water. Transfer the combined filtrate and washings to a glass-stoppered 500-ml separating funnel, and add 50 ml of light petroleum, shake the funnel vigorously for at least 1 minute, releasing the pressure, if necessary, at intervals, and allow the layers to separate for at least 5 minutes or until the lower aqueous layer is clear before running off the lower layer into a flask. Filter the light-petroleum extract through a plug of cotton-wool, and collect the filtrate in a 250-ml separating funnel containing 10 ml of water. Return the aqueous liquid to the 500-ml separating funnel, and repeat the extraction with a further 50 ml of light petroleum; run off the lower aqueous layer and reserve it for the determination of "pyrethrin II," and filter the light-petroleum extract through the same plug of cotton-wool into the 250-ml separating funnel containing the first extract. Rinse the 500-ml separating funnel, the flask used to hold the aqueous layer and the plug of cotton-wool successively with two 10-ml portions of light petroleum, and add the rinsings to the combined light-petroleum extracts. Shake the combined light-petroleum extracts and rinsings with the 10 ml of water for about 30 seconds, allow the layers to separate, and run off the lower aqueous layer; use it to rinse the 500-ml separating funnel, and then add it to the reserved aqueous liquid. Repeat the washing of the light-petroleum solution with a further 10-ml portion of water, and run off the lower aqueous layer as completely as possible and add it to the reserved aqueous liquid as before.

Add 5 ml of dilute sodium hydroxide solution to the washed light-petroleum solution, shake the funnel vigorously for at least 1 minute, releasing the pressure carefully at intervals during the shaking, and allow the layers to separate for at least 5 minutes or until the lower aqueous layer is clear. Run off the lower layer into a 150-ml conical flask, leaving any interfacial "cuff" in the separating funnel and draining the stem of the separating funnel carefully. Repeat the extraction of the light-petroleum solution with 5 ml of dilute sodium hydroxide solution, the lower aqueous layer being added to the first alkaline extract in the conical flask

as before. Finally, extract the light-petroleum solution with 5 ml of water, and add the lower aqueous layer to the combined alkaline extracts as before, a few drops of water being used to rinse the outer side of the tip of the separating funnel.

To the alkaline solution in the conical flask add 10 ml of mercuric sulphate solution, stopper the flask, swirl the liquid, and set aside in complete darkness in a thermostatically controlled bath at 25° ± 0·5° C for exactly 60 minutes from the addition of the mercuric sulphate solution. Add 20 ml of dehydrated ethanol and 3 ml of sodium chloride solution, warm the mixture on a water-bath at about 60° C, and set aside for several minutes until the precipitated mercurous chloride has coagulated and settled. Decant the supernatant liquid through a folded (not fluted) 9-cm Whatman No. 1 filter-paper in a small glass funnel (4·5 cm in diameter), and drain carefully, retaining most of the precipitate in the flask. Wash the precipitate in the flask with 10 ml or more of hot dehydrated ethanol, decanting the ethanol through the same filter-paper as before. Wash the precipitate with three successive 10-ml portions of hot chloroform, decanting each washing through the same filter-paper, and transfer the filter-paper and any precipitate to the flask containing the bulk of the precipitate; wipe the outer part of the neck of the flask with a small piece of filter-paper, and place this also in the flask.

Add to the contents of the flask 50 ml of diluted hydrochloric acid, 5 ml of chloroform and 1 ml of iodine monochloride solution, and titrate with 0·01 M potassium iodate, shaking the flask vigorously for at least 30 seconds after each addition of the iodate solution (see Note 4); take as the end-point that point at which the red colour just disappears from the chloroform layer and does not return within 3 minutes.

Carry out a blank determination by repeating the whole procedure, but omitting the pyrethrum extract (see Note 5). Calculate the percentage of "pyrethrin I" from the equation—

$$\text{"Pyrethrin I"} = \frac{(a - b) \times 0{\cdot}0057 \times 5 \times 100}{W \times 4} \text{ per cent. w/w,}$$

where a = volume, in ml, of 0·01 M potassium iodate required by the sample,

b = volume, in ml, of 0·01 M potassium iodate required by the blank solution and

W = weight, in g, of the sample.

DETERMINATION OF "PYRETHRIN II"—

Transfer the combined aqueous liquids reserved in the determination of "pyrethrin I" (see Note 6) to the calibrated 600-ml beaker, add two or three glass beads as a boiling aid, cover the beaker with a clock-glass, and concentrate the liquid to 50 ml, a glass rod being used to prevent bumping, by placing the beaker on an asbestos plate having a 3-inch diameter hole covered by asbestos gauze and by heating strongly with a bunsen burner (see Note 7); the evaporation should be completed in 35 to 45 minutes. Rinse any droplets of liquid on the underside of the clock-glass into the beaker with not more than 5 ml of water, and filter the liquid through a plug of cotton-wool into a glass-stoppered 500-ml separating funnel. Rinse the beaker with three successive 15-ml portions of water; pass each rinsing through the same plug of cotton-wool into the separating funnel, and allow each rinsing to drain completely through the cotton-wool before the next rinsing is added. Saturate the liquid in the separating funnel with sodium chloride (see Note 8), add 10 ml of concentrated hydrochloric acid (see Note 9) and 30 ml of ether, shake the funnel for 1 minute, releasing the pressure, if necessary, at intervals during the shaking, allow the layers to separate for at least 5 minutes or until the lower aqueous layer is clear, and run off the lower layer completely into another 500-ml separating funnel. Repeat the extraction of the aqueous liquid with a further 50-ml portion and then with two successive 35-ml portions of ether, transfer each ether extract into the separating funnel containing the first extract, and then discard the aqueous liquid.

Run off any aqueous liquid that separates from the combined ether extracts, and then wash the ether layer with three successive 10-ml portions of sodium chloride solution; shake the funnel for 1 minute with each washing and finally remove the lower aqueous layer as completely as possible. Filter the ether layer through a plug of cotton-wool into a 500-ml conical flask, rinse the separating funnel with 10 ml of ether, and pass the rinsings through the plug of cotton-wool into the conical flask. Place two or three glass beads in the flask, attach a condenser, and distil off the ether on a water-bath; remove the last traces of vapour

by passing a gentle current of air through the flask (see Note 10). Dry the residue at 100° C for 10 minutes, and remove any hydrochloric acid fumes with a gentle current of dry air.

Add 75 ml of boiling water to the residue in the flask, filter the liquid through a 9- or 11-cm folded (not fluted) Whatman No. 1 filter-paper, and wash the flask and filter-paper with five successive 20-ml portions of boiling water or until the washings are neutral to litmus paper. Add two drops of neutralised phenolphthalein indicator solution to the combined filtrate and washings, and rapidly titrate with 0·02 N sodium hydroxide to a brownish-pink end-point that persists for 30 seconds, the flask being kept stoppered between additions of alkali.

Carry out a blank determination by repeating the whole procedure on the aqueous liquid reserved in the blank determination for "pyrethrin I" (see Note 5). Calculate the percentage of "pyrethrin II" from the equation—

$$\text{"Pyrethrin II"} = \frac{(a - b) \times 0{\cdot}00374 \times 5 \times 100}{W \times 4} \text{ per cent. w/w,}$$

where a = volume, in ml, of 0·02 N sodium hydroxide required by the sample,
b = volume, in ml, of 0·02 N sodium hydroxide required by the blank solution and
W = weight, in g, of the sample.

NOTES

1. Because loss of "pyrethrins" has been shown to occur from purified extracts by absorption on Filtercel, this preliminary filtration of a light-petroleum solution of the extract should be omitted when a purified extract is being assayed unless a qualitative test shows more than a slight opalescence when the extract is dissolved in the solvent.

2. Do not more than half fill the crucible, in order to avoid loss of liquid by its creeping over the rim.

3. If the assay cannot be completed in a day, the solution may be kept overnight at this stage in an alkaline condition.

4. Potassium iodate reacts with mercury^{I} to form mercury^{II} and iodine; further addition of potassium iodate in the presence of hydrochloric acid causes the iodine to be oxidised to iodine monochloride—

$$2Hg_2Cl_2 + KIO_3 + 6HCl = 4HgCl_2 + ICl + KCl + 3H_2O.$$

The addition of iodine monochloride does not change the volumetric relationship between mercury^{I} and potassium iodate, but aids in determining the end-point when small amounts of mercury are being titrated.

5. The blank titre should not exceed 0·1 ml of potassium iodate solution.

6. If necessary, filter the reserved combined aqueous liquids through an asbestos-packed Gooch crucible.

7. This heating arrangement ensures that the sides of the beaker are not overheated by the high bunsen flame needed to effect the evaporation in the time stated.

8. About 30 to 35 g of sodium chloride are required.

9. This ensures that a small excess of sodium chloride in a finely divided state is present throughout the extraction.

10. The heating should not be continued longer than 5 minutes after the solvent has been removed.

Appendix III

RECOMMENDED METHOD FOR THE ASSAY OF PYRETHRUM FLOWERS

APPARATUS—

Soxhlet extractor.

REAGENTS—

Light petroleum—Boiling-range 40° to 60° C. Commercially available, aromatics-free.

METHOD

EXTRACTION OF SAMPLE—

Grind the flowers to pass a 16-mesh sieve, and weigh 12·5 g of the powdered flowers. Transfer the sample to a Soxhlet thimble, and extract with light petroleum for 7 hours in a Soxhlet apparatus. When extraction is complete, evaporate off the light petroleum from the extract until the volume is reduced to about 40 ml, stopper the flask, and set aside in a refrigerator at 0° to 5° C for 16 hours. Filter the cold extract through a plug of cotton-wool, previously saturated with ice-cold light petroleum, into a 250-ml conical flask, and wash the

Soxhlet flask and plug of cotton-wool with a 20-ml portion and then two successive 10-ml portions of ice-cold light petroleum; use a rubber-tipped glass rod to dislodge resinous material from the walls of the flask, and stand the flask in a mixture of ice and water during the washing procedure to ensure that the light petroleum is kept cold.

DETERMINATION OF "PYRETHRINS"—

Combine the filtrate and washings, and continue the assay as described in Appendix II under "Preliminary Treatment of Sample," beginning at "Place one or two glass beads (1 mm in diameter) . . ." in the third paragraph.

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The Gravimetric Determination of Iron by the Homogeneous Precipitation of the Tris(2-thiopyridine-*N*-oxide)-Iron$^{(III)}$ Complex*

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The use is described of a new organic reagent, "thione," for the gravimetric determination of iron. The reagent, 2-mercaptopyridine-*N*-oxide, is conveniently prepared and used as the bromide of its thiouronium derivative. The salt, which is water-soluble and stable towards air oxidation, is hydrolysed slowly in hot dilute acids to form thione. The inner-complex salt, $(C_5H_4NOS)_3Fe$, is obtained by homogeneous precipitation from solutions of either ferrous or ferric ions in the pH range 2 to 6. The precipitates are crystalline, extremely easy to filter and can be dried in air below 160° C without decomposition.

A preliminary investigation is also described of the selectivity and general properties of thione.

GRAVIMETRIC methods for determining iron are at present less satisfactory than volumetric methods based on the oxidimetric titration of iron^{II}. In the classical method, involving precipitation of ferric hydroxide and its ignition to Fe_2O_3, precautions must be taken to avoid co-precipitation of metal ions, and there is the possibility that reduction may occur during the ignition to give an oxide of variable composition. Procedures that minimise co-precipitation, *viz.*, precipitation with cupferron or as the basic acetate or succinate[1] and the homogeneous precipitation of basic ferric formate,[2] are lengthy and it is still necessary to ignite the precipitates carefully and at high temperature to Fe_2O_3.

In this paper, the general properties of a new organic reagent, 2-mercaptopyridine-*N*-oxide, which we propose to call "thione," are described, and in particular we report the investigation of its use for the rapid gravimetric determination of iron as the tris(2-thiopyridine-*N*-oxide) - iron^{III} complex, $(C_5H_4NOS)_3Fe$. The reagent is used as the stable bromide of its thiouronium derivative (*S*-2-pyridylthiouronium bromide 1-oxide), which is hydrolysed rapidly in alkaline solution and more slowly on heating in dilute acid solutions to form thione *in situ*. The iron complex is thus formed from the thiouronium salt by homogeneous precipitation in buffered solutions at pH 2 to 6. Ferrous ions are spontaneously oxidised in the presence of thione and the total iron in solution is precipitated as the iron^{III} complex. The precipitates are crystalline and extremely easy to filter; they are stable in air up to about 160° C. The method is potentially one of high accuracy and reproducibility; 50 mg of iron can be determined to within $\pm 0{\cdot}2$ per cent. Thione is not particularly selective for iron, but many metals that would be co-precipitated in the classical method, *e.g.*, aluminium, titanium and thorium, do not interfere.

In chloroform solution, the iron complex has a strong absorption band at 520 mμ (ϵ, about 3400) and the value of this for the more selective colorimetric determination of iron with thione is at present under investigation.

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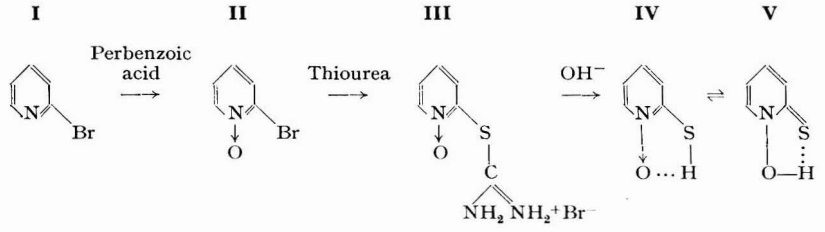
General properties of the reagent

2-Mercaptopyridine-*N*-oxide, **IV**, was prepared from 2-bromopyridine, **I**, purchased from Eastman-Kodak, by the method of Shaw, Bernstein, Losee and Lott[3] thus—



Elemental analysis of the product gave—

Element	C	H	N	S
Found, per cent.	47·6	3·8	11·0	26·1
C_5H_5NOS requires, per cent.	47·2	3·9	11·0	25·2

The melting-point was 68° to 70° C, in agreement with the value given in the literature. The infrared spectrum of the product agreed entirely with the values reported for 2-mercaptopyridine-*N*-oxide by Katritzki and Jones.[4] These authors have reported a pK_a value of 4·65 for 2-mercaptopyridine-*N*-oxide and, on the basis of infrared and ultraviolet spectra, consider that the tautomeric form 1-hydroxypyridine-2-thione, **V**, predominates. This is supported by the observed stability of thione towards air oxidation to the disulphide, and is an advantage in its application as a gravimetric reagent compared with other mercaptans.[5] However, thione has some disadvantages as an organic reagent, since it is only sparingly soluble in aqueous media and we have found that it is light-sensitive and must be stored in dark bottles, although this has not been reported by other workers. The bromide of the thiouronium derivative (*S*-2-pyridylthiouronium bromide 1-oxide), **III**, is more satisfactory for use as an analytical reagent. The solid is completely stable towards air oxidation and light and it can be stored indefinitely. It is appreciably soluble in water, about 6 per cent. w/v, but hydrolyses slowly even in M sulphuric acid and it is preferable to store the reagent as a solid.

Thione forms highly crystalline compounds with several metal ions. Many of these are coloured and can be extracted into chloroform, which suggests that they are chelated inner-complex salts[6] and are probably monomeric. A summary of the reactions of thione with metal ions is given in Table I.

Thione possesses anti-tuberculin properties,[7] which are associated with its stability and the lipid solubility of the iron^{III} complex. Thione and its copper derivative inhibit certain plant diseases,[8] and some molybdenum derivatives have useful fungicidal properties.[9] The zinc salt, $(C_5H_4NOS)_2Zn$, is available commercially as a bactericide - fungicide under the trade-name Vancide ZP.[10] The thiouronium bromide salt (*S*-2-pyridylthiouronium bromide 1-oxide) will be available shortly from Hopkin & Williams Ltd., under the name "Thiurone."

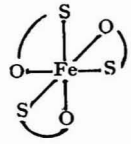
Reactions of thione with iron

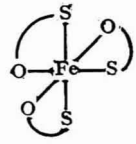
Thione reacts immediately with aqueous solutions of either ferrous or ferric ions over a wide pH range to form an insoluble crystalline black material of empirical formula $(C_5H_4NOS)_3Fe$. It is formed even in solutions of iron^{II} containing an excess of hydroxylamine that have been degassed with nitrogen. Elemental analysis of the material, re-crystallised by Soxhlet extraction from chloroform, gave—

Element	C	H	N	Fe
Found, per cent.	41·2	2·7	9·9	13·0
$(C_5H_4NOS)_3Fe$ requires, per cent.	41·5	2·8	9·7	12·9

The insolubility of the material made it difficult to determine the molecular weight accurately, but an approximate value of 500 $\pm$ 50 in chloroform suggests that it is monomeric. The magnetic susceptibility of 5·9 Bohr magnetons for the solid at room temperature proves that it must be a spin-high complex of iron^{III}. If the reasonable assumption is made that the

thione forms five-membered chelate rings with the iron via sulphur and oxygen, two geometrical isomers are possible for the monomer—





No changes have been detected in the ultraviolet and visible spectra of different preparations, and therefore the isomers appear to be formed in a constant ratio. The molecular extinction coefficient at 520 mμ is much higher than other metal - thione complexes and higher than is normal for d - d transitions in outer-orbital complexes. This exceptional property could be associated with one isomer only.

TABLE I

REACTION OF THIONE, SHOWING COLOUR, EXTRACTION INTO CHLOROFORM AND pH OF PRECIPITATION OF COMPOUNDS FORMED WITH METAL IONS

Element	Form	Precipitation: not below pH	Precipitation: colour	Colour of chloroform extract	Comment
Alkali metal ..	Na^+, K^+		none	none	
Alkaline earth ..	Ca^{2+}, Mg^{2+}		none	none	
Titanium ..	Ti^{4+}	acid*	orange	orange	
Vanadium ..	VO_3^-	acid	mauve	cherry-red	Grey-green precipitate with excess of reagent
Chromium ..	Cr^{3+} / $Cr_2O_7^{2-}$	acid	green	green	Slow precipitation of chromium^{III} complex in each instance
Manganese ..	Mn^{2+}	5 to 6	pale yellow	none	Manganese^{II} complex
	MnO_4^-	acid	dark green	emerald	Manganese^{III} complex is formed
Iron	Fe^{3+} / Fe^{2+}	acid	dark violet	dark violet	Iron^{III} complex formed in both instances
Cobalt	Co^{2+}	4	brown	brown-green	Cobalt^{III} complex can also be prepared
Nickel	Ni^{2+}	3	buff	red	
Copper	Cu^{2+}	acid	green	green-red (dichroism)	
Zinc	Zn^{2+}	4	white	colourless	
Cadmium ..	Cd^{2+}	4	white	colourless	
Mercury	Hg^{2+}	acid	white	—	
Aluminium ..	Al^{3+}		none	none	
Tin	Sn^{2+}	acid	white	—	
Lead	Pb^{2+}	acid	yellow	yellow	
Arsenic ..	AsO_4^{3-}		none	none	
Antimony ..	Sb^{3+}		none	none	
Bismuth ..	Bi^{3+}	4	lime-green	yellow	
Cerium	Ce^{3+}, Ce^{4+}		none	none	
Zirconium ..	ZrO^{2+}	acid	white	—	
Molybdenum ..	$Mo_7O_{24}^{6-}$	acid	yellow	yellow	$(C_5H_4NOS)_2MoO_2$
Tungsten ..	WO_4^{2-}	7	brown	brown	
Rhenium ..	ReO_4^-		none	none	Yellow aqueous solution
Ruthenium ..	Ru^{3+}	acid	brown	green	
Palladium ..	Pd^{2+}	acid	brick-red	brown	
Platinum ..	Pt^{4+}	acid	brown	brown	
Uranium ..	UO_2^{2+}		none	none	
Thorium ..	Th^{4+}		none	none	
Silver	Ag^+	acid	white	—	
Gold	Au^{3+}	acid	dark yellow	yellow	

* N mineral acid

The preferential formation of the iron^{III} complex, which is reminiscent of the well known example of the iron^{III} - thioglycollate complex,[11] has been confirmed by polarographic studies.[12] Oxidation of iron from iron^{II} to iron^{III} by aqueous solutions of thione must be accompanied by reduction in the system. Since there is no evolution of hydrogen it is unlikely

that water acts as the oxidant. *N*-Oxides do not normally act as oxidants, but this might occur because the $iron^{III}$ - $iron^{II}$ reduction potential will be more negative if the $iron^{III}$ complex is more stable than the $iron^{II}$ complex. It has not been possible to detect 2-mercaptopyridine, the reduction product of thione, in reaction mixtures of ferrous ions and thione, but a preliminary investigation of its properties proves that it does not interfere below pH 7.

