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Summaries of Papers in this Issue

Metal - Metallochromic Indicator Complexes as Acid - Base Indicators

When the formation of metal - metallochromic indicator complexes is accompanied by the release of more than one proton per indicator molecule complexed, the colour change at the end-point is sharper than that of a conventional acid - base indicator, when only one proton is released. The larger the number of protons released per indicator molecule, the sharper the colour change. Because complexes of different stabilities are formed between metals and metallochromic indicators, one such indicator can provide a whole range of pH indicators by variation of the metal ion used. The pK value for the indicator system is a conditional constant, and can be lowered by increasing the amount of free metal ion present or by increasing the concentration of the indicator complex. The nature of the metal - indicator complex can be deduced from a study of the absorbance - pH curve for different ratios of metal ion and indicator concentrations. Either component of the indicator system can be determined by potentiometric (pH) titration with the other. Limitations are imposed by the necessity to avoid the presence of species that form more stable complexes with the metal ion, by hydrolysis, and by the acid - base characteristics of the free indicator. In the most favourable instances it is possible to achieve a complete colour change over 0·5 pH unit or less. The pK value is relatively indifferent to changes in ionic strength.

ROBERT A. CHALMERS and FRANK I. MILLER
Department of Chemistry, University of Aberdeen, Old Aberdeen, Scotland.

Analyst, 1971, **96**, 97–105.

The Influence of the Formation of Metal - Indicator Complexes of the M_2I Species on the Accuracy of Complexometric Micro Titrations with Photometric End-point Determination

When micromolar amounts of tervalent and quadrivalent metals such as bismuth, thorium and iron are bound to triphenylmethane dyes and titrated complexometrically, large systematic deviations are found.

There are reasons to suppose that M_2I type complexes are formed. It is shown that if M_2I predominates in solution, the large systematic deviations can be accounted for theoretically. The use of azo-type indicators, such as PAR, TAR and TAN, gives more accurate results.

J. KRAGTEN
Natuurkundig Laboratorium, University of Amsterdam, The Netherlands.

Analyst, 1971, **96**, 106–109.

The Use of Partial-pressure Mass Spectrometry in the Study of the Thermal Desorption and Oxidation of Carbon and Graphite

A partial-pressure mass-spectrometer system is described for measuring thermally desorbed species from solid surfaces. The system evaluates not only the ratio of masses present in the gaseous phase but also relates the specific mass (mg g^{-1} of solid) desorbed or decomposed during thermal treatment. The extension of the method to include oxidation studies is also described.

A study has been made of the initial evolution of gases from graphitic and non-graphitic carbons. These range in properties from a ground graphite of specific surface 103 $m^2 g^{-1}$ to a nuclear-type graphite of 0·6 $m^2 g^{-1}$. A study of a non-graphitic carbon, saran charcoal, of molecular-sieve type is also included.

The formation of surface oxide on a clean surface at low pressures is evaluated together with the resultant thermal decomposition of the surface oxide. The results from this paper together with other published work on graphites are reviewed and used to illustrate the application of the results of thermal desorption to oxidation studies on carbons and graphites.

F. E. AUSTIN, J. G. BROWN, J. DOLLIMORE, C. M. FREEDMAN and B. H. HARRISON
Department of Pure and Applied Physics, University of Salford, Salford 5, Lancashire.

Analyst, 1971, **96**, 110–116.

The Determination of Fluorine in Rock Materials by γ-Activation and Radiochemical Separation

A technique is described for the determination of fluorine in rock materials involving irradiation in a source of high energy γ-photons to induce the ^{19}F (γ,n) ^{18}F reaction. Fluorine-18 is then separated from the radioactive matrix by distillation and its activity measured either in the distillate or as precipitated calcium fluoride and compared with that of irradiated calcium fluoride standards. The technique has been applied to the analysis of standard rock materials G1 (638 p.p.m.), W1 (221 p.p.m.), T1 (476 p.p.m.) and to Apollo 11 lunar fines (76 p.p.m.). Precautions are taken to eliminate interferences. The results obtained for the standard rocks are in good agreement with those of conventional methods but disagree with other activation results. The limit of detection of the method is 0·002 μg.

