The ANALYST

The Journal of the Society for Analytical Chemistry

A monthly International Publication
dealing with all branches of
Analytical Chemistry

Published for the Society by
W. HEFFER & SONS, LTD., CAMBRIDGE

Volume 92

No. 1095, Pages 347 - 410 **June 1967**

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

14 BELGRAVE SQUARE, LONDON, S.W.1.
Telephone: BELgravia 3258

Assistant Editor:
Miss C. M. Richards, B.Sc.

SUBSCRIPTION RATES

(other than for Members of the Society)

Subscriptions to *The Analyst*, including *Analytical Abstracts*, should be sent through a Bookseller or direct to

W. HEFFER & SONS LTD., CAMBRIDGE, ENGLAND

Subscription Rates: *The Analyst*, *Analytical Abstracts* and indexes, £15 per annum, post free

The Analyst, with *Analytical Abstracts* printed on one side of the paper (without index), £15 15s. per annum

The Analyst, with *Analytical Abstracts* printed on one side of the paper (with index), £17 10s. per annum

Subscriptions are NOT accepted for *The Analyst* alone

N.B.—Members send their subscriptions to the Hon. Treasurer.

Single copies £1 10s.

Volume 92. No. 1095 June, 1967

THE ANALYST

The Reversed-phase Thin-layer Chromatography of Metal Ions with Tributyl Phosphate

BY L. S. BARK, G. DUNCAN AND R. J. T. GRAHAM

(*The Department of Chemistry and Applied Chemistry, University of Salford, Salford 5, Lancashire*)

About sixty-five metal-containing ionic species have been chromatographed on thin layers of cellulose impregnated with tributyl phosphate (TBP) at various concentrations of aqueous hydrochloric acid. A mechanism for the chromatography may be explained in terms of the ability of the metal ions to form chloro complexes, *viz.*, that those metal ions which readily complex with chloride ion are readily extracted by the TBP and consequently have low R_F values. Conversely, those metals which do not form chloro complexes are not retained by the TBP and hence have high R_F values.

Attention is drawn to the similarities between the known liquid - liquid extraction behaviour of the metal ions in the TBP - hydrochloric acid systems and the behaviour of these ions in the TBP - hydrochloric acid chromatographic systems. A strong resemblance has also been found between the R_F spectra (R_F *versus* hydrochloric acid) of the metal ions and the behaviour of these ions in resinous anion-exchange - hydrochloric acid systems. The latter similarity has been used as evidence for the suggestion that the TBP on the layers functions as a liquid anion exchanger, *i.e.*, that TBP-solvated protons, ion-associated with chloride ions, can undergo ion exchange with the metal chloro complex—

$$(TBP)_o + (HCl)_{aq} \rightleftharpoons (TBPH^+Cl^-)_o$$

$$(nTBPH^+Cl^-)_o + (MCl_x^{-n}) \rightleftharpoons MCl_x^{-n}(TBPH^+)_{no} + (nCl^-)_{aq}.$$

The suffix "o" refers to the organic phase and the suffix "aq" refers to the aqueous phase.

THE easy detection of metals as both major and trace components is of obvious industrial importance. While solvent extraction and ion exchange have been used for the separation of some of the metal ions, the use of a system that would simultaneously both separate and maintain these ions in a concentrated form is of potential importance. A technique combining solvent extraction, ion exchange and chromatography, obtained by using a suitable liquid ion exchanger or solvent extractant as the stationary phase in a chromatographic system, makes this feasible.

We have previously reported[1,2,3] the separation of some metals, including some of toxicological importance,[3] by using a reversed phase of cellulose impregnated with tributyl phosphate (TBP), with hydrochloric acid as the eluent. This system has been extended to a study of a great many metal ions, including the alkali metals, the alkaline earths, some first, second and third row transition metals, the lanthanides, scandium, yttrium, thorium and uranium.

The separation of some of these metals by reversed-phase paper chromatography, with TBP as the stationary phase, has been previously reported.[4,5,6] Hu and Liu[7] have studied the separation of niobium, tantalum and some noble metals by using TBP-impregnated silica gel, with hydrochloric acid (M and 2 M) as the mobile phases; the R_F values quoted by these authors indicate that the spots obtained were not discrete but were badly streaked. We have previously shown that the TBP loadings used by these workers cannot give chromatograms suitable for comparative purposes. It is necessary to control the loading of the layers to the same rigorous standards that are necessary for the other extramolecular factors to obtain highly reproducible chromatographic values.

METAL IONS—

Solutions were prepared from analytical-grade chloride or nitrate salts of the alkali metals (lithium to caesium), the alkaline earths (beryllium to barium), Al(III), TiO^{2+}, VO_2^+, Cr(III), Mn(II), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Sb(III), ZrO^{2+}, Ru(IV), Rh(II), Pd(II), Ag(I), Cd(II), In(III), Sn(II), Ir(III), Pt(II), Au(III), Hg(II), Tl(I), Pb(II), Bi(III), La(III), Ce(IV), Lu(III), Y(III), Th(IV) and UO_2^{2+}.

Se(IV), Te(IV), Re(VII) and Os(VIII) were used in the form of the sodium or potassium salt of the oxy-anion (SeO_3^{2-}, TeO_3^{2-}, ReO_4^- and OsO_5^{2-}).

Sc(III), Pr(III), Nd(III), Sm(III), Eu(III), Gd (III), Tb(III), Dy(III), Ho(III), Er(III), Tm(III) and Yb(III) ions were obtained from the appropriate metal oxide.

CHROMOGENIC REAGENTS—

3,5,7,2′,4′-Pentahydroxyflavone (Morin), 0·1 *per cent. in ethanol.*
8-Hydroxyquinoline, 2 *per cent. in chloroform.*
1-(2-Pyridylazo)-2-naphthol (PAN), 0·1 *per cent. in ethanol.*
p-Dimethylaminobenzylidenerhodanine, 0·1 *per cent. in ethanol.*
2,7-Bis-(o-arsonophenylazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid, sodium salt. Arsenazo III, 0·1 *per cent. in water.*
Diethyldithiocarbamate (DDTC), 0·1 *per cent. in ethanol.*

EXPERIMENTAL

PREPARATION OF THE METAL-ION SOLUTIONS—

Solutions of the metal ions (5 mg per ml) in suitable solvents were prepared. Water was used as the solvent, except in those instances when the metal salt was insoluble in water, *viz.*, antimony trichloride was dissolved in sodium hydroxide solution, scandium and several of the lanthanide oxides (Sm_2O_3, Eu_2O_3, Tb_2O_3, Er_2O_3 and Yb_2O_3) were dissolved in dilute hydrochloric acid, while others (Pr_2O_3, Gd_2O_3 and Tm_2O_3) were dissolved in about 8 M nitric acid and diluted with water. The pentoxides of niobium and tantalum were converted to the pentachlorides by boiling in sulphur dichloride for several hours. The yellow crystals of the pentachlorides were dissolved in sufficient "A"-grade carbon tetrachloride to give a concentration of 5 mg per ml.

PREPARATION OF THE LAYER—

Cellulose powder (15 g of Machery Nagel MN 300 HR) was mixed with a solution of purified[8] TBP in carbon tetrachloride (70 ml of a 5 per cent. solution) to give a homogeneous slurry that was spread as an even layer, 0·3 mm thick, on five 20 × 20-cm glass plates. The layers were allowed to dry in an air-oven at room temperature for 1 hour to remove the carbon tetrachloride.

APPLICATION OF THE METAL-ION SOLUTIONS—

When the plates were dry, aliquots (1 μl) of the metal-ion solutions to be chromatographed were applied to the layers by using a multiple spotting device[9] at a fixed distance from the base of the plate; the applied spots were allowed to air-dry for a further standard time (15 minutes).

ELUTION OF THE CHROMATOPLATE—

The chromatoplates were developed in a small-volume double-saturation chamber, as previously described,[1] with various concentrations of hydrochloric acid (0·1 to 9 M). By using a vertical development, the eluent was allowed to move a fixed distance (12·5 $\pm$ 0·25 cm) from the point of application of the metal ions. The development times ranged from 1 to 3 hours, depending on the acid concentration of the mobile phase (there was an increase in time with increase in acid concentration).

When the eluent front had moved to the pre-determined distance from the point of application of the spots, the plates were removed from the saturation chamber and heated for a standard time (20 minutes) in an air-oven (temperature 110° $\pm$ 2° C) to remove the acid and most of the TBP.

VISUAL IDENTIFICATION OF THE METAL IONS—

It is necessary to remove most of the TBP as indicated, to allow the chromogenic indicator to form complexes with some of the "free" metal ions.

Each plate was examined in visible light and under ultraviolet radiation. It was then sprayed with one of the chromogenic reagents mentioned above.

Treatment	Appearance	Metal
Visible light	Dark spots on white layer	TiO^{2+}, UO_2^{2+}, Ru
Ultraviolet light	Dark spots quenching background fluorescence	Rh, Ir, Nb, Ta, Sb
Sprayed with morin, exposed to NH_3 and held under ultraviolet light	Yellow fluorescent spots	Alkali metals
Sprayed with 8-hydroxyquinoline, exposed to NH_3 and held under ultraviolet light	Dark spots quenching background fluorescence	TiO^{2+}, UO_2^{2+}
	Yellow fluorescent spots	Alkaline earths and Al
Sprayed with arsenazo III	Green spots on blue background	Sc, Y, the lanthanides, Th, UO_2^{2+}
Sprayed with a 1 + 1 mixture of PAN and *p*-dimethylamino-benzylidenerhodanine and viewed	Red spots	Cr, Mn, Ni, Cu, Zn, ZrO^{2+}, Cd, In, Sn, Hg, Tl
(*i*) in visible light	Blue spots	Co, Pd, Pt
	Grey spots	Fe(II), Fe(III)
	Yellow spots	Ag
(*ii*) in ultraviolet light	Dark spots quenching background fluorescence	Ru, Rh, TeO_3^{2-} ReO_4^-, OsO_5^{2-}
	Bright yellow fluorescent spot	Au
	Blue fluorescent spot	SeO_3^{2-}
Sprayed with DDTC and viewed in visible light	Green spots	Pb, Bi

Results

The mean R_F values of the metal ions chromatographed in the various eluent systems are shown in Tables I to V. The corresponding R_F spectra are shown in Fig. 1. Each R_F value is the mean of at least four determinations, each within $\pm 0{\cdot}02$ of the mean value. Such highly reproducible R_F values were achieved by strictly controlling the experimental conditions.

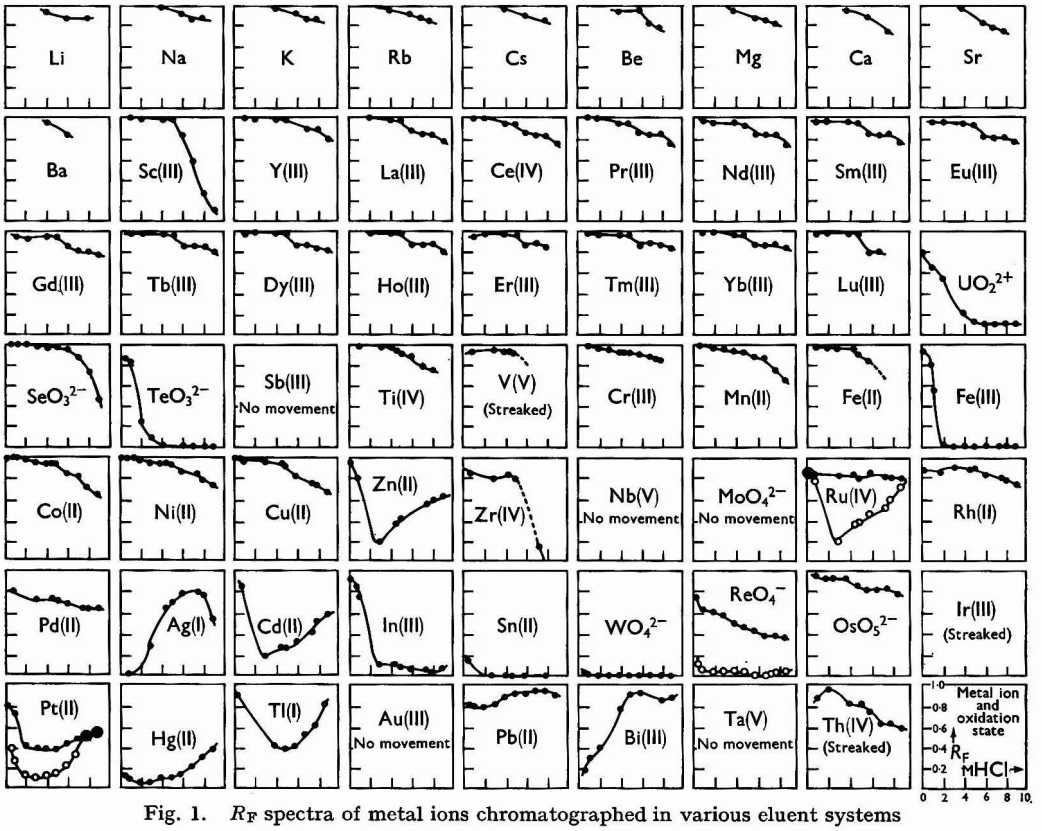


Fig. 1. R_F spectra of metal ions chromatographed in various eluent systems

Discussion of results

It is not possible to obtain a single system that will separate each of the ions from a mixture of the whole. The tables of results have been grouped according to the periodic classification, as often, mixtures requiring separations are of metals that are closely related in the periodic table, *viz.*, transitional or alkali metals.

Table I

Mean R_F values of the alkali and alkaline earth metals and aluminium on cellulose - TBP (5 per cent.) layers at various acid concentrations

Metal ion	4 M HCl	6 M HCl	7 M HCl	8 M HCl
Lithium	0·94	0·87	—	0·88
Sodium	0·98	0·94	0·87	0·89
Potassium	0·98	0·93	0·88	0·87
Rubidium	0·98	0·92	0·88	0·86
Caesium	0·98	0·91	0·88	0·85
Beryllium	0·94	0·95	0·82	0·78
Magnesium	0·94	0·87	0·85	0·81
Calcium	0·94	0·87	0·77	0·73
Strontium	0·94	0·82	0·78	0·70
Barium	0·94	0·82	—	—
Aluminium	1·00	1·00	1·00	1·00

The spots obtained in these systems were generally elongated.

The alkali and alkaline earth metals—

Table I shows the average R_F values for the alkali and alkaline earth metals on layers of 5 per cent. TBP - cellulose. It was possible by using either the 4 M or 6 M eluent, partially to separate lithium from the other alkali metals (sodium, potassium, rubidium and caesium), but none of the other metal ions listed in this table could be separated. The spots, except for lithium and sodium, were generally elongated (2 to 2·5 cm), and the length of the spot increased with atomic weight. Identification of the alkali metal ions after development was difficult, and the spray reagent used (morin) gave the most definite reaction of those tried

Table II

Mean R_F values of the lanthanides, scandium, yttrium, thorium and uranium on cellulose - TBP (5 per cent.) layers at various acid concentrations

Metal ion	M	2 M	4 M	5 M	6 M	7 M	8M	9 M
Scandium(III)	0·99	0·98	0·98	0·97	0·82	0·57	0·25	0·10
Yttrium(III)	0·99	0·98	0·98	0·97	0·90	0·89	0·89	0·78T
Lanthanum(III)	0·99	0·98	0·96	0·96	0·86	0·83	0·84	0·76T
Cerium(IV)	0·99	0·98	0·94	0·94	0·85	0·84	0·83	0·75T
Praseodymium(III)	0·99	0·98	0·95	0·94	0·85	0·84	0·84T	0·74T
Neodymium(III)	0·95	0·97	0·96	0·92	0·85	0·83	0·83T	0·74T
Samarium(III)	0·96	0·97	0·96	0·94	0·85	0·84	0·84T	0·76T
Europium(III)	0·96	0·96	0·96	0·94	0·83	0·82	0·82T	0·78T
Gadolinium(III)	0·95	0·93	0·96	0·95	0·86	0·82T	0·81T	0·78T
Terbium(III)	0·97	0·98	0·97	0·96	0·86	0·86	0·85T	0·80T
Dysprosium(III)	0·97	0·98	0·97	0·97	0·86	0·86	0·83T	0·81T
Holmium(III)	0·95	0·98	0·97	0·97	0·86	0·86	0·88T	0·80T
Erbium(III)	0·95	0·97	0·97	0·96	0·86	0·88	0·84T	0·76T
Thulium(III)	0·98	0·97	0·97	0·97	0·86	0·89	0·87T	0·84T
Ytterbium(III)	0·98	0·99	0·97	0·96	0·87	0·86	0·87T	0·83T
Lutetium(III)	0·98	0·99	0·97	0·97	0·89	0·90	T	T
Thorium(IV)	0·88T 0·65	0·97T	0·84T	0·83T	0·76T	0·64T 0·41T	0·64T 0·38T	0·59T
(Uranyl) UO_2^{2+}	0·65	0·55	0·20	0·13	0·11	0·10	0·12	0·11

The spots obtained were rather large (3 cm), especially for the higher acid concentrations.
T = Tailed or streaked spot.
Multiple values indicate multiple spots.

(zinc uranyl acetate, violuric acid and 8-hydroxyquinoline). Other workers[10] have also found difficulty in identifying the alkali metals on thin layers. Similar difficulties were encountered in finding a sufficiently sensitive reagent for barium; 8-hydroxyquinoline gives only a faint indication of this metal. However, it was decided to retain 8-hydroxyquinoline as a spray reagent for the alkaline earth metals because of its very sensitive reaction with beryllium, and also because of the experimental difficulties in spraying any single metal ion with its individual spray reagent (the metal ions were applied to the layers at 1-cm intervals, and so attempts at spraying individual spots generally led to overlapping of the sprayed bands).

The R_F values quoted in Table II are in agreement with the extraction behaviour of these metal ions in the corresponding TBP - hydrochloric acid system.[8,11] Of the alkali metals, only lithium measurably extracts into TBP.

SCANDIUM, YTTRIUM, THE LANTHANIDES, THORIUM AND URANIUM—

From the results in Table II it can be seen that only scandium, thorium and uranium are appreciably retained by the stationary phase. Except for scandium and uranium, the spots were generally very large (3 cm) and diffused laterally. The thorium spot was badly streaked although well defined and reproducible, and at acid concentrations above 6 M hydrochloric acid separated into two spots, the upper spot being the more intense. The phenomenon of multiple-spot formation will be discussed later. The streaking is probably caused by a slow rate of attainment of equilibrium between the thorium and the hydrochloric acid or between the thorium chloro - aquo complex and the TBP, or both. Similar arguments can be used to explain the tailing of the lanthanides at high eluent acid concentration.

The results show that the separation of scandium from yttrium, the lanthanides and uranium is possible by using 6, 7 and 8 M hydrochloric acid as eluents. Scandium can be separated from uranium at all of the eluent concentrations used, except 9 M hydrochloric acid.

The R_F behaviour of these metals is in agreement with the available extraction results in the corresponding TBP - hydrochloric acid system.[11]

TABLE III

MEAN R_F VALUES OF THE FIRST ROW TRANSITION METALS ON CELLULOSE - TBP (5 PER CENT.) LAYERS AT VARIOUS ACID CONCENTRATIONS

Acid concentration	Ti(IV)	V(V)	Cr(III)	Mn(II)	Fe(II)	Fe(III)	Co(II)	Ni(II)	Cu(II)	Zn(II)
0·1 M	1·00	0·99	0·99	1·00	1·00	0·92	1·00	1·00	1·00	0·94
0·75 M	1·00	0·95T	0·98	0·99	0·99	0·81	0·99	0·99	0·99	0·80
1·0 M	0·99 0·53	0·93T 0·64T	0·98	0·99	0·98	0·55	0·99	0·98	0·97	0·65
2·0 M	—	—	0·97	0·97	0·98	—	0·97	0·97	0·97	0·20
3·0 M	0·89	0·95T T	0·96	0·96	0·97	0·00	0·96	0·96	0·96	0·18
4·0 M	0·97	0·94T T	0·94	0·95	0·97	0·00	0·95	0·95	0·93	0·31
4·5 M	0·94	0·94T	0·93T	0·95	0·97	—	—	—	—	—
5·0 M	0·91	0·93T T	0·93	0·93	0·94	0·00	0·95	0·94	0·91	0·40
6·0 M	0·89	T	0·91	0·89	0·87	0·00	0·85	0·88	0·82	0·47
7·0 M	0·79	T	0·89	0·87	T	0·00	0·83	0·87	0·79	0·52
7·5 M	—	—	—	—	—	0·00	—	0·86	0·76	0·55
8·0 M	0·75	T	0·86	0·76	T	0·00	0·72	0·80	0·74	0·59
9·0 M	—	T	0·84	0·66	T	0·00	0·65	0·75	0·67	0·62

T = Tailed spot.
Multiple values indicate multiple spots.

THE FIRST ROW TRANSITION METALS—

The average R_F values for the first row transition metals are given in Table III. The results for manganese, cobalt, copper and zinc have been reported previously.[1,2] The spots obtained for these materials were, in nearly all instances, small and well defined. Vanadium

and iron(II) showed severe tailing in some eluent systems, although in the case of vanadium, the tail consisted of a series of partly separated spots. These spots are probably caused by the presence of several oxidation states of vanadium in the hydrochloric acid mobile phase; chromatographic separation of these oxidation states will occur, resulting in the tail described above. Titanium showed multiple-spot formation in the M hydrochloric acid eluent system. For acid concentrations below 6 M, none of the first row transition metals, except zinc, is appreciably retained by the stationary phase. Complete separation of titanium, vanadium, manganese, cobalt and copper is not possible in any of the eluent systems used. However, separation of iron(II) from iron(III) is possible in all eluent systems, and separation of chromium, iron(II)and III), nickel and either cobalt or manganese from each other is possible by using 8 M hydrochloric acid as eluent. Zinc can be separated from the other first row transition metals in nearly all of the eluent systems.

TABLE IV

MEAN R_F VALUES OF THE SECOND ROW TRANSITION METALS ON CELLULOSE - TBP (5 PER CENT.) LAYERS AT VARIOUS ACID CONCENTRATIONS

Acid concentration	Zr(IV)	Nb(V)	Mo(VI)	Ru(IV)	Rh(II)	Pd(II)	Ag(I)	Cd(II)	In(III)	Sn(II)
0·1 M	0·92T	T	T	0·86	0·89	0·75	0·00	0·92	0·92	T
0·75 M	0·84T	T	T	0·85 0·78	0·87	0·79	0·00	0·86	0·86	0·14
1·0 M	T	T	0·00	—	0·88	—	—	—	—	—
2·0 M	T	T	0·00	—	0·89	—	—	—	—	—
3·0 M	0·79T	0·00	0·00	0·83 0·19	0·91	0·71	0·29T	0·18	0·10	0·00
4·0 M	—	0·00	0·00	—	0·90	0·72	—	0·22	0·10	0·00
4·5 M	0·83T	0·00	0·00	0·85 0·36	0·89	0·73	0·66T	0·26	0·11	0·00
5·0 M	0·80T	0·00	0·00	0·80 0·38	0·90	0·70	0·72	0·24	0·07	0·00
6·0 M	T	0·00	0·00	0·86 0·45	0·84	0·67	0·80	0·32	0·06	0·00
7·0 M	—	0·00	0·00	—	0·83	—	—	—	—	—
7·5 M	0·13	0·00	0·00	0·82 0·52	0·82	0·63	0·82	0·41	0·06	0·00
8·0 M	0·00	0·00	0·00	0·80 0·60	0·79	0·63	0·79	0·52	0·01	0·00
9·0 M	0·00	0·00	0·00	0·80 0·73	0·74	0·63	0·55	0·59	0·07	0·00

T = Tailed spot.
Multiple values indicate multiple spots.

THE SECOND ROW TRANSITION METALS—

Well defined spots were generally obtained for the second row transition metals. Zirconium was the only metal ion that showed severe tailing (Table IV) over a wide range of acid concentration; niobium, molybdenum and tin showed moderate tailing in the low acid concentration systems.

Several separations of the second row transition metals are possible. At 3 M hydrochloric acid, niobium, molybdenum, ruthenium, rhodium, palladium, cadmium and indium can all be separated from each other. At 6 M hydrochloric acid, rhodium, palladium, silver, cadmium, indium and tin can be separated, and at 7·5 M hydrochloric acid, zirconium can be separated from the other metals.

A comparison of the R_F values in Table IV with those in Table III shows that the second row transition metals are generally much more strongly held by the TBP than the first row transition metals. Thus, separation of the metals within groups of the periodic table is possible in nearly all of the eluent systems. Such separations will be discussed.

TABLE V

MEAN R_F VALUES OF THE THIRD ROW TRANSITION METALS, ANTIMONY, SELENIUM AND TELLURIUM OF CELLULOSE - TBP (5 PER CENT.) LAYERS AT VARIOUS ACID CONCENTRATIONS

Acid concentration	Se(IV)	Te(IV)	Sb(III)	Ta(IV)	W(VI)	Re(VII)	Os(VIII)
0·5 M	1·00	0·87	T 0·00	0·02	0·00 0·04	0·78 0·12	0·93
1·0 M	1·00	0·83	T 0·00	T 0·00	0·00	0·76 0·64	0·84 0·40
2·0 M	1·00	0·24T	T 0·00	T 0·00	0·01	0·63 0·05	0·95
3·0 M	0·98	0·09	T 0·00	0·00	0·00	0·60 0·05	0·95
4·0 M	0·98	0·02	T 0·00	0·00	0·00	0·52 0·05	0·95
5·0 M	0·98	0·02	T 0·00	0·00	0·00	0·49 0·04	0·88
6·0 M	0·85	0·00	T 0·00	0·00	0·00	0·45 0·00	0·84
7·0 M	0·88	0·01	T 0·00	0·00	0·00	0·40 0·02	0·84
8·0 M	0·73	0·00	T 0·00	0·00	0·00	0·40 0·03	0·85
9·0 M	0·46T	0·00	T 0·00	0·00	0·09 0·02	0·37 0·04	0·80

Acid concentration	Ir(III)	Pt(II)	Au(III)	Hg(II)	Tl(I)	Pb(II)	Bi(III)
0·5 M	0·77T 0·70	0·83 0·38T	0·00	0·11	0·90	0·81	0·19
1·0 M	0·85T 0·72	0·71 0·26	0·00	0·09	T	0·80	0·32
2·0 M	T	0·40T 0·11	0·00	0·06	T	0·81	0·40
3·0 M	T	0·38 0·09	0·00	0·05	T	0·83	0·57
4·0 M	T	0·37 0·11	0·00	0·07	T 0·42	0·89	0·79
5·0 M	T	0·36 0·13	0·00	0·10	T 0·39	0·92	0·92
6·0 M	T	0·41 0·20	0·00	0·13	T 0·40	0·93	0·94
7·0 M	T	0·47 0·32	0·00	0·22	T 0·53	0·95	0·95
8·0 M	T	0·50	0·00	0·29	T 0·61	0·95	0·88
9·0 M	T	0·51	0·00	0·38	T 0·80	0·90	0·90

T = Tailed or streaked spots.
Multiple values indicate multiple spots.

THE THIRD ROW TRANSITION METALS, ANTIMONY, SELENIUM AND TELLURIUM—

Table V gives the R_F values of the third row transition metals, antimony, selenium and tellurium. Many of the metal ions listed in this table showed multiple-spot formation. Iridium was particularly troublesome in this respect in that several spots of equal intensity appeared on spraying with indicator, and considerable streaking occurred between these spots. Thus no accurate R_F values could be measured for iridium in systems with acid concentrations above 1 M. Antimony appeared as a series of tailed spots and the positions of these spots on the chromatograms were not reproducible, except for the very small, intense spot that remained at the point of application. This small spot is probably caused by the

reaction of the cellulose layer with the sodium hydroxide in which the antimony trichloride was dissolved; shrinkage of the layer occurred at the point of application of the spot, resulting in the applied spot breaking away from the rest of the layer. The severe tailing may have been caused by a slow rate of hydrolysis of the antimony compound and partial separation of some of the intermediate species of the hydrolysis reaction.

Despite some tailing at intermediate acid concentrations, selenium can be separated from tellurium in all of the eluent systems used. Osmium, platinum, gold and mercury can be separated by using 0·5 or M hydrochloric acid as eluents. Mercury, thallium, lead and bismuth can also be separated at low acid concentrations, although thallium shows streaking at acid concentrations between 1 M and 9 M hydrochloric acid. Rhenium and osmium can be separated in all of the acid systems above 1 M, while rhenium, osmium, platinum, gold, mercury and lead can be separated by using 8 M hydrochloric acid as eluent. Antimony, tantalum, tungsten and gold have R_F values of zero in all of the eluent systems used.

Tables III, IV and V show that the R_F values of the metals chromatographed generally decrease in the order: first row > second row > third row, and thus separations of the metal ions within a group of the periodic table are, in many instances, possible. Some of these separations have already been discussed.

SEPARATION OF TITANIUM(IV) AND ZIRCONIUM(IV)—

Titanium and zirconium are readily separated in any eluent system above 7 M hydrochloric acid.

SEPARATION OF CHROMIUM(III) FROM MOLYBDENUM(VI) AND TUNGSTEN(VI)—

Chromium can be separated from molybdenum and tungsten in any of the eluent systems used.

SEPARATION OF THE PLATINUM METALS (Ru, Rh, Pd, Os, Ir, Pt)—

It is not possible to separate all of the platinum metals by using a single eluent system. However, it is possible to separate the first three metals (ruthenium, rhodium and palladium) in either the 3 or 5 M hydrochloric acid systems and to separate osmium from platinum in all of the eluent systems. Iridium invariably tailed in all of the eluent systems. Ruthenium, palladium, osmium and platinum can be separated in the 3 M hydrochloric acid system.

SEPARATION OF COPPER, SILVER AND GOLD—

Gold invariably has an R_F value of zero, and so this metal can be separated from silver and copper in all systems above 3 M hydrochloric acid. The three metals can be separated from each other in the 3, 4·5 and 5 M hydrochloric acid systems.

SEPARATION OF ZINC, CADMIUM AND MERCURY—

Although the R_F values of zinc and cadmium are very similar in several systems, separation of both of these metals from each other and from mercury can be achieved in any eluent system between 4·5 and 8 M hydrochloric acid.

While a large number of separations within periods or groups, or both, of the periodic table have been discussed, industrial mixtures are, in fact, not so systematic in their composition, *e.g.*, the coinage and jewellery metals, metals of toxicological interest, and ferrous and non-ferrous alloys, etc.

SEPARATION OF COINAGE AND JEWELLERY METALS (Ni, Cu, Ru, Rh, Pd, Ag, Ir, Pt, Au, Sn)—

A complete separation of all of these metals is not possible in a single eluent system. However, if iridium is absent, a separation of the other metals can be achieved by a suitable choice of two or more solvent systems, *viz.*, at 3 M hydrochloric acid, all, except nickel and copper, and gold and tin, may be separated, one from the other. Nickel and copper may be separated from one another, and from the others, by the use of 7·5 M hydrochloric acid as eluent. Gold and tin can be separated by using 0·75 M hydrochloric acid as eluent.