When a solution of the $iron^{III}$ - thione complex in concentrated sulphuric acid is diluted gradually with water, a characteristic series of colour changes is observed from colourless through green to deep blue, and eventually to dark purple. These colours are probably due to the intermediate species $(C_5H_4NOS)Fe^{2+}$, green, and $(C_5H_4NOS)_2Fe^{+}$, deep blue, formed by the gradual re-association of the complex on dilution.

Methods

Reagents—

Thione—Freshly prepare a 5 per cent. w/v solution of thione in ethanol; this is used for heterogeneous precipitation.

Thiouronium bromide salt—Freshly prepare a 5 per cent. w/v solution of *S*-2-pyridylthiouronium bromide 1-oxide in water; this is used for homogeneous precipitation.

Procedure—

Take between 5 and 50 mg of iron, in the $iron^{II}$ or $iron^{III}$ state, in about 100 ml of solution. Adjust the pH, within the range 2 to 6, with 1 per cent. citric acid solution and 2 M sodium hydroxide. Heat the solutions to about 80° C. Then, either—

(*a*) *For heterogeneous precipitation*—add about 50 per cent. molar excess of the thione solution. Heat the solution on a steam-bath with occasional stirring until the supernatant liquid is colourless (about 15 minutes) and digest it for a further 15 minutes:

or

(*b*) *For homogeneous precipitation*—add about 100 per cent. molar excess of the thiouronium bromide salt solution. Heat the solution on a steam-bath with frequent stirring until the supernatant liquid is colourless (see Note) and digest it for another 20 minutes.

Cool the solution to below 50° C, filter the precipitate on to a tared sintered-glass crucible (porosity 4). Wash the precipitate with about 100 ml of warm water (40° C), dry it for 40 minutes at 110° C, cool it and weigh the precipitate as $(C_5H_4NOS)_3Fe$, which contains 12·86 per cent. of iron.

Note—In the precipitation from homogeneous solution, the time taken for the supernatant liquid to become colourless depends upon the rate of hydrolysis of the thiouronium salt and hence varies with pH and the amount of iron present. For 10 mg of iron the time varies from about 60 minutes at pH 2 to about 15 minutes at pH 6.

Investigation of the gravimetric procedure for iron

The extent of precipitation of the $iron^{III}$ - thione complex as a function of pH was investigated by using these procedures with 10 mg amounts of iron, and the results are shown graphically in Fig. 1. The precipitation of the complex is complete over a wide range of pH, from about 1 to 6, and only at higher pH values is the complex hydrolysed. The use of the thiouronium bromide salt raises the pH at which homogeneous precipitation of the complex is complete in a reasonable time. Prolonged hydrolysis eventually gives the same results as those for heterogeneous precipitation.

In the homogeneous-precipitation method the solutions change colour from yellow, through green and deep blue, to purple before the formation of the insoluble complex. This is the same sequence of colour changes that is observed when a sulphuric acid solution of the complex is diluted. In view of this, it is probable that stable thiouronium complexes cannot exist under these conditions. The hydrolysis of the thiouronium salt, which could proceed through nucleophilic attack on the carbon atom of thiourea, may be accelerated by the presence of the metal ions. The rate of deposition of the precipitate is markedly dependent on the temperature and rate of stirring of the solutions. The complex appears to form from colloidal suspension in the homogeneous-precipitation method, but the improved crystallinity of these precipitates is clear from the micrographs shown in Fig. 2.

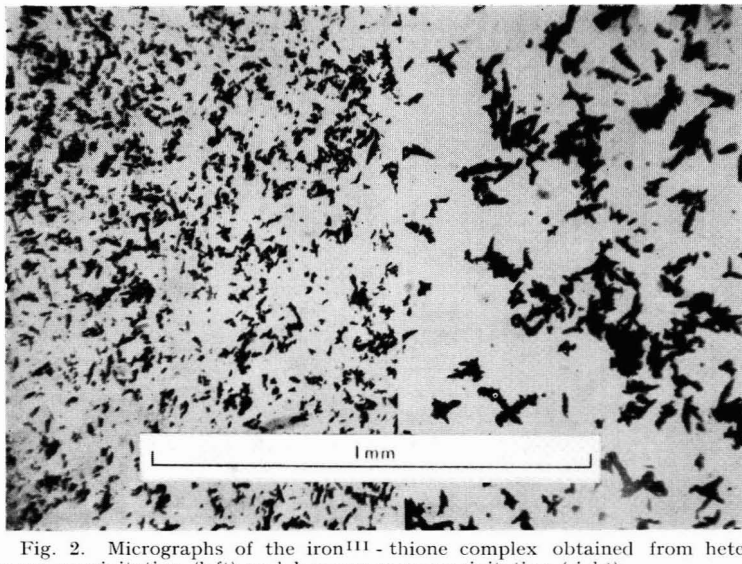


Fig. 2. Micrographs of the iron[III] - thione complex obtained from heterogeneous precipitation (left) and homogeneous precipitation (right)

[*To face page 710*

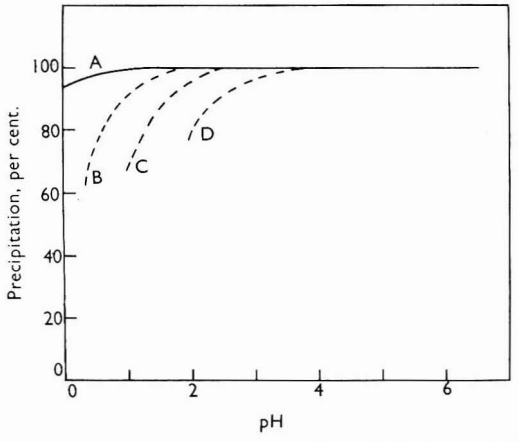


Fig. 1. Curves of extent of precipitation of the iron^{III} - thione complex as a function of pH. Curve A, by heterogeneous precipitation; curves B, C and D, by homogeneous precipitation for 4, 2 and 1 hours, respectively

The filtration characteristics of the complex formed by either method are exceptionally good and this is an important feature of the method. Up to 0·5 g of precipitate in about 150 ml of warm solution can be filtered in a few minutes and there is little tendency for it to "creep" or adhere to the walls of the beaker. The drying temperature of the precipitate has been checked thermogravimetrically by Dr. D. A. Pantony of Imperial College. The weight of the complex remains constant to within one part in five hundred up to about 170° C, at which temperature it suddenly decomposes.

TABLE II

RECOVERY OF IRON^{II} AND IRON^{III} FROM PURE SOLUTIONS

Iron taken, mg	Oxidation state	Iron found, mg	Error, mg
Determination by heterogeneous precipitation—			
12·40	+3	12·20	−0·20
		12·38	−0·02
		12·45	+0·05
		12·35	−0·05
		12·31	−0·09
		12·47	+0·07
		12·39	−0·01
Determination by homogeneous precipitation—			
9·52	+2	9·55	+0·03
		9·54	+0·02
		9·52	0·00
		9·51	−0·01
		9·55	+0·03
		9·52	0·00
12·40	+3	12·24	−0·16
		12·37	−0·03
		12·37	−0·03
		12·30	−0·10
		12·40	0·00
		12·38	−0·02
		12·33	−0·07
24·80	+3	24·90	+0·10
55·25	+3	55·25	0·00
		55·24	−0·01
		55·25	0·00
		55·45	+0·20
		55·22	−0·03
62·00	+3	62·00	0·00

Results for the determination of different amounts of pure iron^{II} and iron^{III} are presented in Table II. The lower precision obtained with smaller amounts of iron is similar to the variation observed in the weights of the empty sintered-glass crucibles. This error is less significant with larger amounts of iron and the method becomes one of high precision, better than $\pm 0{\cdot}2$ per cent. The precision of the results for iron^{II} and iron^{III} are similar.

The interference of other metal ions in the homogeneous precipitation of the complex was determined by weighing the precipitate obtained from a constant weight of iron in the presence of varying amounts of the other metal ion. Table III gives the ratios of other metal ions to

TABLE III

INTERFERENCE BY OTHER IONS IN THE HOMOGENEOUS PRECIPITATION

Interfering ion	Proportion of ion that produces less than 2 per cent. error*	Interfering ion	Proportion of ion that produces less than 2 per cent. error*
Mg^{2+}	>100	Cu^{2+}	0·04
Ca^{2+}	>40	Zn^{2+}	0·1
Al^{3+}	>50	Cr^{3+}	0·1
Mn^{2+}	4	Ti^{4+}	10
Co^{2+}	0·03	Th^{4+}	25
Ni^{2+}	0·1	PO_4^{3-}	>200
		SCN^-	>100

* The proportion is given as the weight ratio of the interfering ion to iron^{III} that gives less than 2 per cent. error.

ferric ion, estimated by interpolation, that will produce a positive error of 2 per cent. in the analysis of the iron. Large amounts of magnesium, calcium, aluminium, titanium and thorium do not interfere with the method and this suggests that the method could be used for analysing certain alloys of these metals and for analysing silicates, dolomites and limestones. It is probable that simple separations can be found to avoid interference from other metals. Thus, it has been found that when copper is separated from iron by thiocyanate precipitation, the excess of thiocyanate does not interfere in the subsequent precipitation of the iron^{III}-thione complex. It has also been noted that the complex is formed in the presence of masking-agents such as citrate and thiourea and reducing agents such as hydroxylamine. Therefore, in spite of its lack of selectivity towards iron, we feel that thione will prove a versatile reagent for the gravimetric determination of iron and moreover that its increased selectivity and sensitivity as a colorimetric reagent is likely to make it a useful reagent over a wide range of concentrations.

We thank the London Transport Board for part-time study leave to one of us (M.T.). We also acknowledge the help of our colleagues, particularly Drs. J. F. McGhie and G. E. Usher who directed our preparative organic chemistry.

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High-pressure Plasmas as Spectroscopic Emission Sources

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An account is given of the authors' preliminary work in the use of high-pressure plasmas as both spectrographic and flame-photometric emission sources. Two types of plasma sources are discussed, *viz.*, the d.c.-arc type and the high-frequency induction type; the latter, having no electrodes, produces little background emission. Tables of the order of detection for some metallic elements are given, but these are not absolute detection limits. The standard deviation obtained with calcium emission at 3933Å is given for both sources.

In the determination of calcium by flame photometry, a depression of the calcium emission occurs in the presence of phosphorus, and more markedly, aluminium, owing to the formation of stable compounds in the flame.[1] The interference of phosphorus can be overcome and that of aluminium diminished by raising the flame temperature. This has been done by injecting oxygen into an air - acetylene flame.[2] It was thought that if the flame temperature were raised even further, the interference of aluminium could be completely overcome and attention was turned to the possibility of using an oxy-cyanogen or a hydrogen - fluorine flame. These flames have been used,[3,4] but were considered too hazardous for general use. Atomic-hydrogen torches[5] and augmented flames[6] were briefly considered, but finally it was decided that d.c.-arc plasma jets offered an elegant approach to this problem.

Several types of these jets were built and operated, and then the apparent advantages of the high-frequency-heated plasma type over the d.c.-arc type, notably the freedom from electrode contamination, became of interest. Also, by this time a spectrograph had been acquired and interest in spectral sources received a fresh impetus. Work on high-frequency plasmas was therefore carried out concurrently with that on d.c.-arc plasmas.

The operating principles of these two sources are different from each other and are briefly described below. A more detailed description can be found in the literature.[7,8]

In the d.c.-arc plasma jet, a closed chamber contains at one end an anode (or cathode) and at the other end a cathode (or anode) that has a small opening in it. A coolant fluid (argon) is introduced tangentially through the chamber wall and swirls around the chamber and out of the cathode hole. When the arc is struck the coolant fluid cools the outer layers of the arc so giving a "thermal pinch" effect, which causes the arc column to contract. The resultant increase in current density gives a higher arc temperature, and the pressure generated leads to the ejection of extremely hot plasma through the cathode opening, where it appears as a flame-like jet. At higher operating currents, the arc suffers a further "pinch" effect called the "magnetic pinch" due to the self-induced magnetic field. For spectroscopic purposes the substance to be analysed is injected into the arc column and is carried out in the plasma flame.

In the high-frequency plasma torch, a stream of ionised gas (argon) contained in a circular quartz tube, surrounded by a coil carrying high-frequency alternating electric current, is heated by induction. Cold gas, being un-ionised, is not an electrical conductor and therefore the plasma torch must be externally initiated, which can be achieved by holding a carbon rod in the mouth of the quartz tube. The high-frequency field heats the carbon rod, which in turn heats and ionises the argon. Once the main discharge has started, the carbon rod is removed and the gas stream carries the ionised gas plasma down the tube away from the coil, where it emerges at the tube mouth as a "flame." To maintain the discharge, a portion of the ionised gas must be re-cycled. This is achieved by feeding the gas tangentially into the tube which causes a vortex, and the partial reduction in pressure at the centre of this causes some of the ionised argon to move back down the tube in the opposite direction to the main gas flow.

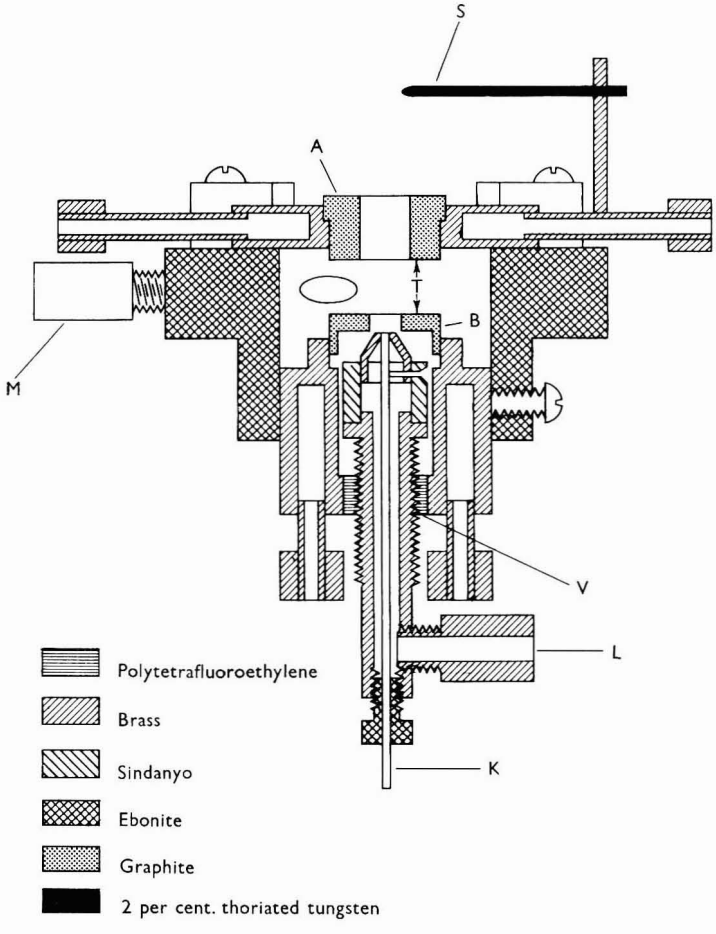
The quartz tube described in this paper is cooled by a secondary stream of argon passing over it. When fully coupled, the high-frequency plasma can be regarded as a transformer, the work coil being the primary and the ionised gas a one-turn secondary.

The factor limiting the maximum temperature of a combustion flame is the dissociation of the gases in the flame. These various dissociations consume an appreciable amount of the thermal energy released by the flame reaction, and thus prevent this energy being used to increase the gas temperature. The hottest flames are those whose combustion products are stable molecules with high dissociation temperatures. For example, the temperature developed by the reaction—

$$C_2N_2 + O_2 \rightarrow 2CO + N_2$$

is 4850° K.

In a thermal plasma, the gas is not heated by energy liberated in a chemical reaction, but by electrical energy. The electric field imparts kinetic energy to the electrons in the plasma, which then share this energy with plasma atoms and ions by colliding with them; as a result electrons, atoms and ions are in thermal equilibrium. The temperature of a thermal



A = Anode opening increased to 6 mm

B = Cathode, 8-mm opening, and projecting 1 mm either side of cooling-jacket

K = Capillary tube, 24 s.w.g. stainless steel

L = Argon inlet, 5 p.s.i., 5 litres per minute

M = Coolant-argon inlet, 2 p.s.i., 7 litres per minute

S = Stabiliser electrode, $\frac{1}{8}$-inch rod, 7 mm from top of cathode

T = 3-mm gap, 10-A d.c. arc, 230-V line voltage

V = Tapped polytetrafluoroethylene insulator

Fig. 1. Diagram of modified Margoshes and Scribner plasma jet

plasma is not limited by the dissociation temperature of the gas, in fact it must be higher than the temperature at which the gas ionises, since the electrical conductivity of the gas depends upon the number of electrons and ions. The temperature of a thermal plasma generally lies between the temperatures required to bring about 10 per cent. and 50 per cent. ionisation. From this it can be seen that the temperature of a plasma must be higher than that of a normal flame. The limit set upon plasma temperatures is the temperature at which increasing ionisation is countered by thermal expansion and the conductivity of the plasma no longer increases with temperature.

D.C.-ARC PLASMA JETS

Our first experiments on the application of this type of source to spectroscopic analysis were carried out with a modified version of the tungsten-electrode plasma jet used for chemical synthesis by Stokes and Knipe.[9] The polytetrafluoroethylene insulating gasket was replaced with polypropylene, and the coolant gas (argon) was fed through a gasket with a $\frac{7}{16}$-inch bore tube. The coolant gas at a rate of 7 litres per minute was supplied from the expansion chamber of a Unicam atomising unit so that a liquid sample could be fed to the plasma jet as an aerosol. An adjustable $\frac{1}{8}$-inch, 2 per cent. thoriated tungsten rod was connected to the tungsten insert electrode (used as the cathode) to act as a stabiliser electrode. The use of a stabiliser electrode improved the operation of the assembly. The plasma jet was powered from a rotary convertor at 22 amps d.c., and was initiated by shorting the two electrodes.