J. S. HISLOP, A. G. PRATCHETT and D. R. WILLIAMS
Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Berks.

Analyst, 1971, **96**, 117–122.

Pyridine-2,3-diol as Metal Indicator in the Chelatometric Determination of Iron(III) with EDTA

Pyridine-2,3-diol can be satisfactorily used as an indicator in the chelatometric determination of iron(III) over the pH range of 1 to 4, the end-point being sharp and distinct. The indicator is effective in the presence of common bivalent metal ions. Interferences from some quadrivalent, tervalent and bivalent metal ions have been prevented by using masking agents, but oxalate and thiocyanate seriously interfere. Borate, tartrate and citrate do not interfere. However, the presence of a large excess of acetate ions must be avoided.

D. P. GOEL and R. P. SINGH
Department of Chemistry, University of Delhi, Delhi-7, India.

Analyst, 1971, **96**, 123–126.

Spectrophotometric Determination of Vanadium with *N*-Benzoyl-*o*-tolylhydroxylamine

The method prescribed by Majumdar and Das for the spectrophotometric determination of vanadium(V) with *N*-benzoyl-*o*-tolylhydroxylamine has been re-examined. The validity of the method originally reported is supported by the further results presented.

A. K. MAJUMDAR and S. K. BHOWAL
Department of Inorganic and Analytical Chemistry, Jadavpur University, Calcutta-32, India.

Analyst, 1971, **96**, 127–129.

The Analysis of Tin Stabilisers Used in Poly(vinyl chloride) Compositions

Schemes are described for the analysis of commercial tin stabilisers commonly used in poly(vinyl chloride) compositions. Methods are given for the chemical breakdown of a sample and subsequent separation and identification of the breakdown products, which may include dialkyltin oxides, alcohols, thiols, carboxylic acids and thioacids. Separations are carried out by precipitation, solvent extraction and thin-layer chromatographic techniques, and the components identified by gas-chromatographic, infrared, nuclear magnetic resonance and mass-spectroscopic methods.

Manual and potentiometric titration procedures for quantitative examination together with recommended methods for the determination of tin are either described in this paper or in the literature cited, and methods for identification of additives and procedures for identification of tin stabilisers in the presence of excess of plasticiser, by means of column chromatography and ion-exchange procedures, are also included.

J. UDRIS
Research Department, Imperial Chemical Industries Limited, Plastics Division, Welwyn Garden City, Herts.

Analyst, 1971, **96**, 130–139.

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Metal - Metallochromic Indicator Complexes as Acid - Base Indicators*

BY ROBERT A. CHALMERS AND FRANK I. MILLER

(Department of Chemistry, University of Aberdeen, Old Aberdeen, Scotland)

When the formation of metal - metallochromic indicator complexes is accompanied by the release of more than one proton per indicator molecule complexed, the colour change at the end-point is sharper than that of a conventional acid - base indicator, when only one proton is released. The larger the number of protons released per indicator molecule, the sharper the colour change. Because complexes of different stabilities are formed between metals and metallochromic indicators, one such indicator can provide a whole range of pH indicators by variation of the metal ion used. The pK value for the indicator system is a conditional constant, and can be lowered by increasing the amount of free metal ion present or by increasing the concentration of the indicator complex. The nature of the metal - indicator complex can be deduced from a study of the absorbance - pH curve for different ratios of metal ion and indicator concentrations. Either component of the indicator system can be determined by potentiometric (pH) titration with the other. Limitations are imposed by the necessity to avoid the presence of species that form more stable complexes with the metal ion, by hydrolysis, and by the acid - base characteristics of the free indicator. In the most favourable instances it is possible to achieve a complete colour change over 0·5 pH unit or less. The pK value is relatively indifferent to changes in ionic strength.