SEPARATION OF THE TOXIC METAL IONS (Bi, Cd, Co, Cu, Hg, Mn, Ni, Pb, Sb, Se, Te, U, Zn)—

The application of the technique to the separation of metals of toxicological interest has been reported previously.[3]

Possible separations of many other industrial mixtures could be discussed. However, the feasibility of separating any given mixture of metal ions can be readily ascertained by reference to the R_F spectra shown in Fig. 1.

MULTIPLE-SPOT FORMATION—

A theoretical treatment of multiple-spot formation has been given by Keller and Giddings,[12] who have shown that slow chemical equilibrium in either one or both phases can be the cause. However, in most instances, the formation of multiple spots from a single applied substance actually involves separation of different forms of the substance, these forms being relatively stable in the chromatographic conditions used.[13] The latter is probably true in TBP - hydrochloric acid systems where several extractable species can co-exist.[14]

THE INFLUENCE OF CHLORO-COMPLEX FORMATION ON THE R_F VALUES AND THE NATURE OF THE SPECIES EXTRACTED INTO TBP

The R_F values of the metal ions show that, in general, those metal ions that readily form chloro complexes have low R_F values, *e.g.*, zinc, cadmium, mercury, platinum and gold, while those metals which do not readily form chloro complexes have high R_F values, *e.g.*, chromium, cobalt and nickel. Much of the literature on the nature of chloro complexes formed by most of the metal ions in different concentrations of hydrochloric acid has been reviewed.[15] From the chloro complexes formulated in the above review, and from the R_F values quoted in the results, it is evident that TBP favours the extraction of both neutral and anionic chloro complexes. In particular, TBP favours the extraction of acido - halo complexes, and those metals which form such complexes generally have R_F values of zero.

Marcus[16] has reviewed the use of TBP in the solvent extraction of metal ions and mineral acids, and has formulated some of the extracted species existing in the organic phase. He has drawn attention to the possibility that TBP can extract in two different ways—

(*i*) by direct co-ordination of the TBP to the metal, *e.g.*, in the TBP - hydrochloric acid system, uranium is reported to be extracted as $UO_2Cl_2.2TBP$[17] at low acid concentration;

(*ii*) by solvating the proton in the extraction of the transition metal halo-acids, *e.g.*, $H(TBP)_2FeCl_2$ exists in the organic phase at high hydrochloric acid concentrations.[18]

Only the first possibility has been considered by most authors, *e.g.*, the cobalt and iron species have been stated to be $CoCl_2.2TBP$ and $FeCl_3.2TBP$.[19] However, it has been shown in many instances that the second possibility is much more likely, and that the metal ions are extracted as "ion-association" complexes, *e.g.*, cobalt may be extracted as $(TBPH^+)_2CoCl_4^{2-}$ and iron(III) as $(TBP)_2H^+FeCl_4$.[19] It can be seen that the TBP-to-metal ratio is the same for both solvated and ion-associated formulae, and thus it is not possible to formulate the species unambiguously from a knowledge of this ratio alone. Morris and Short[14] have shown that the complexes $HZnCl_3.3TBP$ and $H_2ZnCl_4.2TBP$, both of which are present in the TBP phase, exist as ion pairs $[TBPH^+][ZnCl_3.2TBP^-]$ and $[TBPH^+]_2[ZnCl_4]^{2-}$, respectively. It is well known that at high acid concentration, both cobalt and iron(III) exist as their acid chloro complexes, and hence it is reasonable to assume that at high acid concentration the ion-association species predominates. From the similarities in the R_F spectra of zinc, cadmium and mercury, it is also reasonable to assume that the species extracted will be ion-associated, rather than solvated.

At high hydrochloric acid concentration, the acid itself is extracted into the TBP, the amount extracted becoming appreciable at concentrations above 7 M hydrochloric acid.[20] The extraction of hydrochloric acid by TBP has been reviewed and several possible extraction mechanisms have been given.[16] The extraction process is not a simple one and it appears that partly dissociated ion pairs of the type $[H(H_2O)_4]^+[A(TBP\ solvated)]^-$ co-exist in the TBP phase with $TBP.H_2O$ and un-ionised TBP.HA. More TBP is associated with the ion pair at low acid concentration and more hydrochloric acid is associated with the ion pair at very high acid concentration.

From the above discussion, it is evident that the R_F value of a metal ion in any TBP - hydrochloric acid system is dependent on the ability of the metal ion to form chloro complexes in the aqueous phase in order that the TBP can either solvate or ion-associate as $TBPH^+$ with those complexes. The dividing line between solvation and ion-association is not clear,

and it may well be that many of the complexes showing TBP as a solvating molecule are actually ion-associations involving TBP-solvated protons ($TBPH^+$). The similarities between TBP-extraction behaviour and ion-exchange behaviour (discussed below) certainly support this view.

Comparison between the R_F spectra and corresponding liquid - liquid extraction behaviour

Ishimori, Watanabe and Nakamura[11] have plotted the partition coefficient of metal ions in pure TBP - hydrochloric acid systems against the concentration of the hydrochloric acid in the aqueous phase. The curves obtained by these workers compare favourably with the R_F spectra of the same metal ions, *i.e.*, an increase in partition coefficient with acid concentration can be compared with a decrease in R_F value over the same range of acid concentration. In the acid concentration range 4 to 9 M hydrochloric acid, the alkali and alkaline earth metals have very low partition coefficients that increase slightly with increasing acid concentration. This compares favourably with very high R_F values that decrease slightly with increasing acid concentration. The transition metals also compare favourably, and the maxima in the extraction curves of zinc, cadmium, mercury, platinum and lead occur at the same acid concentration as the minima in the corresponding R_F spectra. Those metal ions which have partition coefficients above 100 generally have R_F values of zero, *e.g.*, niobium, tantalum, molybdenum, tungsten, iron(III), tellurium and gold. From the extraction curves for niobium and tantalum, it should be possible to separate these metals at low acid concentration, although no separation was detected by us because of the difficulty in identifying these metals on the layers. Niobium is not readily extracted into TBP at low acid concentrations (M hydrochloric acid), whereas tantalum is. O'Laughlin and Banks[4] obtained an R_F value of 0·1 for niobium on TBP-treated papers eluted with 0·5 M hydrochloric acid, but they did not chromatograph tantalum.

It is of interest to compare the R_F values quoted by O'Laughlin and Banks[4] with those obtained in the present study, and with the extraction results of Ishimori, Watanabe and Nakamura.[11] It has already been shown that there is good agreement between the last two of these. The first of these gives the R_F values of several metal ions on TBP-treated papers eluted with various concentrations of hydrochloric acid. Several of the R_F values show a maximum at 9 M hydrochloric acid (yttrium, titanium, manganese, cobalt, nickel, copper, vanadium and chromium), while others show a minimum at 6 M hydrochloric acid (tin and mercury). Such behaviour is not in agreement with extraction results or the R_F behaviour of the same metal ions in the present work.

Comparison between R_F spectra and ion-exchange behaviour

Few authors have drawn attention to the similarities between the extraction behaviour of metal ions in the TBP - hydrochloric acid system and the anion-exchange behaviour of the same metal ions in a resinous ion exchanger - hydrochloric acid system.[11,21] Ishimori, Watanabe and Nakamura[11] have shown that the shapes of the extraction curves compare favourably with the shapes of the ion-exchange curves and these authors are of the opinion that the mechanism of solvent extraction by TBP is similar to the ion-exchange mechanism. The maxima in the ion-exchange and extraction curves for zinc, cadmium and lead occur at about the same acid concentration as the minima in the R_F spectra of these metal ions; all of the noble metals are strongly retained by the resin, corresponding to high TBP extraction and low R_F values. The alkali and alkaline earth metals are not appreciably retained by the resin, corresponding to poor extraction by the TBP and high R_F values.

Kraus and Nelson[22] explained their results in terms of chloro-complex formation and they classified the metal ions into three groups according to their ion-exchange behaviour—

(*i*) Those not adsorbed at any concentration (the alkali and alkaline earth metals, aluminium, nickel and thorium).

(*ii*) Those which show increasing adsorption with increasing acid concentration and only good adsorption at high acid concentration.

(*iii*) Those which show only decreasing adsorption with increasing acid concentration (metals of the central region of the periodic table), *i.e.*, those metals which readily complex with chloride ion.

The same classifications could equally well be applied to the R_F spectra of the metal ions.

Elution of zirconium from the resin resulted in tailing at low acid concentration. This tailing is in agreement with the tailing that zirconium exhibits in the TBP - hydrochloric acid chromatographic system.

From the above considerations it appears likely that in the chromatographic systems used, the TBP extracts the metal ions by an anion-exchange process in which the metal - chloro complex exchanges with chloride associated with the TBP-solvated proton.

We have previously suggested[3] that the TBP in such a system should be considered to act more as an ion exchanger than as an extractant. $[TBPH^+]Cl^-$, *viz.*, TBP-solvated protons associated in an "ion-association" system with chloride ions can exchange the chloride ion for negatively charged chloro complexes of the metal ions. Thus ion exchange results as the consequence of the ion-association of the $TBPH^+$ and the metal - chloro complexes. Such a mechanism has been suggested by Morris and Short[14] for the extraction of zinc into TBP.

We suggest that the systems studied exhibit behaviour typical of ion-exchange chromatography and are better regarded as such than as purely liquid - liquid extraction systems. Any difference may be one of technique rather than one involving the mechanism of the process. Ion-exchange chromatography may be regarded as a steady-state process and liquid - liquid extraction as an equilibrium phenomenon; whenever TBP and metallochloro complexes are used the $[TBPH]_n^+[M_xCl_y]^{n-}$ complex is probably formed and retained by the TBP. The stability of this complex and hence the degree of retention by the TBP determines the R_F value of the metallochloro anion (and hence of the metal) and the partition coefficient.

The positioning of the TBP and the method by which it is brought into contact with the other phase are all that determine whether the process is ion-exchange chromatography or solvent extraction.

It is thus probable that other materials capable of giving ion-association systems and complex-ion systems of different stabilities may be of use in separating metals from one another.

After the initial submission of this paper for publication, work was published by Pierce and Flint,[23] and by Brinkman and Veltkamp,[24] dealing with some of these metal ions chromatographed in reversed-phase systems with TBP. In general, the results[24] are in agreement with those recorded here; the results for copper(II) and manganese(II) reported by Pierce and Flint[23] do not agree with the general findings. An interpretation of Pierce and Flint's results is not possible in terms of the extraction - ion association described earlier.

References

1. Duncan, G., Bark, L. S., and Graham, R. J. T., *Proc. Soc. Analyt. Chem.*, 1966, **3**, 145.
2. Bark, L. S., Duncan, G., and Graham, R. J. T., *Analyst*, 1967, **92**, 31.
3. ——, ——, ——, "Proceedings of the 4th International Symposium, Chromatography and Electrophoresis," Belgian Pharmaceutical Society, Bruxelles, 1966, in the press.
4. O'Laughlin, J. W., and Banks, C. V., *Analyt. Chem.*, 1964, **36**, 1222.
5. Hu, Z.-T., Shi, S.-C., *Acta Chim. Sin.*, 1964, **30**, 352.
6. Hu, Z.-T., *Kexue Tongbao*, 1966, **17**, 166.
7. Hu, Z.-T., Liu, C.-L., *Acta Chim. Sin.*, 1964, **31**, 267.
8. Healy, T. V., and Brown, P. E., *U.K. Atomic Energy Authority Report*, AERE/C/R 1970, H.M. Stationery Office, London, 1956.
9. Bark, L. S., Graham, R. J. T., and McCormick, D., *Talanta*, 1965, **12**, 122.
10. Hashmi, M. H., Maqbool, A. S., and Ayaz, A. A., *Ibid.*, 1955, **12**, 713.
11. Ishimori, T., Watanabe, K., and Nakamura, E., *Bull. Chem. Soc. Japan*, 1960, **33**, 636.
12. Keller, R. A., and Giddings, J. C., *J. Chromat.*, 1963, **3**, 205.
13. Maček, K., and Hais, I. M., "Paper Chromatography," Academic Press, London, 1963, p. 158.
14. Morris, D. F. C., and Short, E. L., *J. Chem. Soc.*, 1962, 2662.
15. Jørgensen, Ch. K., "Complexes in Inorganic Chemistry," Academic Press, London, 1963, p. 42.
16. Marcus, Y., *Chem. Rev.*, 1963, **63**, 139.
17. Gal, I. J., and Ruvarac, A., *Bull. Inst. Nucl. Sci. Boris Kidrich*, 1958, **8**, 67.
18. Majumdar, S. K., and De, A. K., *Talanta*, 1960, **7**, 1.
19. Chatelet, M., and Nicaud, C., *C. R. Hebd. Séanc. Acad. Sci., Paris*, 1956, **242**, 1471 and 1891.
20. Kertes, A. S., *J. Inorg. Nucl. Chem.*, 1960, **14**, 104.
21. Brinkman, U. A. Th., and De Vries, G., *J. Chromat.*, 1965, **18**, 142.
22. Kraus, K. A., Nelson, F., *Proc. Int. Conf. Peaceful Uses of Atom. Energy, Geneva*, 1958, **7**, 837.
23. Pierce, T. B., and Flint, R. F., *J. Chromat.*, 1966, **24**, 141.
24. Brinkman, U. A. Th., and Veltkamp, H., *Ibid.*, 1966, **24**, 489.

Received *September 29th*, 1966

Analyst, June, 1967, Vol. 92, *pp.* 358–363

A Specific Spectrofluorimetric Determination of Terbium as its EDTA - Sulphosalicylic Acid Complex

By R. M. DAGNALL, R. SMITH and T. S. WEST

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

The ternary complex formed by terbium with ethylenediaminetetra-acetic acid and sulphosalicylic acid has been used as the basis of a spectrofluorimetric determination of between $6{\cdot}4 \times 10^{-3}$ and $3{\cdot}2$ μg of terbium per ml. The system absorbs radiation characteristic of sulphosalicylic acid (about 320 mμ) and emits the band fluorescence of sulphosalicylic acid (410 mμ), together with the sharp line emission characteristic of terbium(III) (545 mμ). The determination is carried out with 4×10^{-3} M EDTA and 2×10^{-3} M sulphosalicylic acid in aqueous solution at a pH of between 11·6 and 11·9. No interference resulted from 50-fold molar excesses of the other rare earth ions, 33 other metal ions or 14 anions. The fluorescence was not subject to oxygen quenching, was stable for several days and had a temperature coefficient of $-0{\cdot}87$ per cent. per °C. Analytical results obtained with prepared samples have been included.

The spectrofluorimetric determination of the rare earth elements dysprosium, europium, samarium and terbium has been the subject of much recent study. This has been made attractive by the line-like fluorescence characteristics of these rare earth ions, thereby increasing the versatility of the analytical methods so that two or more ions can be determined simultaneously. However, these methods are generally susceptible to interference from other rare earth ions, and standard addition procedures must be used or correction factors applied.

Alberti and Massucci[1] have investigated the simultaneous determination of dysprosium, europium, samarium and terbium in 0·6 M sodium tungstate solutions, while Sevchenko and Kuznetsova[2] have used 1,10-phenanthroline in a simultaneous determination of the same elements. Both methods are subject to interference from other rare earth ions.

Kononenko, Lauer and Poluektov[3] have used a 1,10-phenanthroline - salicylic acid system for the extraction of the rare earth ions into benzene in a fluorimetric determination of europium and terbium. The determinations of both elements are subject to interference from some of the other rare earth ions. A similar extraction system that involves the use of 1,10-phenanthroline and thenoyltrifluoroacetone[4] has been used for the fluorimetric determination of europium and samarium. Ballard and Edwards[5] have examined the thenoyltrifluoroacetonate - trioctylphosphate extraction system in the determination of europium. Determinations of europium with 2-phenyl-4-quinoline carboxylic acid[5] and of dysprosium and terbium with 4-sulphophenyl-3-methyl-5-pyrazolone[6] also suffer from interelement effects of other rare earth ions.

McCarthy and Winefordner[7] have examined the possibilities of the simultaneous analysis of dysprosium, europium, samarium and terbium in non-aqueous solutions of aromatic carbonyl compounds. The energy transfer in this instance is said to be caused by the collision of the carbonyl donor with the rare earth ion. Although interelement effects are present, the choice of suitable carbonyl compounds has been shown to increase the selectivity of the method.

Spectrophotometric, fluorimetric and potentiometric studies have been carried out by Charles and Riedel[8] on the terbium - EDTA - sulphosalicylic acid complex in aqueous solution. They reported a high quantum efficiency of fluorescence ($0{\cdot}7 \pm 0{\cdot}1$), a line emission characteristic of the Tb^{3+} ion and an analogous, but non-fluorescent, Eu^{3+} complex. We describe here an analytical exploitation of the same system for terbium by using EDTA both as part of the complex and as a general masking agent. As a result there is no interference from any of the rare earth or other metal ions examined.

Experimental

Reagents—

Terbium solution—Dissolve 0·183 g of terbium oxide (Johnson, Matthey Ltd. 99·9 per cent. Tb_2O_3) in a few drops of concentrated nitric acid and evaporate to dryness. Dissolve the residue in water and dilute to 1 litre. This gave a 10^{-3} M solution of terbium nitrate that was used as stock (equivalent to 159 μg of terbium(III) per ml).

Buffer solution (pH 11·7 to 11·9)—To 400 ml of water add 70 ml of diethylamine (general-purpose reagent) and adjust to pH 11·9 with about 5 ml of concentrated hydrochloric acid. This buffer deteriorates rapidly on exposure to air and must, therefore, be stored in a well stoppered bottle.

Sulphosalicylic acid solution, 10^{-1} M—Dissolve 12·70 g of sulphosalicylic acid (general-purpose reagent, Hopkin & Williams Ltd.), $C_6H_3(OH)COOH.SO_3H.H_2O$, in 500 ml of water containing 20 ml of buffer solution.

Disodium ethylenediaminetetra-acetic acid ($Na_2EDTA.2H_2O$), 10^{-1} M.

Other rare earth ion solutions—Prepare 10^{-3} M solutions in a manner similar to the terbium solution from Johnson, Matthey Ltd. 99·9 per cent. rare earth oxides.

Apparatus—

Farrand Optical Co. spectrofluorimeter described in detail elsewhere,[9] but fitted with an RCA 1P21 photomultiplier.

Procedure—

To each 25-ml calibrated flask add an aliquot of solution containing between 0·2 and 100 μg of terbium. Add 1 ml of 10^{-1} M EDTA solution, 0·5 ml of 10^{-1} M sulphosalicylic acid (SSA) and 1 ml of buffer. Dilute to volume with distilled water.

Measure the fluorescence after 10 minutes at 545 mμ by using 20-mμ half-bandwidth slits on the analysing monochromator. An excitation wavelength of 320 mμ can be used in conjunction with 20-mμ slits on the excitation monochromator, but it was preferable to remove the slits from the excitation monochromator and to use a Corning 7–54(9863) filter instead. This filter transmits radiation between 230 and 400 mμ, giving maximum transmission at 350 mμ (see Limits of Determination).

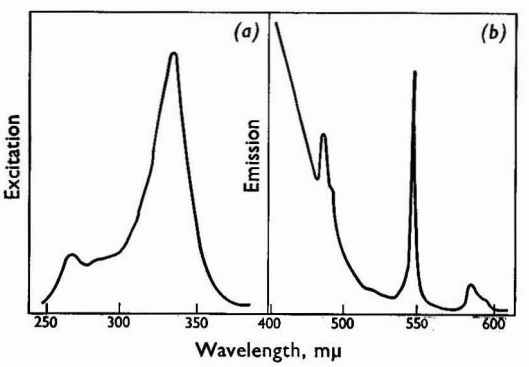


Fig. 1. Uncorrected (*a*), excitation and (*b*), emission spectra of 16 μg of terbium(III) in 4×10^{-3} M EDTA and 2×10^{-3} M sulphosalicylic acid. There is no intensity correlation between excitation and emission spectra

Results and discussion

Spectral characteristics—

Fig. 1 shows the uncorrected excitation and emission spectra of the terbium - EDTA - SSA system as used analytically. The excitation wavelength maximum (320 mμ) coincides with the absorbance maximum for the SSA^{3-} ion, while the emission consists of a broad band,

(not shown completely) characteristic of the SSA^{3-} ion, together with the line-emission characteristic of Tb^{3+}. The line emission of the Tb^{3+} ion has been assigned[8] to transitions from the $^5D_4$ level to the $^7F_6$, $^7F_5$ and $^7F_4$ levels at 485, 545 and 575 mμ, respectively. A fourth emission peak due to the $^5D_4$ - $^7F_3$ transition occurring at longer wavelengths (about 630 mμ) was not observed because of the insensitivity of the photomultiplier in this region. With the apparatus described, only the peak at 545 mμ is analytically useful.

The system appears to exhibit intramolecular energy transfer from the co-ordinated sulphosalicylic acid to the terbium ion. This phenomenon has been reviewed by Crosby[10] and energy transfer mechanisms discussed. In this particular instance, however, the co-ordinated EDTA also affects the fluorescence, and in the absence of EDTA the fluorescence is reduced by a factor of about 10^3. It is likely that the co-ordinated EDTA protects not only the terbium ion but also the co-ordinated sulphosalicylic acid from collisional interference by other rare earth ions or solvent molecules. Thus the SSA triplet state is de-activated chiefly by energy transfer to the adjacent terbium ion. The reported high quantum yield of fluorescence (0·7 in aqueous solution[8]) supports this hypothesis.

Influence of pH—

The fluorescence intensity of a 10^{-5} M terbium solution with 10-fold molar excesses of EDTA and SSA was measured over a range of pH values from 8 to 13, by using ammonia solution and sodium tetraborate as the buffer solution and adjusting the pH with hydrochloric acid or sodium hydroxide solution. Blank solutions containing no terbium were prepared separately. Maximum fluorescence intensity was obtained at a pH of between 11·6 and 11·9 (Fig. 2); a diethylamine - hydrochloric acid buffer is most suitable for this range.[11] The fluorescence is independent of buffer concentration at this pH, and there is adequate buffering capacity if neutral metal ion solutions are used.

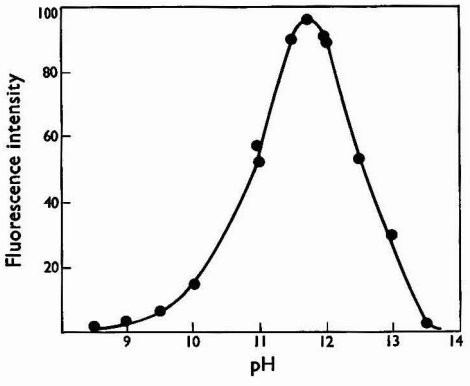


Fig. 2. Variation of fluorescence intensity with pH for 2 μg of terbium(III) in 4×10^{-3} M EDTA and 2×10^{-3} M sulphosalicylic acid

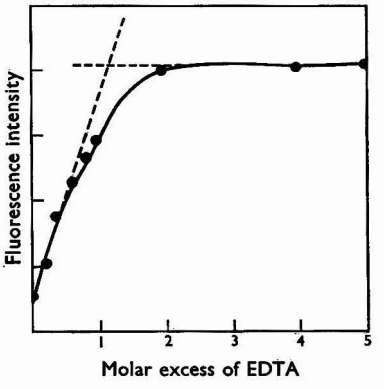


Fig. 3. Influence of excess of EDTA on fluorescence intensity of 10^{-6} M terbium(III) in 2×10^{-3}M sulphosalicylic acid

Reagent excess—

The effect of reagent excess was extensively investigated for both EDTA and SSA. Investigations carried out on 10^{-5} M terbium solutions indicated that the fluorescence intensity increased linearly with EDTA concentration up to a 1 : 1 stoicheiometry, and that excesses of EDTA, *e.g.*, 1000-fold, did not affect the fluorescence further (Fig. 3). Because of the masking action of EDTA, a 10^4 molar excess (which can be increased if necessary) is recommended.

A logarithmic relationship was obtained between SSA concentration and fluorescence intensity (Fig. 4). With lower concentrations of terbium (about 10^{-7} M), no improvement in signal-to-blank ratio was found when the SSA concentration was decreased from the recommended 10^4 molar excess to a 10-fold molar excess.

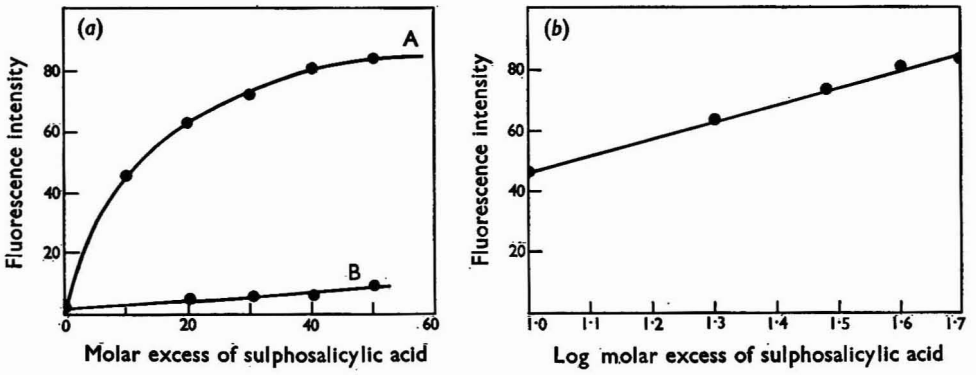


Fig. 4 (*a*). Influence of excess of sulphosalicylic acid on the fluorescence intensity of: curve A, 10^{-6} M terbium(III) in 4×10^{-3} M EDTA; curve B, 4×10^{-3} M EDTA. Fig. 4 (*b*). Log excess of sulphosalicylic acid against fluorescence intensity of 10^{-6} M terbium(III) in 4×10^{-3} M EDTA

OTHER ANALYTICAL PARAMETERS—

The fluorescence intensity of solutions containing 10^{-7} and 10^{-6} M of terbium were measured and related to that of a quinine sulphate standard by using the same excitation filter, but measuring the quinine sulphate fluorescence at 450 mμ. Constant fluorescence intensity was noted from 10 minutes after dilution to 100 hours later, when observations were discontinued. During the first 10 minutes after dilution, the fluorescence decreases by about 5 to 10 per cent.

The effect of temperature on the fluorescence of a 4×10^{-6} M solution of terbium was observed over the range 10° to 60° C. A high terbium concentration was chosen so that blank fluorescence was negligible. Between 15° and 30° C, the temperature coefficient was constant and had the value −0·87 per cent. per °C. Charles and Riedel[9] have found the quantum efficiency of the terbium - EDTA - SSA system in D_2O to be constant between 5° and 25° C.

The effect of dissolved oxygen was investigated by measuring the intensity of fluorescence of a 10^{-7} M solution of terbium, before and after de-gassing with nitrogen for 20 minutes. Oxygen was then bubbled through the solution for 5 minutes and the fluorescence intensity measured again. Three identical readings were obtained, showing that no quenching of fluorescence by dissolved oxygen occurred. Care was taken to make these measurements at the same temperature because bubbling the gases caused some cooling.

LIMITS OF DETERMINATION—

A linear relationship between fluorescence intensity and terbium concentration was obtained for solutions containing from $6{\cdot}4 \times 10^{-3}$ (4×10^{-8} M) to $3{\cdot}2$ (2×10^{-5} M) μg of terbium per ml. The intensity of fluorescence of the blank was equivalent to 6×10^{-2} (4×10^{-7} M)μg of terbium per ml and, therefore, the lowest calibration graph was for the range 0·16 to 1·6 μg of terbium in 25 ml of solution, *i.e.*, 4×10^{-8} to 4×10^{-7} M. The blank at this level is 50 per cent. of the maximum scale deflection and has, therefore, been set as a limiting value. Suitable amplification of the signal would enable the method to detect about 0·03 μg of terbium when using a blank value corresponding to 90 per cent. of the full-scale deflection.

Narrowing the slit width in the analysing monochromator also improves the signal-to-blank ratio, but higher amplification must be used to compensate for this. This is a direct result of the sharp line emission of the terbium chelate being superimposed on the approximately uniform spectral distribution of the blank. However, because the narrow slits (5 mμ) did not isolate the whole of the terbium emission line, it was found that non-linearity of the calibration curves resulted for low concentrations of terbium, *i.e.*, less than 1·0 μg. For this reason, 20-mμ slits are recommended in the analysing monochromator; these isolate all the terbium fluorescence, and the analytical signal is proportional to the area under the fluorescence peak rather than to the height. With 20-mμ slits, linear calibration curves for 0·16 to 1·6 μg of terbium were obtained.

The use of ultraviolet filters in the analysing monochromator to eliminate stray light did not improve the signal-to-blank ratio.

EFFECT OF FOREIGN IONS—

Fifty-fold molar excesses of foreign ions were added, together with 4 μg of terbium as in the recommended procedure. The following tervalent rare earth ions were examined and found to cause no interference: La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm and Yb.

The following metallic ions were also examined in 50-fold molar excesses, but none was found to interfere: Ag(I), Al, As(III), Ba, Be, Ca, Cd, Co(II), Cr(III), Cr(VI), Cu(II), Fe(II), Fe(III), Ga, Hg(I), Hg(II), K, Li, Mg, Mn(II), Mn(VII), Mo(VI), Na, Nb(V), Ni(II), NH_4, Pb, Se(IV), Sr, Ta(V), Te(IV), U(VI) and Zn.

The following anions were examined in 1000-fold molar excess over 4 μg of terbium, but none was found to cause any interference: chloride, bromide, iodide, perchlorate, sulphide, thiosulphate, sulphite, sulphate, carbonate, phosphate, nitrate, acetate, oxalate and tartrate.

One-thousand-fold excesses of lead, however, gave a visible white precipitate, probably lead chloride, which reduced the fluorescence by about 20 per cent. Similar excesses of the mercury(I) ion gave a fine grey suspension that reduced the fluorescence intensity by about 2 per cent. One-thousand-fold excesses of uranium(VI) gave a strongly yellow coloured EDTA complex, which gave rise to inner filter effects and reduced the fluorescence by about 18 per cent.

The analytical results on various prepared mixtures are given in Table I.