However, this type of plasma generator was found to be too unstable for use with a non-integrating type of flame spectrophotometer, such as the Unicam SP900. Further, it was found that sample-to-sample contamination occurred because of condensation in the arc chamber, and that tungsten emission from the electrodes gave high background readings.

With the experience gained from these experiments it was decided that it was necessary to use a plasma jet specifically designed for spectroscopic analysis with direct injection of liquid samples into the plasma jet.

Such a plasma generator had already been used by Margoshes and Scribner.[10] A similar generator was built, and with the modifications shown in Fig. 1 it provided a stable spectrographic source. The modifications were made to improve its operation and to permit the jet to be used with a d.c. source limited to a maximum of 15 amps output.

Attempts to use this unit as the emission source on the Unicam SP900 flame spectrophotometer were unsuccessful because of the instability introduced by flare from the stabiliser electrode, although Webb and Wildy[11] used this type of plasma jet with great success, by screening the stabiliser and using an integrating flame photometer incorporating an additional photomultiplier tube to monitor the background interference.

Some preliminary experiments were carried out by using this plasma jet with aqueous solutions on a Hilger large-quartz spectrograph. The plasma-jet assembly was mounted in the electrode holders of a Hilger FS55 spark stand so that all the usual adjusting controls could be used for alignment.

TABLE I

ORDER OF THE LEVELS OF DETECTION FOR THE D.C.-ARC PLASMA JET

Species	Spectrum line, Å	Concentration of aqueous solution, p.p.m.	Percentage transmission	
			line + background	background
Ca^{II}	3933·666	2	53·7	61·6
Sr^{II}	4077·714	5	49·0	60·3
Ba^{II}	4554·042	5	66·1	69·2
Al^{I}	3961·527	100	42·7	50·1
Mn^{II}	2576·104	10	77·6	95·5
Cu^{I}	3247·540	10	61·7	74·1
Cr^{II}	2835·633	20	72·4	89·1
Mg^{II}	2795·530	1	77·6	91·2
Co^{I}	3453·505	50	54·9	57·5

The results obtained from the preliminary experiments are shown in Tables I and II.

Table I shows the order of the levels of detection obtained. Ilford N.30 plates were used, an exposure of 30 seconds was given with a slit width of 0·01 mm. The atomising rate of sample to the plasma was 0·25 ml per minute. The quartz optical system was used, and the source was focused on the slits so that the top of the cathode and the tip of the stabiliser electrode were excluded.

Table II gives the stability of the d.c.-arc plasma jet as a source. Ilford N.30 plates were used, an exposure of 30 seconds was given with a slit width of 0·015 mm and slit height of 3 mm. The quartz optical system was used with the source focused on the slit. The source was run continuously, and a solution containing 20 p.p.m. of calcium was aspirated at 0·25 ml per minute whilst the 10 exposures were made. The plate was developed in a dish with P.Q. Universal developer for three minutes at 20° C. The plate was calibrated by using a rotating stepped sector and a 3-amp iron arc. The standard deviation of the values for the percentage transmission given in Table II was calculated by using the root mean square method and by assuming normal distribution. The standard deviation for percentage transmission for calcium 3933 is $\pm 0{\cdot}12$, *i.e.*, the coefficient of variation is 4·5 per cent. Considering the high level of background density in this wavelength region, the source stability indicated by the above figure is extremely good.

TABLE II

STABILITY OF THE D.C.-ARC PLASMA JET

Exposure number	Percentage transmission of Ca 3933Å, *plus* background	Percentage transmission, background	Percentage transmission of Ca 3933Å, corrected for background
1	1·30	12·58	2·72
2	1·30	13·18	2·70
3	1·30	13·18	2·70
4	1·45	14·45	2·75
5	1·41	13·80	2·82
6	1·30	13·80	2·58
7	1·30	14·45	2·51
8	1·30	14·45	2·51
9	1·41	14·79	2·75
10	1·41	14·12	2·82

TEMPERATURE OF THE SOURCE—

By using the lines for calcium at 3933·666Å and 4226·728 Å at the 20 p.p.m. of calcium level, the degree of ionisation of the calcium in the plasma jet was found to be 77 per cent. By using the Saha equation,[15] this degree of ionisation indicates an electron temperature of approximately 9500° K.

OPERATION OF THE PLASMA-JET UNIT—

The unit is simple to operate and has been run for periods of up to 30 minutes.

No carry-over contamination between samples was found if a 30-second wash with de-ionised water was made between exposures. Since the plasma-jet column and the sample spray do not touch the electrodes during operation, the electrodes can be used for many exposures. Owing to the burning of the top electrode (cathode) during the initiation of the plasma, this electrode has to be replaced at intervals. If spark initiation of the unit were used, this troublesome erosion of the cathode could no doubt be avoided.

Since the unit uses aqueous samples at a rate of only 0·25 ml per minute, an exposure of 30 seconds with a pre-spray time of 30 seconds will use only 0·25 ml of sample.

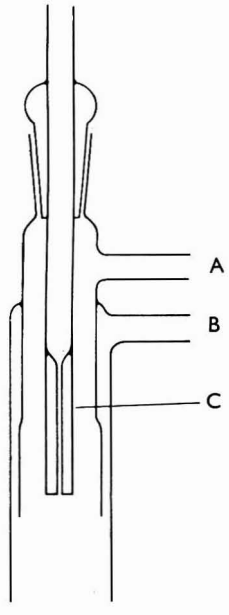
If the atomiser is used under the same operating conditions, the atomising rate for organic solvents such as alcohol, acetone, diethyl ether, chloroform and carbon tetrachloride will be much higher; if necessary the uptake of sample can be reduced by dropping the atomiser pressure or by adjusting the position of the atomiser needle. The above-mentioned solvents have been sprayed into the plasma jet quite satisfactorily by using the same operating conditions as for aqueous solutions.

Attention was now turned to high-frequency thermal plasmas, since it had not been possible to use the d.c. plasma jet on the flame photometer.

HIGH-FREQUENCY THERMAL PLASMA TORCH

The initial experiments were carried out on argon plasmas in single silica tubes with a 4·5-Mc/s, 1·0-kW induction-heating generator. These experiments indicated that powers greatly in excess of 1·0 kW were necessary to maintain a stable source at this frequency and be capable of exciting aqueous aerosols. Ultimately, a 2·5-kW dielectric-heating generator operating at 36 Mc/s and modified to work with inductive coupling was used.

The torch cell evolved is a modified form of the type used by Reed[12] in his experiments on the growth of refractory crystals. The cell with an aerosol injector fitted is shown in Fig. 2, and consists basically of two concentric clear silica tubes with individual gas-entry tubes, A and B, joined tangentially to the main tubes. The cell also carries a silica B14 socket, which takes the sample injectors for gases, vapours, aerosols, liquids, powdered solids and slurries. The sample injectors are all made of Pyrex glass and are sufficiently separated from the plasma to prevent their melting.



A = Plasma-argon tangential inlet
B = Coolant-argon tangential inlet
C = Pyrex-glass aerosol injector

Fig. 2. Diagram of the high-frequency plasma-torch silica cell

The plasma torch was run at 1·5-kW output power. The plasma gas used was argon at a flow-rate of 5 litres per minute via side-tube A, with a coolant gas (argon) flow-rate of 17 litres per minute via side-tube B. The plasma was coupled to the generator output via a work coil consisting of $3\frac{1}{2}$ turns of $\frac{1}{4}$-inch o.d., $\frac{3}{32}$-inch i.d. copper tube. The overall diameter of the coil was $1\frac{3}{4}$ inches, and the separation between turn centres was $\frac{5}{8}$ inch. The live coil was placed nearest the open end of the tube just above the plasma tube. Initiation was by carbon rod.

Since this paper was read before a Joint Meeting of the Society for Analytical Chemistry and The Sheffield Metallurgical Association, in October 1963, descriptions of other high-frequency plasma torches have come to our attention.[13,14] The torch described in this paper differs from these, in that it uses an argon plasma, has no central electrode and is capable of exciting aerosols, liquids or powders. The sample path is of glass or quartz, and contamination of sample is less likely than in any other type of torch so far described in the literature. The radiation from the sample is produced in a "tail-flame" region remote from the main body of the plasma. The torch can be so aligned that the radiation from the main plasma does not enter the spectrograph or monochromater slit, and apart from the OH band system no intense background spectrum is produced.

The torch produces a flame-like plasma $1\frac{1}{4}$ inches long, $\frac{3}{4}$ inch in diameter and annular in form, *i.e.*, it has a hole or low-temperature region in its centre. The sample is injected through this low-temperature region, and if the radiation produced by the sample is in the visible range of the spectrum, a coloured "tail flame" is produced downstream of the main plasma.

The series of experiments carried out on the d.c.-arc plasma jet were repeated with the high-frequency plasma torch as the source on a Hilger large-quartz spectrograph and a Unicam SP900 flame spectrophotometer. The results obtained are given in Tables III and IV. These figures are intended as an indication of the order of sensitivity available with an aerosol injector, which delivers about 10 per cent. of the sample to the plasma. If the torch is used with direct injection of liquids and especially with powders, the levels of photographic detection should be improved by a factor of about ten, and better than this when photomultiplier detection is used.

Table III shows the order of levels of detection obtained. Ilford N.30 plates were used with an exposure of 60 seconds, and the slit width was 0·03 mm. The rate of atomisation into the cyclone chamber of the aerosol injector was 2·5 ml per minute. The quartz optical system was used and the "tail flame" of the plasma flame was focused on the slit.

The background transmission of the N.30 plates for the region 2,300 to 5,000Å is 99·5 to 99·8 per cent. under the exposure conditions given in Table III. Therefore, with such favourable background conditions the figures can be improved by increasing the exposures or by using more sensitive plates. Table IV shows the stability of the high-frequency plasma torch. Ilford N.30 plates were used with an exposure time of 30 seconds. The slit width was 0·01 mm, and the slit height was 3 mm. With the source in continuous operation, a solution containing 100 p.p.m. of calcium was aspirated at 2·5 ml per minute into the cyclone chamber of the aerosol injector, and the ten exposures were made. The quartz optical system was used with the "tail-flame" region focused on the slit. The plate was developed in a dish with P.Q. universal developer for 3 minutes, and calibrated by using a rotating stepped sector and a 3-amp iron arc. No background correction was carried out on the results shown in Table IV because the transmission was 99·5 to 99·8 per cent.

TABLE III

ORDER OF THE LEVELS OF DETECTION FOR THE HIGH-FREQUENCY PLASMA TORCH

Species	Spectrum line, Å	Concentration of aqueous solution, p.p.m.	Percentage transmission	
			line + background	background
Ca^{II}	3933·666	1	85·1	99·5
Al^{I}	3961·527	50	83·2	99·3
Mn^{I}	4030·755	10	89·1	99·5
Cu^{I}	3247·540	10	81·3	99·8
Cr^{I}	4254·366	20	87·1	99·3
Mg^{II}	2795·530	5	85·1	99·3
Ni^{I}	3414·765	5	77·6	99·5
Co^{I}	3453·505	100	93·3	99·3

TABLE IV

STABILITY OF THE HIGH-FREQUENCY PLASMA TORCH

Exposure number	Percentage transmission for Ca 3933 Å	Percentage transmission for Ca 4226 Å
1	33·72	36·31
2	34·20	36·65
3	34·35	37·15
4	35·98	38·38
5	39·44	40·18
6	36·99	37·32
7	39·09	41·68
8	39·00	40·55
9	36·81	39·26
10	39·81	40·72

The standard deviation of the values for percentage transmission given in Table IV was calculated by using the root mean square method and by assuming normal distribution. Thus for the calcium 3933 Å line, the standard deviation of the percentage transmission was $\pm 2{\cdot}3$, *i.e.*, the coefficient of variation was 6·2 per cent., and for the calcium 4226 Å line, the standard deviation of the percentage transmission was $\pm 1{\cdot}9$, *i.e.*, the coefficient of variation was 4·9 per cent. These figures indicate a high degree of source stability.

TEMPERATURE OF THE SOURCE—

As for the d.c.-arc plasma jet, the approximate temperature of this source was again calculated by using the degree of ionisation of calcium at the 20 p.p.m. level.

The source is composed of three more or less distinct regions, *viz.*, the extremely hot plasma region inside the cell in the maximum field of the work coil (temperature, 12,000 to 15,000° K), the cooler plasma region, which is outside the cell, and the "tail-flame" region. Since most of the work done here has been in the "tail flame," the temperature was measured in this region. Thus for 20 p.p.m. of calcium, the degree of ionisation was approximately 46 per cent. This degree of ionisation indicates a sample electron temperature of 8000° K.

OPERATION OF THE HIGH-FREQUENCY THERMAL PLASMA TORCH—

The source is simple to operate, no electrodes are involved and once the unit has been correctly set up the quartz cell is not affected by the plasma flame.

The torch has been operated continuously with an argon plasma for periods of 8 hours without any difficulty.

Most of the preliminary work has been done with an indirect atomiser and an aerosol feed to the plasma. However, direct injection into the plasma of liquids (aqueous and organic), powdered solids and slurries has also been carried out. No quantitative work with direct injection has as yet been carried out, but the "tail flame" produced is extremely intense and appears to be quite stable.

HIGH-FREQUENCY PLASMA TORCH USED ON A FLAME SPECTROPHOTOMETER—

The "tail-flame" region of the torch was roughly aligned on the optical axis of a Unicam SP900 flame spectrophotometer, no lens or mirror being used.

The reproducibility of the calcium line at 422·7 mμ, as plotted on a chart recorder over a period of 1 hour (20 scans of the calcium line being taken in this period), was found to be ± 2 per cent. at the 10 p.p.m. level.

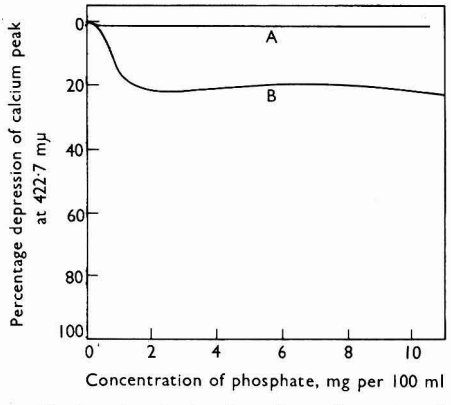


Fig. 3. Graph showing release from phosphate interference on 12 p.p.m. of calcium with a high-frequency torch. Curve A, high-frequency plasma torch; curve B, air - acetylene flame

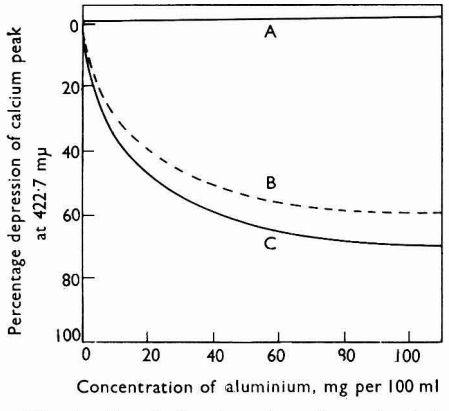


Fig. 4. Graph showing release from aluminium interference on 30 p.p.m. of calcium with a high-frequency plasma torch. Curve A, high-frequency plasma torch; curve B, oxygen-rich air - acetylene flame; curve C, air - acetylene flame

As anticipated, the higher excitation energy available overcame the depressive effects mentioned in the introduction. Figs. 3 and 4 show this release from interference from phosphate and aluminium ions, respectively, on the calcium emission at 422·7 mμ. An indication of the increased sensitivity possible by using the high-frequency plasma torch is shown in Table V. The figures given in Table V are merely comparative, and should not be taken as detection limits.

TABLE V: COMPARISON OF THE HIGH-FREQUENCY PLASMA TORCH AND THE AIR - ACETYLENE FLAME ON THE UNICAM SP900 SPECTROPHOTOMETER

Gain, 6·2; slit width, 0·08 mm; aqueous solutions

Element	Wavelength, mμ	Detection in— air - acetylene flame	Detection in— high-frequency plasma torch
Mercury.. ..	253·6 (atomic line)	10,000 p.p.m. give 5 galvanometer divisions' deflection	50 p.p.m. give 5 galvanometer divisions' deflection
Zinc	334·5 (atomic line)	10,000 p.p.m. cannot be detected	50 p.p.m. give 5 galvanometer divisions' deflection
Aluminium ..	484 (oxide band system)	200 p.p.m. give 5 galvanometer divisions' deflection	—
Aluminium ..	396 (atomic line)	1000 p.p.m. give 5 galvanometer divisions' deflection	20 p.p.m. give 5 galvanometer divisions' deflection

CONCLUSIONS

Our experience with these sources shows them to have advantages that may be conveniently summarised under two headings.

FLAME PHOTOMETRY—

The plasma source has a high degree of stability, has the ability to overcome depressive interference effects caused by the formation of stable compounds, is capable of exciting several elements that are not excited in orthodox chemical flames, and gives increased sensitivity of detection.

SPECTROGRAPHY—

The plasma source is far simpler to operate than the conventional arc and spark methods, especially in solution and liquid analysis, and gives the high degree of stability associated with the a.c. spark combined with the sensitivity of the d.c. arc.

Particular advantages of the high-frequency plasma torch are the lack of electrodes, which gives freedom from contamination, and the extremely low background produced.

The ability to inject powdered samples directly into the plasma, over a period of time, overcomes the problem of sample inhomogeneity and allows longer exposures to be given than with usual powder techniques, with consequent increase in sensitivity. It is hoped to publish shortly some further basic work on the high-frequency plasma torch itself, and some actual analytical methods, some absolute detection limits and results obtained with this source.

We thank Mr. D. I. Spash and Mr. M. R. Yates of Radyne Limited* for their technical assistance and for the loan of high-frequency generators. We also thank Mr. C. L. Brierley for the construction of apparatus.

* Joint patent applications have been made by Messrs. Albright & Wilson (Mfg) Ltd. and Radyne Ltd., for this method and apparatus for exciting substances to emit radiation.

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Photometric Precipitation Titrations*

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Deviation from linearity in the titration curve is to be expected in normal photometric precipitation titrations. The use of such titrations for determining the pH range of precipitation, the number of protons released and the molar ratios of the reactants in precipitation processes is discussed briefly. The rapid determination of calcium in limestone is described; agreement with values of certified samples is better than 1 per cent.

In a previous paper[1] the absence of any intermediate breaks in photometric precipitation titration curves was reported; smooth curves, rather than straight lines with breaks, were always better representations of the experimental results. Simultaneous photometric and potentiometric titrations, however, are extremely useful for determining optimum conditions for performing a precipitation reaction; the final end-points permit the determination of reacting mole ratios and quantitative analysis (between 10^{-2} and 10^{-4} M) with an error usually within 1 per cent. This paper records briefly some applications of photometric precipitation titrations and describes a method for the rapid determination of calcium in limestone.

Experimental

The apparatus and procedure used were as described previously.[1] Light was passed vertically through a stirred sample and fell on to a photocell whose output was fed to a recorder. Portions of reagent were added and the steady-state values of percentage transmission were noted. Simultaneous pH measurements were made with a Beckman combination electrode and a Radiometer 4 pH meter. The photometric titration curves were obtained by plotting a graph of the optical density of the stirred suspension as a function of the volume of reagent added.