ALTHOUGH the theory of metal - metallochromic indicator complexes is well understood (see, for example, Schwarzenbach and Flaschka[1]), and the possible use of these indicators as acid - base indicators is implicit in the theory, they do not seem to have been applied hitherto for this purpose. The idea of investigating this possibility arose when demonstrating how to adjust the pH of a solution of bismuth and lead for consecutive EDTA titrations of the two components. The bismuth must be titrated at pH 1, at which lead does not interfere, and the pH is then raised to about 5 for titration of the lead. The sample solution must be acidic (or the bismuth would hydrolyse) but its pH is not known. If xylenol orange is added to the sample solution and a purple colour is produced, then the pH must be above 1, but its true value will not be known. If acid is now added until the yellow colour of the acid form of the free indicator is produced, the pH will be below 1 (as when the yellow colour appears in the first place). It follows that simple dilution with water to ten times the original volume will raise the pH value by 1 unit, and a colour change will be seen during the dilution. If the original solution was more acidic than 0·1 N, then the metal - indicator will not appear until the solution is sufficiently diluted to raise the pH by the necessary amount. After titration of the bismuth a buffer is used to raise the pH value to 5 for the lead titration, and a colour change again occurs when a particular pH value is reached (dilution to, say, 10 litres is not a practical proposition). Investigation has shown that the colour change occurs almost completely during a 2-fold dilution of the solution, corresponding to a change of 0·3 pH unit.

In a conventional acid - base indicator, the colour change arises because the release of one proton from the indicator molecule results in a species of different colour from the original. The result is that for the transformed fraction of the indicator to change from 10 to 90 per cent. (the limits usually regarded as necessitated by the response of the human eye to mixtures of colours) the pH value must be increased by about 2 units. The form of the equilibrium

* Paper presented at a meeting of the Society for Analytical Chemistry, Birmingham, May 7th and 8th, 1970.

equation shows that if n protons could be released at the same time, the pH interval for the complete colour change would be $2/n$. This is precisely the situation with many metal - metallochromic indicator complexes, especially those which have iminodiacetic acid groups substituted into an acid - base indicator molecule. The purpose of this paper is to explore the possibilities of that situation.

Theory

The general equation for a metal reacting with a metallochromic indicator to form a complex containing only one ligand molecule may be written—

$$m\mathrm{M}^{n+} + \mathrm{H}_x\mathrm{I} \rightleftharpoons \mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}^{m(n-h)+} + mh\mathrm{H}^+ \quad \ldots \quad (1)$$

$$K = \frac{[\mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}^{(n-h)+}][\mathrm{H}^+]^{mh}}{[\mathrm{M}^{n+}]^m[\mathrm{H}_x\mathrm{I}]} \quad \ldots \quad (2)$$

which allows for the possibility of formation of polynuclear and protonated complexes (h is the number of protons released per metal ion complexed). It follows that (omitting charges for simplicity)—

$$\log K = \log \frac{[\mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}]}{[\mathrm{H}_x\mathrm{I}]} - m \log [\mathrm{M}] - mh\,\mathrm{pH} \quad \ldots \quad (3)$$

When the transformed fraction (TF) of the indicator is 50 per cent.—

$$\mathrm{TF} = \frac{[\mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}]}{[\mathrm{H}_x\mathrm{I}] + [\mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}]} \quad \ldots \quad (4)$$

so

$$[\mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}] = [\mathrm{H}_x\mathrm{I}] \quad \ldots \quad (5)$$

and

$$\mathrm{pH}_{50\%} = \mathrm{p}K_{\mathrm{MI}} = -\frac{1}{mh}\log K - \frac{1}{h}\log [\mathrm{M}] \quad \ldots \quad (6)$$

At 10 per cent. TF

$$9[\mathrm{M}_m\mathrm{H}_{(x-mh)}\mathrm{I}] = [\mathrm{H}_x\mathrm{I}] \quad \ldots \quad (7)$$

and

$$\mathrm{pH}_{10\%} = \mathrm{p}K_{\mathrm{MI}} + \frac{1}{mh}\log\frac{1}{9}$$

$$\sim \mathrm{p}K_{\mathrm{MI}} - \frac{1}{mh} \quad \ldots \quad (8)$$

Similarly,

$$\mathrm{pH}_{90\%} \sim \mathrm{p}K_{\mathrm{MI}} + \frac{1}{mh} \quad \ldots \quad (9)$$