TABLE I

ANALYSIS OF SAMPLES TREATED AS UNKNOWNS

Terbium, μg	Foreign ions, μg	Found, μg	Error, μg	Error, per cent.
0·16	—	0·19	+0·03	+18·8
0·16	—	0·16	0·00	0·0
0·80	—	0·80	0·00	0·0
0·80	—	0·83	+0·03	+3·6
1·27	—	1·29	+0·02	+1·6
1·27	—	1·26	−0·01	−0·8
0·95	Eu (159), Gd (158), Sm (150)	0·94	−0·01	−1·1
0·95	Nd (144), Pr (141), Yb (346), Er (334)	0·95	0·00	0·0
0·64	Al (270), Be (90)	0·65	+0·01	+1·5
0·64	$(NH_4)_2S_2O_8$ (210), Cr(VI) (52), Mn(VII) (55)	0·64	0·00	0·0
0·64	Pb (207), Ce (140), La (140)	0·58	−0·06	−10·3
1·19	La, Eu, Gd, Sm, Er, Nd, Dy, Tm (16)	1·24	+0·05	+4·0
1·19	Th (23), Ho (17), Yb (17), Nb (19)	1·21	+0·02	+1·7
1·19	Cu(II) (63), Mn(II) (55), Fe(II) (56), Co(II) (59)	1·18	−0·01	−0·9

STRUCTURE OF THE COMPLEX—

Mole ratio and Job plots have been carried out fluorimetrically for EDTA and SSA; a ratio of 1 + 1 + 1 for the terbium - EDTA - SSA complex was found. This is in complete agreement with the results of Charles and Riedel.[8] Our optimum pH value for fluorescence of 11·6 to 11·9 compares well with pH titration data,[8] showing that the complex is completely formed at this pH. A systematic study of the structure of this complex would require the determination of the co-ordination number of terbium, and this could be greater than, or equal to, six.

We have also carried out fluorescence measurements with other complexones (Table II). The solutions were made up as in the recommended procedure, except that 10 ml of 10^{-2} M complexone was substituted for 1 ml of 10^{-1} M EDTA solution. None of the complexones except 1,2-diaminopropanetetra-acetic acid gave a terbium fluorescence intensity equal to that obtained with EDTA, and some gave no fluorescence even on high amplification. When a measurable fluorescence was observed, no change in wavelength of the line fluorescence was noted, nor was there any appreciable change in the bandwidth of the emitted line. Similar experiments were carried out with europium, dysprosium and samarium solutions in place of terbium, but no characteristic fluorescence for these ions was obtained. This can perhaps be attributed to mismatch of the rare earth excited energy levels with the

TABLE II

RELATIVE FLUORESCENCE OF OTHER TERBIUM - SSA - COMPLEXONE SYSTEMS

Complexone	Fluorescence	Observation
Ethylenediaminetetra-acetic acid	100	—
1,2-Diaminopropanetetra-acetic acid	100	—
Trans-1,2-cyclohexanediaminetetra-acetic acid ..	85	—
Diaminodiethylene ether tetra-acetic acid ..	65	—
Hexamethylenediaminetetra-acetic acid	37	—
Iminodiacetic acid	9	—
2,6-Diaminopyridinetetra-acetic acid	7	No detectable terbium(III) fluorescence. Impure sample.
Ethylenediamine-*NN'*-diacetic acid	4	Weak terbium(III) fluorescence.
Nitrilotriacetic acid	0	No fluorescence from terbium(III) was detected, even on high amplification of the instrument.
Diethylenetriaminepenta-acetic acid	0	—

sulphosalicylic acid triplet level, and an extension of this work is now being undertaken in which energy donors other than sulphosalicylic acid are being examined. Energy transfer from *p*-benzoylbenzoic acid to the europium - EDTA chelate[12] in aqueous solution has been reported, and analytical possibilities of this system are now being examined.

The absolute analytical specificity of this type of energy transfer system in aqueous solution offers a technique of solution spectrofluorimetry that challenges those of atomic absorption and atomic-fluorescence spectroscopy in flame media, and it is, therefore, apparent that such systems should be submitted to close examination.

We are grateful to the Science Research Council for the award of a research studentship to one of us (R.S.) and for a grant for the purchase of the spectrofluorimeter used in these studies.

REFERENCES

1. Alberti, G., and Massucci, M. A., *Analyt. Chem.*, 1966, **34**, 214.
2. Sevchenko, A. N., and Kuznetsova, V. V., *Redkozem. Elementy. Akad. Nauk. SSSR. Inst. Geokhim. i Analit Khim.*, 1963, 358; *Chem. Abstr.*, 1964, **61**, 2474c.
3. Kononenko, L. I., Lauer, R. S., and Poluektov, N. S., *Zh. Analit Khim.*, 1963, **18**, 1468.
4. Kononenko, L. I., Poluektov, N. S., and Nikonova, M. P., *Zav. Lab.*, 1964, **30**, 779.
5. Ballard, R. E., and Edwards, J. W., *in* Shallis, P. W., *Editor*, "Proceedings of the SAC Conference Nottingham 1965," W. Heffer and Sons Ltd., Cambridge, 1965, p. 328.
6. Poluektov, N. S., Vitkun, R. A., and Kononenko, L. I., *Ukr. Khim. Zh.*, 1964, **30**, 629.
7. McCarthy, W. J., and Winefordner, J. D., *Analyt. Chem.*, 1966, **38**, 849.
8. Charles, R. G., and Riedel, E. P., *J. Inorg. Nucl. Chem.*, 1966, **28**, 527.
9. Dagnall, R. M., Smith, R., and West, T. S., *Talanta*, 1966, **13**, 609.
10. Crosby, G. A., Whan, R. E., and Alire, R. M., *J. Chem. Phys.*, 1961, **34**, 743.
11. Meites, L., "Handbook of Analytical Chemistry," McGraw-Hill, New York, 1964.
12. Charles, R. G., Riedel, E. P., and Haverlack, P. G., *J. Chem. Phys.*, 1966, **44**, 1356.

Received *December 28th*, 1966

Analyst, June, 1967, Vol. 92, *pp.* 364–370

The Enthalpimetric Titration of Basic Nitrogen Compounds

By G. A. VAUGHAN and J. J. SWITHENBANK
(*Coal Tar Research Association, Gomersal, Cleckheaton, Yorkshire*)

When aqueous solutions of bases are titrated enthalpimetrically with acid, it has been found that ammonia and aliphatic bases are titrated before pyridine and aniline bases. When aqueous solutions of the mineral acid salts of bases are titrated, the titration order is (*i*) the free acidity, (*ii*) the pyridine and aniline base salts and (*iii*) the ammonia and aliphatic base salts.

In non-aqueous solution, use has been made of the large endothermic heat of dilution when a strong hydrogen chloride solution in isopropyl alcohol is added to a wide range of organic solvents, excluding alcohols. As an enthalpimetric titrant, this solution first gives a temperature rise as a result of the neutralisation of the base in the solvent, followed by a sharp temperature drop that marks the end-point. In solvents other than acetic acid, aliphatic bases are titrated first and are distinguishable from aromatic bases, except those similar to diphenylamine, which are not titrated. In acetic acid solution, aliphatic and aromatic bases are titrated together first and are distinguishable from the weak bases like diphenylamine, which are titrated in this solvent.

Although the enthalpimetric titration of basic substances has been known for a long time,[1] there is only one reference to work on titrations that distinguish between different types of basic nitrogen compounds. Parsons, in a paper given to the American Chemical Society,[2] stated that the titration of pyridine in the presence of ammonia was one of the applications of enthalpimetry that showed promise.

The following is an account of an investigation into the enthalpimetric titration of water-soluble basic nitrogen compounds and also of basic nitrogen compounds in non-aqueous media.

Method

In all instances the titration was carried out with the titrant in a syringe driven by a synchronous motor. A 15-ml tall-form beaker, surrounded by a larger beaker to act as a draught screen, was used as the titration vessel. The titration vessel was fitted with a stirrer, titrant inlet-tube and a thermistor. The thermistor was connected to a Wheatstone bridge, the output of which was fed to a strip chart recorder driven by a synchronous motor, all as described in a previous paper.[3] The titrant was standardised by titration of known substances to give an equivalence in terms of recorder chart length.

TITRATION IN AQUEOUS SOLUTION

Experimental

Direct titration—

A typical titration graph when 5 ml of 0·1 N ammonia solution are titrated with 5 N hydrochloric acid is shown in Fig. 1 (*a*), and a similar titration of 5 ml of 0·1 N pyridine in Fig. 1 (*b*). The difference in slopes of the two titration graphs indicates that the two bases can be titrated separately in admixture. Fig. 1 (*c*) illustrates the titration graph of an equidecimolar mixture of ammonia and pyridine and shows that the two types of bases may be distinguished in aqueous solution, the ammonia being titrated before the pyridine.

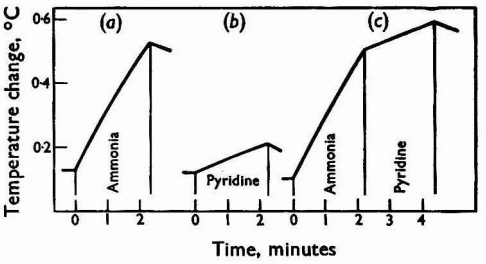


Fig. 1. Graphs of enthalpimetric titrations of aqueous solutions of nitrogen bases with 5 N hydrochloric acid

Aqueous solutions of a wide variety of basic nitrogen compounds were titrated and it was found that, in general, they fell into two categories: those with a steep titration graph which was indistinguishable from that of ammonia; and those with a less steep titration graph which was indistinguishable from that of pyridine, as follows—

Bases with steep graphs: ammonia, methylamine, diethylamine, trimethylamine, pyrrolidine, piperidine, cyclohexylamine, ethylenediamine and ethanolamine.

Bases with less steep graphs: pyridine, 3-picoline, quinoline, aniline and *o*-toluidine.

A probable reason for the difference in slopes is that the strong bases are almost completely ionised and would be expected to give a ΔH value of about $-13{\cdot}5$ kcal. per mol. With weak bases there is less ionisation and, during their titration, the endothermic heat of ionisation lowers the slope of the graph.

Thus it is possible to titrate enthalpimetrically in aqueous solution both aliphatic (including ammonia) and aromatic bases separately in admixture, *e.g.*, mixtures of pyridine with piperidine, and of aniline with cyclohexylamine. When the method is applied to the determination of ammonia - pyridine mixtures, the lower molecular weight of ammonia facilitates the titration of traces of ammonia in the presence of a large excess by weight of pyridine. Conversely, the determination of traces of pyridine in excess of ammonia is difficult because of the large amount of heat evolved before the pyridine is titrated, but this difficulty can be overcome by using the indirect titration method below.

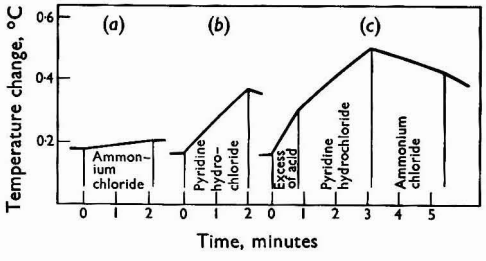


Fig. 2. Graphs of enthalpimetric titrations of aqueous solutions of nitrogen bases with 5 N sodium hydroxide

INDIRECT TITRATION—

Fig. 2 (*a*) is a typical titration graph when 5 ml of 0·1 M ammonium chloride are titrated with 5 N sodium hydroxide. A similar titration of pyridine hydrochloride is shown in Fig. 2 (*b*), and of an equidecimolar mixture of ammonium chloride and pyridine hydrochloride containing a slight excess of hydrochloric acid in Fig. 2 (*c*).

The titration graphs show that the excess acid is titrated first, with a very sharp temperature rise; the pyridine salt second, with a less sharp temperature rise; and finally, the ammonium salt, with a barely discernible rise.

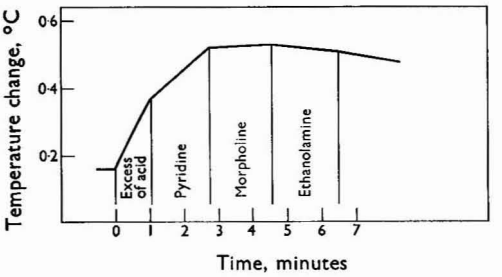


Fig. 3. Graph of enthalpimetric titration of an aqueous solution of pyridine, morpholine and ethanolamine, slightly acidified with sulphuric acid, with 5 N sodium hydroxide

As in the direct method, the bases tested fell into the same two categories, but because there was less restriction caused by water solubility, other base salts behaving similarly to pyridine could be titrated and these included the hydrochloride of *p*-phenylenediamine.

Similar titration curves were obtained with other mineral acid salts, but with the weaker organic bases no distinction could be made between the free and combined acidity of the salts of bases behaving similarly to pyridine. The method can, however, be useful in the determination of the free mineral acidity in organic base salts. For example, the free acidity and total base content can be determined in sulphuric acid extracts of base-containing oils from tar works.

Morpholine was found to be in an intermediate position between the ammonia and pyridine-type bases, and Fig. 3 illustrates the titration of the free acidity and the base salts in a mixture of pyridine, morpholine and ethanolamine, slightly acidified with sulphuric acid.

Results

Direct titration—

The 5 N hydrochloric acid used as titrant was standardised by using 5 ml of 0·1 N sodium hydroxide.

The method has been used to determine the ammonia content of several tar works products, some containing pyridine bases that were also determined. The results in Table I were obtained by using 5 ml for each test.

Table I

Ammonia and pyridine base contents of tar products

Source	Basic nitrogen compounds, per cent. w/v	
	Ammonia as NH_3	Pyridine bases as C_5H_5N
Distillate from phenols plant rectifier (*b*)	2·42, 2·46	0·29, 0·33
Ammonium sulphate liquor from pyridine plant ..	0·78, 0·81	0·11, 0·12
Aqueous condensate from phenols plant	0·077, 0·080	0·25, 0·26
Ammonia liquor from crude tar (*a*)	0·027, 0·027	nil, nil
Ammonia liquor from crude tar (*b*)	0·011, 0·010	nil, nil

Indirect titration—

The 5 N sodium hydroxide used as titrant was standardised by using 5 ml of 0·1 N hydrochloric acid.

The method has been used to determine pyridine bases in a number of tar works products, many of which contained ammonia. The results shown in Table II were obtained by taking 5 ml for each test and acidifying with hydrochloric acid to methyl orange; if interfering acids such as hydrogen sulphide were present, the solutions were boiled and cooled before titration.

TABLE II

PYRIDINE BASE CONTENTS OF TAR PRODUCTS

Source	Basic nitrogen compounds, per cent. w/v. Pyridine bases as C_5H_5N
Distillates from phenol plant rectifiers—	
a	0·60, 0·63 (0·51*)
b	0·33, 0·35 (†)
c	0·15, 0·18
d	0·11, 0·11 (0·14*)
e	0·09, 0·09
Condensates from sulphate plants—	
f	0·31, 0·31
g	0·71, 0·73
Ammonium sulphate liquors from pyridine plants—	
h	5·6, 5·6 (5·1‡)
i	9·2, 9·4
Liquor from tar distillation plant—	
j	0·10, 0·11

* Hydrochloric acid titration after removal of ammonia with formalin.
† Contains 2·5 per cent. of ammonia.
‡ Perchloric acid titration of toluene extract of alkaline distillate.

The indirect method has also been used to determine the free acidity and total pyridine base content of sulphuric acid extracts of base-containing tar oils, with 1 ml of sample diluted to 5 ml for each test. The results are shown in Table III.

TABLE III

FREE ACIDITY AND PYRIDINE BASE CONTENTS OF SULPHURIC ACID EXTRACTS

Base sulphate	Free sulphuric acid, per cent. w/v	Bases, per cent. w/v, as pyridine
a	nil, nil*	39·7, 39·8
b	1·6, 1·6	31·6, 31·2
c	1·9, 2·1	31·4, 30·7
d	16·9	36·4
e	16·9	16·9

* Slight acidification of the sample and re-titration showed the absence of free pyridine bases.

TITRATION IN NON-AQUEOUS SOLUTION

In 1964, Kelly and Hume titrated organic bases enthalpimetrically in anhydrous acetic acid solution. They used perchloric acid in anhydrous acetic acid as titrant and, to eliminate the heats of dilution and mixing, devised a dual titration system with a differential thermistor bridge circuit.[4] We thought that if the heats of dilution and mixing could be made large enough by choosing a suitable titrant - solvent combination, this might give an indicator effect in non-aqueous enthalpimetry, and thus enable simple apparatus to be used. The following describes how this principle has been applied to the non-aqueous titration of organic bases.

EXPERIMENTAL

The solubility and molar heats of solution of hydrogen chloride in alcohols are both high; the figures quoted by Mellor[5] are 40 to 50 g in 100 g and 1·1 to 1·8 cal., respectively. The corresponding figures for other organic solvents are very much lower. The dilution of an alcoholic solution of hydrogen chloride by other solvents should therefore give an appreciable endothermic heat change. Table IV shows the initial temperature change when 5 N hydrogen chloride in isopropyl alcohol is added at the rate of 0·066 ml per minute to 5 ml of a selection of organic solvents, including water.

The results in Table IV were obtained under the conditions described in "Method" and are therefore comparative. They show that the greatest temperature drop is obtained with those organic solvents in which hydrogen chloride is least soluble but, apart from the alcohols that give a slight rise in temperature, all of them give an appreciable temperature drop. The table also shows that water must be absent from those solvents with which it is miscible.

TABLE IV

INITIAL TEMPERATURE CHANGE IN °C PER MINUTE ON ADDITION OF ACID

Solvent	Initial temperature change
Carbon tetrachloride	−2·30
Benzene	−1·56
Nitrobenzene	−1·56
Acetone	−0·90
Dioxan	−0·82
Isobutyl methyl ketone	−0·72
Diethyl ether	−0·72
Acetic acid	−0·70
Petroleum spirit (boiling-range 100° to 120° C)	−0·60
Methanol	+0·12
Isopropyl alcohol	+0·16
Water	+0·65

Fig. 4 (*a*) is a typical graph for the enthalpimetric titration of 5 ml of acetone with 5 N hydrogen chloride in isopropyl alcohol. Figs. 4 (*b*), (*c*) and (*d*) show similar titrations of benzene, of acetone containing 30 mg of pyridine and of benzene containing 30 mg of pyridine, respectively. The graphs show that the organic base is titrated first with a temperature rise caused by the neutralisation of the base. This is followed by a rapid temperature fall at the end-point, the result of the endothermic heat of dilution of the titrant. As would be expected from the results in Table I, benzene gives a greater temperature fall at the end-point than acetone but, even with the latter, the end-point of the titration is extremely sharp. It should be noted that the temperature rise on neutralisation of the pyridine is about five times greater than in the aqueous titration. A wide range of solvents is thus made available for a precise non-aqueous enthalpimetric titration of basic nitrogen compounds.

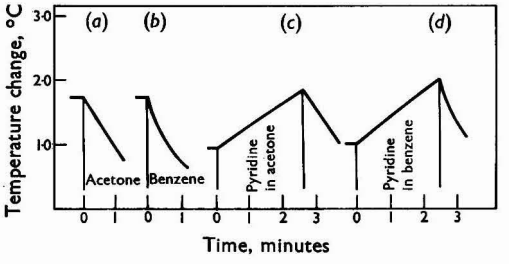


Fig. 4. Graphs of enthalpimetric titrations in non-aqueous solvents with 5 N hydrogen chloride in isopropyl alcohol

MATERIALS TITRATABLE BY THIS METHOD—

The following substances are among those that have been satisfactorily titrated.

Aliphatic amines—

Ethylamine; n-octylamine; monoethanolamine; brucine; diethylamine; piperidine; and trimethylamine.

Aromatic amines—

Primary: aniline; *o*-toluidine; 1-naphthylamine; 2-aminoanthracene; *o*-phenetidine; *o*-aminophenol; *p*-aminobenzoic acid; *m*-nitroaniline (*a*), *p*-nitroaniline (*a*); *p*-phenylenediamine; and benzidine.

Secondary: *N*-ethylaniline; diphenylamine (*a*); diphenylbenzidine (*a*); and benzotriazole (*a*).

Tertiary: *NN'*-diethylaniline; pyridine; 2-picoline; quinoline; acridine; 2,2'-dipyridyl; 2-hydroxypyridine; 8-hydroxyquinoline; quinaldinic acid; 2,2'-pyridylamine; and 2-(2-aminoethyl)pyridine.

Amides—

Urea; thiourea (*a*).

It was found that very weak bases, *e.g.*, diphenylamine could only be titrated in acetic acid solution. These bases are marked (*a*) in the above list. Amides, other than those listed, indole, carbazole, anilides and *o*-nitroaniline could not be titrated. All of the compounds that titrated gave the expected equivalence, except benzotriazole and 2,2′-pyridylamine, both of which behaved as mono-acidic bases.

DIFFERENTIATION OF BASES BY THIS METHOD—

Figs. 5 (*a*) and (*c*) illustrate the titration of a mixture of piperidine and pyridine in acetone and acetic acid solution, respectively. The graphs are typical and show that aliphatic amines have a greater heat of neutralisation, and are titrated before other types of bases in solvents other than acetic acid. The absence of a marked temperature rise during the neutralisation of the bases in acetic acid solution indicates that base acetates are formed and are being titrated. This is a displacement reaction involving little heat change and the bases cannot be distinguished.

Figs. 5 (*b*) and (*d*) illustrate the titration of a mixture of aniline and the weak base *p*-nitroaniline in acetone and acetic acid solution, respectively. The graphs are typical and show that weak bases cannot be titrated in solvents other than acetic acid, in which they are differentiated from the stronger bases. The differentiation can be explained if it is assumed that the weak bases are not ionised in acetic acid solution. The stronger bases will then be titrated with little heat change, as described above, and the neutralisation of the weak base, which follows, is a reaction involving a greater heat change.

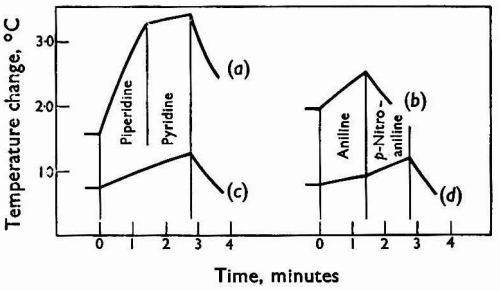


Fig. 5. Graphs of enthalpimetric titrations of mixtures of bases in different solvents, with 5 N hydrogen chloride in isopropyl alcohol: graphs (*a*) and (*b*), in acetone; graphs (*c*) and (*d*), in acetic acid

PREPARATION OF THE TITRANT—

The titrant was prepared by absorbing hydrogen chloride in dry isopropyl alcohol until a 5 N solution was formed. The hydrogen chloride was generated from 35 per cent. w/w hydrochloric acid by a slow stream of concentrated sulphuric acid.

RESULTS

Several titrations of between 64 and 100 mg of 8-hydroxyquinoline in 5 ml of acetone as solvent were made with about 5 N hydrogen chloride in isopropyl alcohol to standardise the titrant. The titrant was added at 0·066 ml per minute, and, with a recorder chart speed of 60 mm per minute, the following results were obtained, expressed as millilitres of N solution per cm: 0·0568; 0·0567; 0·0566; 0·0567; 0·0568; and 0·0566.

Duplicate determinations of the purity of several substances gave the following results—

Substance	Solvent	Purity, per cent. w/w
n-Octylamine	Acetone	100·1, 101·1
2,2′-Dipyridyl	Acetone	102·1, 100·2
1-Naphthylamine	Acetone	99·9, 99·8
Diphenylamine	Acetic acid	99·8, 99·9

The basic nitrogen contents of a variety of tar products have been determined and compared with a potentiometric titration in acetic acid solution, with perchloric acid as titrant. About 0·5 g of sample was dissolved in 5 ml of the solvent for each enthalpimetric test and titrated with the 5 N acid. The results are shown in Table V.

TABLE V

BASIC NITROGEN CONTENTS OF TAR PRODUCTS IN SEVERAL SOLVENTS

Product	Solvent	Basic nitrogen, per cent. w/v	
		Enthalpimetric	Potentiometric
Creosote (*i*)	Acetone	0·31, 0·35	0·35
Creosote (*ii*)	Acetone	0·55, 0·56	0·56
Creosote (*iii*)	Acetone	0·79, 0·79	0·79
Anthracene oil	Acetone	0·39, 0·41	0·40
Road tar (*i*)	Nitrobenzene	0·57, 0·58	0·61
Road tar (*ii*)	Nitrobenzene	0·59, 0·62	0·62
Road tar (*iii*)	Nitrobenzene	0·38, 0·42	0·42
Xylenol distillation residue	Acetic acid	0·44, 0·44	0·47
Gas main condensate	Acetone	6·71, 6·75	6·62
High boiling tar acids A	Acetic acid	0·190, 0·186	0·180
High boiling tar acids A (purified)	Acetic acid	0·019, 0·019	0·019
Cresylic acid	Acetone	0·014, 0·015	0·013
Crude carbolic oil	Acetone	0·63, 0·64	0·63

Because fluid tar products could act as their own "indicator" in the titration it is possible to titrate their basic nitrogen content directly. The results shown in Table VI were obtained by taking 5 ml of the sample for the enthalpimetric titration and are compared with the potentiometric titration, as above.

TABLE VI

BASIC NITROGEN CONTENTS OF TAR PRODUCTS WITH NO SOLVENT

Product	Basic nitrogen, per cent. w/v	
	Enthalpimetric	Potentiometric
Benzene extract of cresylate	1·25, 1·29	1·22
Carbolic oil	0·60, 0·60	0·60
Crude naphtha	0·21, 0·23	0·23
Refined naphtha	0·071, 0·072	0·068
Xylenols*	0·019, 0·020	0·019

* One millilitre of acetone added to reduce the viscosity.

In an investigation into the proportion of different types of basic nitrogen compounds present in a crude light-tar oil, use was made of the differential effect of different solvents. The solvents and the results obtained are given below.

Solvent	Basic nitrogen, per cent. w/v	
Acetone—		
Strong bases	0·14	0·15
Weak bases	0·34	0·34
Total strong and weak bases	0·48	0·49
Acetic acid—		
Total bases	0·51	0·52

The organic base distribution calculated as nitrogen in the oil is, therefore, ammonia and aliphatic bases, 0·145 per cent. w/v; pyridine and aniline bases, 0·485 per cent. w/v; and very weak bases, 0·025 per cent. w/v.

REFERENCES

1. Dutoit, P., and Grobet, E., *J. Chem. Phys.*, 1922, **19**, 324.
2. Parsons, J. S., Abstract of Papers, 132nd Meeting, American Chemical Society, 1957, 12B.
3. Vaughan, G. A., and Swithenbank, J. J., *Analyst*, 1965, **90**, 594.
4. Keilly, H. J., and Hume, D. N., *Analyt. Chem.*, 1964, **36**, 543.
5. Mellor, J. W., "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green and Co., London, 1947, Volume II, p. 197.

Received *December 19th*, 1966

Analyst, June, 1967, Vol. 92, *pp.* 371–374

A Gas-chromatographic Determination of Residues of Picloram

By J. S. LEAHY and T. TAYLOR
(*Huntingdon Research Centre, Huntingdon*)

A method is described for the gas-chromatographic determination of Picloram herbicide in soils and plant material by using an electron-capture detector. The method described for soil has a sensitivity of 0·02 p.p.m. By the use of more rigorous clean-up procedures Picloram can be detected in other plant material at a level of 0·005 p.p.m., and recoveries of added Picloram range from 60 to 110 per cent. over the concentration range of 0·02 to 0·5 p.p.m.

Picloram (Tordon,* 4-amino-3,5,6-trichloropicolinic acid) is a systemic herbicide controlling a wide range of woody plants and perennial herbaceous broad-leaf weeds at dosage rates ranging from ½ to 4 lb of active ingredient per acre. It also controls many seedlings of established annual broad-leaf weeds, although most grass species are not susceptible. Picloram, in combination with other phenoxyacetic or phenoxypropionic herbicides, can be used to control broad-leaf weed seedlings in cereals at rates as low as ¼ oz of active ingredient per acre.

Because of the biological activity of this broad spectrum herbicide, and in particular the susceptibility of certain crops, especially beans and tomatoes, to low levels of Picloram, it became essential to develop a suitable sensitive method of analysis for determining residues in soil and straw. The high degree of susceptibility of beans has been used as a basis for the development of a sensitive bio-assay for residues of Picloram in soils and certain plant materials.[1] This method is capable of detecting and determining the very low levels of Picloram present in soils or plant tissues resulting from trials carried out at low rates of application. This technique can also be used semi-quantitatively for screening purposes to ascertain whether previously treated land can be used for growing these susceptible crops.

The bio-assay has the disadvantage of being time consuming and cumbersome. To obtain accurate quantitative results it can only be used over a fairly limited range of concentrations.

More recently Merkle, Boevy and Hall[2] have devised a gas-chromatographic method for determining Picloram in soil samples from trials in which the herbicide was applied at rates of 2 and 8 lb per acre to control brush in woodland. The Picloram molecule containing three chlorine atoms might be expected to show a good response with an electron-capture detector, and advantage of this response has been taken by these authors and ourselves.

The method described here was originally devised to determine low levels of Picloram in soil, and was later developed further to determine residues in grains, straw, oil, oil seeds and oilseed cake. The sensitivity of the method of analysis is about the same as that of the bio-assay, namely, 0·005 p.p.m.

Picloram, as the free acid, is a white crystalline solid with a melting-point of 210° C. It is only slightly soluble in water (430 p.p.m.), virtually insoluble in non-polar organic solvents, slightly soluble in acetone and isopropyl alcohol and appreciably soluble in methanol and ethyl acetate. The potassium salt is highly soluble in water and the herbicide is normally applied as the potassium salt.

The basis of the method of analysis is as follows: Picloram is extracted from the soil or crop with dilute potassium hydroxide solution. After acidification of the extract the free acid is partitioned into ethyl acetate. After a further clean-up stage, the residue is esterified with diazomethane by Schenk and Gellerman's method[3] and dissolved in benzene.

With most soil samples, portions of this solution can be applied directly to the gas-chromatographic column. With cereals and straw samples in particular, and soils with high organic content, the benzene solution is washed with alkali and purified by absorption

* Trade mark of The Dow Chemical Company.

chromatography on a column of Florisil. The Picloram ester is eluted with benzene - ether mixtures. Portions of the concentrated eluate are then examined by gas - liquid chromatography by using an electron-capture detector.

Experimental

Reagents—

All reagents are of recognised analytical-reagent grade.

Ethyl acetate, re-distilled.

Potassium hydroxide, 0·05 N, *in* 10 *per cent. potassium chloride.*

Sulphuric acid, N.

Diethyl ether, re-distilled over sodium.

Sodium sulphate, anhydrous granular.

Methanol.

Ethanol, absolute.

N-*Methyl* N-*nitroso* p-*toluene sulphonamide.*

Potassium hydroxide, 60 *per cent. w/v, aqueous solution.*

Benzene—Refluxed over sodium and re-distilled.

Sodium hydroxide, N.

Florisil—Florisil, as bought, is standardised by adding graded amounts of water (usually between 2 and 10 per cent.) so that a standard of 10 nanograms of Picloram methyl ester is eluted in 20 ml of 2 per cent. diethyl ether in benzene.

Diethyl ether in benzene—Prepare freshly, a 2 per cent. v/v solution of re-distilled diethyl ether in re-distilled benzene.

Gas-chromatographic apparatus—

A Perkin-Elmer model 801 gas chromatograph fitted with glass injection ports and an electron-capture detector was used for these analyses. The column was 1 m long, $\frac{1}{8}$ inch o.d., of stainless-steel, and packed with 2·5 per cent. neopentyl glycol adipate on silanised Chromosorb W, 80 to 100 mesh.