Shapes of titration curves—

The shapes of titration curves in photometric precipitation titrations to a maximum turbidity cannot be calculated with any accuracy owing to uncertainties in the nucleation and growth processes. Fischer, Yates and Batts[2] have attempted to correlate the optical density of a suspension with the number and size of particles present, and they predicted that a linear relationship is obtained only when, for an equal volume of reagent added, an equal mass containing particles of the same size is formed. Any factor that enhances the formation of nuclei rather than the growth of the particles, therefore, should help in forming particles of uniform shape and size. Unfortunately, the linear relationship is seldom found in practice.

A series of experiments was performed, in the present work, in which the relationship between the weight of substance present and the optical density of the suspension was investigated; results are essentially in agreement with those of Fischer, Yates and Batts.[2] In the experiments, weights of powdered glass of known mesh size (60 to 100, 140 to 200, 200) were added to water, which was stirred at a constant speed in the heterometer; a few drops of Triton X were added as a wetting agent. The optical densities were plotted as a function of the weight of glass for different mesh sizes. For a given weight of glass, the optical density was greatest for the most finely divided particles; for any one mesh size, even though the particles were essentially the same, a curve was obtained and the deviation from linearity became greater as the particle size increased.

The results of a similar series of experiments in which the powdered glass was replaced by carbon were somewhat different. In all experiments with carbon, straight-line graphs were obtained; for a given weight of carbon, the optical density was again greatest for the most finely divided particles and much less for the largest. The difference between the curves for powdered glass and those for carbon can be attributed to the difference in the

* This paper reports part of the work carried out by St. J. H. Blakeley for his Ph.D. thesis, Dalhousie University, 1964.

absorbing and reflecting power of the two substances. Carbon absorbs rather than scatters light, and is thus more like a coloured solution in its action; carbon, in effect, obeys Beer's Law, in that the optical density is proportional to the "concentration" of the carbon present. Glass on the other hand is reflective and the light that is not back-reflected, rather than a true absorption, is measured.

These experiments suggest that, in photometric precipitation titrations, a graph of the optical density against volume of reagent added will not result in a linear curve. Deviation from linearity is to be expected in precipitation processes, since the number and size of particles formed for equal volumes of added reagent are seldom constant (both nucleation and crystal growth are undoubtedly involved). Even when particles are essentially the same in size, a normal precipitate would exhibit behaviour intermediate to the extremes of carbon and powdered glass; one might expect some deviation from linearity as a result of the absorption characteristics of the precipitate, particularly if the particle size is relatively large.

pH RANGE OF PRECIPITATION—

Many metal ions are completely precipitated by organic reagents only over a narrow range of pH; the determination of this range by performing the precipitation at various values of pH, testing for completeness of precipitation and analysing the product can be tedious and time consuming. A simultaneous photometric and potentiometric titration, however, will often give the desired information quickly. The general method is to mix the metal ion with an excess of reagent in an acidic or a basic solution in which no precipitate is formed, and to titrate with base or with acid. The steady-state values of the optical density, the pH of the solution and the volume of titrant added are measured.

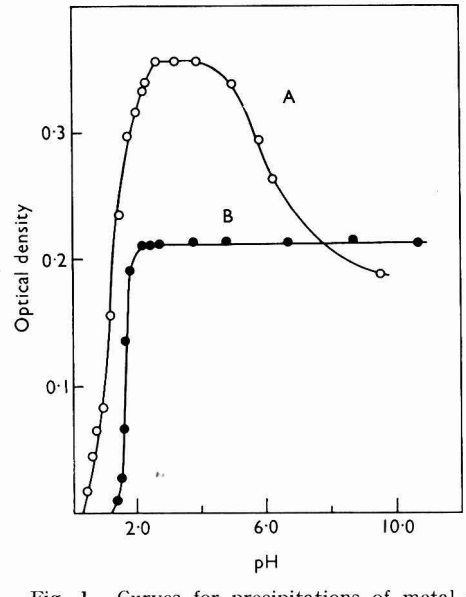

Fig. 1. Curves for precipitations of metal ions with pyromellitic acid. Curve A, iron; curve B, lead

The results can be presented in two ways—

(*a*) A graph is plotted of the optical density as a function of pH; the horizontal part of the curve is the range of complete precipitation. Fig. 1 shows two typical results for pyromellitic acid (1,2,4,5-benzenetetracarboxylic acid), which was found to precipitate lead completely above pH 2 (curve B); the precipitate was stable and did not dissolve in an excess of sodium hydroxide. Iron, however, was completely precipitated over the pH range 2·6 to 3·9 (curve A); above pH 4 the optical density decreased as the light-brown precipitate changed to red-brown hydrated ferric oxide, indicating that the iron - pyromellitic acid complex was rather unstable.

(*b*) The optical density and the pH of the solution are plotted as a function of the volume of acid or base added. Fig. 2 shows the titration curves for a mixture of oxine and aluminium (molar ratio, 3 to 1) in acetic acid titrated with sodium hydroxide solution. The pH range of precipitation differed from that reported by Bobtelsky and Welwart,[3] in that the precipitation started at pH 4·42 and was complete at pH 5. After pH 6·5, on the addition of more sodium hydroxide, the pH rose sharply to above 9 and the precipitate started to dissolve. The molar ratio of protons released to aluminium ions reacting, as calculated from the difference between the volume of sodium hydroxide solution needed to titrate the acetic acid alone and the volume needed to titrate the acetic acid and the liberated protons, was found to be 3 to 1.

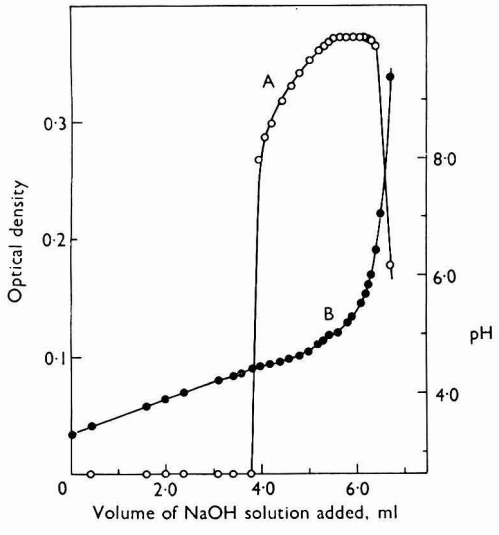

Fig. 2. Titration curves for a mixture of oxine and aluminium titrated in acetic acid with sodium hydroxide solution. Curve A, photometric curve; curve B, potentiometric curve

Another method that may sometimes be used for determining the number of protons released upon reaction simply involves the simultaneous measurement of optical density and pH as metal ion is titrated by reagent, or *vice versa*. The molar ratios of the reactants are obtained from the photometric end-point, and if protons are released in the reaction the number of protons involved is calculated from the initial pH and the final pH of the solution at the photometric end-point. An example is the titration of pyromellitic acid with lead solutions (see Fig. 3). When 4·98 ml of 0·01469 M pyromellitic acid and 10 ml of water were titrated with 0·04936 M lead perchlorate solution, the molar ratio of lead to pyromellitic acid at the photometric end-point was found to be 1·97 to 1. From the initial pH (2·37), the final pH (1·71) and the volume of the solution, the number of protons released per mole of lead reacting was found to be 1·98. In this reaction it was therefore readily shown by simply recording the pH at the beginning and end of the reaction, that two moles of lead react with one mole of pyromellitic acid, with the liberation of four moles of proton.

DETERMINATION OF THE MOLAR RATIOS OF THE REACTANTS—

The determination of the molar ratios of the reactants in precipitation processes involving organic reagents is not always easy. Weighing the product obtained from a varied amount of reagent and a fixed amount of metal for this purpose is not only time-consuming and necessitates a product stable to drying, but it can lead to misleading interpretation if contamination occurs by excess of reagent, or reagent products (if an intermediate oxidation - reduction takes place) or if anions are also part of the precipitated product. The end-point of a photometric precipitation titration, however, readily shows the molar ratios of metal ion to reagent.

The titration of tin^{II} with benzoylphenylhydroxylamine (BPHA), for example, gave a molar ratio for tin to reagent of 1 to 4, whereas the titration of tin^{IV} gave a molar ratio of 1 to 2 for tin to reagent. As the two products are identical,[4] $[Sn(BPHA)_2Cl_2]$, the titrations show directly that two moles of benzoylphenylhydroxylamine are used to oxidise the tin^{II} to tin^{IV}. It is interesting to note that cupferron reacted with tin^{IV} to give a molar ratio of 1 to 4 for tin to reagent; this suggests that the extra steric hindrance of the benzoyl group, which replaces the nitroso group in cupferron to give benzoylphenylhydroxylamine, favours the formation of the 1-to-2 complex.

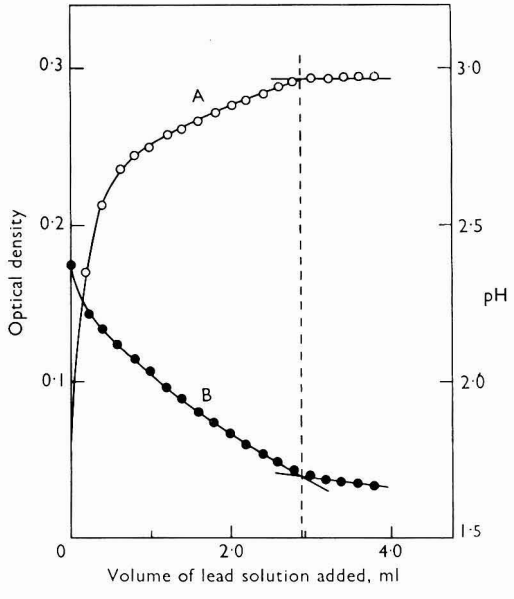

Fig. 3. Titration curves for the titration of pyromellitic acid with lead solutions. Curve A, photometric curve; curve B, potentiometric curve

Similarly, although direct analysis is both difficult and time consuming in determining the number of moles of organic material involved in such precipitations as palladium with *o*-phenanthroline, platinum with dimethylphenylbenzylammonium chloride and lead with phytic acid, the photometric titrations readily showed that the molar ratios of metal ion to reagent in these reactions were 1 to 1, 1 to 2 and 9 to 1, respectively.

Quantitative analysis—

The use of the final end-point in a photometric precipitation titration for quantitative analysis is obvious; Bobtelsky[5] has shown that many ions can be quantitatively determined in the presence of large amounts of other ions. However, since a true test of the technique is the quantitative determination of a particular element in an ore or a mineral, the determination of calcium in limestone by a photometric precipitation titration technique was investigated.

Determination of calcium in limestone

Principle of the method—

The limestone sample is dissolved in acid, filtered and diluted to a known volume. Portions of the solution are then titrated in an acetic acid - acetate buffer at pH 4·5 to 5 with a standard solution of sodium oxalate. The percentage of calcium (reported as oxide) is calculated from the end-point of the photometric precipitation titration.

REAGENTS—

Limestone samples—Having certified calcium contents.

Sodium oxalate—Prepare a 0·1 M solution, and standardise it.

Buffer solution—Mix 0·05 M acetic acid and sodium acetate to give a buffer solution of pH 4·5 to 5.

PROCEDURE—

Dry the limestone sample at 100° C for one hour. Warm 0·4 to 0·5 g of the sample with 5 ml of 4 N hydrochloric acid, ensuring complete acid attack. Filter off the insoluble matter, almost neutralise the excess of acid in the filtrate with sodium hydroxide and dilute the solution accurately to 100 ml. Add 8 ml of the buffer solution to 10 ml of the sample solution and titrate it with a standard solution of 0·1M sodium oxalate.

RESULTS—

The results obtained for a series of limestone samples are given in Table I; the samples contained 4 to 10 per cent. of SiO_2, 1 to 4 per cent. of R_2O_3, and 4 to 9 per cent. of MgO. The time for a titration was about 10 to 20 minutes. An acid leach is sufficient to dissolve all the calcium in most limestone samples, and a preliminary separation of silica and hydrated oxides was not necessary. The results indicated that there was no interference from the co-precipitation of magnesium oxalate; the agreement with the analysed values was better than 1 per cent.

TABLE I

RESULTS OF DETERMINATION OF CALCIUM IN LIMESTONE

The values given are the average of three titrations

Weight of sample in 100 ml of solution, g	Concentration of oxalate, M	Percentage CaO found	Percentage CaO present
0·84534	0·1168	$55·9_6$	56·00
0·37887	0·1168	$46·6_5$	46·97
0·39529	0·1168	$44·3_8$	44·45
0·44580	0·1171	$39·2_0$	38·90
0·45895	0·1171	$41·8_1$	41·71
0·44092	0·1171	$40·5_6$	40·61

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A Field Method for Determining Benzene Hexachloride in Timber Dips

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A simple and reliable method is described for determining benzene hexachloride in timber dips containing Mixol 20. It involves extraction of the insecticide with toluene from a sample solution and subsequent dehydrochlorination with alkali at room temperature. The final colorimetric procedure is based on displacement of the thiocyanate from mercuric thiocyanate by hydrolysable chlorine and reaction between the liberated thiocyanate and ferric ion to give an intense red colour. The method is applicable to concentrations up to 0·5 per cent. w/v of BHC.

The early preservation of newly sawn timber against attacks by insect pests, such as powder-post beetles and pinhole borers, is of great economic importance in tropical regions such as West Africa. One of the most effective treatments, widely used by sawmills, is dipping the timber in a bath containing an insecticide, such as benzene hexachloride (BHC).[1,2] This dip consists of a 2·5 per cent. v/v aqueous dispersion of an emulsifiable concentrate of γ-BHC* together with a fungicide, sodium pentachlorophenate. The dipping practice adopted by sawmills varies with locality and variety of timber treated. The bath is usually topped up with water and further amounts of the BHC formulation during repeated use, and renewed after 3 to 4 weeks. The common dosage of BHC in a freshly prepared bath is about 0·5 per cent. w/v, and a concentration below 0·2 per cent. is considered as seriously depleted. One of the difficulties confronting mills was not knowing when the bath needed replenishing owing to the lack of a simple method for determining BHC. A reliable field method for rapid assessment of BHC in timber dips was sought.

In ethanolic alkaline solution, BHC is dehydrochlorinated to yield 1,2,4-trichlorobenzene as the principal product.[3] Methods are available for determining BHC based on titration of the hydrolysable chlorine with standard silver nitrate and potassium thiocyanate solutions according to the Volhard method, or on electrometric titration with standard silver nitrate solution.[4,5,6] For a field method as applied to timber dips, a procedure with a colorimetric final determination of hydrolysable chlorine would be a decided advantage. In 1952, Iwasaki, Utsumi and Ozawa[7] developed an absorptiometric method for determining chloride, which depended on displacement of the thiocyanate from mercuric thiocyanate by chloride and subsequent addition of iron^{III} to form a red complex. This method was adopted for analysing water[8] and concentrated hydrogen peroxide.[9] By using a procedure based on this colour reaction, Paulini and Reis[10] determined small amounts of DDT and BHC residues on sprayed surfaces after decomposition of the insecticide in benzene extracts with alcoholic sodium hydroxide.

The procedure described by Paulini and Reis has been modified for determining BHC in timber dips. Owing to its toxic nature, benzene was considered unsuitable for use as an extractant in this work, toluene (solubility of BHC, 27·6 per cent.[11]) being chosen as a substitute. To obtain the reliability required, conditions for extracting BHC from timber dips and for developing the colour were critically explored. For accurate assessment of the method, optical densities were measured at 460 mμ with a Unicam SP600 spectrophotometer.

* Available commercially as Mixol 20.

Method

Reagents—

Sodium nitrate solution, saturated.

Toluene.

Sodium hydroxide, 0·1 N, alcoholic—Dilute 2·0 ml of 5 N sodium hydroxide to 100 ml with chloride-free 95 per cent. alcohol; prepare a fresh solution each day.

Ammonium ferric sulphate solution, 1 per cent. w/v in 15 per cent. v/v nitric acid—Dissolve by heating 1 g of ammonium ferric sulphate in water containing 15 ml of concentrated nitric acid, cool the solution, and dilute it to 100 ml with water.

Mercuric thiocyanate solution, 0·1 per cent. w/v, alcoholic—Dissolve by stirring 0·1 g of mercuric thiocyanate in 100 ml of chloride-free 95 per cent. alchool.

Stock BHC solution—Dissolve 0·50 g of lindane (99 per cent. γ-BHC) in toluene, and dilute the solution to 100 ml with toluene. Then—

1 ml of solution $\equiv$ 5 mg of BHC.

Standards A, B, C, D and E, containing respectively 0·2, 0·4, 0·6, 0·8 and 1·0 mg of BHC per ml of solution and equivalent to 0·1, 0·2, 0·3, 0·4 and 0·5 per cent. w/v of BHC in timber dips, are prepared by diluting 2·0, 4·0, 6·0, 8·0 and 10·0 ml of stock solution to 50 ml with toluene. The standard solutions are stable under normal laboratory conditions.

Procedure—

Transfer 10·0 ml of the well mixed sample of timber dip into a 250-ml separating funnel, and add 50 ml of saturated sodium nitrate solution and 50·0 ml of toluene. Shake the contents vigorously for 1 minute, then allow the phases to separate. Reject the aqueous solution, and transfer the toluene extract to a 100-ml conical flask.

Transfer by pipette, 1·0 ml of the extract into a 6-inch $\times$ $\frac{3}{4}$-inch glass-stoppered test-tube. At the same time, measure 1·0 ml of standards A, B, C, D and E into five test-tubes. Add 10·0 ml of 0·1 N sodium hydroxide to each test-tube, mix the contents thoroughly, then set them aside for 15 minutes. Add 2·0 ml of 1 per cent. ammonium ferric sulphate solution and 2·0 ml of 0·1 per cent. mercuric thiocyanate solution to each tube, mixing after each addition.

Compare visually the colour of the sample solution with standards. If the colour is the same as or paler than standard B, the dipping solution under test contains less than 0·2 per cent. w/v of BHC, and is seriously depleted.

Discussion of method

Dehydrochlorination—

Gunther and Blinn[3] found that chlorine atoms at the 3, 5 and 6 positions of BHC were readily removed in ethanolic alkaline solution under reflux. The extent of dehydrochlorination of BHC at room temperature in 0·1 to 0·3 N alcoholic sodium hydroxide was investigated. Dehydrochlorination took place almost immediately after sodium hydroxide had been added, and the approximate maximum colour in the final solution was produced with 0·1 N sodium hydroxide. When 0·3 N sodium hydroxide was used, the final solution was cloudy owing to partial precipitation of sodium hydroxide. The colour intensity was reduced by about 35 per cent. when the alcohol in the reagent was decreased from 95 to 80 per cent.

Nitric acid concentration—

In the presence of an excess of ferric ions in acid solution, the thiocyanate liberated from mercuric thiocyanate immediately produced a red complex. To find the most suitable reagent, solutions of 10 to 30 per cent. v/v of nitric acid (each containing 1 per cent. w/v ammonium ferric sulphate) were used for acidifying the toluene - alcoholic sodium hydroxide solution containing BHC. The sensitivity of the method was found to be increased with increased acid concentration. Addition of 2 ml of 15 per cent. nitric acid containing 1 per cent. of ammonium ferric sulphate provides sufficient excess of acid after the alkali has been neutralised to give adequate sensitivity for visual comparison of colour over the range 0·1 to 0·5 per cent. of BHC.

Ammonium ferric sulphate and mercuric thiocyanate concentrations—

Optical densities for both the reagent blank solution and the final solution containing BHC increased with ammonium ferric sulphate concentration from 0·5 to 2 per cent. w/v.