For fixed initial concentrations of metal ion (C_{M}) and indicator (C_{I}), with ratio $r = C_{\mathrm{M}}/C_{\mathrm{I}}$ and the condition $C_{\mathrm{M}} \geqslant mC_{\mathrm{I}}$, if the stability constant of the indicator complex is reasonably high and all m metal ions are complexed simultaneously

$$[\mathrm{M}]_{50\%} = C_{\mathrm{M}} - 0{\cdot}5\,mC_{\mathrm{I}} = (r - 0{\cdot}5\,m)C_{\mathrm{I}} \quad \ldots \quad (10)$$

$$[\mathrm{M}]_{10\%} = C_{\mathrm{M}} - 0{\cdot}1\,mC_{\mathrm{I}} = (r - 0{\cdot}1\,m)C_{\mathrm{I}} \quad \ldots \quad (11)$$

and

$$[\mathrm{M}]_{90\%} = C_{\mathrm{M}} - 0{\cdot}9\,mC_{\mathrm{I}} = (r - 0{\cdot}9\,m)C_{\mathrm{I}} \quad \ldots \quad (12)$$

Therefore

$$\mathrm{pH}_{10\%} = -\frac{1}{mh}\log K - \frac{1}{h}\log (r - 0{\cdot}1\,m)C_{\mathrm{I}} - \frac{1}{mh} \quad \ldots \quad (13)$$

and

$$\mathrm{pH}_{90\%} = -\frac{1}{mh}\log K - \frac{1}{h}\log (r - 0{\cdot}9\,m)C_{\mathrm{I}} + \frac{1}{mh} \quad \ldots \quad (14)$$

Therefore

$$\mathrm{pH}_{90\%} - \mathrm{pH}_{10\%} = \Delta\mathrm{pH}_{90/10} = \frac{2}{mh} - \frac{1}{h}\log \frac{(r - 0{\cdot}9\,m)}{(r - 0{\cdot}1\,m)} \quad \ldots \quad (15)$$

TABLE I

EFFECT OF VARIOUS PARAMETERS ON

$\Delta pH_{90/10}$ AND ΔpK_{MI}

		Δ pH$_{90/10}$		Δ pK_{MI}‡
h	r*	$m = 1$	$m = 2$†	$m = 1$
1	1	2·95	—	0
	2	2·24	1·95	−0·3
	3	2·15	1·37	−0·4
	4	2·10	1·24	−0·6
	5	2·08	1·18	−0·7
	10	2·04	1·08	−1·0
Limiting value		2·0	1·0	
2	1	1·48	—	0
	2	1·12	0·98	$-0{\cdot}1_5$
	3	1·07	0·68	−0·2
	4	1·05	0·62	−0·3
	5	1·04	0·59	$-0{\cdot}3_5$
	10	1·02	0·54	−0·5
Limiting value		1·0	0·5	
3	1	0·99	—	0
	2	0·75	0·65	−0·1
	3	0·72	0·45	$-0{\cdot}1_5$
	4	0·70	0·41	−0·2
	5	0·70	0·39	$-0{\cdot}2_5$
	10	0·68	0·36	−0·3
Limiting value		0·67	0·33	