The carrier gas was nitrogen at a flow-rate of 55 ml per minute. The diluent gas to the detector was nitrogen at a flow-rate of 40 ml per minute. Other operating parameters were column temperature, 185° C; injector temperature, 260° C; and detector temperature, 200° C.

The electron-capture detector was used in the d.c. mode with an applied potential of 20 volts. Under these conditions the retention time of Picloram methyl ester was about 20 minutes.

Procedure—

For grain, straw, soil and feed cake, shake mechanically 10 g of finely ground and mixed sample with 200 ml of 0·05 N potassium hydroxide in 10 per cent. potassium chloride solution for 30 minutes, and filter the mixture through a sintered-glass Buchner funnel. Wash the residues in the funnel twice with 100-ml portions of water. Combine the extract and washings and transfer them quantitatively to a separating funnel.

For oils, dissolve 10 g of well mixed sample in 50 ml of hexane and extract with 50 ml of 0·05 N potassium hydroxide in 10 per cent. potassium chloride. After the phases have separated, run off the lower aqueous layer and extract the hexane twice more with 25 ml of 0·5 N potassium hydroxide in 10 per cent. potassium chloride. Wash the combined aqueous extracts with a small volume of hexane and discard the hexane.

In either case wash the alkaline extracts by shaking them with 50 ml of ethyl acetate for 30 seconds. After the phases have separated transfer the upper ethyl acetate layer into a centrifuge tube and break up any emulsions by gentle centrifugation. Return any water that separates after centrifugation to the alkaline extracts. Discard the ethyl acetate. Repeat the process with a further 50 ml of ethyl acetate and discard the organic phase as before. Return the aqueous extract to the separating funnel.

Acidify the aqueous extracts to pH 2 with N sulphuric acid (about 12 ml). Extract with one 50-ml and two 25-ml portions of ethyl acetate. Break up any emulsions that are formed by gently spinning them in a centrifuge. Combine the clear ethyl acetate extracts and dry over sodium sulphate. Filter, wash the sodium sulphate with a little ethyl acetate and add the washing to the filtrate. Evaporate the solution to dryness on a rotary film evaporator.

Dissolve the dry residue in 25 ml of 0·05 N potassium hydroxide in 10 per cent. potassium

chloride, and transfer the solution to a separating funnel. Wash the flask with a small amount of the alkaline solution and add the washings to the separating funnel. Wash the alkaline solution with 25 ml of diethyl ether by shaking the solutions for 30 seconds. Discard the ether. Acidify the aqueous layer to pH 2 with about 3 to 5 ml of N sulphuric acid and extract with one 20-ml portion followed by two 10-ml portions of diethyl ether. Combine the ethereal extracts and wash with about 5 ml of water.

Discard the aqueous layer and dry the ethereal extracts by standing them over anhydrous sodium sulphate. Filter, and evaporate the solvent just to dryness on a rotary film evaporator. Dissolve the residue in 0·4 ml of methanol and 4 ml of diethyl ether.

Prepare a solution of 400 mg of *N*-methyl *N*-nitroso *p*-toluene sulphonamide in 4·6 ml of ether and 6 ml of absolute ethanol. Add 1 ml of 60 per cent. potassium hydroxide and bubble the diazomethane generated into the ethereal solution of the residue. Remove the excess of diazomethane and solvent under a stream of nitrogen.

Dissolve the dry residue in 20 ml of benzene. Transfer the benzene quantitatively to a separating funnel and wash the benzene solution three times with 10-ml portions of N sodium hydroxide. Discard the sodium hydroxide layer. Wash the benzene with one 10-ml and one 5-ml portion of water and discard the aqueous washings. Dry the benzene solution with sodium sulphate. Filter, wash the sodium sulphate with a small amount of benzene and evaporate the benzene and washing to dryness under reduced pressure. Dissolve the residue in about 0·5 ml of benzene and transfer the solution quantitatively to a 2-ml calibrated flask. Rinse the evaporating flask with successive portions of benzene and transfer the washings to the calibrated flask. Adjust the volume of the solution to 2 ml with benzene.

Plug a chromatographic tube with a small pledget of cotton-wool and prepare a column, about 1·5-cm high, from 200 mg of Florisil. Apply 1 ml of the benzene solution to the column and allow the benzene to percolate through. Wash the column with 2 ml of benzene and discard the percolate and the washings. Elute the Picloram methyl ester from the column with 20 ml of 2 per cent. diethyl ether in benzene, collecting the eluate in a small flask. Evaporate just to dryness under reduced pressure. Dissolve the residue in benzene and transfer it quantitatively to a 1-ml calibrated flask. Rinse the evaporating flask with several small portions of benzene and add the rinsings to the calibrated flask. Adjust the contents of the flask to the mark with benzene.

One microlitre of this solution is equivalent to 5 mg of the sample, and is suitable for direct injection on to the gas chromatograph; 10 μl of the final extract solution (50-mg sample) were routinely injected on to the column.

While the response of the electron-capture detector was linear up to 20 nanograms of the methyl ester of Picloram injected, small changes in column efficiency occurred with ageing, which precluded the use of calibration curves for accurate quantitative determination. Aliquots of the final extracts were "spiked" with a known amount of the methyl ester of Picloram, and these were chromatographed under the same conditions as the test sample. The concentration of Picloram in the sample was then calculated by reference to this internal standard.

Results

The above method of analysis has been applied to several samples derived from a variety of field trials. In cereals only a small amount of Picloram has been detected, for example, between 0·02 and 0·09 p.p.m. in grain. No peak was observed at the retention time of Picloram methyl ester in the untreated samples of plant material that were analysed, and the limit of detection was defined by the signal-to-noise ratio of amplifier. With an amplifier sensitivity of 5×10^{-10} amp for full scale deflection the noise level was found to be about 0·5 per cent. of full scale deflection. This allowed a limit of detection corresponding to 0·005 p.p.m. on a 50-mg sample equivalent injected with a signal-to-noise ratio of 4 to 1. Seven samples of wheat straw, five samples of barley grain and three samples of oat grain showed a "blank" value below the level of detection (less than 0·005 p.p.m.). Samples of untreated rape seed oil, seed cake and whole seeds also showed the same value of less than 0·005 p.p.m. Blank values of untreated soils of low organic content (8 samples) which were analysed by the "short" procedure showed an average of 0·017 p.p.m. (range 0·010 to 0·026 p.p.m.). The analysis of crops and soil samples from sites treated with a mixture of Picloram and either CMPP [(±)-2-(4-chloro-2-methylphenoxy)propionic acid], MCPA (4-chloro-2-methylphenoxyacetic acid) or 2,4-dichlorophenoxyacetic acid showed no evidence whatsoever of interference

from these herbicides under the gas-chromatographic conditions used. Recovery experiments of Picloram added to soil and plant material have been performed. The results of these experiments are given in Table I.

TABLE I

RECOVERIES OF PICLORAM ADDED TO SOIL AND PLANT MATERIAL

Sample	Added, μg	Level, p.p.m.	Found, μg	Recovery, per cent.
Soil	0·50	0·10	0·45	90
	0·33	0·07	0·36	109
	0·33	0·07	0·34	103
	0·28	0·06	0·25	89
	0·25	0·05	0·22	88
	0·14	0 03	0·10	71
	0·10	0·02	0·07	70
Rape seed oil	4·0	0·40	4·4	110
	4·0	0·40	4·2	105
	4·0	0·40	3·9	98
Rape seed cake ..	5·0	0·50	4·8	96
	2·5	0·25	2·4	96
Whole rape seed ..	1·0	0·10	0·88	88
Straw	2·5	0·25	2·2	88
	1·3	0·13	1·0	75
	1·0	0·10	0·72	72
	0·67	0·07	0·40	60
	0·40	0·04	0·26	65
Barley grain	1·65	0·17	1·25	76
	1·0	0·10	0·78	78
	1·0	0·10	0·76	76
	0·83	0·08	0·65	78
Oat grain	0·50	0·05	0·34	68
	0·17	0·02	0·10	59

DISCUSSION

A method for the detection and determination of low levels of Picloram has been developed and applied to several samples derived from field trials. The method was devised originally for soil samples and very little clean-up is needed for soil samples of low organic content. The ether partition, column chromatography and the alkali wash of the benzene solution after methylation can be omitted for these samples. The time taken for the soil analysis can be reduced further by using a 1-m column packing of 2 per cent. Versamid 900 at a nitrogen flow-rate of 40 ml per minute. Picloram methyl ester is eluted from this column after about 4 minutes with good peak shape. The factor limiting the use of this column packing is the number of interfering peaks in the chromatogram. These interferences are noted mainly in soils of high organic-matter content, and in such cases it is necessary to use the more rigorous clean-up procedure devised for crops before gas chromatography. When this "short" method was applied to crop samples, this column showed poor selectivity as interfering materials present in the final extract caused overlapping or masking of the Picloram methyl ester peak. There was gross contamination of the electron-capture detector with consequent fall of standing current. Some improvement of the resulting chromatograms could be obtained by altering the operating parameters of the gas chromatograph. However, it was decided to investigate other column packings for a more selective column. Picloram methyl ester runs well on polar columns; of those that we have investigated neopentyl glycol adipate has proved to give the most satisfactory resolution with a reasonable retention time. The column that we have used has been quite efficient with 1000 theoretical plates per metre.

We thank Mr. H. N. Lawson of Dow Chemical Company (U.K.) Limited for his interest in this work and for providing the samples of Picloram.

REFERENCES

1. Leisure, J. K., *Weeds*, **1964**, **12**, 232.
2. Merkle, M. G., Boevy, R. W., and Hall, R., *Ibid.*, **1966**, **14**, 161.
3. Schenk, H., and Gellerman, J., *Analyt. Chem.*, 1960, **32**, 1412.

Received *September 9th*, 1966

Analyst, June, 1967, Vol. 92, *pp.* 375–381

Spectrophotometric Determination of Diquat and Paraquat in Aqueous Herbicide Formulations

By S. H. YUEN, J. E. BAGNESS* and D. MYLES†

(*Imperial Chemical Industries Limited, Agricultural Division, Jealott's Hill Research Station, Bracknell, Berkshire*)

Methods are described for determining diquat and paraquat, singly and in admixture, in formulations. For determining diquat, ultraviolet absorptiometry at 310 mμ in a sodium acetate buffer solution at pH 4·05 is adopted. Paraquat is determined in a diluted solution by measuring the optical density, at 600 mμ, of the blue free radical produced by reduction with alkaline sodium dithionite. For analysing mixtures containing both diquat and paraquat, these methods are combined and use is made of a base-line correction procedure to compensate for interference of diquat in the determination of paraquat. The error for these methods is established to be within ±2 per cent.

Diquat and paraquat are the common names for 1,1′-ethylene-2,2′-bipyridylium and 1,1′-dimethyl-4,4′-bipyridylium cations, respectively, which are manufactured in the form of diquat dibromide and paraquat dichloride or di(methyl sulphate), and are the active components of commercial aqueous preparations known as Reglone‡ and Gramoxone.‡ A preparation incorporating both herbicides is known as Preeglone Extra.‡ The unique herbicidal properties of these compounds, their uses and advantages over conventional total herbicides have been described elsewhere.[1]

Methods have been reported for determining diquat and paraquat residues in water by ultraviolet absorptiometry,[2] in food crops by spectrophotometry of the reduced ions[3,4,5] and by polarography.[6] Ultraviolet absorptiometry at 310 mμ has been found to be specific and suitable for determining diquat in aqueous formulations, either alone or in the presence of paraquat. On reduction with alkaline sodium dithionite, both herbicides are converted to coloured free radicals that are relatively stable in an excess of reducing agent.[7,8] Solutions of the radical ions from diquat are green, the light absorption spectrum showing a sharp peak at 378 mμ, tailing off through the visible region with an inflection from 410 to 440 mμ. Reduced paraquat is blue and the light absorption spectrum of the radical ions has a sharp peak at 394 mμ, and a broad one at 600 mμ. The blue colour of reduced paraquat is sufficiently stable in solution to enable paraquat to be determined differentially at 600 mμ. In formulations containing both diquat and paraquat, the latter can be determined if a base-line correction is applied to compensate for the absorption caused by the reduced diquat, which is virtually linear in the region from 520 to 700 mμ.

Experimental

Ultraviolet spectra of diquat and paraquat—

Fig. 1 shows the ultraviolet spectra for diquat and paraquat in the buffer solution at pH 4·05, which contains 5·44 g of sodium acetate trihydrate and 9·5 ml of glacial acetic acid per litre, and is preferable to water in ensuring maximum stability and reproducibility of the peaks.

At 310 mμ, the maximum for diquat, the $E^{1\%}_{1cm}$ values for diquat and paraquat were found to be 1045·0 and 7·0, respectively, indicating that for a mixture containing equal cation weights of these herbicides, diquat should be determinable with as little as +0·7 per cent. error, caused by paraquat. Interference from a range of additives used in aqueous formulations, such as "wetters," anti-foaming agents and corrosion inhibitors, has been found to be negligible at this wavelength. Consequently, ultraviolet absorptiometry at 310 mμ has

* Present address: Plant Protection Limited, Yalding, Kent.
† Present address: Imperial Chemical Industries Limited, Dyestuffs Division, Blackley, Manchester.
‡ Registered trade marks of Plant Protection Limited.

been used for determining diquat alone and in the presence of paraquat. The calibration graph over the range 0 to 0·6 mg was consistent and rectilinear, 0·6 mg of diquat in 100 ml of solution giving an optical density of 0·63 in a 1-cm optical cell.

The absorption maximum of paraquat occurs at 257 mμ, but this wavelength is unsuitable for determining paraquat, as in mixed formulations there is considerable overlap in this region from the absorption of diquat, and interference from certain additives, such as "wetters."

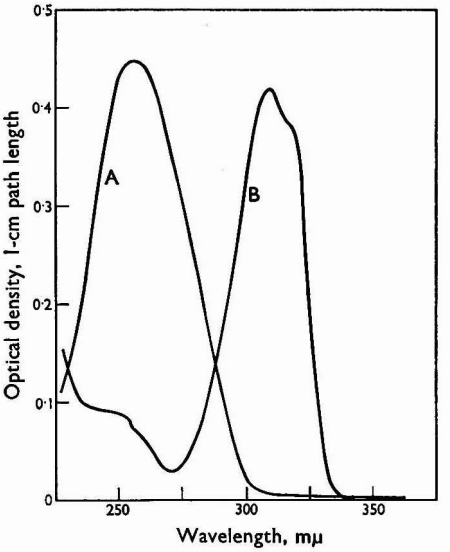


Fig. 1. Ultraviolet spectra of diquat and paraquat in sodium acetate buffer solution: curve A, 0·4 mg of paraquat in 100 ml; curve B, 0·4 mg of diquat in 100 ml

VISIBLE SPECTRA OF SOLUTIONS CONTAINING REDUCED DIQUAT AND PARAQUAT—

The free radicals from diquat and paraquat, formed on reduction with alkaline sodium dithionite, are stable only in an excess of the reducing reagent. The colour may fade on standing owing to depletion of the dithionite in the immediate vicinity of the radical ions, which are then oxidised back to the parent cations. The maximum colour intensity for reduced paraquat can be restored immediately by gently swirling the solution. Vigorous shaking of the reduced solution causes rapid discharge of colour, owing to oxidation of the radical by atmospheric oxygen. On the other hand, if the sodium dithionite concentration is increased to above 2 per cent., over-reduction can occur with paraquat, leading to the formation of a less highly coloured dihydrobipyridyl derivative by uptake of 2 electrons. The stability of the colour produced on reduction is enhanced by decreasing the strength of the sodium hydroxide in the final solution.

With solutions containing paraquat only, the optimum conditions for reduction have been found to require 1 per cent. sodium dithionite in 0·1 N sodium hydroxide (reagent I). However, work with formulations containing both diquat and paraquat has shown that rapid fading may occur unless a reagent consisting of 1 per cent. sodium dithionite in N sodium hydroxide (reagent II) is used. Reagent I or II for appropriate analyses gives rise to paraquat radical colours that are stable for up to 3 hours from the time of mixing. The spectra produced by diquat and paraquat from 400 to 700 mμ are shown in Fig. 2.

In analysing solutions containing only paraquat, a calibration graph relating optical densities at 600 mμ to concentrations has been found to be linear over a wide range. The colour is sufficiently stable to permit the use of differential absorptiometry. An optical density of 0·43 was obtained in a 1-cm optical cell with 1 mg of paraquat in 100 ml of reduced solution, measured against the 0·4-mg standard.

Instead of ultraviolet absorptiometry, diquat, in the absence of paraquat, may be determined absorptiometrically by reduction with 1 per cent. sodium dithionite in 2 N sodium hydroxide and measurement of the optical density at 430 mμ.

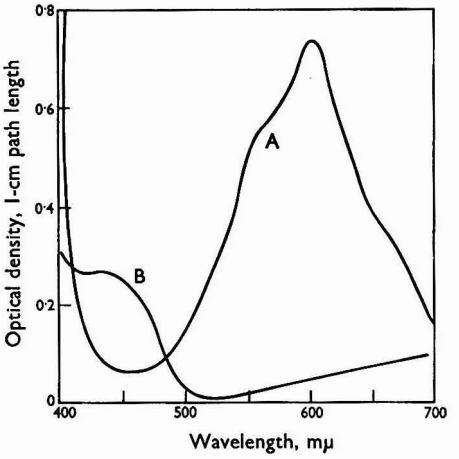


Fig. 2. Visible spectra of diquat and paraquat in reduced solution: curve A, 1 mg of paraquat in 100 ml; and curve B, 1 mg of diquat in 100 ml

DETERMINATION OF PARAQUAT IN THE PRESENCE OF DIQUAT—

As the spectrum of a reduced diquat solution is virtually linear from 520 to 700 mμ, a base-line correction may be applied when paraquat is determined absorptiometrically at 600 mμ, which involves the measurement of optical densities at three wavelengths.[9] Wavelengths at 550, 600 and 650 mμ were selected, the shortest and longest lying at equal distances from the paraquat peak, 600 mμ, and chosen so as to be as far apart as possible on either side of the maximum, consistent with the linear portion of the diquat spectrum. The corrected value of the observed optical density at 600 mμ, designated E_{600M}, is then calculated from the equation—

$$E_{600M} = E_{600} - \tfrac{1}{2}(E_{550} + E_{650})$$

It should be noted that E_{600M} is not the true, corrected optical density for paraquat, but a smaller value; this is immaterial as the calibration graph constructed from E_{600M} is rectilinear and reproducible. A corrected optical density of 0·35, as compared with 0·88 when uncorrected, was given by a solution containing 1·2 mg of paraquat in 100 ml of reduced solution, measured in a 1-cm optical cell against the reagent blank.

To assess the specificity of the base-line correction procedure for determining paraquat, mixtures containing 0·5 mg of paraquat and increasing amounts of diquat were reduced and measured at 550, 600 and 650 mμ in a 1-cm optical cell. Results found for E_{600M} are shown in Table I, which indicates that for a mixture containing equal amounts of diquat and paraquat the error in the paraquat determination was insignificant, but a negative error would be expected when the diquat content greatly exceeded that of paraquat.

TABLE I

DETERMINATION OF E_{600M} VALUES FOR MIXTURES CONTAINING DIQUAT AND PARAQUAT

Paraquat added, mg per 100 ml	Diquat added, mg per 100 ml	E_{600M} found	Error, per cent.
0·5	0	0·154	–
0·5	0·05	0·154	0
0·5	0·1	0·154	0
0·5	0·5	0·155	0
0·5	2·0	0·148	−3·9
0·5	5·0	0·141	−8·4

METHODS

APPARATUS—

Spectrophotometer—A Unicam SP500 is used.

REAGENTS—

Buffer solution, pH 4·05—Dissolve 5·44 g of sodium acetate trihydrate in water, add 9·5 ml of glacial acetic acid and dilute to 1 litre with water.

Sodium dithionite, reagent I—Prepare a 1 per cent. w/v solution of sodium dithionite in 0·1 N sodium hydroxide.

Sodium dithionite, reagent II—Prepare a 1 per cent. w/v solution of sodium dithionite in N sodium hydroxide.

These reagents should not be kept for more than 3 hours. Sodium dithionite is unstable in the presence of moisture and should be stored in small bottles with tightly screwed lids in a desiccator. Take one bottle at a time for current use.

Standard diquat solution—Prepare a stock solution by dissolving 0·1968 g of pure diquat dibromide monohydrate ($C_{12}H_{12}N_2Br_2.H_2O$, molecular weight 362·1; 50·87 per cent. cation) in the buffer solution, diluting to 500 ml with buffer solution and mixing. Dilute 10·0 ml of the stock solution to 100 ml with buffer solution.

1 ml of solution ≡ 0·02 mg of diquat.

Standard paraquat solution—Dissolve 0·1097 g of pure paraquat di(methyl sulphate) ($C_{14}H_{20}N_2S_2O_8$, molecular weight 408·4; 45·59 per cent. cation) or 0·0691 g of pure paraquat dichloride ($C_{12}H_{14}N_2Cl_2$, molecular weight, 257·2; 72·40 per cent. cation) in water, dilute to 500 ml with water and mix (paraquat salts are hygroscopic and should be dried at 100° C for 5 hours, then cooled in a desiccator before use).

1 ml of solution ≡ 0·10 mg of paraquat.

Both standard diquat and paraquat solutions should be prepared as required.

PROCEDURE FOR ANALYSING FORMULATIONS CONTAINING PARAQUAT ONLY—

Weigh accurately a portion of the well mixed sample containing about 1 g of paraquat into a 250-ml calibrated flask, dilute to the mark with water and mix. Call this solution A. Transfer 5·0 ml of this solution to a 200-ml calibrated flask, dilute to the mark with water and mix. Call this solution B.

Transfer 10·0 ml of solution B, and 4·0, 6·0, 8·0 and 10·0 ml of standard paraquat solution, equivalent to 0·4, 0·6, 0·8 and 1·0 mg of paraquat, respectively, to five 100-ml calibrated flasks and dilute the content of each flask to about 80 ml with water. Add to each flask, by a fast-running pipette, 10 ml of sodium dithionite, reagent I, dilute to the mark with water and mix by inverting the flask end-over-end three times. Mix each solution again in a similar way just before transferring it to the optical cell.

Within 15 minutes of adding the reducing reagent, measure the optical densities of the solutions at 600 mμ in a 1-cm optical cell against the 0·4-mg standard as reference. Draw the calibration graph relating optical densities of standards to paraquat contents in milligrams, and read off the paraquat content of solution B; alternatively, compute the paraquat content by interpolation. Call this amount X mg.

$$\text{Paraquat content, per cent. w/v} = \frac{100 \times X \times S}{\text{Weight of sample in grams}}$$

where S is the specific gravity of the sample.

PROCEDURE FOR ANALYSING FORMULATIONS CONTAINING DIQUAT ONLY OR MIXTURES OF DIQUAT AND PARAQUAT—

Determination of diquat—Transfer 10·0, 20·0 and 30·0 ml of standard diquat solution, equivalent to 0·2, 0·4 and 0·6 mg of diquat, respectively, to three 100-ml calibrated flasks, dilute each to the mark with buffer solution and mix. Measure the optical densities of standards at 310 mμ in a 1-cm silica cell against the buffer solution as reference, and draw the calibration graph relating optical densities to diquat contents in milligrams.

Weigh accurately a portion of the well mixed sample containing about 0·5 g of diquat into a 250-ml calibrated flask, dilute to the mark with buffer solution and mix. Call this

solution C. Transfer 10·0 ml of this solution to a 200-ml calibrated flask, dilute to the mark with buffer solution and mix. Call this solution D (solution D is also required for determining paraquat, if this is present). Transfer 5·0 ml of this solution to a 100-ml calibrated flask, dilute to the mark with buffer solution and mix. Call this solution E.

Measure the optical density of solution E at 310 mμ in a 1-cm silica cell, against the buffer solution as reference, and read off from the prepared calibration graph the diquat content of solution E; alternatively, compute the diquat content by interpolation. Call this amount Y mg.

$$\text{Diquat content, per cent. w/v} = \frac{100 \times Y \times S}{\text{Weight of sample in grams}}$$

where S is the specific gravity of the sample.

Determination of paraquat—Transfer 4·0, 8·0 and 12·0 ml of standard paraquat solution, equivalent to 0·4, 0·8 and 1·2 mg of paraquat, respectively, to three 100-ml calibrated flasks, and add water to each flask, and to a fourth flask, to about 80 ml. Add in turn to each flask 10 ml of sodium dithionite, reagent II, from a fast-running pipette, dilute to the mark with water and mix by inverting the flask end-over-end three times. Transfer the solution to a 1-cm optical cell and measure the optical densities at 550, 600 and 650 mμ against the reagent blank as reference. Call these optical densities E_{550}, E_{600} and E_{650}, respectively.

Calculate the corrected optical densities, E_{600M}, at 600 mμ by the equation—

$$E_{600M} = E_{600} - \tfrac{1}{2}(E_{550} + E_{650})$$

Draw the calibration graph relating E_{600M} to paraquat contents in milligrams.

Transfer 10·0 ml of solution D to a 100-ml calibrated flask and dilute to about 80 ml with water. Add 10 ml of sodium dithionite, reagent II, dilute to the mark with water and measure the optical densities at 550, 600 and 650 mμ, as described above.

Calculate the corrected optical density, E_{600M}, and read off from the prepared calibration graph the paraquat content in 10 ml of solution D; alternatively, compute the paraquat content by interpolation. Call this amount Z mg.

$$\text{Paraquat content, per cent. w/v} = \frac{50 \times Z \times S}{\text{Weight of sample in grams}}$$

where S is the specific gravity of the sample.

Results and discussion

The accuracy of the recommended method was established by carrying out recovery experiments on laboratory made, formulated samples, consisting of appropriate corrosion inhibitors, "wetters" and anti-foaming agents, namely, 8 samples, each containing 20·0 per cent. w/v of diquat; 16 samples, each containing 20·0 per cent. w/v of paraquat; and 6 samples containing varying amounts of diquat and paraquat. These samples were analysed by the recommended methods (except the diquat formulations that were also assayed by differential absorptiometry, involving reduction of the herbicide with 1 per cent. sodium dithionite in 2 N sodium hydroxide and measurements of optical density at 430 mμ in a 2-cm optical cell against the 0·5-mg standard, the calibration graph ranging from 0·5 to 2 mg of diquat in 100 ml of solution). Results obtained are shown in Tables II, III and IV.

Table II

Determination of diquat in laboratory made, formulated samples

Formulation	Diquat added, per cent. w/v	Diquat found, per cent. w/v	
		Ultraviolet method	Dithionite method
Diquat dibromide	20·0	20·1	20·0
	20·0	20·4	20·2
	20·0	19·8	19·6
	20·0	20·2	19·8
Diquat dichloride	20·0	20·2	19·7
	20·0	19·5	19·8
	20·0	20·3	19·9
	20·0	19·8	20·3

TABLE III

DETERMINATION OF PARAQUAT IN LABORATORY MADE, FORMULATED SAMPLES

Formulation	Paraquat added, per cent. w/v	Paraquat found, per cent. w/v
Paraquat di(methyl sulphate)		
Without "wetter" ..	20·0	19·9
		20·0
		19·7
		20·0
With "wetter"	20·0	20·1
		19·1
		19·9
		20·6
Paraquat dichloride		
Without "wetter" ..	20·0	19·4
		19·8
		20·0
		20·4
With "wetter"	20·0	19·5
		19·8
		20·3
		19·6

Table II shows that the percentage recoveries for diquat by ultraviolet absorptiometry ranged from 97·5 to 102·0, with a mean of 100·2 (standard deviation, ±1·5 per cent.). The dithionite method gave 98·5 to 101·5 per cent. recoveries, with a mean of 99·6 per cent. (standard deviation, ±1·2 per cent.). The results obtained by these two methods are practically identical. Table III shows that the percentage recoveries for paraquat ranged from 95·5 to 103·0, with a mean of 99·4 (standard deviation, ±1·9 per cent.). With suitable adjustment of the dilution factors, these methods have been satisfactorily applied to a range of formulations, including water-soluble granules, and to technical liquors, and the precision has been found to be adequate for this type of analysis.

TABLE IV

DETERMINATION OF DIQUAT AND PARAQUAT IN LABORATORY MADE, FORMULATED SAMPLES

Sample No.	Diquat added, per cent. w/v	Diquat found, per cent. w/v	Paraquat added, per cent. w/v	Paraquat found, per cent. w/v
1	9·00	9·00, 8·96, 9·00, 9·00	9·00	9·20, 8·80, 8·90, 9·00
2	9·00	8·96, 9·00, 8·96, 9·05	8·99	9·05, 8·85, 8·90, 8·90
3	4·50	4·80, 4·80, 4·84, 4·77	8·99	9·25, 9·05, 9·03, 9·23
4	12·0	11·9, 11·8, 11·8, 11·9	2·95	2·80, 2·85, 2·88, 2·75
5	7·98	8·13, 8·08, 8·08, 8·10	9·99	9·40, 9·45, 9·45, 9·30
6	10·0	10·1, 10·1, 10·1, 10·2	7·97	8·01, 8·01, 8·05, 7·90

Table IV shows that for formulations containing equal amounts of diquat and paraquat recoveries were better than 98 per cent. for both herbicides. When the paraquat contents greatly exceeded those of diquat, or *vice versa*, the results were less satisfactory. The recommended methods have been routinely applied to the commercial preparation, Preeglone Extra, which incorporates these herbicides at a 1 : 1 ratio, and agreement amongst replicate results usually lies within 0·3 per cent. of the mean.

While the absorption band at 310 mμ in sodium acetate buffer solution may be taken as specific for diquat, the possibility of interference by substances other than paraquat must not be disregarded, particularly in technical liquors containing coloured impurities. Experience has, so far, shown such interference to be negligible. Likewise, no significant background absorption in the region of 310 mμ has been observed in formulations as a result of the presence of a range of additives, including "wetters," anti-foaming agents and corrosion inhibitors.

Similar considerations apply to the procedure involving reduction by alkaline sodium dithionite, which appears to be highly specific for the bipyridylium ions. Any coloured impurities in aqueous formulations derived during manufacture from the technical diquat and paraquat concentrates have invariably been found to be diluted to negligible amounts before the final determination.

We thank Dr. A. Calderbank for helpful criticism of the manuscript.

References

1. Springett, R. H., *Outl. Agric.*, 1965, **4**, 226.
2. Faust, S. D., and Hunter, N. E., *J. Amer. Wat. Wks Ass.*, 1965, **57**, 1028.
3. Calderbank, A., Morgan, C. B., and Yuen, S. H., *Analyst*, 1961, **86**, 569.
4. Calderbank, A., and Yuen, S. H., *Ibid.*, 1966, **91**, 625.
5. ——, ——, *Ibid.*, 1965, **90**, 99.
6. Engelhardt, J., and McKinley, W. P., *J. Agric. Fd Chem.*, 1966, **14**, 377.
7. Michaelis, L., and Hill, E. S., *J. Gen. Physiol.*, 1931, **16**, 859.
8. Homer, R. F., and Tomlinson, T. E., *Nature*, 1959, **184**, 2012.
9. Allen, W. M., *J. Clin. Endocr. Metab.*, 1950, **10**, 71.