Concentrations higher than 2 per cent. produced a cloudy final solution. A reagent consisting of 1 per cent. of ammonium ferric sulphate is considered adequate.

There was no appreciable difference between optical densities of final solutions obtained by using 0·1 and 0·2 per cent. w/v mercuric thiocyanate solutions, but a 0·05 per cent. reagent gave a decidedly lower colour intensity. Mercuric thiocyanate is not readily soluble in 95 per cent. alcohol; for ease of preparing the reagent without sacrificing the sensitivity of method, use of 0·1 per cent. mercuric thiocyanate in 95 per cent. alcohol is recommended.

STABILITY OF COLOUR—

A period of 5 minutes was required for developing the maximum colour, which was stable for at least 1 hour. The colour, however, fades rapidly in sunlight. The time for visual comparison of colour is therefore not critical provided that exposure of the final solution to strong light is avoided.

EXTRACTION OF BHC FROM TIMBER DIPS—

In preliminary experiments, BHC in timber dips was repeatedly extracted with toluene, the ratio of timber dip to toluene being 1 to 5 and the time of shaking 1 minute. Removal of BHC from timber dips was found to be incomplete, recoveries ranging from 20 to 30 per cent. in the first extraction, about 15 per cent. in the second and 5 per cent. in the third. Separation of phases was slow, and filtration was required to obtain a clear extract before colour development. Extraction was improved, but by no means complete, when a greater proportion of toluene was used, but this is inconvenient in practice. The incomplete removal of BHC by toluene is apparently owing to formation of emulsions. Addition of an inorganic salt, such as sodium sulphate or nitrate, improved the partition ratio of BHC between toluene and timber dip by the salting-out effect. Use of 50 ml of saturated sodium nitrate solution effected complete extraction of BHC and rapid separation of phases.

INTERFERENCE—

Inorganic chlorides present in timber dips do not affect the method, as they are not extracted into toluene. Organo-chlorine compounds, such as sodium pentachlorophenate (61·5 per cent. chlorine), cause no interference under the experimental conditions. However the apparatus and reagents used, particularly alcohol and nitric acid, must be free from chloride, or a high background will be found. Absolute alcohol may be used, instead of 95 per cent. alcohol, provided that 5 ml of water is added to each 100 ml. Absolute alcohol alone should not be used in the preparation of reagents, as precipitates form in the final solution, owing to low solubilities of sodium nitrate, ammonium ferric sulphate and mercuric thiocyanate.

RESULTS

To establish the accuracy of the method, BHC contents of 18 samples of laboratory-made timber dips containing 0 to 0·5 per cent. of BHC were determined. Three groups of dips were prepared—

(*i*) with tap water, but containing neither fungicide nor borax,

(*ii*) with Thames water and containing 2 per cent. each of sodium pentachlorophenate and borax, and

(*iii*) with Thames water and containing 2 per cent. each of sodium orthophenylphenate and borax.

Recovery results for BHC are shown in Table I.

TABLE I

DETERMINATION OF BHC IN LABORATORY-MADE TIMBER DIPS

	Group (*i*)		Group (*ii*)		Group (*iii*)	
BHC added, per cent.	BHC found, per cent.	Recovery, per cent.	BHC found, per cent.	Recovery, per cent.	BHC found, per cent.	Recovery, per cent.
0	0	—	0·005	—	0·005	—
0·1	0·100	100	0·095	95	0·105	105
0·2	0·200	100	0·195	98	0·192	96
0·3	0·295	98	0·290	97	0·305	101
0·4	0·385	96	0·382	96	0·390	98
0·5	0·460	92	0·487	97	0·485	97

The percentage recoveries of BHC ranged from 92 to 105 with a mean of 98 (standard error $\pm$ 1·1), based on 15 determinations.

The proposed method was applied to a sample of timber dip obtained from Lagos, Nigeria. It was taken from a bath that had been used for 2 weeks and consisted of Mixol 20, sodium pentachlorophenate and borax, which was frequently topped up with water, but not with BHC. Duplicate results for the amount of BHC present were 0·070 and 0·073 per cent. This is considerably lower than the accepted minimum of 0·2 per cent. for adequate timber treatment. The average recovery, based on 0·2 and 0·4 per cent. of BHC added to this sample, was found to be 87 per cent., which is considered satisfactory.

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A Sensitive Method for Determining Nitrate in Water with 2,6-Xylenol

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Use of 2,6-xylenol in place of 2,4-xylenol in a common procedure for the determination of nitrate yields a fourfold increase in sensitivity. A method is described in detail. The linearity of the calibration graph and the reproducibility of the method are established. Interference from nitrite is removed by sulphamic acid, and chloride interference is prevented by means of mercuric sulphate. Good recoveries are obtained from natural waters.

THE use of substituted phenols as reagents for determining nitrates is well established, the procedures in general being based on the nitration of the phenols in the presence of a high concentration of sulphuric acid. Phenol-2,4-disulphonic acid and 2,4-xylenol have been used widely for several years, and recently, methods based on 2,6-xylenol, involving determinations of optical density at ultraviolet wavelengths, have been proposed.[1,2,3] 3,4-Xylenol has also been used.[4]

If measurements are to be made in the visible region of the spectrum it is necessary to convert the nitrophenol to the alkali metal or ammonium salt. In the phenoldisulphonic acid method, this is achieved by prior evaporation of the sample to dryness, so that only a small amount of sulphuric acid is required to produce a suitable nitrating mixture; the acid is then neutralised by an excess of alkali hydroxide solution.[5,6] When 2,4-xylenol or 3,4-xylenol is used, the nitroxylenol may be separated from the acid mixture by steam-distillation,[4,7] or by solvent extraction[8]; no evaporation of the sample is necessary.

Although the steam-distillation procedure is not applicable to 4-nitro-2,6-xylenol,[4] solvent extraction may be used.

Experimental

In the work described here, the high sensitivity of a 2,6-xylenol method with solvent extraction has been established.

An aqueous solution of the sodium salt of 4-nitro-2,6-xylenol, obtained by this method, was examined by using the Unicam SP 500 spectrophotometer with water as the reference solution, and the spectrum was found to have a broad absorption maximum at 432 mμ.

The factor for converting optical densities, measured in 1-cm cells, to the weight in milligrams of nitrate nitrogen (nitrate-N) per litre in the original solution was 3·47 mg per litre per unit optical density. This corresponds to an optical density of 1·15 per mg of nitrate-N per litre in the final solution, compared with 0·27 per mg of nitrate-N per litre obtained by using 2,4-xylenol in a similar procedure.

The blank solution, obtained by carrying out the analytical procedure on a portion of demineralised water, had an optical density of 0·014, with a reproducibility corresponding to $\pm$0·007 mg of nitrate-N per litre in the original portion.

Interference by nitrite was overcome, when necessary, by adding sulphamic acid solution. For a sodium nitrite solution containing 5 mg of nitrite-N per litre, twice the theoretical equivalent of sulphamic acid was found adequate for the complete destruction of the nitrite. Nitrites interfere by reacting with the 2,6-xylenol to form 4-nitroso-2,6-xylenol,[1,2] whose sodium salt showed an absorption maximum at 393 mμ. However, owing to the incomplete recovery of this compound by the extraction procedure, and to the relatively low optical density at 432 mμ, nitrite concentrations up to 0·05 mg of nitrite-N per litre would not introduce a significant error.

Interference by chlorides at concentrations up to 700 mg of chloride per litre was prevented by including 0·1 per cent. of mercuric sulphate in the sulphuric acid reagent. Chlorides, under the conditions of the determination, reduce nitrates probably to nitrosyl chloride,[9] which reacts with the 2,6-xylenol to form 4-nitroso-2,6-xylenol,[1,2,3] and hence leads to a serious loss in the observed optical density. In the presence of an excess of mercuric sulphate, soluble, undissociated mercuric chloride is formed, and the formation of nitrosyl chloride is prevented.[10] It was found that the mercuric sulphate was slowly precipitated from the sulphuric acid on standing; the sulphuric acid reagent therefore was prepared daily from a stock mercuric sulphate solution.

By using these refinements, the recovery of added nitrate was determined at two levels in the 0 to 1·0 mg of nitrate-N per litre range in each of five drinking waters and one effluent, with initial nitrate concentrations of 0 to 2·5 mg of nitrate-N per litre. In eleven of these twelve results, the recovery differed from the amount added by not more than 0·02 mg of nitrate-N per litre; the twelfth, being the highest total concentration determined, was low by 0·04 mg of nitrate-N per litre.

Two recoveries in the 0 to 9 mg of nitrate-N per litre range, from an effluent containing 11 mg of nitrate-N per litre initially, were made by using a tenfold dilution of the final solution and correcting the blank value accordingly. These recoveries were in error by 0·2 and 0·3 mg of nitrate-N per litre.

As each recovery is given by the difference of two determinations, individual determinations on most natural waters should be within about 0·01 mg per litre of the true value in the range 0 to 3 mg of nitrate-N per litre.

METHOD

REAGENTS—

All materials should be of recognised analytical-reagent grade.

2,6-Xylenol reagent—Dissolve 1 g of 2,6-xylenol in 100 ml of glacial acetic acid.

Mercuric sulphate stock solution—Dissolve 2 g of mercuric sulphate in 400 ml of water and 20 ml of concentrated sulphuric acid.

Sulphuric acid reagent—Add 158 ml of concentrated sulphuric acid to 42 ml of the mercuric sulphate stock solution with stirring. Allow the mixture to cool This solution should be prepared daily.*

Sodium hydroxide solution—Dissolve 80 g of sodium hydroxide in 1 litre of water.

Toluene.

Potassium nitrate standard solution—Dissolve 0·722 g of dried potassium nitrate in water and dilute it to 1 litre. This solution contains 100 mg of nitrate-N per litre; suitable dilutions are used for preparing a calibration graph.

Sulphamic acid solution—Dissolve 0·07 g of sulphamic acid in 100 ml of water. This solution is stable for 3 months.

PROCEDURE—

Transfer by pipette a 5-ml portion of the sample containing not more than 15 μg of nitrate-N (*i.e.*, 3 mg per litre) into a 100-ml beaker provided with a cover glass. (Alternatively, small conical flasks may be used.) Add 15 ml of sulphuric acid reagent slowly from a burette, mix the reagents by swirling to avoid overheating, and place the beaker in a cold-water bath to cool.

Add, from a microburette, 1 ml of the 2,6-xylenol reagent, mix the reagents by swirling, and place the beaker in a water-bath at 35° C for 30 minutes. Remove the beaker, and cool it to room temperature.

Wash the contents of the beaker into a 250-ml glass-stoppered separating funnel with 80 ml of water. Add 10 ml of toluene, shake the funnel for 1 minute and allow the phases to separate. Discard the lower, aqueous phase, and wash the toluene extract with a further 10 ml of water; an extremely careful separation should be made at this stage. Add 20 ml of the sodium hydroxide solution from a pipette, shake the funnel for 1 minute, and set it aside for 20 minutes to allow the aqueous phase to clear.

* The mercuric sulphate begins to precipitate from the sulphuric acid reagent after about 24 hours. Reagent that has been stored for more than a day is therefore less effective in preventing interference. In the absence of chloride, the mercuric sulphate may, of course, be omitted.

Flush out the tap and funnel stem with a few millitres of the aqueous alkaline extract, and filter the remainder through a cotton-wool plug inserted into the funnel stem, discarding the first few millitres. Measure the optical density at 432 mμ, by using 1-cm cells with water as a reference solution.

Prepare a calibration graph by treating 5-ml aliquots of a series of solutions, containing 0 to 3 mg of nitrate-N per litre, in the manner described above.

If nitrite is present at a level greater than 0·05 mg of nitrite-N per litre, add twice the equivalent volume of sulphamic acid solution (1 ml of solution ≡ 100 μg of nitrite-N) before carrying out the determination.

Results

Wavelength of maximum absorption—

The wavelength of maximum absorption was found to be 432 mμ by using a Unicam SP500 spectrophotometer. Water was used as the reference solution.

Calibration graph—

A calibration graph was prepared by measuring the optical density of standard solutions of nitrate at 432 mμ in 1-cm cells with water as the reference solution. The results from which the graph was plotted are given in Table I.

Table I

Calibration results

Concentration of nitrate-N, mg per litre	Optical density	Factor, mg per litre: optical density *minus* blank value	Nitrate-N, mg per litre, calculated by using the mean factor
0	0·014	—	—
0·5	0·160	3·42	0·51
1·0	0·301	3·48	1·00
1·5	0·448	3·46	1·51
2·0	0·586	3·50	1·98
2·5	0·737	3·46	2·51
3·0	0·875	3·48	2·99

Mean factor = 3·47

From Table I it can be seen that the concentrations of nitrite-N calculated by applying the mean conversion factor to the observed optical densities of the standard solutions differed from the true values by amounts giving a standard deviation of ±0·011 mg of nitrate-N per litre.

Reproducibility of blank value—

The optical densities of replicate blank solutions were measured at 432 mμ and the results obtained are given below—

Optical density of blank solutions	0·012, 0·013, 0·012, 0·012, 0·015, 0·017
Mean value	0·0135
Standard deviation	±0·007 mg of nitrate-N per litre

Nitrite interference and removal—

Nitrite interference and its removal by adding sulphamic acid were studied and the results are given in Table II.

Table II

Effect of nitrite

Nitrite-N, mg per litre	Sulphamic acid, nitrite-N equivalent, mg per litre	Nitrate-N found, mg per litre*
5	0	0·26
5	5	0·13
5	10	0·04
5	20	0·02
5	50	0·03

* After subtraction of the appropriate blank value for sulphamic acid.

The small residue of nitrate found in the presence of an excess of sulphamic acid must be assumed to be derived from the sodium nitrite used to prepare the standard nitrite solution: 0·025 mg of nitrate-N per litre would correspond to 0·5 per cent. of the total nitrogen being as nitrate ions.

CHLORIDE INTERFERENCE AND REMOVAL—

Chloride interference and its removal by adding mercuric sulphate to the sulphuric acid were studied, and the results are given in Table III.

TABLE III

RECOVERY OF NITRATE IN THE PRESENCE OF CHLORIDE

Reagent	Nitrate-N added, mg per litre	Chloride added, mg per litre	Nitrate-N recovered, mg per litre
80 per cent. sulphuric acid	2·0	0	2·01
	2·0	100	1·79
	2·0	10,000	0·22
80 per cent. sulphuric acid *plus* 0·1 per cent. mercuric sulphate ..	2·0	0	1·99
	2·0	200	2·02
	2·0	500	2·02
	2·0	700	1·99
	2·0	1000	1·41
	2·0	2000	0·29

RECOVERIES FROM NATURAL WATERS—

The proposed method was used to determine the nitrate-N content of several natural waters. The results are given in Table IV.

TABLE IV

RECOVERY OF NITRATE-N FROM NATURAL WATERS

Sample number	Nitrate-N, mg per litre				
	Present	Added	Found	Recovered	Difference
137	0·70	0·47	1·17	0·47	0·00
		0·93	1·64	0·94	+0·01
138	0·78	0·46	1·23	0·45	−0·01
		0·92	1·71	0·93	+0·01
139	2·34	0·15	2·49	0·15	0·00
		0·38	2·73	0·39	+0·01
146	2·50	0·38	2·88	0·38	0·00
		0·75	3·21	0·71	−0·04*
197	0·60	0·47	1·05	0·45	−0·02
		0·94	1·52	0·92	−0·02
E22	0·05	0·69	0·72	0·67	−0·02
		1·18	1·21	1·16	−0·02
E37†	11·0	4·4	15·2	4·2	−0·2*
		8·8	19·5	8·5	−0·3*

* These results were omitted when the standard deviation was calculated.

† Determinations on this sample were made by diluting the final solution to 200 ml in a calibrated flask, the reagent blank value subtracted being reduced by the appropriate factor.

The standard deviation was found to be ±0·013 mg of nitrate-N per litre.

ROUTINE ANALYSIS OF SAMPLES—

The characters of the waters in Table IV are indicated by the results of routine laboratory analysis given in Table V.

TABLE V

ANALYSIS OF SAMPLES

Sample		Content, mg per litre			
Number	Location	Nitrite-N	Chloride	Permanganate value, 4-hour	B.O.D.
137	Public supply..	0·001	14	0·8	—
138	Public supply..	absent	14	1·1	—
139	Public supply..	absent	155	0·1	—
146	Spring and borehole	absent	58	0·8	—
197	Borehole	0·004	183	0·4	—
E22	Outlet of settling tank ..	0·04	51	27	108
E37	Final effluent	0·12	26	6·1	10

REPRODUCIBILITY—

Six replicate determinations were carried out on each of two drinking-water samples. The results are given in Table VI.

TABLE VI

REPRODUCIBILITY

Sample		Replicate determinations: nitrate-N, mg per litre
Number	Location	
153	Spring ..	1·81, 1·77, 1·79, 1·81, 1·79, 1·80
354	Borehole ..	0·61, 0·61, 0·62, 0·61, 0·62, 0·61

Permission to publish this paper has been given by the Government Chemist, Department of Scientific and Industrial Research.

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The Effect of Glycerol Added to Tobacco on the Constituents of Cigarette Smoke

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The use of an absolute isotopic-dilution method for determining benzo[*a*]pyrene in cigarette smoke has shown that the addition of glycerol to tobacco up to the 6 per cent. w/w level fails to effect any major change in the production of benzo[*a*]pyrene in the mainstream smoke. Evaluation of the smoke nicotine and whole tar (oven-baked) confirmed that the humectant does not effect any significant change in the particulate phase of cigarette smoke.

THERE have been several reports[1,2,3] of the effect of additives to tobacco in reducing the benzo[*a*]pyrene content of cigarette smoke. Most of these reports concerned the effects of inorganic salts. Bentley and Burgan,[2] however, also reported that glycerol and ethylene glycol added to tobacco at the 3 per cent. w/w level, lowered the benzo[*a*]pyrene content of cigarette smoke by over 50 per cent. Since glycols are widely used throughout the tobacco industry as humectants,* the implication is that a means of reducing benzo[*a*]pyrene in cigarette smoke is readily available.

Benzo[*a*]pyrene levels found in the smoke of non-filter cigarettes containing a typical Canadian blend of flue-cured tobaccos, analysed by an isotopic-dilution method developed in this laboratory (see "Methods," p. 737), have been in close agreement with the published results[4,5,6,7] for American non-filter blended cigarettes with respect to the levels of this polycyclic hydrocarbon (3·5 μg per 100 cigarettes) and to its ratio to whole tar (0·97 to 1·2 p.p.m.). Yet American cigarette tobaccos contained polyhydric alcohol humectants whereas the Canadian blend did not. This similarity in results might be considered surprising if humectants such as glycerol could effect a reduction of benzo[*a*]pyrene in mainstream smoke of over 50 per cent. This laboratory sought confirmation of the effect on benzo[*a*]pyrene as well as on whole tar (oven-baked) and nicotine, for a typical blend of flue-cured tobaccos containing glycerol at two distinct levels. Table I summarises the results obtained.

Discussion

As can be seen from Table I, no major change, owing to the effect of added glycerol, was observed in the mainstream smoke for any of the three constituents measured. All differences are within the experimental error of the methods used for their analysis. In this experiment, the manufacture and selection of the various cigarettes was purposely made at different weight levels, proportional to the level of glycerol applied. Thus, the basic tobacco weight remained essentially the same for all cigarettes. Any major differences in the smoke could then be attributed directly and solely to the presence of the additive.