* $C_I = 1 \times 10^{-5}$ M.
† Calculated on the assumption that both metal ions are complexed simultaneously.
‡ $\Delta pK_{MI} = pK_{MI(r=x)} - pK_{MI(r=1)}$

$$= -\frac{1}{mh}\log K - \frac{1}{h}\log [M]_{(r=x)} + \frac{1}{mh}\log K + \frac{1}{h}\log [M]_{(r=1)}$$

$$= \frac{1}{h}[pM_{(r=x)} - pM_{(r=1)}] = \Delta pM/h.$$

For a fixed value of m, the difference in $\Delta pH_{90/10}$ at two r values can be calculated ($\delta\Delta$pH), and comparison of experimental and calculated values of $\delta\Delta$pH will permit evaluation of m and h. The values of $\Delta pH_{90/10}$ are shown in Table I. If an absorbance - pH curve is plotted for $r = 1$ and 10, it will at once be obvious from the $pH_{10\%}$ and $pH_{90\%}$ values (*i.e.*, the pH values for absorbances equal to 10 and 90 per cent. of the maximum net absorbance) whether m is 1 or 2 and whether the complex is protonated or not. It also follows that addition of an increasing excess of metal ions narrows the transition interval, with a limiting value of 1·0 for $m = 1$ and $h = 2$, and of 0·7 for $m = 1$ and $h = 3$; the limits become narrower if a binuclear complex is formed with both metal ions complexed simultaneously. If there is successive formation of MI and M_2I, the latter will be formed at a higher pH value and the pH - absorbance curve will show two steps (*cf.* Fig. 1).

A graph of pK_{MI} *versus* r shows that the former decreases as the latter increases (which is to be expected because of the increased competition by metal ion for the indicator anion). This effect permits fine control of the pH range covered by the indicator colour change. The concentration ratio of metal – indicator complex to free indicator is constant at pH equal to pK_{MI} (equation 6) but the free metal-ion concentration will depend on the total indicator concentration and the concentration ratio (r); because the value of pK_{MI} for a given concentration ratio decreases with increasing free metal-ion concentration (see Table I), it follows that pK_{MI} should decrease with increasing total metal - indicator complex concentration. The situation in which complexes of the type M_mI_i are formed, where i is greater than unity, has not been considered, for two reasons: it is not amenable to simple logarithmic treatment, and if i is greater than m the indicators would be no better than conventional acid - base indicators.

Applications and Limitations

By varying the metal and metallochromic indicator, and their concentrations, indicators can be obtained for almost any pH range. Limitations are imposed, however, by the nature of the system. The solution to be tested must not contain ligands that form more stable complexes than the indicator with the metal ion, nor must it contain metal ions that form more stable complexes with the indicator than the metal ion chosen unless the difference in stability is small enough for the change in pK to be tolerated. The metal - indicator complex must be sufficiently stable to avoid hydrolytic effects.

To determine the pH value of a system, the metallochromic indicator is added first, and then different metal ions are added in order of increasing stability of metal - indicator complex, until one is found which gives a colour change. The pH value can then be assessed more accurately by adding a known amount of free indicator and "titrating" with metal ion (see Table I). For example, for $C_I = 1 \times 10^{-5}$ M, the zinc - xylenol orange complex is orange at pH 4·5 and $r = 1$, but the orange colour does not form until $r = 17$ if the pH value is 3·9.

For acid - base titrations the appropriate end-point pH is selected and the corresponding indicator - metal combination is used. For titration of polyprotic acids it is best to begin with the normal salt and titrate it with a strong acid, by using a single metallochromic indicator and a series of metals in order of increasing stability of indicator complexes. More than one metallochromic indicator can be used, provided the colours of the free indicators and their metal complexes do not give a masking effect. If the successive dissociation constants of the acid are sufficiently well separated, it should be possible to titrate stepwise in this way.

When metal ion is added to free metallochromic indicator, the pH value will decrease rapidly as protons are released, then gradually as r increases. A graph of pH against volume of metal solution added gives two straight lines that intersect at the equivalence point. Either component of the indicator system can therefore be determined by titration with the other, provided the initial pH value of the indicator solution is about 7 and the metal ion does not undergo strongly hydrolytic interaction with water. For example, xylenol orange can be determined by potentiometric titration with zinc sulphate solution (Fig. 4).