Received *January 4th*, 1967

Analyst, June, 1967, Vol. 92, *pp.* 382–386

Detection and Determination of Hexoestrol in Meat

By P. J. COOPER, M. J. de FAUBERT MAUNDER and G. J. McCUTCHEON

(*Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.*1)

A gas-chromatographic procedure for the detection of hexoestrol at the $0{\cdot}4 \times 10^{-9}$ g level is described. Other oestrogenic substances can be detected at similar levels by the same method. A method based on this procedure has been devised, which enables hexoestrol to be determined in meat samples at levels down to 0·1 p.p.m.

Hexoestrol is a hormonal substance with an oestrogenic activity similar to, although slightly less marked than, that of stilboestrol. It is used as an aid to fattening in intensive beef production, and in other forms of intensive animal management aimed at producing meat for human consumption. The manner of its introduction into the animal may be by way of a pellet implanted subcutaneously at a suitable site on the body, or in the animal feeding stuff. Methods for the determination of hexoestrol exist, but these are mostly either concerned with relatively large amounts, such as may be met in animal feeding stuffs, or are biological and involve the use of laboratory animals. The level at which hexoestrol residues may be present in meat is in the region of 0·001 to 0·01 p.p.m., *i.e.*, 1×10^{-9} to 1×10^{-8} g per g,[1] and at this level suitable chemical techniques are limited. Unlike stilboestrol, hexoestrol does not fluoresce in ultraviolet light and it cannot therefore be detected by this technique.[2] Colorimetric methods[3] are of limited sensitivity and lack specificity. The biological assay of stilboestrol is well described[4] and is sufficiently sensitive in the required range, but in common with other biological assays of this type, it suffers from the disadvantages of requiring a plentiful supply of laboratory animals, and having a lack of specificity and a lengthy procedure.

Gas chromatography was thought likely to be the most fruitful approach in that it combines specificity with adequate sensitivity, provided suitable halogenated derivatives for electron-capture detector devices could be prepared.

EXPERIMENTAL

Preparation of a suitable halogenated hexoestrol derivative

Bromination—

Direct bromination of hexoestrol with aqueous bromine yielded a fairly well characterised compound having an infrared spectrum corresponding to that of a fully ring-substituted molecule of octabromohexoestrol. The volatility of this derivative was, however, too low to enable it to be conveniently gas chromatographed at moderate temperatures, and therefore its use was not pursued.

Chloracylation—

Lansdowne and Lipsky[5] suggested that the chloracetyl esters were particularly useful derivatives for the gas-chromatographic examination of sterols. The diesters of hexoestrol were readily prepared by refluxing hexoestrol with either the acid chlorides or anhydrides of chloroacetic and trichloroacetic acids in an inert solvent, with pyridine as catalyst. The volatilities of these derivatives were, like that of octabromohexoestrol, too low at normal temperatures for satisfactory gas-chromatographic analysis. At higher temperatures a response could be observed, but the sensitivity of the detector was limited by an excessive loss of stationary phase. The use of different stationary phases, of columns prepared with

low loadings of stationary phase, of ballotini columns, etc., was not investigated, it being thought preferable to obtain a derivative of sufficient volatility at normal temperatures to suit the generality of columns and columnar materials.

HEPTAFLUOROBUTYLATION—

Clark and Wotiz[6] reported good results with heptafluorobutyrate esters of sterols. A satisfactory ester of hexoestrol was prepared, but the high cost of the reagent, together with an unsatisfactory blank on the sample examined, suggested that it was not the most suitable derivative in a method intended for routine use.

TRIFLUOROACYLATION—

Trifluoroacetate esters of a number of sterols were examined by Van den Heuvel, Sjovall and Horning[7] and found to be satisfactory derivatives for gas-chromatographic analysis. The di-trifluoroacetate was found to be the most suitable derivative for gas chromatography in the present work, and was used in preference to the other derivatives described. It was prepared by refluxing hexoestrol with an excess of trifluoroacetic anhydride for 2 hours in the presence of a pyridine catalyst, or by allowing the reactants to stand overnight. The yield of hexoestrol di-trifluoroacetate from 100 mg of hexoestrol, reacted with 10 ml of trifluoroacetic anhydride, was 96 per cent. of the theoretical. Gas-chromatographic examination of the product on an Apiezon column, similar to that described under Method, showed only a single peak, with a retention time of about 7 minutes, in a total running time of 4 hours. Infrared spectra of hexoestrol and its trifluoroacetate were also recorded. The hexoestrol spectrum showed an intense absorption at about 3 μ caused by –OH stretching, and this absorption was completely absent from that of the esterified product. Between 5 and 15 μ the ester spectrum shows a strong carbonyl absorption at about 5·57 μ due to the trifluoroacetate group. In the 8 to 9-μ region of the ester spectrum there were bands at 8·1 and 8·6 μ due to –COOR and $-CF_3$ absorptions, respectively. The C–O and O–H absorptions in these regions, visible in the spectrum of hexoestrol, had disappeared. These spectral changes are all consistent with a fully esterified product and, taken with the gas-chromatographic evidence and the yield (based on the diester), pointed to a single, well defined reaction product.

The effectiveness of the esterification procedure at the microgram level was tested by comparing the gas-chromatographic response of hexoestrol esterified in milligram amounts and subsequently diluted to a concentration of 1 μg per ml, with the response from hexoestrol esterified directly in microgram amounts. The standard solution of 1 μg per ml was derived from a 10-mg sample of hexoestrol dissolved in 5 ml of diethyl ether and refluxed with 2 ml of trifluoroacetic anhydride for 2 hours, with 1 drop of a 1 per cent. solution of pyridine in toluene as catalyst. Ten-microgram samples of hexoestrol were esterified under the same conditions with 0·2 ml of trifluoroacetic anhydride. The results are shown in Table I.

TABLE I

EFFECTIVENESS OF TRIFLUOROACYLATION AT THE MICROGRAM LEVEL

	Sample	Amount injected, μl	Peak height, mm	Esterification, per cent.
	Standard, 1 μg per ml	5	45	—
1.	10 μg per 10 ml	5	43	96
2.	..	..	44	98
3.	..	..	49	109
4.	..	..	42	93
5.	..	..	46	102
		Mean	44·8	99·6

Reproducibility of the 5-μl injection was tested by making repeated injections of sample 1. Peak heights recorded were 44, 43, 44, 42 and 43 mm. Esterification of hexoestrol with trifluoroacetic anhydride is quantitative under the conditions used.

The limit of detection of the method is $0{\cdot}4 \times 10^{-9}$ g in a 5-μl injection. On the basis of a final analytical solution of 10 ml and a meat sample of 50 g, this would correspond to a level of detection of 0·016 p.p.m. in the meat itself, provided that the same sensitivity could be maintained. In fact, in meat extracts the sensitivity of the method was reduced and the lower limit of determination was 0·1 p.p.m. Reduction of the volume of the final

analytical solution leads, of course, to effectively lower limits of detection. Other oestrogenic substances were also examined, and the sensitivities relative to lindane, together with the limits of detection, are shown in Table II.

TABLE II

RELATIVE RETENTION TIMES AND LIMITS OF DETECTION OF SOME SYNTHETIC OESTROGENS

Substance	Relative retention time	Relative sensitivity	Limit of detection for a 5-μl injection, g
Lindane	100	100	$1{\cdot}0 \times 10^{-12}$
Hexoestrol	112	1·35	$0{\cdot}4 \times 10^{-9}$
Stilboestrol (*a*)	65	2·18	$0{\cdot}25 \times 10^{-9}$
(*b*)	98	4·96	$0{\cdot}11 \times 10^{-9}$
Dienoestrol	104	2·04	$0{\cdot}25 \times 10^{-9}$

The gas chromatogram of stilboestrol shows two peaks, probably corresponding to the two geometrical isomers.

EXTRACTION OF HEXOESTROL FROM MEAT

The method involves a preliminary degradation of the meat sample with orthophosphoric acid, followed by a diethyl ether extraction in a liquid - liquid extractor. This extract is washed with water and a weak base to remove acidic substances and is then extracted with aqueous alkaline solution. After acidification of the alkaline solution, the hexoestrol present is re-extracted into ether and is esterified by the method already described after suitable concentration. Recoveries by this method were fairly consistent, but only a little over half of the theoretical. This loss was traced to the liquid - liquid extraction stage, and is a problem that remains to be solved. The recoveries are shown in Table III.

TABLE III

RECOVERY OF HEXOESTROL FROM 50 g OF BEEF

Hexoestrol added, μg	Hexoestrol recovered, μg
100	58, 55, 54, 55, 55, 55
10	6·2, 5·5, 7·4, 5·6, 6·0, 6·0, 8·0, 6·6, 6·4 6·0, 5·5, 5·5
8·7	5·3

A 5-μl injection from 10 ml of toluene solution was used throughout. Provided that the meat extracts are free from background interference, the volume of toluene can be reduced to 5 ml.

METHOD

APPARATUS—

Isothermal gas chromatograph—An all-glass system with a 60-cm column (6·5 mm o.d. × 3 to 4 mm i.d.). The injection must be either into the top of the glass column or directly "on column."

Detector—Electron capture with tritium source.

Injection syringe—Use a 0 to 10-μl Hamilton syringe fitted with guides.

Homogeniser—A high speed food mixer with stainless-steel fittings.

Hirschsohn flasks—Use 50-ml flasks with 10-ml graduations. Flasks for the Institute of Petroleum method IP145/65 (available from Technico Ltd.) are suitable.

Separating funnels—Use 2 × 250-ml and 2 × 100-ml funnels fitted with ground-glass stoppers. The taps must not be greased.

Liquid - liquid extractors—200 ml for liquids less dense than water. 150-ml flasks and electric heating mantles.

Sintered-glass filter funnel—About 5 cm in diameter, and of porosity 0.

REAGENTS—

Analytical-reagent grade materials are used except when stated.

Nitrogen—Should be oxygen free. Pass through untreated molecular sieve No. 5A to remove electron-capturing impurities.

Apiezon grease—Grade "L."
Epikote resin 1001.
Inert support—Chromosorb G, graded 100 to 120 and silanised.
Chloroform—General-purpose reagent.
Orthophosphoric acid, concentrated, sp.gr. 1·75.
Ether—Re-distil until free from gas - liquid chromatographic impurities on trifluoracylation. Store in the dark.
Sodium hydrogen carbonate, 5 per cent. w/v in water.
Alkaline sodium sulphate—Prepare a solution of 1 per cent. sodium hydroxide and 10 per cent. anhydrous sodium sulphate w/v in water.
Hydrochloric acid—Dilute the concentrated acid 1 + 1 v/v with water.
Sodium sulphate, anhydrous, granular.
Pyridine.
Trifluoroacetic anhydride—Pure.
Toluene—Re-distil until free from gas - liquid chromatographic impurities.

PREPARATION OF GAS - LIQUID CHROMATOGRAPHIC COLUMN—

Prepare a solution of 2·5 per cent. w/v Apiezon L and 0·5 per cent. w/v Epikote 1001 in chloroform. Add a sufficient volume of this solution to a suitable weight of Chromosorb G inert support to produce a slurry. Allow to stand for a few minutes and then filter it through the sintered-glass filter funnel. Draw air through the support until it is dry and free running. Heat for half an hour at 110° C, and cool to room temperature.

Pack the glass column as densely as possible with the prepared packing, plugging the ends with glass-wool. Connect it into the gas chromatograph and condition overnight at a temperature between 200° and 225° C, and at a gas flow-rate of 100 ml per minute. Cool to a suitable temperature, maintaining the gas flow. Connect the detector and associated electronics and allow the temperature of the column to equilibrate at about 160° C, with a gas flow-rate of 100 ml per minute.

The response to the hexoestrol derivatives may be poor for some hours after commissioning a new column. It reaches a maximum response after 3 to 4 days and then maintains its sensitivity for a prolonged period, sometimes with a gradual increase over the next 10 to 20 days. The absence of a response to large amounts of derivative on the first day of commissioning does not necessarily mean that a column will be of no subsequent value. If no response is obtained by the second day make a new batch of packing material and pack a fresh column.

PROCEDURE—

Extraction—Weigh, to the nearest 0·5 g, about 50 g of boned meat into the food mixer vessel, add 50 ml of concentrated orthophosphoric acid and allow the sample to thaw if it is frozen. Comminute the sample gently to break down the bulk of the meat fibres; complete breakdown is unnecessary. Heat the vessel in boiling water or in a steam-bath for 20 minutes with regular swirling of the contents. Cool and transfer the contents of the vessel to a 200-ml liquid - liquid extractor, washing it in with about 50 ml of water and 50 ml of diethyl ether. Connect the distillation flask, add a further 100 ml of diethyl ether and extract for 2 hours. Retain the ether layer and discard the aqueous residue.

Purification—Wash the ether extract in a 250-ml separating funnel with three 25-ml portions of water, followed by three 25-ml portions of sodium hydrogen carbonate solution, allowing any emulsions formed to clear. Extract the ether layer with three 25-ml portions of alkaline sodium sulphate solution, shaking for 2 minutes with each extraction. Combine the alkaline extracts (which may be cloudy or hazy) and wash with successive 25-ml portions of 40° to 60° C light petroleum until no further solid matter collects at the liquid interface. Retain the clear aqueous phase. Combine the light petroleum wash liquors together with the separated solids, and wash with 25 ml of alkaline sodium sulphate solution. Discard the light petroleum wash liquors and combine the aqueous phases. Add 15 ml of hydrochloric acid (1 + 1 v/v) and extract with 15 and 10-ml portions of re-distilled diethyl ether. Pass the combined ether extracts through a column of 5 g of granular anhydrous sodium sulphate into the Hirschsohn flask. Wash the separating funnel and the sodium sulphate column

with re-distilled ether, collecting a total volume of not more than 45 ml in the flask. Add a granule of carborundum (30 mesh) or a glass bead, and distil off the ether until a residue of 3 to 5 ml remains.

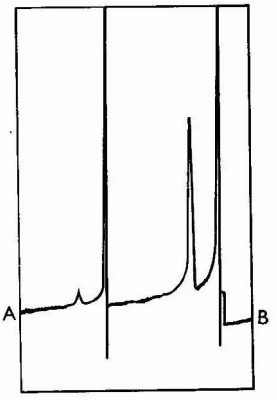


Fig. 1. Chromatographic responses of A, a 5-μl injection of a 0·1 μg per ml solution of hexoestrol di-trifluoroacetate in toluene; and B, a 5-μl injection of a 1 μg per ml solution of hexoestrol di-trifluoracetate in toluene

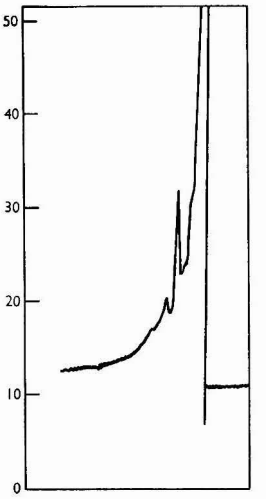


Fig. 2. Chromatographic response of a 5-μl injection of a 1 μg per ml solution of hexoestrol di-trifluoracetate recovered from 50 g of beef
Peak height ≡ 60 per cent. recovery

Esterification and gas chromatography—Add 0·2 ml of trifluoroacetic anhydride and 1 drop of pyridine solution to the ether solution and reflux for 30 minutes. Evaporate to dryness, cool and add 10 ml of sodium hydrogen carbonate solution and a small amount of toluene. Shake the solution for 2 minutes. Allow the phases to separate, and make up to the graduation mark with water. Add a suitable volume of toluene (Note 1). Inject 5 μl of this toluene solution into the gas chromatograph and determine the hexoestrol content from a standard injection. Fig. 1 shows the chromatographic responses obtained from 1 and 10-μg samples of esterified hexoestrol extracted into 10 ml of toluene, and Fig. 2 shows the response obtained from 10 μg of hexoestrol recovered from 50 g of meat by the method described.

Note 1. The actual volume of toluene used depends upon the amount of hexoestrol present in the meat sample, and on the extent of interference from other substances.

The authors thank the Government Chemist for permission to publish this paper.

References

1. Glascock, R. F., and Hoekstra, W. G., "Peaceful Uses of Atomic Energy," United Nations, New York, 1958, Volume 27, p. 104.
2. Analytical Methods Committee, *Analyst*, 1963, **88**, 925.
3. Tompsett, S. L., *J. Pharm. Pharmac.*, 1964, **16**, 207.
4. Umberger, E. J., Gass, G. H., and Surtis, J. M., *Endocrinology*, 1958, **63**, 806.
5. Lansdowne, R. A., and Lipsky, S. R., *Analyt. Chem.*, 1963, **35**, 532.
6. Clark, S. J., and Wotiz, H. H., *Steroids*, 1963, **2** (5), 535.
7. Van den Heuvel, W. J. A., Sjovall, J., and Horning, E. C., *Biochim. Biophys. Acta*, 1961, **48**, 596.

Received *December 9th*, 1966.

Analyst, June, 1967, Vol. 92, *pp.* 387–390

The Colorimetric Determination of Molybdenum in Soils and Sediments by Zinc Dithiol

By R. E. Stanton and Mrs. A. J. Hardwick
(*Department of Geology, Imperial College, London, S.W.7*)

A method is presented for determining molybdenum in soils, sediments and rocks. The sample is decomposed by fusion with potassium hydrogen sulphate and the molybdenum is taken into solution with hydrochloric acid. Interference by iron is prevented by reduction to the iron(II) state and the reaction with copper by adding potassium iodide. Tungsten interference is suppressed by careful control of the time allowed for complex formation.

The molybdenum - dithiol complex is extracted into light petroleum and determined either by visual comparison or spectrophotometry.

North's[1] procedure for the determination of molybdenum has been in use in geochemical exploration and research studies for some years, but it is slow and imprecise. The methods of Marshall[2] and Baker,[3] although more rapid, involve the use of dilute hydrochloric acid digestion for the sample attack and have a poor tolerance towards copper, which is particularly undesirable as molybdenum and copper are often associated in soils and sediments. The procedure described below is a modification of these two methods that overcomes such defects.

Method

Reagents—

Potassium hydrogen sulphate—Fused and powdered.
Hydrochloric acid, sp.gr. 1·18—Analytical-reagent grade.
Hydrochloric acid, 6 M.
Reducing solution—Dissolve 75 g of citric acid and 150 g of ascorbic acid in water and dilute to 1 litre.
Potassium iodide solution, 50 per cent. w/w.
Zinc dithiol.
Thioglycollic acid, sp.gr. 1·33.
Ethanol, absolute.
Sodium hydroxide—Pellets, analytical-reagent grade.
Dithiol solution—Add 2 ml of ethanol to 0·3 g of zinc dithiol, followed by 4 ml of water, 2 g of sodium hydroxide and 1 ml of thioglycollic acid. Mix well, and when clear dilute the solution to 50 ml with water, when it will become cloudy again. Mix with 50 ml of potassium iodide solution (50 per cent. w/w), and store in a refrigerator when not in use.
Petroleum spirit, sp.gr. 0·72—Boiling range 80° to 100° C, analytical-reagent grade.
Iron solution—Dissolve 5 g of ammonium iron(III) sulphate crystals in 500 ml of 6 M hydrochloric acid.
Sodium molybdate—$Na_2MoO_4.2H_2O$, analytical-reagent grade.
Standard molybdenum solutions—Dissolve 0·1261 g of sodium molybdate in 6 M hydrochloric acid and dilute to 500 ml with this acid in a calibrated flask to give a solution containing 100 μg of molybdenum per ml. From this solution prepare dilute solutions containing 1 and 10 μg of molybdenum per ml in 6 M hydrochloric acid.

Procedure—

Weigh 0·25 g of sample into a borosilicate test-tube and fuse it with 1 g of potassium hydrogen sulphate until a quiescent melt is obtained; continue heating for a further 2 minutes. Leach on a sand-tray with 5 ml of 6 M hydrochloric acid, then add another 5 ml of 6 M hydrochloric acid, mix and leave to settle. Transfer by pipette 5 ml of the clear sample solution into a test-tube (16 × 150 mm), add 2 ml of reducing solution, mix and leave to stand for

2 minutes. Add 2 ml of potassium iodide solution and, mixing after each addition, 1 ml of dithiol solution and leave to stand for 2 minutes. Add 0·5 ml of petroleum spirit, stopper the tube with a silicone rubber bung, shake it vigorously for 90 seconds and then compare the intensity of colour in the solvent phase with that of a standard series.

PREPARATION OF THE STANDARD SERIES—

To each of thirteen test-tubes (16 × 150 mm) add 2 ml of iron solution, followed in order by 0, 0·2, 0·4, 0·6, 0·8, 1·0, 1·5, 2·0, 3·0, 4·0, 5·0, 7·0 and 10·0 μg of molybdenum. Dilute to 5 ml with 6 M hydrochloric acid and treat the solutions as described in the "Procedure" for a sample solution.

DISCUSSION OF THE METHOD

The use of a different sample weight was checked over the range 0·05 to 0·50 g, there being no significant difference in results. The only alteration necessary in the procedure is the use of 1·5 g of potassium hydrogen sulphate for a sample weight greater than 0·25 g. If a sample aliquot of less than 5 ml is used, 2 ml of iron solution must be added and the volume made up to 5 ml with 6 M hydrochloric acid.

As alternative solvents, petroleum spirit with a boiling range of 120° to 160° C and white spirit (B.S. 245) were similar to the one recommended, except that there was a tendency to give a slight turbidity in the solvent phase and at the interface.

Variation in acidity of the final aqueous phase over the range 0·5 to 5 N hydrochloric acid had no effect upon the intensity of colour of the molybdenum complex, which was fully developed during the 2 minutes' standing period before solvent extraction, but tolerance towards copper improves with increasing acidity. The standard series shows an increasing intensity of green colour in the solvent phase.

There was complete recovery of molybdenum when standard solutions were incorporated in the sample leach solution.

The inclusion of potassium iodide in the dithiol solution serves to increase the density of this solution, thus promoting rapid mixing when it is added to the sample solution. In its absence, when dithiol solution was added slowly enough to remain at the top of the sample solution, a heavy grey precipitate was formed that inhibited the formation of the molybdenum complex and obscured the colour of the subsequent solvent phase. This precipitate is believed to be a dithiol complex of iron.

A spectrophotometric finish could be adopted, the molybdenum - dithiol complex exhibiting an absorption maximum at 670 mμ.

INTERFERENCE FROM OTHER ELEMENTS—

The elements found to interfere were iron, copper, tungsten, arsenic, antimony and selenium, the effects of elements other than the first named being shown in Table I.

Interference from iron(III) is prevented by reducing it to the iron(II) state, and the procedure is applicable even to the analysis of iron(III) oxide. However, in the presence of iron(II) there is a decrease of about 10 per cent. in the intensity of the molybdenum - dithiol complex, and consequently, when the iron content of the sample aliquot is less than 2 mg, some modification is necessary to ensure that standards and samples are comparable. If all of the samples are known to be virtually iron-free, it is convenient to omit the iron solution when preparing the standard series. Otherwise, a 2-ml addition of iron solution should be made to the sample aliquot. A minimum standing period of 2 minutes is necessary for complete reduction to the iron(II) state, but it is not harmful to exceed this time.

Copper is suppressed by the addition of iodide, the order of addition of reagents being critical. When iodide is added after the iron(III) ions have been reduced, 1000 μg of copper in the sample aliquot can just be tolerated. Above this level, precipitation of the grey copper complex occurs at the solvent interface and formation of the molybdenum complex is suppressed. Tolerance towards copper can be improved further by adding more potassium iodide.

Tungsten is masked by citric acid, and up to 2000 μg in the sample aliquot may be tolerated, but it is important not to exceed the period of 2 minutes allowed for complex formation after the addition of the dithiol solution. After a standing period of 10 minutes, even 50 μg of tungsten showed a trace of its blue complex in the solvent phase.

TABLE I

EFFECTS OF VARIOUS ELEMENTS ON THE DETERMINATION OF MOLYBDENUM

Element	Amount added, mg	Molybdenum, μg Present	Molybdenum, μg Found	Element	Amount added, mg	Molybdenum, μg Present	Molybdenum, μg Found
Aluminium ..	30·0	0	<0·05	Manganese ..	1·25	0	<0·05
	30·0	2·0	2·0		1·25	2·0	2·0
Antimony ..	1·25	0	—*	Mercury ..	1·25	0	<0·05
	1·25	2·0	—*		1·25	2·0	2·0
	0·2	0	<0·05				
	0·2	2·0	2·0	Nickel ..	1·25	0	<0·05
	0·1	0	<0·05		1·25	2·0	2·0
	0·1	2·0	2·0				
				Potassium ..	30·0	0	<0·05
Arsenic ..	1·25	0	—*		30·0	2·0	2·0
	1·25	2·0	—*				
	0·2	0	<0·05	Selenium ..	0·25	0	<0·05
	0·2	2·0	2·0		0·25	2·0	2·0
	0·1	0	<0·05		0·02	0	<0·05
	0·1	2·0	2·0		0·02	2·0	2·0
Calcium ..	30·0	0	<0·05	Sodium ..	30·0	0	<0·05
	30·0	2·0	2·0		30·0	2·0	2·0
Chromium ..	1·25	0	<0·05	Ti anium ..	30·0	0	<0·05
	1·25	2·0	2·0		30·0	2·0	2·0
Cobalt ..	1·25	0	<0·05	Tungsten ..	3·0	0	—†
	1·25	2·0	2·0		3·0	1·0	—†
					2·0	0	<0·05
Copper ..	2·0	0	<0·05		2·0	1·0	1·0
	2·0	4·0	3·0		1·0	1·0	1·0
	1·0	4·0	4·0				
	0·5	4·0	4·0	Vanadium ..	1·25	0	<0·05
					1·25	1·0	1·0
Lead ..	1·25	0	<0·05	Zinc ..	1·25	0	<0·05
	1·25	2·0	2·0		1·25	1·0	1·0
Magnesium ..	30·0	0	<0·05				
	30·0	2·0	2·0				

* Solvent phase coloured yellow.
† Solvent phase coloured blue.

Both in the presence and absence of molybdenum, 1·25 mg of arsenic((III) caused a yellow-coloured solvent phase. In the presence of 0·2 mg of arsenic there was a slight yellow colour, but it was possible to assess correctly the presence of 2 μg of molybdenum. The effect of antimony(III) was similar, except that a turbidity also occurred.

Selenium was troublesome because of its reduction to the red elemental stage, which occurred with amounts greater than 20 μg. However, although difficult, it was possible to determine correctly the amount of molybdenum present.

RESULTS

Many samples have been analysed by both North's method and the proposed procedure, and the comparison of the results obtained is presented in Table II. The two main defects of North's method are tendencies towards the inadequate leaching of the sample fusion, and the incomplete formation and extraction of the molybdenum complex in samples and standards, both of which defects are avoided in the proposed procedure.

Replicate aliquots were taken from each of two sample solutions and by the proposed method gave mean values of 79 and 53 p.p.m., with standard deviations of ±1 and ±5 p.p.m., respectively. Replicate analysis of one sample by both North's and the proposed procedure gave mean values of 106 and 101 p.p.m., respectively, and their standard deviations were ±35 and ±3 p.p.m.

TABLE II

COMPARISON OF RESULTS BY DIFFERENT METHODS

Sample No.	Molybdenum, p.p.m. North's method	Molybdenum, p.p.m. Proposed procedure	Sample No.	Molybdenum, p.p.m. North's method	Molybdenum, p.p.m. Proposed procedure
1	30	50	13	80	74
2	48	58	14	10	12
3	12	26	15	2	<2
4	50	76	16	90	56
5	40	56	17	80	120
6	50	46	18	36	90
7	14	12	19	30	46
8	26	34	20	100	120
9	54	74	21	38	36
10	40	36	22	20	20
11	14	18	23	84	96
12	34	66	24	4	<2

The U.S. Geological Survey standard samples G–1 and W–1 were analysed by the proposed procedure, molybdenum values of 7·6 and 1·0 p.p.m. being obtained, respectively. These values are in satisfactory agreement with those obtained by Hamaguchi, Kuroda, Shimuzu, Tsukahara and Yamamoto,[4] with neutron activation, who reported 7·0 and 1·3 p.p.m., respectively.

This work forms part of the programme of the Applied Geochemistry Research Group under the direction of Professor J. S. Webb.

REFERENCES

1. North, A. A., *Analyst*, 1956, **81**, 660.
2. Marshall, N. J., *Econ. Geol.*, 1964, **59**, 142.
3. Baker, W. E., *Bull. Australas. Inst. Min. Metall.*, 1965, No. 214, 125.
4. Hamaguchi, H., Kuroda, R., Shimuzu, T., Tsukahara, I., and Yamamoto, R., *Geochim. Cosmochim. Acta*, 1962, **26**, 503.

Received *July 27th*, 1966

Analyst, June, 1967, Vol. 92, *pp.* 391–395

Volumetric Determination of Styphnates with Methylene Blue

By E. KURZ and G. KOBER

(Central Laboratory for Explosives and Ammunition, Israeli Military Industries, Tel-Aviv, Israel)

A modification of Bolliger's extraction titration of *o*-polynitrophenols with methylene blue is described. Because of its better solvent power, nitrobenzene has been used as the extracting agent instead of chloroform, which would necessitate repeated renewal during the titration. The method is applied to the determination of lead styphnate and of the intermediates and effluents in its production, as well as to the analysis of priming compositions. Nitrate interference is removed by extracting the styphnic acid with isobutyl methyl ketone before the titration.

The use of time-consuming and inaccurate gravimetric methods, of expensive instruments needed in polarographic and spectrophotometric methods, and the necessity of strict exclusion of air in reduction methods were avoided when Bolliger's volumetric procedure[1,2] for the fast, simple and accurate determination of styphnates was examined.

Experimental

In Bolliger's method *o*-polynitrophenols, mainly picrates and picrolonates, are titrated with methylene blue and the addition compound formed is extracted with frequently renewed portions of chloroform.

Similarly, in the proposed method, styphnates are titrated with a standardised solution of methylene blue, but the dark green 1 : 1 molar addition compound formed is extracted into nitrobenzene instead of chloroform. Because of the better solvent power of nitrobenzene complete extraction of the methylene-blue styphnate from the water phase is possible without renewal of the extracting agent.

The end-point of the titration is indicated by a change of the yellow colour of the water phase to a faint blue, which is compared with a colour standard. After subtraction of a blank value for the small excess of methylene blue required to attain this colour, the amount of titrant used is proportional to the amount of styphnic acid taken.

The optimum pH for the titration was established to be between 2 and 3, which gives a more distinct end-point than lower values, while in alkaline solutions decomposition of the addition compound occurs.

The acidification necessary for the determination of styphnates is effected with sulphuric acid, which gives a sharper end-point in the titration of lead styphnate than hydrochloric acid, probably as a result of the precipitation of lead. Nitric acid interferes with the titration.