In fact, a less exhaustive drying of the collected smoke such as vacuum drying by using a water-aspirator at 50° to 60° C (as used in the determination of benzo[*a*]pyrene), showed that the weight of mainstream smoke can increase with the level of glycerol—

Glycerol in tobacco, per cent. w/w ..	0	3·3	6·1
Whole tar (vacuum-dried)	28·7	36·2	37·0

* Glycols are not used in the United Kingdom where Customs' regulations prohibit the sale of cigarettes containing added glycerol. In the North American continent and in most countries there are no restrictions on its addition.

This increase must be due to the entrainment in smoke of a portion of the humectant. When the whole smoke is evaluated by oven drying for 7 hours at 105° C, the differences in weight disappear, indicating that the entrained glycerol has been removed. The equality of the results shown in Table I indicates, therefore, that the weight of smoke, determined by oven-drying, as well as the levels of benzo[*a*]pyrene and nicotine are essentially unaltered by the presence of glycerol on the tobacco when the same weight of additive-free tobacco is smoked.

TABLE I

EFFECT OF GLYCEROL ON SMOKE CONSTITUENTS

Glycerol in tobacco, per cent. w/w	Weight of cigarette, mg		Benzo[*a*]pyrene, μg per 100 cigarettes	Whole tar (oven-baked), mg per cigarette	Nicotine, mg per cigarette
				30·0	2·12
				31·7	2·21
0	1090		3·44	31·6	2·23
			3·26	31·0	2·19
		Mean ..	*3·35*	*31·1*	*2·18*
				31·5	2·10
				32·3	2·02
3·3	1125		3·41	31·6	2·06
			3·54	31·8	2·06
		Mean ..	*3·48*	*31·8*	*2·06*
				31·1	2·04
				30·6	2·05
6·1	1155		3·63	30·5	1·99
			3·50	30·3	1·99
		Mean ..	*3·51*	*30·6*	*2·02*

These results differ significantly from the 62 per cent. reduction in polycyclic hydrocarbon reported by Bentley and Burgan,[2] obtained by adding 3 per cent. of glycerol to tobacco. The major difference from our work lies in the collection device and smoking régime. Bentley and Burgan[8] collected the smoke by electrostatic precipitation after smoking their cigarettes with a 15-ml puff volume of 2 seconds' duration taken 4 times a minute. In this laboratory the smoke was condensed in cold traps after the cigarettes had been smoked with a 35-ml puff, also of 2 seconds' duration, but taken only once a minute. This latter smoking regime is considered standard on the North American continent, being close to human practice as observed by Sclur and Rickards[9] and Keith and Newsome.[10,11]

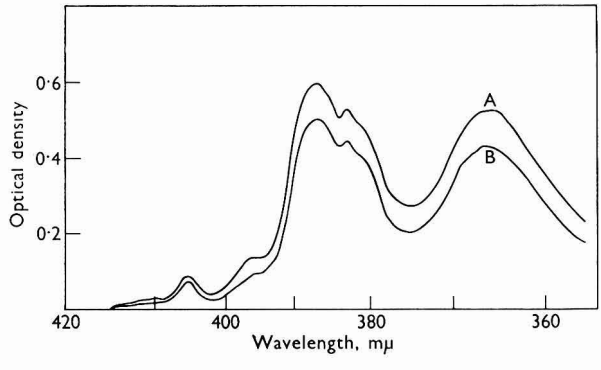

Fig. 1. Absorption spectra of benzo[*a*]pyrene in cyclohexane and measured in 10-cm cells: A, obtained from cigarette smoke; B, a pure solution.

It is nevertheless difficult to reconcile the divergencies on the basis of smoking régimes alone. Although different smoking parameters would yield different levels of smoke constituents, any changes due to different tobacco treatments should show essentially the same proportional effect. Rather, the explanation may be that the reproducibility of the method used by Bentley and Burgan[7] was insufficient to reflect the true effect of glycerol.

The method in use in our laboratory permits the precise assessment of the benzo[*a*]pyrene recoveries by radioactive tracer techniques and the successful isolation of the pure polycyclic hydrocarbon (see Fig. 1). The confidence level for the mean of duplicate runs is 0·22 μg per 100 cigarettes at the 95 per cent. level of significance. This precision is sufficient to establish a 10 per cent. difference in the benzo[*a*]pyrene content of cigarette smoke.

Methods

Preparation and smoking of samples—

A 50-lb sample of a typical flue-cured blend of tobaccos was divided into three equal portions. Two portions were sprayed with a 5 or 10 per cent. w/v alcoholic solution of glycerol in a rotating cylinder to yield, respectively, a 3 and a 6 per cent. w/w (wet basis) level of humectant. After removal of the alcohol by air-drying, the tobacco was re-equilibrated at 69° F and 60 per cent. relative humidity. The final levels of glycerol obtained were confirmed by a gas-chromatographic method (see below). Cigarettes were then manufactured from each sub-sample of tobacco with the same dimensions (74-mm length and 25-mm circumference) and with the same type of paper, but at target weights that were increased in proportion to the level of glycerol present.

All cigarettes were smoked to a 23-mm butt length by the régime described above on a constant-volume, falling-water column type of machine similar to that of Bradford, Harlan and Hanmer,[12] but equipped with solenoid valves.

Determination of benzo[*a*]pyrene—

The method used in this laboratory is similar in many respects to those described by Wynder and Hoffman[7] and Barkemeyer[13] although developed independently in 1959. It is fully described here as it contains several detailed improvements, particularly with respect to the determination of recoveries by isotopic dilution and in the isolation of pure benzo-[*a*]pyrene by column chromatography and a single paper-chromatographic development that permits quantitative determination by ultraviolet spectroscopy without resort to more specific but involved fluorescence methods.

Cigarette smoke was collected in glass spiral traps cooled with an acetone - dry ice mixture. A measured amount of purified 7,10-^{14}C-benzo[*a*]pyrene (Bio-Rad Laboratories, Richmond, California, U.S.A.) of known specific activity (6·3 μC per mg) was added to the condensed smoke, which was then taken up in a 17 + 3 methylene chloride - methanol mixture. After the solution had been dried overnight over anhydrous sodium sulphate, it was filtered and evaporated to dryness under vacuum in a rotary evaporator so that a gross weight of dry smoke was obtained. For a gross enrichment of the polycyclic aromatic fraction, the dry residue was extracted by heating it under reflux with, and decanting off, three 20-ml portions of benzene, and subsequently by processing the concentrated extract (2 to 3 ml) through a 15 × 4·5-cm column of 200-mesh neutral alumina (activated at 200° C for 24 hours). n-Hexane was used as eluant until the first yellow-green fluorescent band was eluted. This ensured the removal of waxes and squalene-type compounds that would interfere in subsequent steps. n-Hexane was then replaced by benzene, and the elution continued until the bulk of the blue fluorescent materials had been removed (in up to 3 litres of benzene). The benzene eluate, containing the polycyclic hydrocarbons, was concentrated by careful distillation and finally reduced to dryness. To obtain a preliminary separation and purification of benzo-[*a*]pyrene, the dry residue was processed with n-hexane through a 20 × 1·5-cm column of "through 200 mesh" silica gel (activated at 110° C for 6 hours). Fractions (25 ml) were collected, and all those with inflexions in their spectra at 365 and 385 mμ (in a 4-cm cell) were combined, and the combined solution reduced to dryness. Pure benzo[*a*]pyrene was finally separated from associated polycyclics by paper chromatography on Whatman 3 MM paper, acetylated by Spotswood's[14] method, with a methanol - ether - water (4 + 4 + 1, v/v) developing system. By an ascending-solvent "stop and start" technique (*i.e.*, the development was stopped after 3 hours, the paper air-dried and the development re-initiated for a further 6 to 7 hours), benzo[*a*]pyrene was obtained with an R_F value of 0·2 to 0·3. The blue fluorescent spot at this R_F was cut out and the benzo[*a*]pyrene extracted with hot benzene in a micro Soxhlet apparatus. The extract was then concentrated and eluted with benzene through a short (1·0 × 2·0-cm) silica-gel column to remove paper fibres and ultraviolet-absorbing impurities. The eluate was finally reduced to dryness and diluted to 10 ml with cyclohexane.

The benzo[*a*]pyrene concentration of this solution was determined by measurement of the ultraviolet absorption at 384 mμ in a 10-cm cell (by using the base-line technique with tangents between 374 and 400 mμ) and comparison with a previously established calibration curve: $C = 1{\cdot}40\,A$, where C in μg per ml is the concentration and A is the optical density.

Determinations of radioactivity were made at "infinite thinness" by plating suitably sized portions (usually 0·2 μl) in triplicate on stainless-steel planchets and counting them in a Baird-Atomic Automatic Sample Changer equipped with an ultra-thin (less than 70 μg per sq. cm) window proportional-flow detector. The absolute recovery of benzo[*a*]pyrene was calculated from a knowledge of the amounts and levels of radioactivity of the compound isolated and that added to the smoke before processing. The original weight of benzo[*a*]-pyrene in the collected smoke was obtained from the equation—

$$W_c = \frac{100\,W_o}{R} - W_s$$

where W_c, W_o and W_s are respectively: the weight of benzo[*a*]pyrene present in the original smoke sample; that determined by spectrophotometry; and that of 7,10-^{14}C-benzo[*a*]pyrene added to the original smoke sample, and R is the per cent. recovery of radioactivity.

In the above procedure certain specific points were considered essential to ensure adequate recovery levels and the isolation of pure benzo[*a*]pyrene—

(*a*) Light was excluded as much as possible to minimise photo-decomposition.

(*b*) All steps involving reduction to dryness were made with a current of dry nitrogen at 50° to 60° C to avoid degradation.

(*c*) Halogenated-hydrocarbon vapours were excluded from the atmosphere surrounding the radioactive counter to eliminate a progressive count decay.

(*d*) The amount of labelled benzo[*a*]pyrene added to the collected smoke was kept at a level approximately equal to that expected in the smoke sample, to provide portions for sufficiently reliable counts as well as to minimise the magnitude of error.

The purity of the benzo[*a*]pyrene isolated by this procedure (see Fig. 1) and the consistency of the results obtained were further established by fluorescence analyses with an activation wavelength of 363 mμ and an emission wavelength of 403 mμ (see Table II).

TABLE II

COMPARISON OF BENZO[*a*]PYRENE MEASUREMENT TECHNIQUES

	Concentration of benzo[*a*]pyrene, μg per ml										
Smoke sample . .	A	B	C	D	E	F	G	H	I	J	K
Ultraviolet . .	0·29	0·36	0·55	0·58	0·60	0·68	0·72	0·73	0·76	0·79	0·82
Fluorescence . .	0·28	0·37	0·57	0·58	0·61	0·68	0·72	0·73	0·75	0·79	0·82

The method described was developed initially for smoke samples from several hundred cigarettes. However, it proved readily adaptable to scaling down to permit analyses of the smoke from as few as 20 to 30 cigarettes without loss of precision.

DETERMINATION OF GLYCEROL—

Glycerol levels were analysed essentially by the method of Friedman and Raab.[15] The second clarification with charcoal was found unnecessary. The gas-chromatographic separations were carried out isothermally at 220° C on a 2·3-metre stainless-steel column, having a $\frac{1}{4}$-inch internal diameter with a 10-to-90 weight ratio of Carbowax 20 M on 40-mesh Haloport F. Injector and detector temperatures were 280° C. The thermal-conductivity filament current was 150 mA. With a helium flow-rate of 60 ml per minute (outlet), the elution time for glycerol was 11·7 minutes.

DETERMINATION OF WHOLE TAR (OVEN-BAKED) AND NICOTINE—

The smoke from 11 cigarettes was collected in an absorption train similar to that described by Laurene and Harrell[16] in which the Kjeldahl flask and the two bubbler traps contained 11 ml of 0·05 N ethanolic hydrochloric acid, 5 ml of 0·5 N aqueous hydrochloric acid and 5 ml of 0·5 N ethanolic hydrochloric acid, respectively. The absorption train was

then washed out alternately with ethanol and warm water and the smoke solution was diluted to the mark in a 110-ml calibrated flask. Nicotine was evaluated from a 10-ml aliquot by a micro-scale adaptation of the Laurene and Harrell method.[16] Whole tar was determined on the remaining 100 ml of solution by removing the solvents in a steam-bath under a stream of air and oven-drying the residue at 105° C for 7 hours.

The confidence levels of the methods for the means of four results at the 95 per cent. level of significance are $\pm 1{\cdot}6$ and $\pm 0{\cdot}18$ mg per cigarette, respectively, for whole tar and nicotine.

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A Thin-layer Chromatographic Method for Identifying Annatto and Other Food Colours

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A simple thin-layer chromatographic technique is described for separating and identifying eleven yellow food dyes. Annatto colour and curcumin can be separated from other fat-soluble and water-soluble dyes examined, on glass slides coated with silicic acid containing Plaster of Paris, or silica gel G with amyl acetate as a developing solvent. The fat-soluble and water-soluble dyes can be further separated on glass slides coated with calcium carbonate containing starch if the slides are treated with liquid paraffin. The separation of the dyes is achieved by using a solvent mixture of methanol, water and ammonia solution in the ratio of 20 + 5 + 1. This technique permits the separation of various components in about 15 to 20 minutes. The various dyes are identified by running standards with the unknown mixture.

The colouring of food products is strictly governed by food laws, and annatto and β-carotene are the only dyes permitted in India to be added to butter to impart a pleasant yellow colour to it.[1] Other natural or synthetic dyes may deliberately be substituted for annatto colour. Since the dyes used in butter are present in small amounts, their separation and identification require methods of great sensitivity. Column- and paper-chromatographic methods[2 to 11] generally used for their identification are often time consuming.

The thin-layer chromatographic technique as described by Stahl, Schröter, Kraft and Renz[12] occupies a unique position among the recent developments in the field of separation methods. This new technique has found wide application in the separation and identification of both organic and inorganic compounds and has recently been reviewed by Mahapatra.[13] In the present study the separation of annatto colour from some permissible fat-soluble and water-soluble food colours has been achieved by using thin-layer chromatography and is described in this paper.

Experimental

Individual dyes and their mixtures were chromatographed on glass slides coated with silica gel G or a mixture of silicic acid and Plaster of Paris, and the chromatogram was developed with amyl acetate as a flowing solvent. Fig. 1 illustrates the separation of annatto colour from curcumin, other fat-soluble dyes and water-soluble dyes. It was observed that all the fat-soluble dyes except annatto and curcumin migrated to the solvent front, whereas the water-soluble dyes remained at the point of application. In this way, annatto colour was found to be separated from the other colours, curcumin having a slightly higher R_F value than annatto colour. Yellow OB, Yellow AB, Oil orange E, Oil orange S and Ethyl bixin appeared in the solvent front, whereas Tartrazine and Sunset yellow FCF remained at the point of origin. This method is thus useful in proving the presence of any other food colours in the group examined that may have been added to the annatto colour. It was further observed that water-soluble annatto colour showed two spots, one at the point of origin and the other corresponding to the fat-soluble annatto colour. β-Carotene moves with the solvent front and is separated from annatto colour.

In order to achieve the separation of oil-soluble colours, slides were coated with a material containing calcium carbonate and starch and were immersed in a 10 per cent. solution of liquid paraffin in light petroleum. A solvent system containing methanol, water and ammonia solution in a ratio of 20 + 5 + 1 gave a good separation of different fat-soluble colours, as is shown in Fig. 2 (*a*). It was observed that Yellow AB, Yellow OB, Oil orange E, Oil orange S and ethyl bixin gave distinct spots. Annatto colour on the same plate remained at the point of application and thus could not be separated from Oil orange S. When the amount of water in the solvent system was increased, the rate of migration of the different dyes was slow, the separation was poor and there was tailing of the spots. If the water were excluded, separation of Yellow AB and Yellow OB could not be achieved.

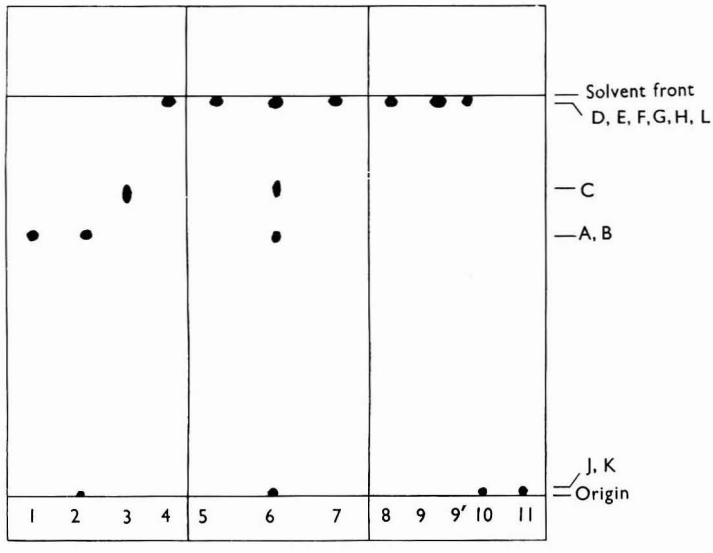

Fig. 1. Chromatograms showing separation of food colours on silica-gel slides

Position	Food colour	Code letter	Position	Food colour	Code letter
1	Annatto (fat-soluble)	A	7	Oil orange E	F
2	Annatto (water-soluble)	B	8	Yellow OB	G
3	Curcumin	C	9	Yellow AB	H
4	Oil orange S	D	9′	β-Carotene	L
5	Ethyl bixin	E	10	Tartrazine	J
6	Mixture of different food colours	—	11	Sunset yellow FCF	K

The separation of colouring matter present in butter had to be carried out in two stages. The annatto colour and curcumin were adsorbed on alumina by passing the ethereal solution of butter through a column packed with alumina. The other synthetic colours were isolated by saponifying the eluate and extraction with ether. If the original coloured butter is saponified, the unsaponifiable material containing the dyes could be extracted with ether, while annatto remained in the soap solution. The colouring materials isolated from butter were examined by thin-layer chromatographic techniques and were found to give spots similar to those shown in Figs. 1 and 2(*a*) for different fat-soluble dyes. No attempt was made to add water-soluble dyes to butter.

The water-soluble dyes Sunset yellow FCF and Tartrazine could be separated on slides coated with a thin layer of calcium carbonate and starch. The separation of water-soluble dyes is shown in Fig. 2 (*b*). In the absence of water in the developing solvent, the water-soluble dyes remained at the point of origin.

Method

Apparatus—

Glass slides—1 × 3 inches.
Chromatographic jars—4 inches tall × 1½ inches in diameter.
Micropipette.

Reagents—

Silicic acid, 100 to 200 mesh—Obtainable from E. Merck & Co. Inc.
Plaster of Paris—Obtainable from E. Merck & Co. Inc.
Silica gel G—Obtainable from E. Merck & Co. Inc.
Calcium carbonate B.P.—Precipitated type.
Starch—Obtainable from E. Merck & Co. Inc.

Liquid paraffin B.P.
Light petroleum—Boiling-range 40° to 60° C.
Ammonia solution, sp.gr. 0·88.
Alumina—Aluminium oxide standard (for absorption chromatography, prepared according to Brockmann). Obtainable from E. Merck & Co. Inc.