Experimental

Stock solutions (2×10^{-4} M) of metal ions and indicators were made from the purest materials readily available. No attempt was made to purify the indicators, because those indicators prepared by condensation of iminodiacetic acid groups on to conventional acid - base indicators would function equally well whether they contained one or two iminodiacetic acid groups (see Results and Discussion below). Suitable volumes were mixed and diluted to give an initial indicator concentration of about 10^{-5} M, and these solutions were titrated with acid or base, pH and spectrophotometric measurements being made to determine the pH range over which the indicators changed colour, and the sharpness of the change.

Results and Discussion

Indicator complexes—

The pH ranges for traverse of the acid - base end-point are shown in Tables II to VI for various metallochromic indicator - metal combinations. The ranges are those for the colour changes shown, which were judged visually. The volume of base required to effect the colour change is also shown, and serves as an estimate of the sharpness of the transition. The total volume of solution used was usually 20 ml.

Figs. 1 and 2 show the pH - absorbance curves for the xylenol orange complexes of bismuth, lead and zinc, the bismuth curve being calculated from results given by Kotrlý and Vřeštál[2] for other purposes, and the other two being obtained experimentally. The curves for zinc clearly show evidence of stepwise formation of the zinc - xylenol orange and $(zinc)_2$ - xylenol orange complexes shown to exist by Murakami, Yoshino and Harasawa.[3] The effect of changing the concentration ratio of metal to indicator is shown for zinc and lead, and the $\Delta pH_{90/10}$ values are $1{\cdot}0 \pm 0{\cdot}1$ for all four curves, which are in agreement with those in Table I for a mononuclear unprotonated complex of a bivalent metal. The effect of increasing the charge on the metal ion and hence the number of protons released per indicator molecule completed is clearly shown by comparison of the bismuth and zinc curves.

TABLE II

METAL - PYROGALLOL RED COMPLEXES

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$ M	pH		V/ml
			Orange	Pink	
Nickel	1·03	0·98	5·5	5·9	$0·04_0$
Bismuth	1·00	0·98	1·7	2·0	—
Lead	1·05	0·98	5·0	5·3	$0·03_4$
Calcium	1·06	0·98	4·2	4·6	$0·08_0$
Zinc	1·13	0·98	5·7	6·1	$0·05_4$
Cadmium	1·06	0·98	5·9	6·1	$0·02_0$
Copper	1·05	0·98	4·5	4·8	$0·03_8$
Molybdenum	1·08	0·98	3·2	3·9	0·80
Aluminium	1·02	0·98	3·5	3·9	0·37
Thallium	1·07	0·98	4·9	5·6	$0·05_4$

C_M is the concentration of metal before titration.
C_I is the concentration of indicator before titration.
V is the volume of 10^{-2} M sodium hydroxide required to traverse the end-point for 10 ml of titration solution.

TABLE III

METAL - METHYLTHYMOL BLUE COMPLEXES

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$ M	pH			V/ml
			Yellow	Almost colourless	Blue	
Lead	1·05	0·99	4·2	4·6	4·8	$0·02_2$
Zinc	1·13	0·99	4·7	4·9	5·1	$0·00_9$
Copper	1·05	0·99	4·5	5·1	5·4	$0·01_0$
Bismuth	1·00	0·99	1·2	1·3	1·5	—

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$ M	pH				V/ml
			Yellow	Very pale green	Green	Blue	
Cadmium	1·06	0·99	5·5	5·5	6·3	6·7	$0·02_0$
Nickel	1·03	0·99	4·0	4·8	5·1	5·7	$0·01_2$

TABLE IV

METAL - ALIZARIN RED S COMPLEXES

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$ M	pH		V/ml
			Almost colourless	Pink	
Iron	1·05	0·99	5·2	5·5	$0·02_2$
Nickel	1·03	0·99	5·6	5·8	$0·01_0$
Zinc	1·13	0·99	5·7	6·1	$0·02_5$
Copper	1·05	0·99	4·6	5·0	$0·02_2$
Lead	1·05	0·99	5·2	5·6	$0·01_8$