For the titration of free acids in a concentration of a few thousandths molar, the addition of sulphuric acid may be omitted, as a distinct end-point that is not changed by the addition of sulphuric acid up to a concentration of about 0·01 N is obtained.

Although the titration can be carried out with a concentration of the titrant as low as 0·0002 M for the determination of a few tenths of a milligram of styphnic acid, the optimal concentration of methylene blue is 0·001 M, high enough to produce a distinct colour change at the end of the titration and sufficiently low to avoid the formation of emulsions with the nitrobenzene.

Styphnic acid, being hygroscopic,[3] is unsuitable for the standardisation of the methylene-blue solution, and therefore non-hygroscopic picric acid[4] is used as a primary standard at a concentration of 0·002 M to avoid undue dilution.

If kept in the dark, the factor of the methylene-blue solution is stable for several weeks. Solutions that have deteriorated on long exposure to light give no distinct end-point, and should be discarded.

Methylene blue tends to form addition compounds with many substances and also reacts easily with various oxidising or reducing agents, nitrates being the most important in this case.

By taking steps to overcome this interference, the range of application of the method is extended from lead styphnate and its intermediates to priming compositions and effluents.

Styphnic acid is separated from the bulk of the nitrates by extracting the acidified solution with isobutyl methyl ketone. The acid is then titrated in the solution that remains after water has been added and the ketone evaporated.

A rather high concentration of sulphuric acid, about 4 N, is necessary for the fast decomposition of lead styphnate, and for the satisfactory extraction and complete separation of the phases.

As gas evolution may start from priming compositions on longer standing in contact with the acid solution, the styphnic acid extract is separated from the water phase as soon as possible.

In the optimal range of concentration of mineral acid used, the partition coefficient of the styphnic acid between the water and the ketone phase is in the range of 1 : 100 to 1 : 200, as established by spectroscopic measurements. By applying a 5-fold volume of ketone, the styphnic acid remaining in the water phase after a single extraction can be reduced to about 0·2 per cent. of the whole amount present.

A larger loss of styphnic acid occurs by volatilisation during the evaporation of the ketone, mainly in the last stage of the procedure; it is therefore necessary to discontinue evaporation immediately after the disappearance of the ketone layer.

An empirical correction for the loss of styphnic acid can be applied to the analysis of priming compositions by determining the relative error of the procedure on a sample of lead styphnate, which is titrated once by the method given under Procedure for purity of lead styphnate, and once by the procedure for priming compositions including the ketone-extraction step.

As a certain amount of nitric acid as well as styphnic acid is extracted by the isobutyl methyl ketone, the end-point of the titration is less distinct for the determination of the styphnate content of effluents, owing to their higher ratio of nitrate to styphnate ions compared with priming compositions.

Method

Reagents—

All reagents used are of analytical grade unless otherwise specified.

Standard solution of picric acid, 0·002 M—Prepare a 0·4582 g per litre solution of C.P.-grade picric acid, re-crystallised from ethanol.

Methylene-blue stock solution—Prepare a solution containing about 1·9 g of methylene blue chloride trihydrate per litre. Store in the dark.

Methylene-blue working solution, about 0·001 M—Dilute the stock solution (1 + 4). Store in the dark.

Nitrobenzene.

Sodium hydroxide solution, N.

Sulphuric acid solutions, 0·1 *and* N.

Sulphuric acid, diluted (1 + 1) *and* (1 + 9).

Isobutyl methyl ketone.

Standardisation of 0·001 M methylene-blue solution—

Transfer 25 ml of nitrobenzene and, by pipette, 5 ml of the 0·002 M picric acid standard solution into a 50-ml graduated cylinder with ground-glass stopper. Titrate with the methylene-blue solution, adding it dropwise or half dropwise towards the end-point, which is indicated by a change of the yellow colour of the water phase to a faint blue.

After adding each increment, mix by tilting the cylinder to and fro several times until the colour of the water phase remains constant. Avoid shaking the cylinder.

Continue adding methylene blue until the colour of the water phase matches that of a colour comparison standard, prepared by adding one drop of methylene blue to 20 ml of water. (Prepare this comparison standard freshly each day.)

Subtract a blank value obtained by titrating 15 to 20 ml of water in the presence of 25 ml of nitrobenzene to the same hue.

PROCEDURE FOR MAGNESIUM OR SODIUM STYPHNATE SOLUTIONS—

Dilute the styphnate solution to about 0·002 M and transfer 5 ml into a 50-ml glass-stoppered cylinder, containing 25 ml of nitrobenzene. Add 1 ml of 0·1 N sulphuric acid. Proceed with the titration as given under Standardisation.

PROCEDURE FOR PURITY OF LEAD STYPHNATE—

Dissolve an accurately known amount (0·24 to 0·28 g) of the dry sample in about 50 ml of warm water and 25 ml of N sodium hydroxide solution in a 250-ml measuring flask. Add, with swirling, 27·5 ml of N sulphuric acid to bring the final acid concentration to 0·01 N and, after cooling, dilute to the mark and mix.

When the bulk of the lead sulphate has settled, take a 5-ml aliquot of the supernatant turbid liquid without filtration and proceed with the titration as given under Standardisation.

PROCEDURE FOR PRIMING COMPOSITIONS—

Transfer an accurately weighed amount of the priming composition containing about 0·1 to 0·15 g of lead styphnate into a 50-ml Erlenmeyer flask with a ground-glass stopper. Add about 5 ml of sulphuric acid (1 + 9) and, by pipette, 25 ml of isobutyl methyl ketone. Shake the flask for about 5 minutes until all of the lead styphnate is decomposed and the styphnic acid completely extracted. Leave the mixture to settle for 1 to 2 minutes, and then cautiously pour off part of the ketone layer into a small stoppered flask to become completely clear.

Transfer, by pipette, an aliquot of 10 ml of the clear extract into a 50-ml measuring flask, add about 30 ml of boiling water and immerse the flask in a glass beaker containing water maintained at a temperature of 70° to 80° C. To hasten evaporation of the ketone, pass a stream of air over the surface of the liquid by inserting through the neck of the flask a small, bent glass tube, ending 1 or 2 cm above the surface of the ketone, and applying suction by a water pump. Discontinue suction immediately after the last drop of ketone disappears; the whole operation requires about 15 to 20 minutes. Rinse the glass tube back into the measuring flask, add sufficient water to dissolve any crusts of styphnic acid formed, and after cooling dilute to the 50-ml mark.

Use a 5-ml aliquot of this solution for the titration and proceed as given under Standardisation.

PROCEDURE FOR SINGLE PRIMER CAPS—

Proceed with the extraction as above, but use a 25-ml Erlenmeyer flask, a sample containing 5 to 10 mg of lead styphnate, 2 ml of sulphuric acid (1 + 9) and 10 ml of isobutyl methyl ketone.

Transfer a 5-ml aliquot of the clear extract into a glass-stoppered test-tube of similar dimensions to the 50-ml cylinder used for titration and add 3 to 4 ml of water. Evaporate the ketone as described above. Add water to bring the volume of the solution to be titrated to about 5 ml, add 25 ml of nitrobenzene and proceed with the titration in the usual way.

PROCEDURE FOR EFFLUENTS CONTAINING NITRATES—

For a high lead styphnate content of 20 to 30 g per litre, add 1 ml of sulphuric acid (1 + 1) to 5 ml of the sample, bringing the sulphuric acid concentration to about 4 N, and 25 ml of isobutyl methyl ketone. Proceed with the extraction and titration as described for priming compositions.

For a lower lead styphnate content, the desirable final concentration of styphnic acid, 0·002 M, can be attained by varying either the volume of ketone for the extraction or the aliquot taken for evaporation, or both.

RESULTS

The accuracy of the procedure for the determination of the purity of lead styphnate was checked as follows.

The titration of a certain amount of styphnic acid, both alone and after the addition of all other ions in the same concentration as present in the titration of the lead styphnate solutions ("synthetic" solution), gave results that agreed to within 0·2 per cent., *i.e.*, the limits of the titration error of half a drop when using about 10 ml of titrant.

The determination of the lead styphnate content of a sample of normal lead styphnate of exactly stoicheiometric composition, prepared by the method of Zingaro,[5] gave 100·1 per cent., which includes the small positive error of 0·05 per cent. caused by neglecting the volume of the precipitated lead sulphate.

The reproducibility of this procedure was checked by parallel determinations of the purity of one production sample of lead styphnate from separate weighings. Each result given below is the mean of two parallel titrations (generally not differing by more than 0·2 per cent.).

Lead styphnate found, % ..	98·95	99·13	99·37	99·26	99·49	99·47	99·44	Mean: 99·30
Deviation from mean, % ..	−0·35	−0·17	+0·07	−0·04	+0·19	+0·17	+0·13	

The accuracy of the procedure for magnesium styphnate solutions, checked by a method similar to that given in the second paragraph of Results above, was the same.

The check of the reproducibility on two production samples gave the following results—

Styphnic acid, g per litre	Sample (*a*)	201·0	201·4			
	Sample (*b*)	221·9	221·9	222·1	221·2	221·2

To check the accuracy of the method, including extraction with isobutyl methyl ketone, synthetic priming compositions were prepared that contained in addition to lead styphnate barium nitrate, lead dioxide, antimony sulphide, calcium silicide and tetracene (Table I).

TABLE I

DETERMINATION OF LEAD STYPHNATE CONTENT IN PRIMING MIXTURES AND LEAD STYPHNATE BY THE EXTRACTION PROCEDURE WITH ISOBUTYL METHYL KETONE

Determination	Total weight of mixture, mg	Lead styphnate		Relative error, per cent.	Percentage of lead styphnate in mixture		
		Calculated,* mg	Found, mg		Calculated*	Found	Difference
1	352·3	116·1	115·1	−0·86	32·95	32·67	−0·28
2	347·0	119·0	117·5	−1·25	34·28	33·86	−0·42
3	331·2	116·0	114·7	−1·11	35·02	34·63	−0·39
4	382·0	119·0	118·4	−0·50	31·15	30·99	−0·16
5	372·6	133·5	132·1	−1·04	35·83	35·45	−0·38
6	309·8	62·4	62·1	−0·48	20·14	20·05	−0·09
7	262·5	90·4	88·8	−1·76	34·44	33·83	−0·61
8†	711·3	263·8	261·4	−0·91	37·09	36·75	−0·34
9†	671·9	241·0	239·4	−0·66	35·87	35·63	−0·24
10	29·48	9·19	9·12	−0·76	31·17	30·93	−0·24
11	17·60	6·00	5·90	−1·66	34·09	33·52	−0·57
12	29·44	8·79	8·70	−1·02	29·86	29·55	−0·31
13	23·82	10·07	10·00	−0·69	42·28	41·98	−0·30
14	—	102·7	101·9	−0·78	—	—	—
15	—	122·4	121·7	−0·57	—	—	—
16	—	113·2	112·1	−0·97	—	—	—

Determinations 1 to 9 relate to priming compositions;
10 to 13 relate to single caps; and
14 to 16 relate to lead styphnate.

* Calculated on the basis of 99·3 per cent. purity of the sample of lead styphnate used.
† Double amounts of reagents were used for the extraction step.

To minimise sampling errors, the lead styphnate and the rest of the mixture were weighed separately for each determination. The weights of the lead styphnate given in Table I are corrected for the lead styphnate content of 99·3 per cent. of the industrial sample used, as determined above.

For determinations 1 to 9, the procedure described for priming compositions was applied, while for 10 to 13, the procedure adapted to the smaller amounts in single caps was used. For determinations 14 to 16, the same procedure as applied to priming compositions, *i.e.*, including the isobutyl methyl ketone extraction step, was used in the determination of known amounts of lead styphnate alone.

In all of the determinations the value of the lead styphnate found was too low, the relative average error being about −1 per cent. (calculated from determinations 1 to 9).

The extraction procedure with isobutyl methyl ketone was applied also to one sample of waste solution, where the lead styphnate content found by two separate extractions was 25·2 and 25·3 g per litre as compared to 25·4 g per litre by a spectroscopic determination.

These values, however, represent for each extraction the mean of three titrations differing by as much as 2 to 3 per cent. Their good agreement with the spectroscopic determination probably results from the compensation of two errors of opposite sign; the negative error caused by volatilisation of styphnic acid, and the positive error by somewhat overstepping the end-point of the titration, which was less distinct because of the high nitrate content of the solution.

The authors thank Mr. Z. Dar, Director General of the Israeli Military Industries, for his permission to publish this paper.

References

1. Bolliger, A., *J. Proc. Aust. Chem. Inst.*, **1935**, **2**, 312.
2. ——, *Analyst*, **1939**, **64**, 416.
3. Auberstein, P., and Gauthier, J., *Mém. Poud.*, **1957**, **39**, 17.
4. Kirk, R. E., and Othmer, D. F., *Editors*, "Encyclopedia of Chemical Technology," Interscience Publishers Inc., New York, **1951**, Volume 6, p. 48.
5. Zingaro, R. A., *J. Amer. Chem. Soc.*, **1954**, **76**, 816.

Received *August 8th*, **1966**

Analyst, June, 1967, Vol. 92, *pp.* 396–397

A Pre-reaction Attachment for the Karl Fischer Cell

By M. D. LACK and B. E. FROST
(*Analytical Laboratory, Novadel Ltd., Gillingham, Kent*)

A pre-reaction attachment to a standard Karl Fischer cell is described for use in determining water in organic peroxide solutions. The attachment can also be used in other determinations in which interferences must be eliminated without the formation of water, or in which liberated water is used as a means of determining other functional groups.

The determination of water in many compounds requires preliminary elimination or masking of interfering groups. Other compounds may be determined by measurement of the water formed when they are treated with suitable reagents.

The attachment to a standard Karl Fischer cell, described below, was devised for use in the determination of water in organic peroxide solutions, which is possible only if interference from the peroxy group can first be eliminated without the formation of water. This can be achieved by reduction with sulphur dioxide in pyridine. The attachment has since found use in other reactions of the type mentioned above.

The pre-reactor allows the Karl Fischer cell to be set up in the usual way and the pre-reaction to be carried out without interfering with the prepared cell. Transfer of the sample solution from the pre-reactor into the cell is effected rapidly without the solution coming into contact with the atmosphere. A potential source of error, especially with hygroscopic materials, is therefore avoided.

Modification of the Karl Fischer cell

The standard Karl Fischer cell, supplied by Messrs. Baird & Tatlock Ltd. for the B.T.L. "Analmatic" instrument, is modified to allow easy attachment of the pre-reactor. The cell may still be used in the normal way if required.

The side tubulure is reduced in diameter, and a C14 socket is fused on as close to the cell body as possible. The original tubulure angle is maintained.

Pre-reaction attachment—

The body of the reactor is a smaller version of the Karl Fischer cell, formed from a B40 socket with the ground area reduced in height to about $\frac{1}{4}$ inch. A drain tube which carries a C14 cone is attached to the tap outlet by a flexible tube. The drain tube reaches about one-third of the way across the Karl Fischer cell. The side tubulure is terminated in a B10 socket.

The reactor lid is formed from a B40 cone, the ground area of which has been reduced in height to about $\frac{1}{4}$ inch. A gas-inlet tube, a 10-ml tap funnel and a glass sleeve, in which a washing tube is free to rotate, are formed on the lid.

The construction of the reactor is shown in Fig. 1.

Use of the pre-reactor—

The apparatus is assembled as shown, the Karl Fischer cell being mounted in its normal position on the titration apparatus and the pre-reactor suitably supported.

The Karl Fischer cell is prepared in the normal way. The pre-reactor is purged with dry nitrogen, which passes in through the gas-inlet tube and out through the washing tube. The sample is introduced into the reactor from a Lunge - Rey pipette (if it is a liquid), which has a B10 cone on its stem. The required reagent is placed in the tap funnel and allowed to run slowly into the reactor where it is mixed with the sample by a magnetic stirrer.

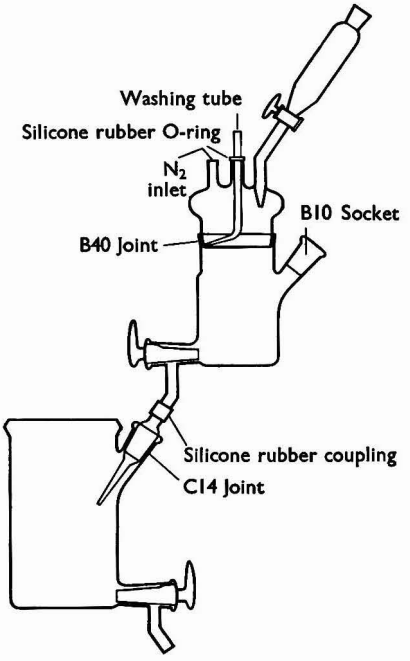


Fig. 1. Pre-reaction attachment and the Karl Fischer cell

When reaction is complete, the sample solution is transferred to the prepared cell. A 10-ml tap funnel is attached to the washing tube and a measured volume of wash solvent placed in it. The solvent is run into the reactor while the washing tube is rotated, so that the reactor walls are washed down. The wash solvent is finally transferred to the Karl Fischer cell where, after mixing, the moisture content is determined in the usual way. A blank determination must be carried out on the reagents and wash solvent.

We thank the Board of Novadel Ltd. for its permission to publish this paper, and Mr. C. L. Bond who did the necessary glass-blowing.

Received *November 28th*, 1966

The Recovery of Trace Elements after the Oxidation of Organic Material with 50 per cent. Hydrogen Peroxide

By J. L. DOWN* and T. T. GORSUCH†

(*United Kingdom Atomic Energy Authority, The Radiochemical Centre, Amersham, Buckinghamshire*)

The recovery of several elements, at the p.p.m. level, from various organic materials after oxidation with sulphuric acid and 50 per cent. hydrogen peroxide has been studied. Most of the elements investigated could be recovered quantitatively, but germanium, arsenic, selenium and ruthenium suffered losses under some or all of the conditions examined. The causes of these losses are discussed.

The last few years have seen the publication of several papers dealing with the oxidation of organic materials with 50 per cent. hydrogen peroxide.[1,2] The advantages claimed for the procedure include speed of oxidation, ability to deal with difficult materials such as plastics, low blank values and the fact that the only decomposition products are water and oxygen. So far, little information has appeared regarding the behaviour of specific trace elements during this oxidation procedure. The work reported here was carried out in an attempt to fill this gap.

Experimental

Apparatus—

The work was carried out with the apparatus shown in Fig. 1, and which has been described elsewhere.[3,4] With the tap in position 1 the solution in the flask can reflux indefinitely. With the tap in position 2 the solution in the flask will distil into the reservoir, and when in position 3, liquid collected in the reservoir can be run out through the side-arm.

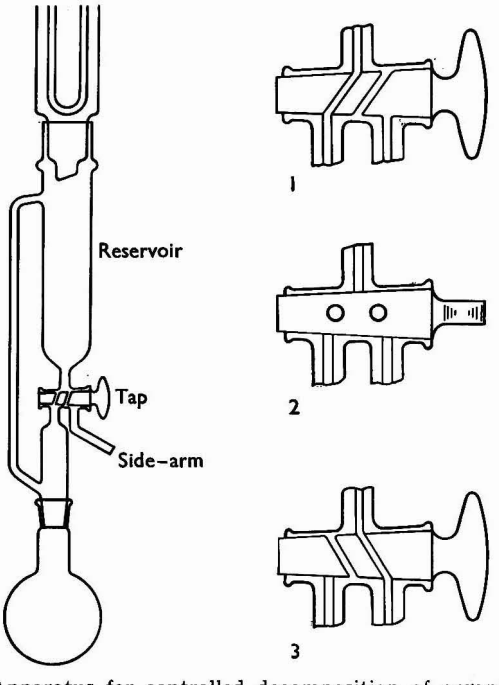


Fig. 1. Apparatus for controlled decomposition of organic material

* Present address: Barking Regional College of Technology, Longbridge Road, Dagenham, Essex.
† Present address: Ranks Hovis McDougall (Research) Limited, Cressex Laboratories, Lincoln Road, High Wycombe, Bucks.

Counting equipment—All the determinations of activity were made by γ-counting with an Ekco scintillation counter, type N664A. This has a thallium-activated sodium iodide crystal, about $\frac{3}{4}$ inch high $\times$ $\frac{1}{2}$ inch diameter, as detector, and the sample, in solution, is contained in an annular polythene cup surrounding the crystal.

REAGENTS—

Sulphuric acid, concentrated, sp.gr. 1·84.

Hydrogen peroxide, 50 per cent.—Supplied by British Drug Houses Limited.

Radioactive tracers—Solutions were prepared of several nuclides, with radioactive concentrations ranging between 1 and 30 μC per ml, and chemical concentrations of about 2 μg per ml. The nuclear and chemical data for the tracers used are given in Table 1.

TABLE I

NUCLEAR AND CHEMICAL DATA FOR TRACERS

Nuclide	Chemical form	Half-life	Principal photon emission			
			MeV	per cent.	MeV	per cent.
Antimony-124 ..	Antimony chlorides	60 days	0·60	98	1·69	48
Arsenic-74	Sodium arsenate	18 days	0·59	60	0·51	59
			0·63	14·5	*plus* X-rays	
Bismuth-207 ..	Bismuth chloride	28 years	0·57	98	1·06	76
					plus X-rays	
Cadmium-109 ..	Cadmium chloride	470 days	0·088	4	0·022	X-rays
Chromium-51 ..	Sodium chromate	27·8 days	0·32	9	0·005	X-rays
Chromium-51 ..	Chromic chloride	27·8 days	0·32	9	0·005	X-rays
Germanium-68 ..	Germanium chloride	280 days	0·51	174	1·08	4
(*plus* gallium-68)					0·009	X-rays
Indium-114*m* ..	Indium chloride	50 days	0·19	19	0·024	X-rays
(*plus* indium-114)						
Manganese-54 ..	Manganese chloride	314 days	0·84	100	0·005	X-rays
Ruthenium-106 ..	Ruthenium chloride	1 year	0·51	21	0·62	11
(*plus* rhodium-106)						
Selenium-75 ..	Sodium selenite	121 days	0·27	56	0·14	54
			0·28	23	0·12	15
			0·40	13	*plus* X-rays	
Tellurium-132 ..	Sodium tellurite	78 hours	0·67	100	0·23	95
(*plus* iodine-132)			0·78	84	0·65	26
			0·52	22	and others	
Tin-113	Tin(II) chloride	118 days	0·39	67	0·024	X-rays
(*plus* indium-113*m*)						
Vanadium-48 ..	Vanadyl chloride	16 days	0·51	112	0·99	100
			1·31	98	*plus* X-rays	
Zinc-65	Zinc chloride	245 days	1·12	49	*plus* X-rays	
Zirconium-89 ..	Zirconyl chloride	78 hours	0·9	100	0·51	50
					plus X-rays	

NOTE—The X-rays produced by elements of atomic number below 30 will be of too low an energy to be recorded by the counting equipment used. For elements of higher atomic number the X-rays will make an increasing contribution to the counts recorded.

PROCEDURE—

Weigh 2 g of organic material into the 250-ml round-bottomed flask shown in Fig. 1, add, by pipette, 1 ml of radioactive tracer solution and assemble the apparatus as shown. Add 20 ml of concentrated sulphuric acid by way of the condenser and reservoir. With the tap in position 1, heat the flask until the organic material chars, and continue heating for between 30 minutes and 1 hour. Add 10 ml of 50 per cent. hydrogen peroxide to the mixture in small amounts through the condenser. When the vigorous reaction has subsided continue refluxing for a few minutes, then turn the tap to position 2. Continue heating for 30 minutes, turn the tap to position 3 and collect the liquid that has distilled into the reservoir. Return the tap to position 1 and add a further 10 ml of 50 per cent. hydrogen peroxide by way of the condenser. Again turn the tap to position 2 and continue heating until white fumes of sulphuric acid appear in the flask. Run the distillate out of the reservoir and combine it with the distillate previously obtained. Make the combined distillates up to 50 ml. Dilute the acid solution remaining in the flask and make this up to 100 ml. Dilute 1 ml of the tracer solution to 100 ml with 10 per cent. sulphuric acid to give

a reference solution corresponding to 100 per cent. of the radioactivity used. Compare the activities present in the distillate and the residue with the activity of the reference solution by counting 10-ml aliquots of each. Calculate the percentage of the original activity to be found in each fraction.

Results

The results of experiments in which all of the tracers and several organic materials were used are listed in Tables II and III. Table II shows the nuclides with which no difficulties were experienced, and Table III shows those where some losses were found.

Table II

Elements showing good recoveries after oxidation

Nuclide	Organic material	Recovery, per cent.	
Cadmium-109	PVC	94, 95, 97, 97	
Cadmium-109	Polythene	95	97
Bismuth-207	Cocoa	101	103
Vanadium-48	Cocoa	100	101
Vanadium-48	Cocoa + NaCl	98	101
Chromium-51 (chromic chloride)	PVC	99	101
Chromium-51 (sodium chromate)	Cocoa + NaCl	100	103
Tin-113	Cocoa + NaCl	100	103
Zinc-65	Cocoa + NaCl	100	102
Antimony-124	Cocoa + NaCl	97	98
Zirconium-89	Cocoa + NaCl	101	100
Manganese-54	PVC	99	102
Indium-114	PVC	97	101
Tellurium-132* (sodium tellurite)	PVC	100	100

*The tellurium-132 solutions were allowed to stand for 20 hours before counting to permit equilibrium with the radioactive daughter, iodine-132, to be re-established.

Table III

Elements not completely recovered after oxidation

Nuclide	Organic material	Recovery, per cent.	
Germanium-68	None	97	92
	Cocoa	69	48
	Cocoa + NaCl	10	13
	Urea	100	101
	Urea + NaCl	12	9
	Polythene	92	94
	PVC	3	3
Ruthenium-106	None	55	27
	Cocoa	72	51
	Polythene	8	3
	PVC	37	33
Arsenic-74	None	98	99
	Cocoa + NaCl	66	59
	Cocoa + NaCl	62	53
	Polythene	97	99
	PVC	3	3
Selenium-75	None	98	99
	PVC	66	72
	Polythene	11	16

Discussion

The apparatus and procedure used in this investigation were selected for their ability to provide the maximum amount of information, rather than for speed. With this technique it is possible to investigate the behaviour of the tracers in considerable detail; this was not done in the present survey but a standard procedure has been established. One advantage of the relatively complex apparatus shown in Fig. 1 is that the risk of mechanical loss during the very vigorous reaction, which occurs upon addition of 50 per cent. hydrogen peroxide to hot sulphuric acid solution, is minimised.

The radioactive concentrations of the tracer solutions were selected to give count-rates of between 30,000 and 80,000 c.p.m. in the 10-ml aliquot of the standard taken for measurement. The concentration chosen was governed by the efficiency with which each nuclide was detected by the scintillation counter used: this varied from about 0·5 per cent. for chromium-51 to nearly 15 per cent. for bismuth-207.

The results in Tables II and III show that most of the tracers used were recovered in yields of well over 90 per cent.; in fact, of the tracers listed in Table II, only the recovery of cadmium seems to be significantly below 100 per cent.

Although the chemical nature of the tracers used varied somewhat, the initial period of heating with sulphuric acid should have been adequate to prevent differences of behaviour arising from this cause. Although the results cannot necessarily be used to predict the behaviour of the elements studied if they occur in organic combination, it should be a good indication of the behaviour to be expected if they occur in inorganic form.

The results in Table III show that with four of the elements studied, germanium, arsenic, selenium and ruthenium, losses occurred under some or all of the conditions studied, but that no single explanation can cover all four.

When the tracers were carried through the oxidation cycle in the absence of organic matter, only ruthenium suffered serious loss. This is readily explicable by the formation of the volatile ruthenium tetroxide on treatment with 50 per cent. hydrogen peroxide. This element showed serious losses in all the experiments, and osmium, although it was not itself studied, might reasonably be expected to behave similarly.

Of the other three elements, selenium was the only one showing large losses when both PVC and polythene were the organic materials being oxidised. A mechanism involving reduction to a selenium hydride or simple alkyl during the early charring stage has been proposed previously[3] to explain such a loss.

The two remaining elements, arsenic and germanium, show large losses in the presence of PVC but little or none in the presence of polythene. Further, with germanium, large losses were shown in the presence of urea and sodium chloride, while there was none in the presence of urea alone. The addition of sodium chloride to cocoa also caused a large drop in the percentage of germanium recovered.

The obvious and reasonable explanation is to attribute the loss to the formation of volatile chlorides of arsenic and germanium when material containing ionic or covalent chlorine is heated with sulphuric acid. To reconcile the loss of germanium in the presence of cocoa with this explanation, tests were carried out in which 2 g of cocoa were charred with dilute sulphuric acid, and the distillate collected and tested for chloride ion. This was readily demonstrated.

It is worth recording one further experiment, even although it is not concerned with the recovery of trace elements. It has been reported[2] that the oxidation of liquid paraffin with sulphuric acid and hydrogen peroxide is dangerous, being accompanied by flashes of flame and small explosions. To investigate this, 2 g of liquid paraffin were oxidised by the procedure described above, but the initial charring period was extended to the full hour. Under these circumstances the oxidation proceeded quite smoothly, although more 50 per cent. hydrogen peroxide was required than is usual.

TABLE IV

BOILING-POINTS OF VOLATILE CHLORIDES

Chloride	Boiling-point, °C
$SbCl_3$	223
$SbCl_5$	79
$AsCl_3$	130
CrO_2Cl_2	117
$VOCl_3$	127
$GeCl_4$	83
$SnCl_4$	114
$TeCl_2$	327
$TeCl_4$	390

Most of the results obtained are very much as would be expected. The volatility of ruthenium tetroxide is well known, the loss of selenium is similar to previously reported losses, and the loss of arsenic and germanium is reasonable in view of the volatility of their

chlorides (see Table IV). However, the figures in Table IV show that antimony, chromium, vanadium and tin also have very volatile chlorides, yet these elements were recovered in good yield. This is probably due to either reduction of the elements, during the original charring stage, to the lower valency states or to hydrolysis of chlorides to oxy compounds, which are less volatile. For antimony, hydrolysis of chloride occurs readily. Similarly, vanadium halides and oxy halides are easily hydrolysed giving rise to involatile products, and it has been reported[5] that tin chloride will not distil from chloride - sulphuric acid solutions, which is precisely the system that has been under investigation.

Although this study has not been exhaustive, it is believed that, with the exception of mercury, most of the elements that are likely to cause difficulty have been included. As the apparatus used should effectively prevent mechanical loss, it is most probable that the losses found are caused by the formation of genuinely volatile species, always assuming that retention of radioactive material on the glassware is not a serious source of error. That this assumption is valid is supported by the fact that with all the volatile elements, excluding ruthenium, complete recoveries were obtained when no organic material was present. This indicates that absorption of the active material upon the glassware is unlikely from the strongly acid solution remaining after digestion.