FOOD DYES TESTED—

(A) Annatto colour (fat-soluble).
(B) Annatto colour (water-soluble).
(C) Curcumin.
(D) Oil orange S.
(E) Ethyl bixin.
(F) Oil orange E.
(G) Yellow OB.
(H) Yellow AB.
(J) Tartrazine.
(K) Sunset yellow FCF.
(L) β-Carotene.

The annatto colour was prepared in the laboratory from the seeds of *Bixa orellana*, whereas the other food colours were commercial products. The dye solution was prepared by dissolving 0·1 g of the dye in 100 ml of methanol.

EXTRACTION OF COLOURING MATTER FROM BUTTER—

Dissolve 10 g of butter containing colouring matter in diethyl ether and dilute the solution to 50 ml. Pass the solution through an alumina column (7·5 cm × 1·5-cm diameter). (Annatto and curcumin are adsorbed on the column, and the other fat-soluble dyes pass through the column with the fat solution.) Elute the colour adsorbed on the column with 25 ml of a 2 + 1 mixture of ethanol and ammonia solution. Make the eluate slightly acidic with 2 N hydrochloric acid, dilute it with water and extract it three times with diethyl ether. Wash the extract with water, dehydrate it over anhydrous sodium sulphate and concentrate it to a small volume. Use this for identifying annatto and curcumin.

Concentrate the eluate obtained after the adsorption of annatto on the alumina column by evaporating the ether, and then saponify it with alcoholic potassium hydroxide solution. After saponification, extract the colour three times with diethyl ether. Mix the ethereal extracts, wash the mixture with water and dehydrate it over anhydrous sodium sulphate. Concentrate the mixture by evaporating the ether and examine the concentrate for the fat-soluble dyes by the thin-layer chromatographic method.

PROCEDURE—

Adsorbent material (*i*)—Prepare this by mixing 20 g of silicic acid with 0·4 g of Plaster of Paris. Make the mixture into a slurry with twice its weight of water. Pour 1 ml of the slurry on a glass slide and spread it with a glass rod, tapping the slide gently to obtain a uniform layer. Allow it to set for about 15 minutes. Dry the slides in an air oven at 100° C for 30 minutes and store them in a desiccator. Use these slides for separating annatto colour from other food colours. Silica gel G (which was available to us at a later date) may also be used for preparing the slides.

Adsorbent material (*ii*)—Mix 10 g of calcium carbonate with 4 g of starch, make it into a slurry with 44 ml of water and apply it on the glass slides. Dry the slides at 100° C for 30 minutes, cool them and then dip them in a 10 per cent. solution of liquid paraffin in light petroleum. Dry the slides at 80° C for 15 minutes and store them in a desiccator. Use them for separating fat-soluble or water-soluble dyes.

Chromatography—Apply the dye solution about 1 cm from the base of the glass slides with the aid of a micropipette. Repeat the application until a distinctly coloured area is obtained. (The spot should contain approximately 10 μg of the dye. If more than one dye is present in a sample, the spot should contain at least 5 μg of each dye.) Develop the chromatogram in an ascending manner. A development time of 15 to 20 minutes is sufficient for the solvent front to move approximately 6·5 cm. Under suitable conditions, the material migrates from the point of application and the mixture is wholly or partially separated into its components. Since the different dyes show as clear, compact coloured spots, no indicator is required to locate their position on the chromatogram.

Discussion

In the paper chromatography of food colours, the separation of food colours takes approximately 24 hours.[8,11] Thin-layer chromatography permits a rapid separation of annatto colour from other food colours within 15 to 20 minutes. Only a small amount of the material is required for this method, compared with other standard methods of column and paper chromatography, to obtain a good separation of the various components. This new technique is useful in rapid identification of the presence of possible synthetic food colours that may have been added to annatto dye sold in the market for use in colouring butter. The proposed method has the distinct advantage that there is no "tailing" of spots as is usually observed in the paper chromatography of these dyes. The various fractions can be eluted from the chromatogram by extracting the scraped material with methanol, for subsequent identification and further fractionation. In our experiments, the fat-soluble and water-soluble dyes that could not be separated on silica-gel slides were eluted with methanol, concentrated and re-chromatographed on calcium carbonate slides and were found to give good separation (see Figs. 2*a* and *b*).

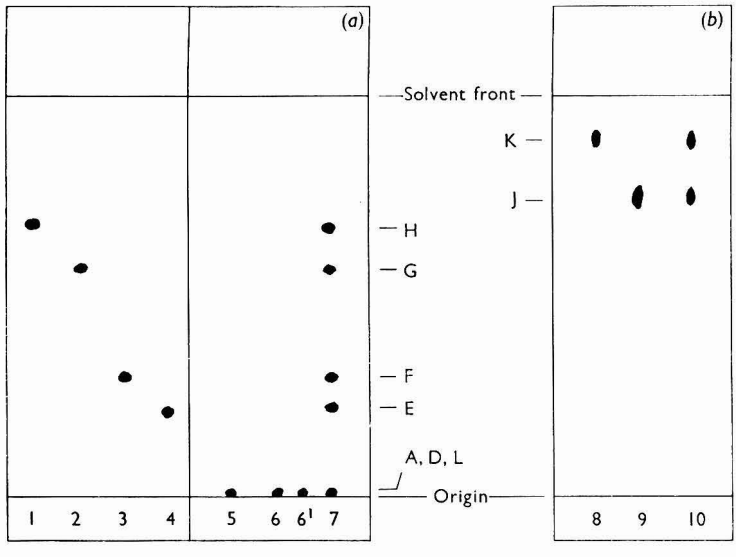

Fig. 2. Chromatograms of (*a*) fat-soluble colours and (*b*) water-soluble colours, on slides coated with calcium carbonate and starch and impregnated with liquid paraffin

Position	Food colour	Code letter	Position	Food colour	Code letter
1	Yellow AB	H	6′	β-Carotene	L
2	Yellow OB	G	7	Mixture of A, D, E, F, G and H	—
3	Oil orange E	F	8	Tartrazine	K
4	Ethyl bixin	E	9	Sunset yellow FCF	J
5	Annatto (fat-soluble)	A	10	Mixture of J and K	
6	Oil orange S	D			

The R_F values could not be relied upon for identification of the various spots because of the variation in different factors, *e.g.*, the thickness of the coating, the composition of the solvent system and the temperature of the room. It is, however, possible to reproduce the results if all the conditions are strictly adhered to. It is desirable that standards should be run simultaneously with the unknown mixtures for the purpose of identification.

We thank Dr. K. K. Iya, Director of Dairy Research, and Dr. N. N. Dastur, Principal, Dairy Science College, for their valuable suggestions during this study.

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SHORT PAPERS

A Modified Burner Base for the Unicam SP900 Flame Photometer

By R. MACKISON

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The scope of the SP900 flame photometer (manufactured by Unicam Instruments Limited) is limited by the possibility of an explosion in the "expansion" chamber when inflammable liquids are atomised. This often imposes a need to wet oxidise organic samples, with consequent waste of time and loss of sensitivity. The burner has therefore been modified[1] so that the test solutions are atomised in nitrogen. Oxygen for burning the acetylene is introduced separately at the base of the burner.

The direct determination of metals in organic liquids is a valuable extension of flame photometry, and further extensions are also made possible. For instance, the whole range of organic solvents is now available for the examination of solids in solution. Selectivity can be increased by the prior use of a solvent-extraction procedure and advantage can be taken of the enhanced sensitivity often found in organic solutions.[2]

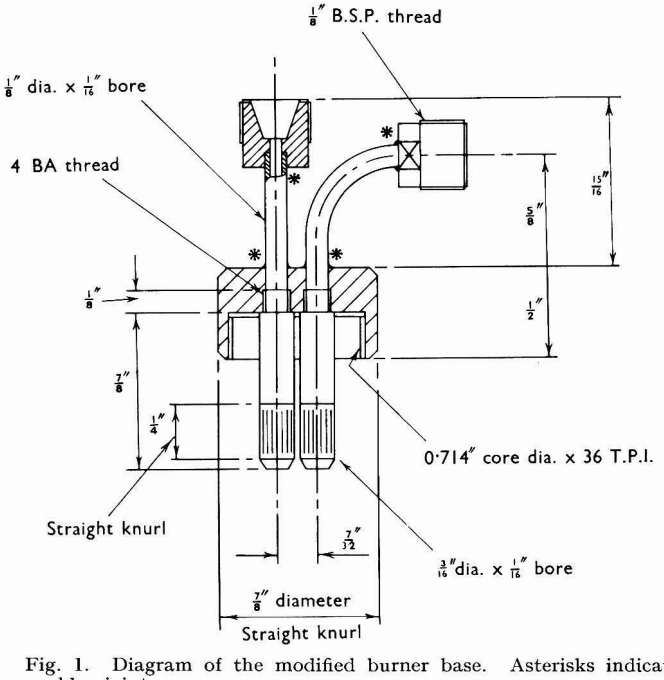

Fig. 1. Diagram of the modified burner base. Asterisks indicate silver-solder joints

Details and operation of apparatus

Modified burner base—

Fig. 1 shows a detailed drawing of the modified burner base. This part is made from stainless steel and screws into the main burner barrel in place of parts 16d and 16c shown in the SP900 Operating Maintenance and Service Manual, Appendix IV, number 6.

Acetylene is fed to one of the inlet ports of the burner base in the conventional way. Oxygen from a cylinder is regulated by means of a reducing valve to give an outlet pressure of 5 p.s.i. This is connected to the other inlet port of the burner base. The flow-rate of oxygen to the burner is controlled with a Drallim valve (manufactured by Drallim Tube Couplings Limited, Whyteleafe, Surrey), and is measured with a Drallim rotameter calibrated from 0 to 2 litres of air per minute at S.T.P.

A nitrogen line at a pressure of 28 p.s.i. is supplied to the atomiser. The pressure is regulated at a reducing valve fitted to the nitrogen cylinder.

Operation—

The sequence below should be adopted to ignite the flame—

(*i*) Turn on the nitrogen and set the pressure to 28 p.s.i.

(*ii*) Turn on the oxygen at the cylinder and adjust the outlet pressure to 5 p.s.i. Turn on the Drallim valve and adjust the flow-rate to give 1·5 litres of air per minute at S.T.P.

(*iii*) Slowly turn on the acetylene and ignite the flame when the manometer reads 9 cm.

(*iv*) Adjust the final oxygen flow-rate to give a blue flame free from excess of fuel.

Stability of the flame—

For measuring the stability of the flame, it was considered advisable to use an emitter present in the flame rather than one introduced by atomisation. Thus errors due to variable atomisation caused by partial blocking of the atomiser were avoided.

The prominent OH band at approximately 310 mμ was chosen for this purpose. Continuous recording at the peak for 30 minutes showed that the flame was stable. The reproducibility of the intensity of the OH band during this period was ± 1 per cent. relative.

Discussion

Although a direct-injection burner of the Beckman type can be used for the determination of metals in organic liquids, the advantages of using the described apparatus with a SP900 are as follows. No modification to the instrument is required to hold the burner. The modified part is cheap to make compared to the price of a direct-injection burner and housing. When a direct-injection burner is used with the SP900, it is necessary to have complete access to the rear of the instrument to permit atomisation of the sample. This makes operation difficult. The modified system can be retained for use with the atomic-absorption attachment now being marketed by Unicam Instruments Limited. The use of an "expansion" chamber is favoured by atomic-absorption practitioners.[3]

Uses

The modified burner has been used to determine, (*a*) lead in petrol, (*b*) lithium in glacial acetic acid and (*c*) manganese and copper after extraction as the diethyldithiocarbamates as in the method of Dean and Cain.[4]

I thank the Directors of The Distillers Company Limited for permission to publish this paper and Mr. E. E. Archer for helpful advice and constructive criticism.

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An Ion-exchange Column for Use with Atomic-absorption Analysis

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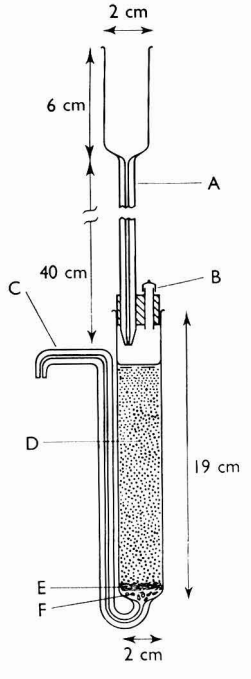
In the determination of strontium in biological materials by atomic absorption,[1] phosphate is removed by means of an anion-exchange column to overcome its depressive effect, in combination with calcium, on strontium absorption. In order to attain the efficiency claimed for the column (the collection, in 25 ml of effluent, of all the strontium injected in a 10-ml sample solution) and to obtain a column well adapted to the handling of large batches of samples, it is necessary to pay attention to several factors in its design and operation.



A = Pyrex-glass funnel
B = Vent tube
C = Outlet tube
D = Resin bed
E = Pyrex glass-wool pad
F = Glass chips

Fig. 1. Diagram of resin column

The column is shown diagrammatically in Fig. 1. It consists of a long-stemmed (40 cm) Pyrex-glass funnel, A, having a capacity in the reservoir slightly greater than 10 ml and a 1·5-mm internal-diameter stem that is sealed into the resin chamber with a rubber stopper. The stopper also carries a vent tube, B, sealed with a removable polyethylene cap. The resin chamber has a Pyrex glass-wool pad, E, supported by glass chips, F, at the base and an outlet tube, C, of internal diameter 1·5 mm, bent back in such a manner as to discharge at a level of 2 to 3 cm below the top of the main chamber, *i.e.*, the approximate height of the top of the resin bed, D. A 17-mm diameter disc of polyethylene sheet with a central hole of 6-mm diameter is placed loosely on the surface of the resin bed to prevent disturbance of the bed by impinging drops of liquid. The column and discharge tube are filled with 0·1 N acetic acid until the top of the bed is just awash, care being taken to avoid entrapping air.

The flow-rate of the column when 100- to 200-mesh De-Acidite FF anion-exchange resin (acetate form) is used, is 10 to 12 ml per minute. The amount of resin in the column will safely process ten 10-ml volumes of biological ash dissolved in 0·02 N hydrochloric acid[1] before regeneration of the resin is necessary.

Place a 10-ml sample solution in the funnel reservoir. This displaces air downward from the neck of the funnel, which may force the level of the liquid in the main chamber below the top of the resin bed and stop the flow. Flow can be restored by releasing a volume of air from the vent tube, B, equal to that displaced from the neck of the funnel and the liquid in the column will then flow, without change in the level of the liquid in the main chamber, until the funnel is empty. Then place successive 3-ml portions of 0·1 N acetic acid wash solution in the funnel reservoir, taking care to wash down the walls of the reservoir, and run each through the column as described for the sample solution until the required volume of effluent is collected. At no time should the depth of free liquid above the top of the resin bed be allowed to exceed 1 mm.

A test run on the column with 10 ml of 3·0 p.p.m. strontium (as the chloride) solution under the operating conditions described gave the results shown in Table I. Each successive millilitre after the ninth was collected and analysed by atomic absorption against strontium chloride standards. Summation of the concentrations in Table I indicates a recovery of 32·1 μg of strontium. The apparent 7 per cent. excess of strontium recovered can be explained by a 2 per cent. enhancement of strontium absorption by acetate (evident from the apparent peak concentration of 3·06 p.p.m.) and by experimental error consequent on the use of 10-ml measuring cylinders to collect the 1-ml portions of effluent.

TABLE I

CONCENTRATIONS OF STRONTIUM IN SUCCESSIVE 1-ml PORTIONS OF EFFLUENT FROM THE ANION-EXCHANGE COLUMN

Millilitre number	Strontium, p.p.m.	Millilitre number	Strontium, p.p.m.	Millilitre number	Strontium, p.p.m.
1 to 9	Discarded	17	2·94	25	0·66
10	<0·002	18	3·00	26	0·27
11	0·18	19	3·06	27	0·15
12	0·90	20	3·06	28	0·06
13	1·51	21	3·03	29	0·04
14	2·04	22	2·67	30	<0·002
15	2·52	23	1·98	31	<0·002
16	2·79	24	1·29		

As can be seen in Table I, all the strontium in the 10 ml of test solution was contained in 19 ml of effluent and the 25 ml normally collected was chosen to allow a 1-ml margin of safety at the beginning of collection and a 5-ml margin at the end. Tests showed that the most critical factor controlling the efficiency of the column was the depth of free liquid above the resin bed while the column was in operation. If this depth was allowed to exceed 1 mm, the collection of 25 ml or more of effluent was found necessary to recover all of the strontium injected.

This column is particularly suited to routine application because it combines a fast flow-rate with high efficiency resulting from the small and controllable volume of liquid in the column itself. Further, the fact that, after a small initial adjustment, the column can be left, without attention or fear of air entering the resin bed, until after all of an applied volume of liquid is exhausted facilitates the operation of 10 or more columns simultaneously.

The column described was made from materials commonly at hand in a laboratory. It could be improved by the use of a ground-glass joint in place of the rubber stopper, a needle valve in place of the polyethylene cap on the vent tube, and, perhaps, sintered glass in place of the glass wool provided that the free space under the sintered-glass plug were kept to the minimum possible volume. Rubber stoppers were found to deteriorate after about 6 months even though they were never in direct contact with the resin.

The results in Table I and results published by Hinson[2] and David[1] indicate that the use of the acetate form of De-Acidite FF is an effective means of removing posphate to prevent its interference with the determination of both calcium and strontium by atomic-absorption analysis.

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A Modification of the Curcumin Method for Determining Boron, for Possible Application to Continuous Analysis of Aqueous Solutions

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In a continuous method of analysis, it is normally desirable that the chemical processing of the sample before measurement should be rapid (not longer than 2 to 5 minutes) and as simple as possible. However, the most sensitive methods for determining trace amounts of boron either require an evaporation to dryness, as in the curcumin - oxalic acid method, or else have long waiting-periods (2 to 6 hours), as in the dianthrimide and methylene blue methods. Even less sensitive methods involving the use of reagents such as quinalizarin and carminic acid have waiting-times of the order of 1 hour to ensure maximum colour development, although, of course, in a continuous method of analysis it is not essential that a reaction should go to 100 per cent. completion, and much shorter waiting-times might be acceptable. However, it is improbable that any of these methods could be adopted to continuous analysis of aqueous solutions for boron contents at the 0·01 to 1 p.p.m. level.

Hayes and Metcalfe[1] have proposed a modified curcumin method. In their procedure, aqueous solutions are either evaporated to dryness, or an aliquot containing not more than 0·25 ml of water is used (if more than 0·25 ml of water is present, the sensitivity falls off rapidly). Their procedure will detect 0·05 μg of boron readily, so that 0·2 p.p.m. of boron should be readily detected by using 0·25 ml of aqueous solution. They state that if 0·25 ml of water is present, then sensitivity is not impaired; however, if the reaction time is reduced from 15 to 5 minutes we have found that this amount of water leads to a 20 per cent. loss in sensitivity, suggesting that the water reduces the rate of reaction.