References

1. Whalley, C., *in* West, P. W., Macdonald, A. M. G., and West, T. S., *Editors*, "Analytical Chemistry 1962," Elsevier Publishing Company, Amsterdam, London and New York, **1963**, p. 397.
2. Taubinger, R. P., and Wilson, J. R., *Analyst*, **1965**, **90**, 429.
3. Gorsuch, T. T., *Ibid.*, **1959**, **84**, 135.
4. Analytical Methods Committee, *Ibid.*, 1960, **85**, 643.
5. Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffmann, J. I., *Editors*, "Applied Inorganic Analysis," Second Edition, John Wiley & Sons Inc., New York, **1953**, p. 285.

Received *September 26th*, **1966**

Analytical Methods Committee

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

The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter

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

REPORT

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. W. C. Johnson (Chairman), Dr. J. C. Gage, Dr. T. T. Gorsuch (resigned November, 1966), Dr. R. A. Hoodless, Miss E. M. Johnson, Dr. H. Liebmann, Dr. R. F. Milton, Dr. E. J. Newman, Mr. W. L. Sheppard (resigned August, 1965) and Mr. G. B. Thackray, with Mr. P. W. Shallis as Secretary.

In 1960, a Report[1] was published from the Metallic Impurities in Organic Matter Sub-Committee that described several procedures for the destruction of organic matter as a preliminary to the determination of trace metal impurities. These procedures were selected so as to provide for the wide variety of circumstances in which organic matter has to be destroyed, and various mixtures of nitric, perchloric and sulphuric acids were used as the oxidising reagents.

Since the appearance of that Report, 50 per cent. hydrogen peroxide, of reagent purity, has become available, and two publications,[2,3] together with an earlier publication from the U.S.A.,[4] have drawn attention to its use in the oxidation of many types of organic matter. Certain members of the Sub-Committee have also acquired experience with the reagent, and one member has had routine experience with the oxidation of a large variety of foods and drinks with hydrogen peroxide, dealing with about 100 digestions per week over a period of 2 years. Further information on its use was also obtained in response to a notice in the "Proceedings of the Society." The Sub-Committee felt that these methods should be reviewed as an important supplement to those contained in the earlier Report.

ADVANTAGES IN THE USE OF 50 PER CENT. HYDROGEN PEROXIDE

The rapid but smooth oxidation of a wide variety of materials is possible. The reagent has been found particularly valuable in the rapid decomposition of some synthetic plastics that are resistant to the action of the oxidising acids.

No objectionable fumes are evolved, except those from the sulphuric acid that must always be used with the reagent. Water is the only side-product from the reagent.

Extremely low blank values can be expected in the determination of heavy metals. The reagent-grade peroxide may contain a trace (0·05 p.p.m.) of aluminium and silica; phosphate (a few p.p.m.) is added as a stabiliser and a trace of sodium (1 to 1·5 p.p.m.) is introduced incidentally.

HAZARDS AND SAFETY PRECAUTIONS

Fifty per cent. hydrogen peroxide should be stored in a cool place in polythene bottles. If the bottles are not provided with pressure vents, the stoppers or caps should be left loose. Contamination can lead to more or less vigorous evolution of oxygen, and completely sealed

bottles would then burst. Decomposition is catalysed by the presence of various metallic compounds, especially the oxides and hydroxides of the heavy metals, and by contact with precious metals, and it is accelerated by alkaline conditions.

Whalley[2] has given more detailed information on the decomposition of hydrogen peroxide and has provided a resumé of its chemical and physical properties.

The reagent should be stored and used under such conditions that, through spillage, it does not come into contact with combustible materials. Spilled peroxide solution should be washed away at once with water, or wiped up with dilute ammonia solution, as higher concentrations are produced on evaporation, and spontaneous ignition of inflammable materials can result.

Rubber or plastic gloves should be worn when handling the reagent. In contact with the skin it immediately produces the "white burns" that are obtained with 100-volume hydrogen peroxide, but the effect is more severe. Any burns must be washed immediately with water or dilute potassium permanganate solution, otherwise they will become painful and may cause blistering.

Eye protection is essential, and oxidation procedures should be carried out behind a safety screen in a fume cupboard.

For the purposes in consideration, 50 per cent. hydrogen peroxide must always be used in conjunction with a sufficient amount of sulphuric acid. Whalley[2] refers to the part played by Caro's acid (peroxymonosulphuric acid) in the oxidations. A Sub-Committee member produced explosions deliberately by evaporating large volumes (about 50 ml) of peroxide with small volumes (less than 5 ml) of sulphuric acid. Though noisy, the explosions did not produce mechanical fracture of the glassware involved. In the methods described below a much greater proportion of sulphuric acid is always present.

As with most methods for the destructive oxidation of organic matter, it is an elementary precaution to observe the behaviour of any new material in a small-scale preliminary experiment.

Methods

It is perhaps obvious that no precisely standardised procedure can be laid down for application in all circumstances. The procedures described in the following paragraphs are drawn from the various sources acknowledged at the beginning of the Report and the variations arise, in the main, from the nature of the samples with which the contributors were concerned and their ease or difficulty of oxidation with hydrogen peroxide. Users will adapt the detail of the procedures to their own particular circumstances.

Apparatus—

Oxidations can be conveniently carried out in Kjeldahl flasks and one of 100-ml capacity is generally suitable for the oxidation of 2-g amounts of organic material. When such a flask is used it is convenient to add the hydrogen peroxide from a small tap funnel, but as grease of any kind must not be used the stopcock must be fitted with a PTFE plug. The stem of the funnel should be bent in such a way that condensation of the vapours on the funnel does not occur.

If there is a possibility that constituents subsequently to be determined may be lost through volatilisation, the apparatus described by Whalley[5] should be used with a condenser. Alternatively, the apparatus of Bethge,[6] or its modified form,[2] may be used.

Procedure A[2,7] (*for plastics and other materials*)—

Place the sample (2 g) in the flask and add 5 to 25 ml of concentrated sulphuric acid. Heat until charring occurs, and then add 50 per cent. hydrogen peroxide dropwise until a colourless solution is obtained. With some substances alternate charring and addition of hydrogen peroxide is necessary, but care must be taken to ensure that the amount of sulphuric acid is not greatly reduced by evaporation (see "Hazards" above).

Excessive charring should be avoided, as the carbon so formed will be difficult to disperse and will subsequently cause trouble. If this point is reached it is best to discard the experiment and start again with a fresh sample.

TABLE I

WET DESTRUCTION OF ORGANIC MATTER WITH 50 PER CENT. W/W HYDROGEN PEROXIDE

Material (2 g taken in each experiment)	Sulphuric acid fuming when hydrogen peroxide added			Sulphuric acid not fuming when hydrogen peroxide added		
	Volume of H_2O_2, 50% w/w, ml	Volume of H_2SO_4 (sp.gr. 1·84), ml	Time for complete destruction, minutes	Volume of H_2O_2, 50% w/w, ml	Volume of H_2SO_4 (sp.gr. 1·84), ml	Time for complete destruction, minutes
Soya bean oil	15 to 20	10	15 to 20	10 to 15	5	20
Lubricating oil	15 to 20	10	20	20 to 25	10	30
Sawdust	15 to 20	10	10 to 15	10 to 15	5	10 to 15
Cabbage	5 to 10	10	5 to 10	5	5	10
Wool	10 to 15	10	10	10 to 15	5	10
Cotton	10 to 15	10	10	10 to 15	5	10 to 15
Nylon	15 to 20	20	10 to 15	20	10	15
Terylene	15 to 20	15	10	20 to 25	10	25
Poly(vinyl chloride)	20 to 25	10	10 to 15	20 to 25	10	20 to 25
Polythene	20 to 25	20	20	25 to 30	20	30 to 35
Cheese	15 to 20	10	10	10 to 15	5	10 to 15
Meat	10 to 15	10	10	10 to 15	5	10
8-Hydroxyquinoline	10 to 15	10	10	15 to 20	5	10 to 15

Table I summarises the experience of Whalley[2] with a variety of organic materials. Procedure A was used with the initial addition of a catalytic ion, such as manganese or vanadium. Later contributors do not appear to have found catalysts necessary, and Whalley[7] now uses them only when decomposing materials that are not themselves likely to contain catalytic metals.

A member of the Sub-Committee has applied Procedure A to some plastics and has provided the information given in Table II.

TABLE II

DESTRUCTION OF ORGANIC MATTER BY PROCEDURE A

Plastic	Volume of peroxide, ml	Time taken, minutes	Comments
Polythene	20	10	—
PVC for foodstuffs	20	10	—
Polypropylene	60	60	At one stage the mixture was allowed to cool and 2 to 3 ml of 50 per cent. H_2O_2 were added. This brought about a large reduction in the amount of carbon present
Urea - formaldehyde	8	1½	Colourless solution when heated to white fumes
Melamine - formaldehyde	8	2 to 3	—
Perspex piece	8	Largely unaffected after 20 minutes	Small pieces became coated with carbon
Perspex powder granules	35	60	—
Butyl rubber	60	Incomplete after 60 minutes	—

A correspondent has used the same type of treatment for samples (2 to 10 g) of formic, acetic, propionic, succinic, fumaric and sorbic acids, as a preliminary to trace metal determinations.

Taubinger and Wilson[3] adopted similar conditions using always 20 ml of concentrated sulphuric acid. They oxidised successfully several synthetic plastic materials, and the details of the amounts of hydrogen peroxide consumed and the times taken for the complete oxidation of each substance are tabulated in their paper.[3] These authors found that carbon black and polytetrafluoroethylene were not attacked by hydrogen peroxide, and liquid paraffin

reacted so vigorously that its oxidation under these conditions was considered dangerous. Tritolyl phosphate and olive oil frothed excessively and were more satisfactorily treated by Procedure B.

PROCEDURE B (*for readily oxidisable materials*)—

Add 20 ml of concentrated sulphuric acid to 2 g of the organic material and, to the cold mixture, add 50 per cent. hydrogen peroxide dropwise until the reaction slows up (the heat produced will be sufficient to maintain the reaction) or until the solution becomes colourless. Then heat the solution until fumes of sulphuric acid are evolved, adding more hydrogen peroxide as necessary until a colourless solution is obtained.

Taubinger and Wilson[3] oxidised tritolyl phosphate and olive oil by this procedure.

A Sub-Committee member uses this type of procedure for the oxidation of solid fruits, vegetables, meat, and milk products, and sometimes finds it necessary to initiate the reaction by gentle warming, which is applied if the reaction does not begin within 2 minutes after the addition of hydrogen peroxide. This contributor recommends the addition of a few glass beads at the outset of the experiment.

PROCEDURE C (*for soft drinks, beers, etc.*)—

To a 250-ml flask add several glass beads and 5 ml of concentrated sulphuric acid. Add the sample (not more than 50 ml) and then 20 ml of 50 per cent. hydrogen peroxide, and heat gently until the initial reaction has ceased. Then heat until fumes of sulphuric acid are evolved. If at any stage charring occurs, add further 1-ml portions (not greater) of hydrogen peroxide. Digestion is complete when the fuming sulphuric acid remains colourless. If at any stage it seems that the sulphuric acid may approach dryness, cool, add 2 to 3 ml of sulphuric acid, and continue.

PROCEDURE D (*for syrups*)—

To a 500-ml flask containing the sample (not more than 50 ml) add several glass beads, 50 ml of water, 5 ml of concentrated sulphuric acid and then 20 ml of 50 per cent. hydrogen peroxide. Heat gently until the initial reaction has ceased. If the solution is dark, repeat the peroxide addition, several times if necessary, and, when reduced to a small bulk, heat strongly to fumes of sulphuric acid. If charring occurs, add further 1-ml portions (not greater) of hydrogen peroxide until the fuming sulphuric acid remains colourless. If at any stage it seems that the sulphuric acid may approach dryness, cool, add 2 to 3 ml of sulphuric acid, and continue.

The member who provided this method found it especially suitable for preventing excessive carbonisation of carbohydrates.

PROCEDURE E (*for herbs, spices, gums, etc.*)—

Mix the sample (2 to 5 g) with the minimum amount of water to form a slurry, and add concentrated sulphuric acid (3 to 5 ml). Heat the mixture gently and add 50 per cent. hydrogen peroxide dropwise, ensuring that all the peroxide has decomposed before making further additions. When the bulk of the sample has decomposed, add more peroxide until a clear fuming liquid is obtained.

The contributor who provided these details has used this procedure for curry powder, acacia, tragacanth and chlorophyll. With certain oils and balsams, *e.g.*, oil of copaiba balsam and Peru balsam, 50 per cent. hydrogen peroxide was found to be too vigorous for safe and accurate work.

CONCLUSIONS

From the information made available, the Sub-Committee was satisfied that the advantages set out on p. 403 had been established, and that 50 per cent. hydrogen peroxide provides a convenient and effective means of destroying organic matter.

Although it is known that the reagent has been used as a preliminary to the determination of several metals, the Sub-Committee has not conducted a systematic examination of the possible effects of treatment with hydrogen peroxide on the subsequent recovery and determination of trace metals. It is intended to take this aspect into account in future reports

from the Sub-Committee. However, since the preparation of this Report the Sub-Committee has become aware of a paper[8] giving the results of a study of the recovery of several elements, at the parts per million level, from organic materials after oxidation with sulphuric acid and 50 per cent. hydrogen peroxide.

References

1. Analytical Methods Committee, *Analyst*, **1960**, **85**, 643.
2. Whalley, C., *in* West, P. W., Macdonald, A. M. G., and West, T. S., *Editors*, "Analytical Chemistry 1962," Elsevier Publishing Company, Amsterdam, London and New York, 1963, p. 397.
3. Taubinger, R. P., and Wilson, J. R., *Analyst*, 1965, **90**, 429.
4. Polley, D., and Miller, V. L., *Analyt. Chem.*, 1955, **27**, 1162.
5. Whalley, C., *Paint Technol.*, 1956, **20**, 47.
6. Bethge, P. O., *Analytica Chim. Acta*, 1954, **10**, 317.
7. Whalley, C., personal communication to the Sub-Committee.
8. Down, J. L., and Gorsuch, T. T., *Analyst*, 1967, **92**, 398.

Book Reviews

OILS, FATS AND FATTY FOODS. THEIR PRACTICAL EXAMINATION. Fourth Edition. By K. A. WILLIAMS, B.Sc., Ph.D., M.Inst.Pet., A.Inst.P., F.R.I.C. Pp. viii + 488. London: J. & A. Churchill Ltd. 1966. Price 100s.

We welcome the new edition of this book, which to many chemists needs no introduction. Being a person of conservative tastes I find it pleasing that in the revision the lay-out of the book remains the same as that which has been familiar to us for many years, but for the benefit of those who are not familiar with this work a brief description of the contents may be helpful. The earlier chapters are devoted to a description of the general chemical and physical tests that are used in the analysis of oils and fats, and these are followed by a chapter on the interpretation of results. It is in this early part of the book that we obtain the full benefit of the author's lifetime of experience in the practical examination of oils and fats.

The full title of the book is "Oils, Fats and Fatty Foods—Their Practical Examination." The book undoubtedly lives up to this ancillary title, and is enriched by much practical know-how that can only have been acquired by long experience of the snags involved from a practical point of view. The chapter on interpretation similarly reflects the author's appreciation of the need for guidance on this subject, as the mere determination of the constants for an oil or fat can be of little value without a knowledge of how they are related to composition; this is an aspect of the examination of oils and fats that is often omitted in books on this subject.

Chapters on the industrial production of oils and fats and hardened oils follow, and then important fats such as butter, margarine and lard each receive extensive individual treatment. The description and properties of vegetable oils occupy a substantial proportion of the book, some 140 different oils being described together with botanical details of the nuts, seeds, etc., from which they are derived. This section of the book would be a work of reference in its own right without the other sections. It includes oils that are infrequently used in commerce and on which it is difficult to find detailed information in the literature. The composition of the cakes remaining after extraction of the oil, many of which are used for animal feeding stuffs, are also given. The closing chapters deal with miscellaneous fatty foods such as cocoa, milk products and animal feeding stuffs.

The entire book has been revised and brought up to date as far as is possible in an age of rapidly changing techniques and rapid accumulation of new knowledge, and many new methods of analysis for oils and fats have been introduced since the last edition of this book, of which mention may be made of gas and thin-layer chromatography and lipase hydrolysis. In view of the valuable information that can be obtained from these techniques one would have liked a larger proportion of the book to have been devoted to them, but it may be that the author feels that the results are as yet not sufficiently firmly established to warrant the methods being discussed in greater detail.

Fats and fatty foods are the subject of a number of Government Standards and full details of the latest requirements are given in the text. The book also includes a list of standards for oils and fats in some of the more important countries in a special appendix. This appendix also includes a list of possible new sources of oil which have been investigated in recent years.

We are indebted to Dr. Williams for a new edition of a book that has been proved by long experience to be invaluable to all concerned in the analysis of oils and fats. J. H. HAMENCE

MODERN TRENDS IN ACTIVATION ANALYSIS. Proceedings of the 1965 International Conference at College Station, Texas, U.S.A. April 19–22, 1965. Pp. 390. Texas A & M University Press. Price $15; 128s.

At a time when activation analysis and related methods are being investigated by scientists of a variety of persuasions, chemists, physicists, metallurgists, instrumental specialists, etc., it is becoming increasingly difficult to follow developments in applications and techniques without an extensive survey of the literature. "Modern Trends in Activation Analysis" contains more than seventy papers on a variety of aspects of activation analysis which were presented to the Texas Conference, in many cases by established workers in their own fields, and provides, within one volume, much useful information and a guide to the fields of research attracting attention at present. Subjects of the papers include reactor neutron-activation analysis, computer techniques,

fast neutron, γ-photon and charged particle activation, secondary reactions, prompt radiation techniques and electron activation.

The largest number of papers are concerned with reactor neutron-activation, and methods for the determination of many elements in a variety of different matrices are described.

In spite of the increasing emphasis on sophisticated instrumental techniques, the high decontamination factors that can be obtained by chemical separation still make this an attractive method of isolating the activity to be determined from all others induced in the sample, particularly for trace element determination, and several papers on chemical separation techniques are included, which in some cases describe automatic systems designed to free laboratory staff from repetitive operations. Instrumental methods receiving attention are not only techniques of γ-ray spectroscopy, such as coincidence methods and the use of germanium counters, but also the handling and processing of data in computer systems. Oxygen determinations figure largely in papers on fast-neutron activation analysis, but automated and sealed-tube systems are also described. Of the less widely applied activation techniques that are the subjects of papers, helium-3, proton, deuteron and γ-photon activation and secondary reactions are discussed as a means of determining light elements that cannot be assayed by conventional reactor neutron activation, and examples are given of methods based on the measurement of "prompt" radiation emitted as a result of such processes as Rutherford scattering, inelastic scattering and radiative capture. The appendix of the book contains a recommended method for the measurement of neutron flux emitted by a neutron generator by the T (d,n) ^{4}He reaction, and is based on assay of the activity induced in a copper foil.

This book is a straightforward collection of research papers delivered to the Conference, and, as such, will appeal primarily to those who are already familiar with activation techniques. In spite of the fact that a few of the papers have appeared in thinly disguised forms elsewhere in the scientific literature, the largest amount of information in this book will recommend it to those interested in keeping abreast of developments in nuclear analytical techniques. T. B. PIERCE

ELEMENTARY PRACTICAL ORGANIC CHEMISTRY. PART 1: SMALL SCALE PREPARATIONS. By ARTHUR I. VOGEL, D.Sc., D.I.C., F.R.I.C. Second Edition. Pp. xx + 435. London: Longmans, Green and Co. Ltd. 1966. Price 35s.

ELEMENTARY PRACTICAL ORGANIC CHEMISTRY. PART 2: QUALITATIVE ORGANIC ANALYSIS. By ARTHUR I. VOGEL, D.Sc., D.I.C., F.R.I.C. Second Edition. Pp. xvi + 431. London: Longmans, Green and Co. Ltd. 1966. Price 35s.

A complete course of practical organic chemistry would necessarily include the preparation of organic compounds including, in advanced stages, the synthesis of compounds of complicated structure; the qualitative identification of compounds including the fragments obtained by the degradation of complex molecules and their quantitative determination alone and in mixtures.

Our brief span limits organised courses to about 3 years and restricts them generally to what we call elementary practical organic chemistry, but the elementary of today was the recondite of a previous generation. Hence the need for the constant renewal of text-books for the student.

This need has been met in Dr. Vogel's well known trilogy consisting of the two volumes now reviewed and the companion volume on Quantitative Organic Analysis. The first two, issued in 1957, and running into five impressions, have now appeared in a second edition, which will long preserve the memory of their author among both teachers and students.

Part 1, Small Scale Preparations, is characterised by the inclusion of new apparatus and the new experimental techniques of paper, thin-layer and ion-exchange chromatography and by descriptions of the mechanisms of the reactions described in the various preparations. New preparations are also included.

Part 2. Qualitative Organic Analysis has been completely re-arranged and extended. The first three chapters deal with the fundamental bases, *viz.*, determination of physical constants, the elementary composition, and solubility as a criterion of classification. Then follow three chapters on class and functional group reactions and the preparation of derivatives for identification. A chapter on the analysis of mixtures is then followed by an important new chapter dealing with the practical essentials of ultraviolet and infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry, techniques and apparatus which have already largely become tools of every-day use in both teaching and industrial laboratories. Finally, 100 pages are devoted to tables of physical constants of compounds and their derivatives, which constants form an integral part of organic analysis in contrast to inorganic analysis.

Both parts are written with the logic and lucidity that is characteristic of Dr. Vogel's well known text-books and fully justify their claims to meet the requirements up to the honours degrees of the universities, and are equally valuable in industrial laboratories. J. I. M. JONES

FORMULA INDEX TO NMR LITERATURE DATA. Volume 2: 1961–1962 References. Edited by M. GERTRUDE HOWELL, ANDREW S. KENDE and JOHN S. WEBB. Pp. x + 516. New York: Plenum Press Data Division, 1966. Price $22.50.

This is the second volume of literature references to high resolution nuclear magnetic resonance spectra to come from the research laboratories of the American Cyanamid Company. Volume 1 covered references prior to 1961 whereas Volume 2 is confined to references in the literature for 1961–62. The format of the new volume is identical with the previous one. Compounds are listed in order of empirical molecular formula and also by structural formula when this is known. The fact that this volume is twice the size of the previous one illustrates the remarkable development of the n.m.r. technique in recent years. It is hoped that the contributors will not feel disillusioned at this increasing pace in the development of the subject and that further volumes are planned, otherwise these present two volumes will lose much of their usefulness.

The high price of this second volume is not unexpected in a reference book of this type, and the book is certainly recommended for any n.m.r. spectroscopy laboratory. K. G. ORRELL

HANDBUCH DER ANALYTISCHEN CHEMIE. Edited by W. FRESENIUS and G. JANDER. Part 3: QUANTITATIVE BESTIMMUNGS- UND TRENNUNGS-METHODEN. BAND IV $\alpha\beta$. ELEMENTE DER VIERTEN HAUPTGRUPPE II · IV GERMANIUM · BLEI. By DR. GUNTHER KRAFT. Pp. xvi + 222. Berlin, Heidelberg and New York: Springer-Verlag. 1966. Price (Linen covered) DM 64; (Hard backed) DM 59.

This book comprises two monographs on the determination of germanium and lead. Both follow the same pattern: gravimetric, titrimetric, photometric, polarographic, spectrochemical and radiochemical methods are discussed in turn; useful chapters on the separation of these elements from probable interferences are included. Methods for the analysis of elemental germanium, and germanium oxide and chloride are given, because of the great importance of these materials in semiconductors.

The volumes of this Handbuch have no real parallel in the English literature. The information given is much more extensive than that in any comparable text in English; for example, there is full experimental detail for no fewer than twenty three photometric methods of determining germanium and many other methods are mentioned. The desirability of this proliferation of detail is arguable, but it does mean that, without recourse to the original literature, the careful reader can choose a method on the basis of his own evaluation of the various possibilities, rather than on the prejudices of the author.

The whole book is a fine example of the assiduous Germanic approach. It should be of great value to anyone engaged either in analysis for germanium and lead or in the production of lesser compilations. A. M. G. MACDONALD

VACUUM MICROBALANCE TECHNIQUES. Volume 5. Proceedings of the Princeton Conference September 27–28, 1965. Edited by KLAUS H. BEHRNDT. Pp. xx + 264. New York: Plenum Press. 1966. Price $13.50.

The justification for the annual appearance of volumes such as this should be that they survey progress in rapidly advancing areas of science. However, even more than preceding volumes, "Vacuum Microbalance Techniques," Volume 5, leaves the impression that the field covered is too restricted for a worthwhile annual publication, and it is poor value at $13.50.

For analytical chemists the book contains little new information likely to be of value in the application of microbalances. Seven of the papers are detailed theoretical analyses of particular aspects of microbalance performance. Knudsen forces in the intermediate pressure range are the subject of three papers, and the importance of avoiding the difficulties of making reliable corrections for their effect is emphasised. The effects of gas pressure and added-mass on quartz crystal microbalances are discussed at length by Stockbridge. Applications of microbalances to studies of the impulse of an ion engine for space propulsion, sputtering yields, magnetic susceptibility, and problems in thin-film deposition are described in other papers. D. W. BASSETT

Summaries of Papers in this Issue

The Reversed-phase Thin-layer Chromatography of Metal Ions with Tributyl Phosphate

About sixty-five metal-containing ionic species have been chromatographed on thin layers of cellulose impregnated with tributyl phosphate (TBP) at various concentrations of aqueous hydrochloric acid. A mechanism for the chromatography may be explained in terms of the ability of the metal ions to form chloro complexes, *viz.*, that those metal ions which readily complex with chloride ion are readily extracted by the TBP and consequently have low R_F values. Conversely, those metals which do not form chloro complexes are not retained by the TBP and hence have high R_F values.

Attention is drawn to the similarities between the known liquid - liquid extraction behaviour of the metal ions in the TBP - hydrochloric acid systems and the behaviour of these ions in the TBP - hydrochloric acid chromatographic systems. A strong resemblance has also been found between the R_F spectra (R_F *versus* hydrochloric acid) of the metal ions and the behaviour of these ions in resinous anion-exchange - hydrochloric acid systems. The latter similarity has been used as evidence for the suggestion that the TBP on the layers functions as a liquid anion exchanger, *i.e.*, that TBP-solvated protons, ion-associated with chloride ions, can undergo ion exchange with the metal chloro complex—

$$(TBP)_o + (HCl)_{aq} \rightleftharpoons (TBPH^+Cl^-)_o$$
$$(nTBPH^+Cl^-)_o + (MCl_x^{-n}) \rightleftharpoons MCl_x^{-n}(TBPH^+)_{no} + (nCl^-)_{aq}.$$

The suffix "o" refers to the organic phase and the suffix "aq" refers to the aqueous phase.

L. S. BARK, G. DUNCAN and R. J. T. GRAHAM

The Department of Chemistry and Applied Chemistry, University of Salford, Salford 5, Lancashire.

Analyst, 1967, **92**, 347–357.

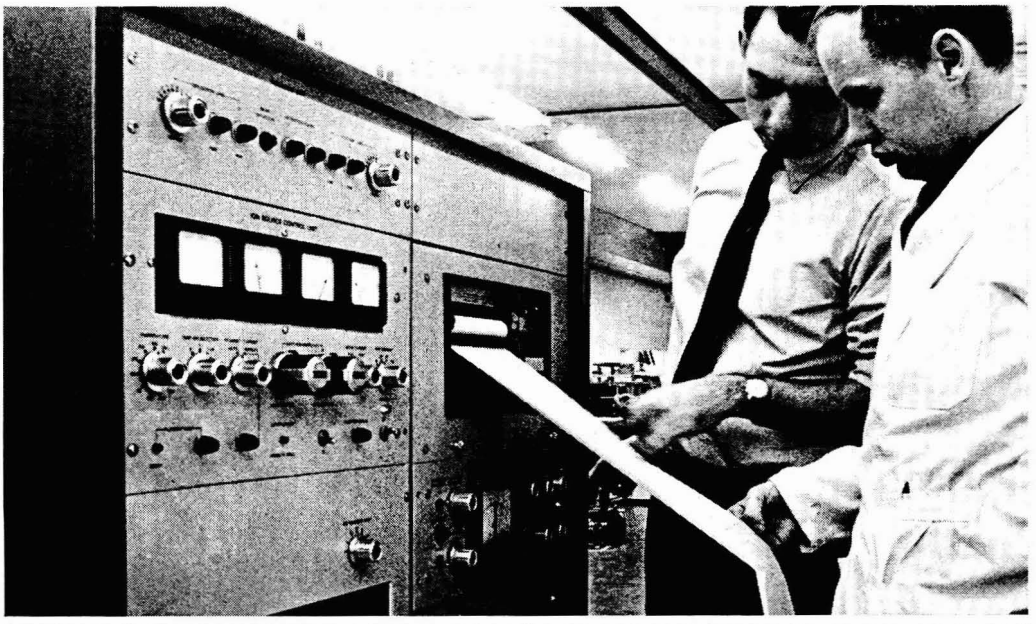
A Specific Spectrofluorimetric Determination of Terbium as its EDTA - Sulphosalicylic Acid Complex

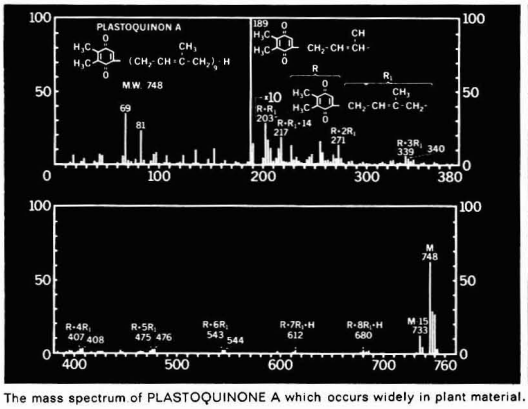
The ternary complex formed by terbium with ethylenediaminetetra-acetic acid and sulphosalicylic acid has been used as the basis of a spectrofluorimetric determination of between $6{\cdot}4 \times 10^{-3}$ and $3{\cdot}2\ \mu g$ of terbium per ml. The system absorbs radiation characteristic of sulphosalicylic acid (about 320 mμ) and emits the band fluorescence of sulphosalicylic acid (410 mμ), together with the sharp line emission characteristic of terbium(III) (545 mμ). The determination is carried out with 4×10^{-3} M EDTA and 2×10^{-3} M sulphosalicylic acid in aqueous solution at a pH of between 11·6 and 11·9. No interference resulted from 50-fold molar excesses of the other rare earth ions, 33 other metal ions or 14 anions. The fluorescence was not subject to oxygen quenching, was stable for several days and had a temperature coefficient of −0·87 per cent. per °C. Analytical results obtained with prepared samples have been included.

R. M. DAGNALL, R. SMITH and T. S. WEST

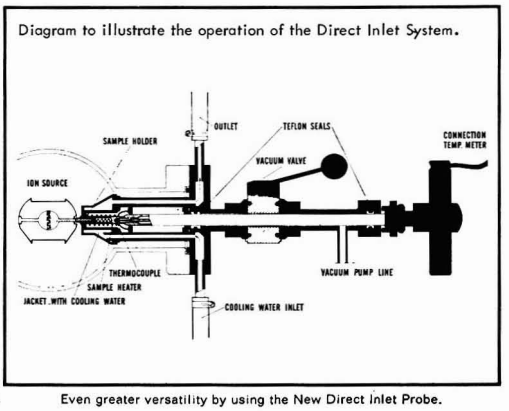
Chemistry Department, Imperial College, London, S.W.7.

Analyst, 1967, **92**, 358–363.





The mass spectrum of PLASTOQUINONE A which occurs widely in plant material.



Even greater versatility by using the New Direct Inlet Probe.

LKB 9000 Produces the Right Answer

The ANSWER—provided by the first commercial instrument to integrate a gas chromatograph, a molecule separator and a mass spectrometer—to analytical problems in chemistry dealing with amino acids, sterols, steroids and bile acid esters, biological toxins and narcotics, barbiturates, tranquilizers, pesticides, carbohydrate derivatives and many others.

The ANSWER to the demand for Speed, Accuracy and Flexibility of operation.

The ANSWER to temperature sensitive or unstable samples as well as to samples with low vapour pressure—the Direct Inlet System.

The ANSWER to effluent containing unresolved compounds—The Accelerating Voltage Alternator.

The ANSWER in fact to everything a scientist could ask for in performance, convenience and dependable, trouble-free operation—a new dimension in GC-MS.

We have given only a few ANSWERS.

If you have som QUESTIONS why not write today to the address below for All the ANSWERS on the LKB 9000 Gas Chromatograph-Mass Spectrometer.

LKB INSTRUMENTS LTD. • LKB HOUSE, 137 ANERLEY ROAD • LONDON S.E. 20

LKB

OTHER HEADQUARTERS FOR SALES AND SERVICE

SWEDEN
LKB-Produkter AB
Box 76,
Stockholm—Bromma 1

USA
LKB Instruments Inc.
12221 Parklawn Drive,
Rockville Md 20852

NETHERLANDS
LKB-Produkten N.V.
Zeekant 35,
The Hague

DENMARK
LKB Instrument A/S
Amagerbroçade 34,
Copenhagen S

The Enthalpimetric Titration of Basic Nitrogen Compounds

When aqueous solutions of bases are titrated enthalpimetrically with acid, it has been found that ammonia and aliphatic bases are titrated before pyridine and aniline bases. When aqueous solutions of the mineral acid salts of bases are titrated, the titration order is (*i*) the free acidity, (*ii*) the pyridine and aniline base salts and (*iii*) the ammonia and aliphatic base salts.

In non-aqueous solution, use has been made of the large endothermic heat of dilution when a strong hydrogen chloride solution in isopropyl alcohol is added to a wide range of organic solvents, excluding alcohols. As an enthalpimetric titrant, this solution first gives a temperature rise as a result of the neutralisation of the base in the solvent, followed by a sharp temperature drop that marks the end-point. In solvents other than acetic acid, aliphatic bases are titrated first and are distinguishable from aromatic bases, except those similar to diphenylamine, which are not titrated. In acetic acid solution, aliphatic and aromatic bases are titrated together first and are distinguishable from the weak bases like diphenylamine, which are titrated in this solvent.

G. A. VAUGHAN and J. J. SWITHENBANK

Coal Tar Research Association, Gomersal, Cleckheaton, Yorkshire.

Analyst, 1967, **92**, 364–370.

A Gas-chromatographic Determination of Residues of Picloram

A method is described for the gas-chromatographic determination of Picloram herbicide in soils and plant material by using an electron-capture detector. The method described for soil has a sensitivity of 0·02 p.p.m. By the use of more rigorous clean-up procedures Picloram can be detected in other plant material at a level of 0·005 p.p.m., and recoveries of added Picloram range from 60 to 110 per cent. over the concentration range of 0·02 to 0·5 p.p.m.

J. S. LEAHY and T. TAYLOR

Huntingdon Research Centre, Huntingdon.

Analyst, 1967, **92**, 371–374.

Spectrophotometric Determination of Diquat and Paraquat in Aqueous Herbicide Formulations

Methods are described for determining diquat and paraquat, singly and in admixture, in formulations. For determining diquat, ultraviolet absorptiometry at 310 mμ in a sodium acetate buffer solution at pH 4·05 is adopted. Paraquat is determined in a diluted solution by measuring the optical density, at 600 mμ, of the blue free radical produced by reduction with alkaline sodium dithionite. For analysing mixtures containing both diquat and paraquat, these methods are combined and use is made of a base-line correction procedure to compensate for interference of diquat in the determination of paraquat. The error for these methods is established to be within ± 2 per cent.

S. H. YUEN, J. E. BAGNESS and D. MYLES

Imperial Chemical Industries Limited, Agricultural Division, Jealott's Hill Research Station, Bracknell, Berkshire.

Analyst, 1967, **92**, 375–381.

Detection and Determination of Hexoestrol in Meat

A gas-chromatographic procedure for the detection of hexoestrol at the $0{\cdot}4 \times 10^{-9}$ g level is described. Other oestrogenic substances can be detected at similar levels by the same method. A method based on this procedure has been devised, which enables hexoestrol to be determined in meat samples at levels down to 0·1 p.p.m.

P. J. COOPER, M. J. de FAUBERT MAUNDER and G. J. McCUTCHEON

Ministry of Technology, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1967, **92**, 382–386.

The Colorimetric Determination of Molybdenum in Soils and Sediments by Zinc Dithiol

A method is presented for determining molybdenum in soils, sediments and rocks. The sample is decomposed by fusion with potassium hydrogen sulphate and the molybdenum is taken into solution with hydrochloric acid. Interference by iron is prevented by reduction to the iron(II) state and the reaction with copper by adding potassium iodide. Tungsten interference is suppressed by careful control of the time allowed for complex formation.

The molybdenum - dithiol complex is extracted into light petroleum and determined either by visual comparison or spectrophotometry.

R. E. STANTON and Mrs. A. J. HARDWICK
Department of Geology, Imperial College, London, S.W.7.

Analyst, 1967, **92**, 387–390.

Volumetric Determination of Styphnates with Methylene Blue

A modification of Bolliger's extraction titration of *o*-polynitrophenols with methylene blue is described. Because of its better solvent power, nitrobenzene has been used as the extracting agent instead of chloroform, which would necessitate repeated renewal during the titration. The method is applied to the determination of lead styphnate and of the intermediates and effluents in its production, as well as to the analysis of priming compositions. Nitrate interference is removed by extracting the styphnic acid with isobutyl methyl ketone before the titration.

E. KURZ and G. KOBER
Central Laboratory for Explosives and Ammunition, Israeli Military Industries, Tel-Aviv, Israel.

Analyst, 1967, **92**, 391–395.

A Pre-reaction Attachment for the Karl Fischer Cell

A pre-reaction attachment to a standard Karl Fischer cell is described for use in determining water in organic peroxide solutions. The attachment can also be used in other determinations in which interferences must be eliminated without the formation of water, or in which liberated water is used as a means of determining other functional groups.

M. D. LACK and B. E. FROST
Analytical Laboratory, Novadel Ltd., Gillingham, Kent.

Analyst, 1967, **92**, 396–397.

The Recovery of Trace Elements after the Oxidation of Organic Material with 50 per cent. Hydrogen Peroxide

The recovery of several elements, at the p.p.m. level, from various organic materials after oxidation with sulphuric acid and 50 per cent. hydrogen peroxide has been studied. Most of the elements investigated could be recovered quantitatively, but germanium, arsenic, selenium and ruthenium suffered losses under some or all of the conditions examined. The causes of these losses are discussed.

J. L. DOWN and T. T. GORSUCH
United Kingdom Atomic Energy Authority, The Radiochemical Centre, Amersham, Buckinghamshire.

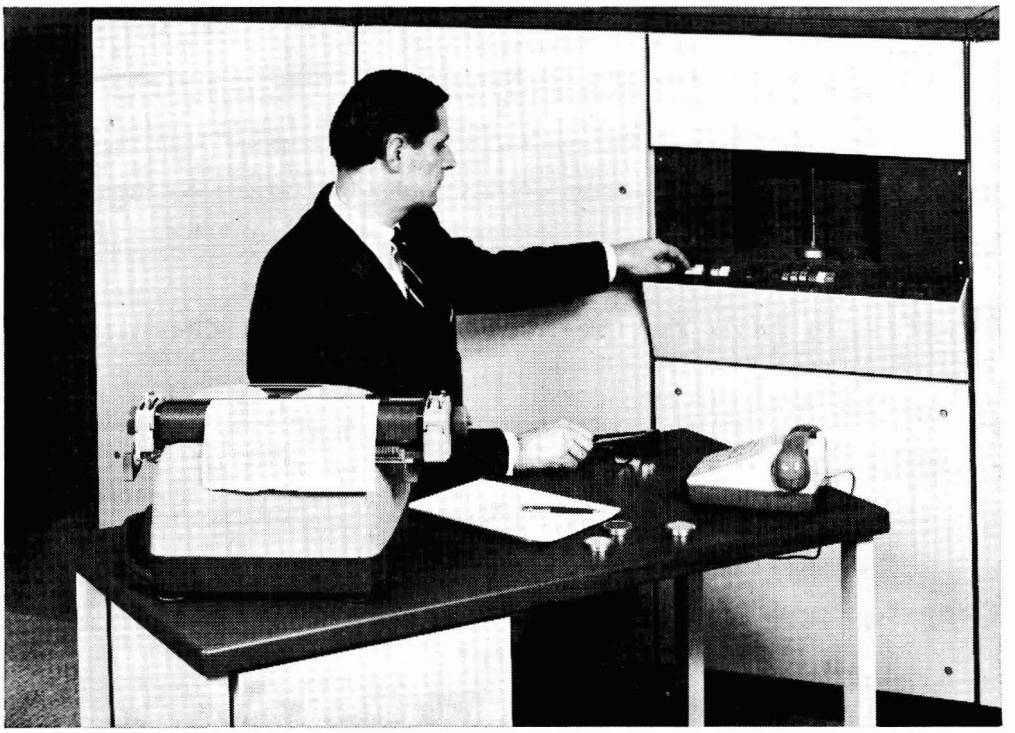
Analyst, 1967, **92**, 398–402.

The Use of 50 per cent. Hydrogen Peroxide for the Destruction of Organic Matter

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

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

Analyst, 1967, **92**, 403–407.



Accurate production control analysis at the touch of a button with the new Philips PW 1250 automatic simultaneous spectrometer

The new Philips PW 1250 Automatic Simultaneous X-ray Spectrometer performs fast, accurate analyses for routine production control of such materials as cement, glass, steel, oil and non-ferrous materials. Once the machine has been set up, it performs multi-element analysis automatically at the touch of a button.

- Up to 7 elements - above atomic number 11 - for analysis in each sample.
- Three programmes may be preset, for different types of substances.
- Allows choice of measuring method: absolute, ratio or monitor.
- Manual or automatic specimen changing of up to 9 or 160 samples at a time - vacuum airlock allows the next sample to be loaded and brought into vacuum while the previous one is under analysis.
- Facilities for computer operation - either with an integral data processor or separate computer so that analytical results can be presented as concentrations, intensities, or both. With data processing, accurate analyses may be accomplished in 20-30 seconds.
- Air, vacuum or helium path.

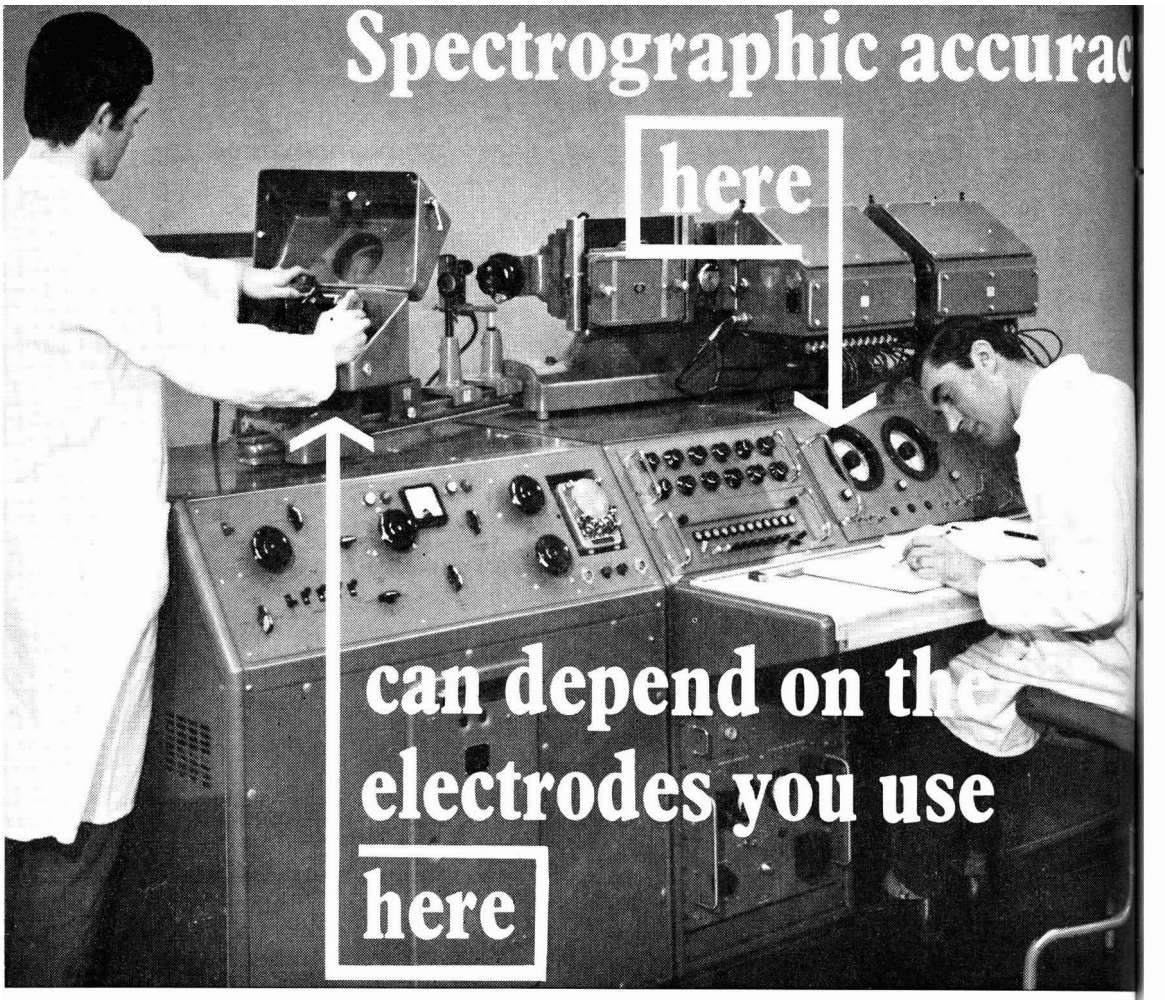
With the PW 1250, extreme stability exists for high voltage and tube current. Maximum power is 3 kW. Use of printed circuit boards ensures easy servicing and rugged dust-proof design facilitates efficient operation in industrial environments.

Further information. A 16-page brochure gives comprehensive details; copies of it are available on request.

N.V. Philips' Gloeilampenfabrieken,
Scientific and Analytical Equipment Department,
Eindhoven, The Netherlands.

PHILIPS

Scientific Equipment



Your spectrographic technique may be faultless, your standards may be exactly right, but the accuracy of your results may hinge on the grade of carbon or graphite electrode you choose

Wise spectrographers the world over know that there is absolutely no point in taking chances in the selection of the carbon and graphite they use for electrodes.

That is why they always specify JMC carbon and graphite products. They know that only the finest grades of carbon and graphite are supplied that have uniform high quality both chemically and in physical properties. And they know that these same grades are used by Johnson Matthey Chemicals themselves in their analytical, research and production control laboratories!

Following the practice pioneered by Johnson Matthey Chemicals many years ago, a detailed analytical report is provided with every consignment stating the quantity of each impurity detected spectrographically in the particular batch.

Write for our publication JMC Spectrographic Carbon and Graphite.

JMC Spectrographic carbon and graphite

90767

Johnson Matthey Chemicals Limited
74 Hatton Garden, London EC1 Telephone: 01-405 6989 Telex: 21465

CLASSIFIED ADVERTISEMENTS

The rate for classified advertisements is 7s. a line (or space equivalent of a line), with an extra charge of 2s. for the use of a Box Number. Semi-displayed classified advertisements are 80s. for single-column inch.

Copy required not later than the 20th of the month preceding date of publication which is on the 16th of each month. Advertisements should be addressed to The Analyst, 47 Gresham Street, London, E.C.2. Tel.: MONarch 7644.

STAFFORDSHIRE COUNTY COUNCIL

APPOINTMENT OF COUNTY ANALYST

Applications are invited for the above-mentioned appointment from candidates possessing the qualifications prescribed by, or pursuant to, the Food and Drugs Act, 1955, and the Fertilisers and Feeding Stuffs Act, 1926.

The salary will be in accordance with the County Council's Principal Officers Special Grade (£3,435—£3,795 per annum).

The appointment will be terminable by three calendar months' notice in writing on either side and subject to the provisions of the Local Government Superannuation Act, 1937, in which connection the successful candidate will be required to pass a medical examination and submit his birth certificate.

The appointment will be subject to the approval of the Minister of Agriculture, Fisheries and Food and the successful candidate must accept appointments as Public Analyst by such other autonomous Food and Drugs Authorities as the County Council may approve. He will also be required to devote the whole of his time to the public offices to which he is appointed with the approval of the County Council and to pay all monies received into the County Fund.

Canvassing, either directly or indirectly, will be a disqualification and candidates should state in their applications whether or not they are related to any member of the County Council.

Applications, stating age, qualifications and experience, accompanied by the names and addresses of three referees, should be forwarded to reach the undersigned not later than June 29th, 1967.

T. H. Evans,
Clerk of the County Council

County Buildings,
Stafford.

CHEMIST/TECHNOLOGIST

Small company/Hertfordshire, with excellent technical reputation require chemist/technologist for varied work including non-routine analysis, technical investigation of product processes, and development of new products. Candidates should be graduates with several years experience in Food, or Fermentation Industries. Contributory Pension Scheme.

Apply stating age, experience and salary required to Box No. 4091.

PERSONNEL

Junior and Senior Chemists including those of exceptional ability generally available. Enquiries from experienced personnel also welcomed.

Miss J. R. Hortin, G. K. Bureau Limited, 196, Oxford Street, London, W.1. MUSeum 6858.

SENIOR ANALYST required by very busy Public Analysts and Consulting Chemists in the Midlands.

Bostock Hill and Rigby, 37–39 Birchfield Road, Birmingham 19.

ANALYST for the makers of Anadin and wide range of proprietary products, laboratories N.16. Excellent conditions, prospects. Non-contributory pension/life assurance; staff canteen. Male candidates 25+ with B.Sc., or L.R.I.C. (minimum qualification H.N.C.) with some industrial experience are invited to write with full details to Chief Chemist, I.C.C. Limited, Braydon Road, N.16.

WEST HAM COLLEGE OF TECHNOLOGY

CHEMISTRY AND BIOLOGY DEPARTMENT

M.Sc. of University of London
Analytical Chemistry

PART-TIME OR FULL-TIME COURSES
October, 1967

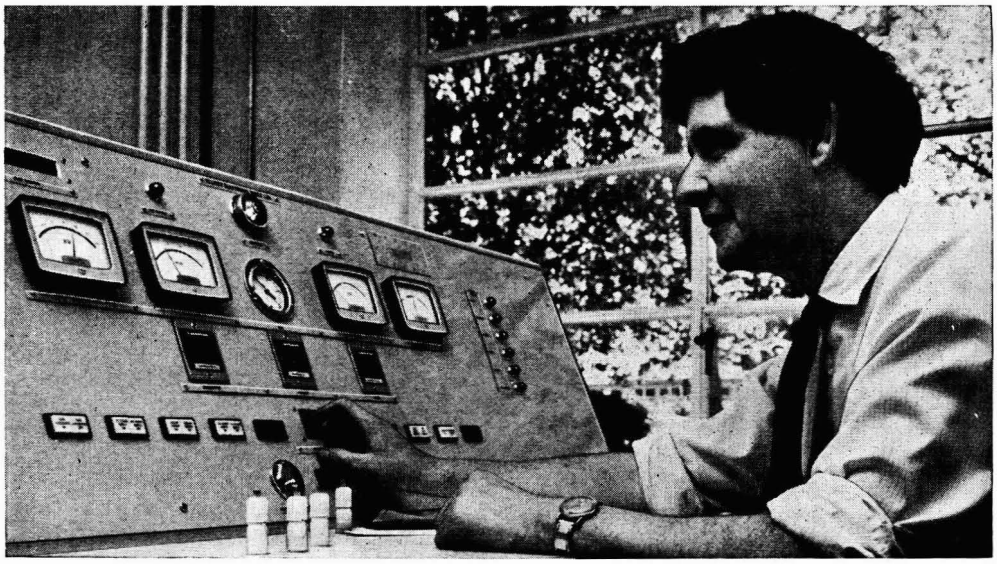
Applications are invited from candidates who hold a B.Sc. degree, Diploma of Technology or Grad. R.I.C.

The course provides a thorough grounding in the principles and practise of analytical chemistry with particular emphasis on special and electroanalytical techniques.

A special qualifying course is offered to some candidates with degrees from overseas universities.

Apply to the Registrar, West Ham College of Technology, Romford Road, London, E.15.

An ANALYST is required in the Department of Biochemistry at Imperial College to take charge of a section carrying out routine chemical and biochemical assays. Applicants should be graduates in chemistry or possess equivalent qualifications. Experience in the use of auto-analyzers would be an advantage. There will be opportunities for the development of new analytical methods. Salary in the range £1040 to £1745 p.a. according to qualifications, age, and experience. Applications in writing to Departmental Superintendent, Biochemistry Department, Imperial College, London, S.W.7.



The Elliott installation at Atomic Energy Research Establishment Harwell

Elliott *integrated* activation analysis systems feature sealed tube

Elliott integrated activation analysis systems save space, reduce shielding costs, and offer automatic routine operation in the factory or laboratory. Elliott sealed neutron tubes provide constant output up to 10^{10} neutrons per second and carry a 100 hour warranty.

ELLIOTT ELECTRONIC TUBES LIMITED

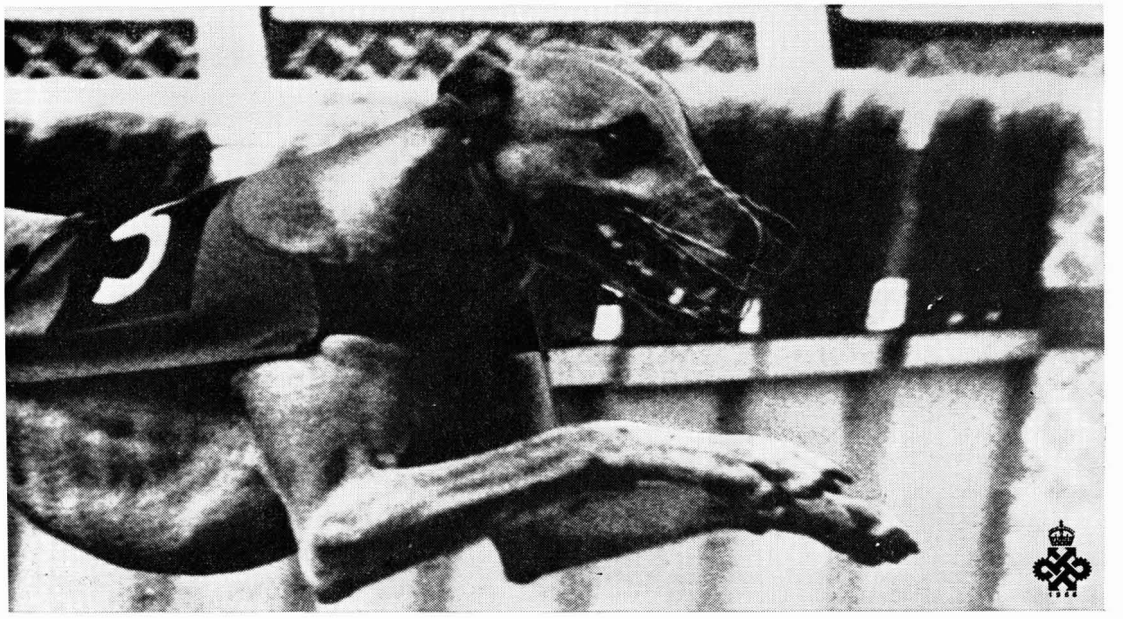
Neutron Division, Elstree Way, Borehamwood, Hertfordshire, England
Tel: Elstree 2040, Ext 459

In the USA: Baird-Atomic Inc.,
33 University Road, Cambridge 38, Mass.
Tel: University 4-7420

ELLIOTT NEUTRON DIVISION

A Member of the Elliott-Automation Group

EN.2



We mean it. Hilger and Watts do not know of any other spectrophotometer made in Britain which can take five cells in one loading—and analyse as *quickly*, *simply* and as *accurately* as the Spectrochem.

In addition, its low cost means that organisations with *low* budgets for capital equipment can benefit from a highly efficient, *high*-speed instrument. Spectrochem has been designed for fast, easy operation in the busiest of laboratories. It takes the labour out of laboratory analysis. Use Spectrochem for routine quantitative analysis or the plotting of detailed absorption spectra through the visible into the ultra violet. Spectral range is either 340 to 750 mμ or 340 to 820 mμ.

Among the names in the pedigree of the Spectrochem is the famous Spekker—*this* is breeding from Hilger and Watts.

And here's a chance to prove us right

Pin this to your letter heading

We would welcome the opportunity to try the Spectrochem for ourselves—when and how can we do this? Please send full details. We are engaged in the field of:

_____

Name_____

Status_____

Company or Institution_____

Address_____

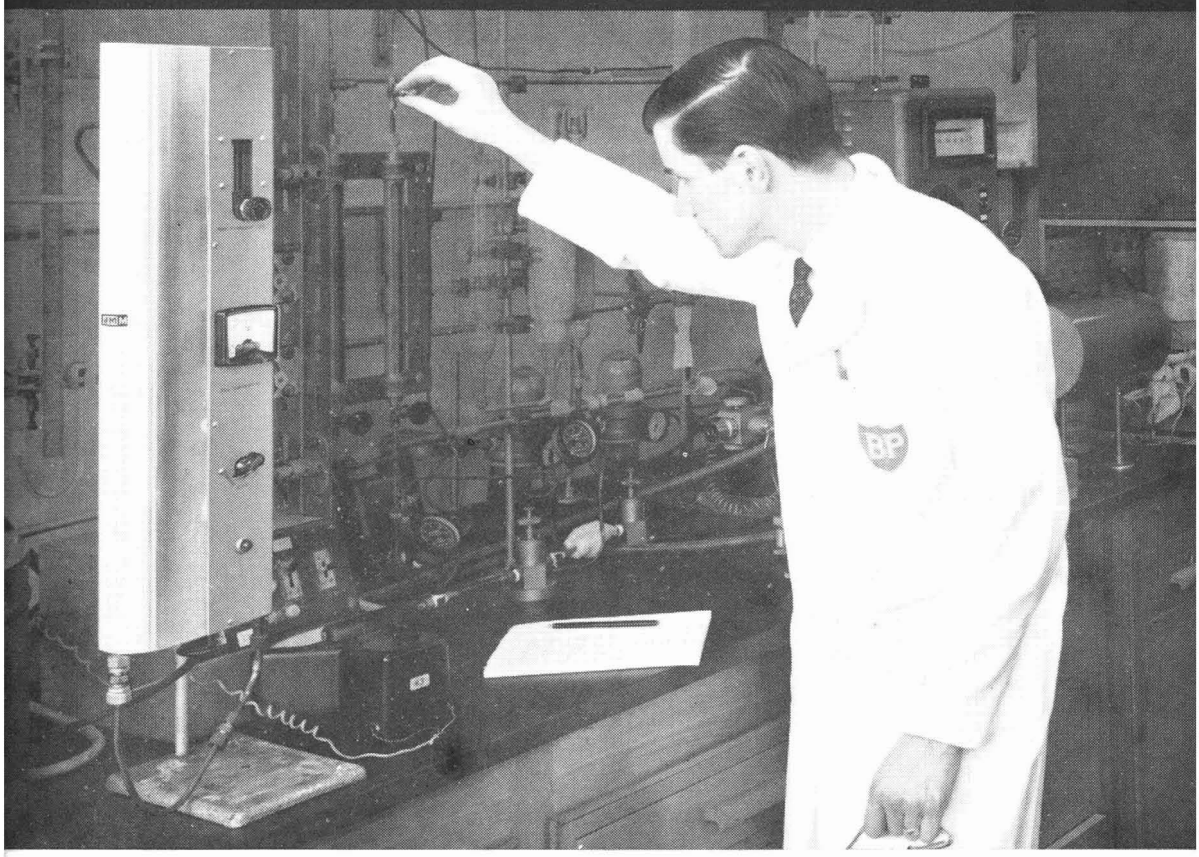
_____ A1

HILGER & WATTS

HILGER & WATTS LTD
98 St. Pancras Way London NW1
Tel: Gulliver 5636
INSTRUMENTS FOR ANALYSIS AND QUALITY CONTROL IN INDUSTRY AND RESEARCH

Why take chances with the purity of the hydrogen you use?

–ultra high purity is so easy to produce!



More and more of the country's leading research and development laboratories are finding distinct advantages in using JMM Hydrogen Diffusion Units, for they afford the simplest and cheapest means of obtaining hydrogen of the highest possible degree of purity.

Simply by installing one of these units you will never again need to worry about the effect on your processes of the impurities present in some commercial grades of hydrogen.

A.1 Diffusion Units, producing up to 40 litres per hour of ultra-pure hydrogen, are ideal for general laboratory work, including catalytic hydrogenations and gas chromatography.

The higher output rate of A.5 Diffusion Units, 150 litres per hour, makes them particularly suitable for use in small-scale production units in such applications as the heat treatment of metals and semiconductor materials.

Operation of the units is simple—they only require connecting to a 220/250 volt a.c. supply, via a power regulator, and to a hydrogen cylinder. Each unit is 22 in. (56 cm) high, weighs under 15 lb (7 kg) and is supplied on a retort stand.

Write today for full technical information.

JMM Hydrogen Diffusion Units

JOHNSON MATTHEY METALS LIMITED

81 Hatton Garden, London, E.C.1 **Telephone: 01-405 6989** **Telex: 21465**

Vittoria Street, Birmingham 1 Telephone: 021-236 8004

75 Eyre Street, Sheffield 1 Telephone: 23121

DIF 771

Volume 92. No. 1095, Pages 347 - 410 June, 1967

THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

CONTENTS

Printed and Published for the Society for Analytical Chemistry by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. B. Attrill, 14 Belgrave Square, London, S.W.1.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47 Gresham Street, London, E.C.2.

Entered at Second Class at New York, U.S.A., Post Office.