In recent modifications of Hayes and Metcalfe's procedure for determining boron in zirconium, hafnium and titanium[2,3] the authors take care to avoid the use of water in the preparation of their samples. However, if the determination of boron in an aqueous solution is required, it is necessary either to use an aliquot containing not more than 0·25 ml of water, or to reduce the amount of water present in a larger portion. Since an evaporation technique would be difficult to apply in a continuous method of analysis, the use of acetic anhydride as a reagent for eliminating free water has been investigated. The reaction between acetic anhydride and water is rather slow, and if acetic anhydride is added to the aqueous solution, and the curcumin reagent and sulphuric acid - acetic acid mixture is then added as in the normal procedure, the heat of reaction at the final stage decomposes the boron - curcumin complex. An alternative order of reagent addition was therefore investigated. The sulphuric acid - acetic acid mixture was first added to the aqueous solution and acetic anhydride (sufficient to react with 90 per cent. of the water) was then added. After the solution had been cooled, the curcumin reagent was added. By using this procedure, the boron - curcumin complex was obtained, but even the optimum amounts of reagents only gave a 50 per cent. yield. These optimum conditions were achieved by adding 4 ml of 1 + 1 sulphuric acid - acetic acid mixture and 4 ml of acetic anhydride to 1 ml of aqueous solution. When the water - acetic anhydride reaction is complete (a few seconds) cool the solution and add 3 ml of 0·125 per cent. curcumin in acetic acid. Set the solution aside for 15 minutes, and then dilute it to 100 ml with ethanol or methanol and measure the optical density at 5500 Å in the usual manner.

It was clear that this order of adding the reagents led to a less sensitive reaction. This was confirmed by reversing the order of adding the reagents in the original Hayes and Metcalfe procedure, when a similar effect was found.

The reaction between acetic anhydride and water was further studied. It was found that this reaction was catalysed by a few drops of hydrochloric acid, when it became almost explosive. However, by adding acetic acid as a diluent and controlling the temperature, a controlled reaction was possible. It was then possible to add the curcumin and mixed acid as in the normal procedure and obtain almost 100 per cent. formation of the boron - curcumin complex, even although the total volume of reactants was 15 ml instead of 6 ml as in the Hayes and Metcalfe procedure, and the reaction time was 5 minutes instead of 15 minutes.

Procedure

To 1 ml of aqueous solution in a platinum dish, floating on a water-bath at 40° C, add 2 drops of hydrochloric acid (from a polythene dropping bottle) followed by 2 ml of acetic acid and 5 ml of acetic anhydride. After a few seconds delay, a vigorous reaction takes place. Then cool the solution to room temperature, add 3 ml of 0·125 per cent. curcumin in acetic acid and 4 ml of 1 + 1 sulphuric acid - acetic acid mixture. Set the solution aside for 5 minutes, dilute it to 100 ml with ethanol or methanol and measure the optical density at 5500 Å in the usual manner.

Discussion

This procedure will detect 0·05 μg of boron readily, or 0·05 p.p.m. in a 1-ml sample, and it is considered that it would provide the basis for a method for continuous analysis of trace amounts of boron in aqueous solutions with a Technicon AutoAnalyzer or comparable equipment. It is possible that, by "backing off" the reagent blank and electrical amplification, a sensitivity of 0·01 p.p.m. could be achieved.

It is anticipated that the interferences found in the Hayes and Metcalfe procedure will apply in this modified procedure.

I thank the English Electric Co. for permission to publish this paper.

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Loss of Strychnine in the Purification of Visceral Extracts with Concentrated Sulphuric Acid

By B. Lilliman and W. H. Trezise
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It has long been asserted in toxicological textbooks[1,2,3] that strychnine is resistant to attack by hot concentrated sulphuric acid.

During quantitative examinations of the purification of strychnine extracts from toxicological cases, large losses were observed.

We treated 1 mg of strychnine with 2 ml of sulphuric acid under various conditions and obtained the results given in Table I. The strychnine was determined colorimetrically.[4]

Table I

Recovery of strychnine under various conditions

Acid strength	Conditions	Time of treatment	Weight of strychnine recovered, mg	Strychnine recovered, per cent.
AnalaR concentrated sulphuric acid	Room temperature	2 minutes	0·99	99
		17 hours	0·90	90
AnalaR concentrated sulphuric acid	Steam-bath	15 minutes	0·31	31
		60 minutes	0·05	5
		120 minutes	0·02	2
80 per cent. v/v AnalaR concentrated sulphuric acid	Steam-bath	15 minutes	0·87	87
		60 minutes	0·70	70
		120 minutes	0·58	58

These figures suggest that treatment with hot concentrated sulphuric acid is not a satisfactory method of purifying strychnine visceral extracts.

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Book Reviews

RESIDUE REVIEWS: RESIDUES OF PESTICIDES AND OTHER FOREIGN CHEMICALS IN FOODS AND FEEDS. Volume 4. Edited by FRANCIS A. GUNTHER. Pp. iv + 175. Berlin, Göttingen and Heidelberg: Springer-Verlag. Distributed in U.S.A. by Academic Press, Inc.: New York. 1963. Price DM 24.

Although, as in other volumes in the series to date, the fourth volume of *Residue Reviews* deals almost solely with pesticide residues, it is good value both for variety of approach and for the general quality of the constituent chapters. There are accounts of the legal position in three countries: Great Britain (Hubert Martin, pp. 16), Japan (I. Suzuki, pp. 8) and Austria (F. Beran, pp. 14). Dr. Martin gives a full historical background to the voluntary pesticide-notification scheme to 1962–63, and of the events which, during the course of nearly a century, lead ultimately to statutory limits being laid down for residual arsenic in foods. In all of these matters, as in the final commentary on the adequacy of the British system, a close study is made of government reports. Dr. Suzuki discusses the 1960 official Japanese standards for food additives, together with the Food Sanitation, the Control of Pesticides and the Control of Poisons and Powerful Substances laws; and Dr. Beran, whose contribution is in the German language, deals with the Austrian federal laws on plant protection and food residues.

The remaining contributors to the volume are American. W. F. Durham's account of pesticide residues in food in relation to human health (pp. 49) is particularly well documented. After considering various standard pharmacological responses of man and animals to pesticides and the dose relationships deducible from both controlled experimental and from accidental ingestion, the harmful effects of some natural food constituents are briefly reviewed. Residues in food and water and cases of human poisoning from pesticides (none have occurred in either the U.S. or the U.K. from "normal" uses) are also reviewed: while the safety record is generally good, the continued need for studying long-term exposures is one of the main conclusions. C. W. Wilson and W. E. Baier put forward an interesting, but not always convincing, account of the advantages of expressing tolerance values either on a non-aqueous matter basis or on a calorific-value basis (pp. 8). Two chapters are concerned with the degradation of residues. J. P. Martin, who also contributes a well documented account, deals with the influence of residues on soil properties (pp. 34), considering particularly microbial degradation and effects on soil microbes and the effects of pesticides on the chemical properties of soil such as the availability of soluble nutrients. L. E. Mitchell and L. Lykken discuss practical aspects of measuring residue decay in crops to which pesticides have been applied (pp. 20); difficulties in interpreting analytical results when very low residues are measured or zero residues are established. In the final chapter K. P. Dimmick and H. Hartmann describe theoretical and practical aspects of electron-capture detectors for gas-chromatographic residue analysis, with particular reference to a commercial instrument (pp. 23).

As in other volumes of the series, chapter summaries are given in English, French and German, trivial and other common names are carefully defined and a general index is provided.

H. EGAN

RESIDUE REVIEWS: RESIDUES OF PESTICIDES AND OTHER FOREIGN CHEMICALS IN FOODS AND FEEDS. Volume 5. Edited by FRANCIS A. GUNTHER. Pp. viii + 176. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1964. Price DM 26.

The fifth volume of *Residue Reviews* differs from the others so far published, in that it is a symposium volume, presenting papers read at the meetings of the American Chemical Society on instrumentation for the detection and determination of pesticides and their residues held in Los Angeles in April, 1963. The opening contribution, from Dr. H. Frehse (pp. 20), is on the broad subject of residue analysis and food control, surveying in particular the usefulness and limitations of general chemical methods, *e.g.*, for phosphorus and sulphur, compared with physical methods, such as chromatography or infrared spectrophotometry, and with esterase and bioassay methods. The chapter concludes with a useful account of the method of expressing residue results in relation to the precision and sensitivity of the analytical methods used.

The remainder of the volume is devoted to different practical aspects of modern methods of pesticide-residue analysis, but with occasional references to othes residues. Four chapters deal with gas chromatography; the use of the electron-absorption detector (L. K. Gaston, pp. 11), a comparison of this detector with flame ionisation detectors for the evaluation of herbicide residues

(H. S. Segal and M. L. Sutherland, pp. 8), the quantitative interpretation of results (S. J. Clark, pp. 13), and the use of microcoulometric titration detection systems (J. A. Challacombe and J. A. McNulty, pp. 16). These are all timely contributions, valuable to the practising residue analyst concerned with both qualitative and quantitative aspects. They are supported by chapters on aspects of specificity. T. Johns and C. H. Braithwaite, jun., (pp. 12) describe a conductivity detector for use in gas chromatography in conjunction with a preliminary account of an infrared technique requiring only 50 μg of residue after gas-chromatographic clean-up. R. C. Blinn (pp. 9) gives a brief though comprehensive survey of both ultraviolet and infrared spectrophotometry in residue analysis; complete infrared spectra needing only 25, down to 2·5, μg of pesticide are possible by using 5-mm cavity cells. Attenuated total reflectance techniques are unlikely to be very useful in general residue work, however. D. McDougall (pp. 11) extends the review of the general scope of fluorescence analysis published in the first volume of the series, pointing out that though restricted in its application, the method can be an extremely sensitive one. Some non-fluorescent compounds can be converted into highly fluorescent materials suitable for assay; examples are drawn both from pesticides and other kinds of residues.

Two further papers deal with polarography: applications to the detection and determination of pesticide residues (R. J. Gagan, pp. 11) and of organic feeding-stuffs additives (P. T. Allen and H. Beckman, pp. 29). The former is a general introductory review with special reference to the value of the method for organo-phosphorus residues; the latter is designed to show how polarography can usefully supplement other analytical methods for additives and in fact also reviews spectrophotometric and gas-chromatographic methods at some length. V. P. Guinn and R. A. Schmitt consider neutron-activation analysis for pesticides (pp. 27); the method is extremely sensitive and (if required) non-destructive, but its specificity may be somewhat limited. G. D. Winter and A. Ferrari describe the application of a commercial system to some aspects of the automation of wet methods of residue analysis (pp. 9).

As explained in the foreword, the papers have been released for publication in the manner described by the American Chemical Society. In common with previous volumes in the series, each contribution is concluded by summaries in English, French and German; a subject index for the whole volume is provided. H. EGAN

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Volume 3. B to CALCIUM. Edited by HERMAN F. MARK, JOHN J. MCKETTA, jun., DONALD F. OTHMER and ANTHONY STANDEN. Second Edition. Pp. xvi + 927. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1964. Price £16 18s.; price per volume for subscribers to the complete set of 18 volumes £13.

The third volume of this important work fully maintains the standards of its predecessors (see *Analyst*, 1963, **88**, 899 and 1964, **89**, 502). It is the third to appear in twelve months, a rate of publication that in itself is a commendable achievement having regard to the size of the encyclopedia in its completed form. Once a work of this nature has been launched, promptitude of publication of successive volumes is a matter of some importance, since the first volumes to appear inevitably become out of date compared with the last. In the present instance, even if the publication frequency of the first year of publication is maintained, it will be at least 6 years before the complete set of volumes is available; and almost time to think about a revision of the earlier sections.

The third volume contains some important entries. The principal of these not mentioned elsewhere in this review are: Barbituric acid and Barbiturates (17 pages), Barium and Barium compounds (22 pages), Beryllium and its alloys and compounds (30 pages), Bismuth and its alloys and compounds (23 pages), Bleaching agents (27 pages), and Bromine and its compounds (16 pages). As an indication of the scope and method of classification of articles on organic chemistry, it may be mentioned that the prefix syllable Benz- is represented by separate articles on Benzaldehyde, Benzene, Benzenesulfonic acids, Benzene and related diaminophenyls, Benzoic acid, Benzophenone, and on Benzyl alcohol and β-phenylethyl alcohols. Similarly, But - is represented only by Butadiene, Butanes, Butylalcohol, Butyraldehyde and Butyric acid.

Some minor and unusual entries are Batteries and electric cells (primary), Bearing materials, Blood, animal (but not human) and Blood fractionation, and Bacterial, rickettsial and mycotis injections, chemotherapy. The volume finishes with Calcium and its alloys, so that if the scheme adopted with the elements already dealt with is followed in this case, calcium compounds should appear at the beginning of Volume 4.

This present volume contains only one article of a purely analytical character and this is rather of allied interest, namely that entitled Bioassay. In 29 pages it gives a sound and comprehensive survey of the subject starting from what is regarded as the first recorded bioassay, namely Cleopatra's use of her handmaidens as test-subjects for the suitability of an asp bite for suicide purposes! The almost purely statistical nature of the latter part of the article provides a striking contrast, which should appeal to students of the development of research methods.

Apart from this, the only treatment of an analytical nature is the almost two pages (with references to six special reviews of the subjects) given under Beryllium; and one page under Cadmium, although there are some notes, with references to the literature, on analysis, tests and specifications in the principal articles such as Benzoic acid (1 page), Bromine (1 page) and Bentonite.

The rather strange and sometimes inconsistent system of subject headings referred to in the earlier reviews (*Analyst, loc. cit.*) is still apparent. Thus, under Bread (the product) the reader is referred to Bakery (the process); but there is no entry under the term brewing, beer and brewing being dealt with under the heading of the product, Beer. Incidentally, the latter is an excellent article and one of the few that takes full regard of English practice; it is interesting to note that 33 per cent. of the beer produced in the United States nowadays is canned, and in consequence this branch of the subject is dealt with at appropriate length.

Bagasse, which, after all, is a general term for the residue after removal of soluble matter from a plant residue and not necessarily from sugar cane residues only, is dealt with under the heading of Bagasse (unqualified); as the article refers exclusively to sugar-cane bagasse, many would have looked for it under sugar cane. The reference to the use of bagasse for paper pulps gives the impression that the addition of a long-fibred pulp is unnecessary, and it does not deal with the important question of the cost of replacing it as a fuel, which is the crux of its economic utilisation in this way. There is a long and comprehensive section on Batteries, again a somewhat unexpected heading; and also on Bile antibiotics. Strangest of all is the entry "Blue printing," under which the reader is referred to printing.

These pecularities will no doubt be ironed out in the final Index, but they give rise to some difficulty in using the work to the best advantage in the 5 to 6 years that remain before this Index is likely to appear. The editors must undoubtedly have prepared in advance a plan of the Encyclopedia as a whole. If this could be supplied in leaflet form with each volume published, it would be of great help to the user, who would know just where he might expect to find the article on any particular subject.

In a review of a monumental work of this nature there is always the danger that the reviewer will tend to over-emphasise the minor criticisms that will inevitably arise. These must be kept in their correct perspective, and it is only fair to say that the general good impressions obtained from the first two volumes remain unchanged. JULIUS GRANT

IR THEORY AND PRACTICE OF INFRARED SPECTROSCOPY. By HERMAN A. SZYMANSKI, Ph.D. Pp. xiv + 375. New York: Plenum Press. 1964. Price $15.00.

Many laboratories are now equipped with some form of infrared spectrometer: technical colleges provide short courses on the subject and students attending them are mostly drawn from laboratories of industrial organisations making their first excursion into the field. Inevitably the question is asked regarding the best possible text-book for the beginner with this sort of background. The choice has been limited and obviously no one book satisfies all conditions. Differences also exist between the requirements of analysts whose interests are mainly quantitative compared with those who hope to become specialists in diagnostic qualitative analysis. It is for the latter group that this text-book is particularly suitable. It is intended primarily for the beginner whose requirements are for a single-volume ready reference.

Early chapters, written by Dr. Alpert of the Perkin Elmer Corporation, are concerned with instrumentation and laboratory techniques. Emphasis is not placed on any one make of instrument, but the subject matter has been condensed into 85 pages. Much useful information is given concerning optimum instrument performance and details are described for production of best possible spectra.

Chapter 4 deals with the theory of infrared spectroscopy and here the author admits great difficulty in achieving a balance between a completely theoretical explanation, understandable only by qualified scientists, and the simplest, but perhaps useless, approach to be understood by all. Much has been omitted; a fair understanding of mathematical principles has been assumed, but few will quarrel with the authors selection in view of the intended coverage. The point

symmetry - point group system is fully discussed. It is pleasing to note that the author repeatedly states the limitation of his treatment, lists omissions, and refers the avid reader to more comprehensive sources.

Chapter 5, covering the use of characteristic group frequencies in structural analysis, forms the backbone of the book. The treatment follows the now familiar pattern with the text liberally supplemented by tables, charts, graphs and spectra. The text claims to deal with infrared spectra in the 2 to 50 μ region; double-grating filter instruments capable of recording to 45 μ are discussed, but no details of the special techniques or characteristic spectra applicable to the 15 to 50 μ region are given.

Chapter 6 deals with the use of infrared instruments for quantitative analysis from both practical and theoretical aspects. The book concludes with a brief chapter on the various systems for documentation of spectra and an Appendix of Character Tables of Point Groups. References to further sources of information are given at the end of each chapter. Several full-length spectra are enclosed loosely by a pocket in the rear cover: how long they will remain intact in a practical manual of this type is open to conjecture.

It should be emphasised that the book is not written for the specialist in any particular branch of spectroscopy and it is not surprising to find little space devoted to such a modern technique as attenuated total reflectance. With these limitations the volume can be thoroughly recommended.

W. L. SHEPPARD

NEUTRON IRRADIATION AND ACTIVATION ANALYSIS. By DENIS TAYLOR, M.Sc., Ph.D., F.Inst.P., M.I.E.E. Pp. x + 185. London: George Newnes Ltd. 1964. Price 50s.

This book provides a condensed account of the techniques and applications of neutron-activation analysis and of analysis depending on the measurement of γ-rays emitted in nuclear reactions induced by bombardments with neutrons. The author is Chief Scientist of Plessey Company (U.K.) Ltd., and was formerly Head of the Electronics Division, Atomic Energy Research Establishment, Harwell. It is not surprising, therefore, that in the book considerable emphasis is given to items of equipment. This is an important feature since many of the recent advances in analysis based on irradiation with neutrons have stemmed from developments in instrumentation and automation.

Although a nuclear reactor is still generally required for activation analysis of trace elements where extremely high sensitivity is essential, in recent years generators in which the reaction $^{3}H(d,n)^{4}He$ is used to produce neutrons have been quite widely used in analysis. When employed in conjunction with sophisticated γ-ray spectrometers, these small accelerator sources can provide a very convenient and rapid non-destructive means of determining a number of elements in appropriate matrices. The basis of such methods and their applicability are well illustrated in the present volume, and this is particularly welcome as they are not discussed very extensively in other recent books on radioactivation analysis. As well as dealing with several interesting special applications, Dr. Taylor's book indicates future trends; these include such exciting possibilities as the use of bombardment with neutrons to determine constituents of the crust of the moon.

The author has stated that the book has been written with the needs of the student in mind, and appendixes summarising the elements of radioactivity and a glossary of terms are provided. In this connection it is unfortunate that some passages in the book are written rather loosely and could possibly give rise to confusion to the reader who is not well acquainted with the subject. Despite this criticism, this compact volume with its wealth of clear diagrams should help to incite and extend interest in analysis dependent on irradiation with neutrons. D. F. C. MORRIS

Erratum

JULY (1964) ISSUE, p. 490, 6th line under "REAGENTS." *For "pH 5·5" read "pH 5·0".*

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