TABLE V

METAL - XYLENOL ORANGE COMPLEXES

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$ M	pH			V_1/ml	V_2/ml
			Yellow	Orange	Red		
Copper	1·05	0·92	4·5	4·7	5·0	$0·02_0$	$0·01_7$
Cadmium	1·06	0·92	5·5	5·6	5·7	$0·00_7$	$0·00_8$
Nickel	1·03	0·92	4·2	4·5	4·8	$0·07_0$	$0·03_0$
Lead	1·05	0·92	3·6	3·7	3·9	0·12	$0·05_7$
Zinc	1·13	0·92	4·4	4·5	4·7	$0·01_8$	$0·02_0$
Bismuth	1·00	0·92	1·1	1·2	1·3	—	—

V_1 is the volume of 10^{-2} M sodium hydroxide required to traverse the yellow to orange end-point.
V_2 is the volume of 10^{-2} M sodium hydroxide required to traverse the orange to red end-point.

TABLE VI

METAL - PYROCATECHOL VIOLET COMPLEXES

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$M	pH: Yellow	pH: Pale green	pH: Blue	V_1/ml	V_2/ml
Bismuth	1·0	0·93	—	5·3	6·2	—	$0·06_2$
Calcium	1·06	0·93	4·7	5·2	5·5	$0·08_0$	$0·03_8$
Copper	1·05	0·93	5·2	5·4	6·0	$0·01_0$	$0·03_0$
Lead	1·05	0·93	5·8	5·9	6·7	$0·01_2$	$0·04_8$
Aluminium	1·02	0·93	—	4·8	5·2	—	$0·07_5$
Zinc	1·13	0·93	5·9	6·5	6·9	$0·02_0$	$0·05^1$

V_1 is the volume of 10^{-2} M sodium hydroxide required to traverse the yellow to pale green end-point.
V_2 is the volume of 10^{-2} M sodium hydroxide required to traverse the pale green to blue end-point.

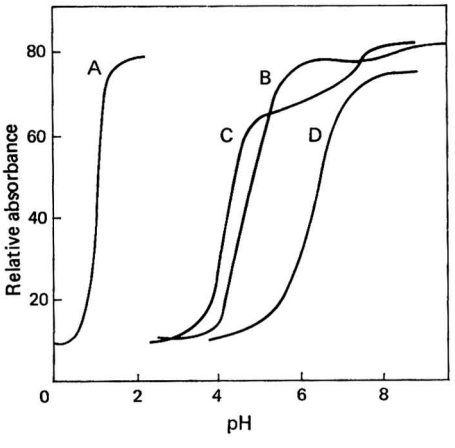

Fig. 1. Absorbance *versus* pH curves for A, bismuth - xylenol orange; B, zinc - xylenol orange, $r = 2$; C, zinc - xylenol orange, $r = 11$; and D, a conventional acid - base indicator

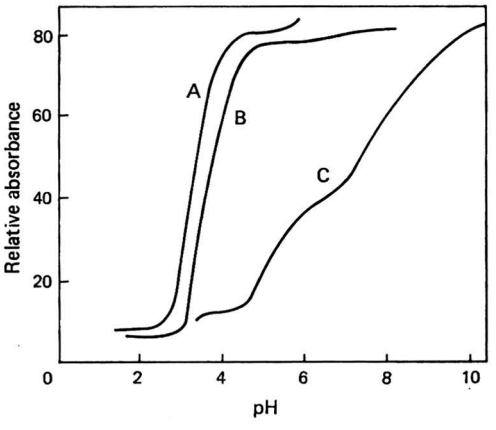

Fig. 2. Absorbance *versus* pH curves for A, lead - xylenol orange, $r = 10$; B, lead - xylenol orange, $r = 2$; and C, free xylenol orange

The results shown in Fig. 3 for the pyrogallol red complexes of copper(II) and nickel indicate that protonated complexes are formed.

Some curves for free indicators and a conventional acid - base indicator are given for comparison purposes.

Table VII shows the effect of increasing the total indicator concentration, and the results are in accordance with theory (Table I) provided the decrease in the pH value resulting from the excess of metal ions is also taken into account (*cf.* results 4 and 5 for nickel).

EFFECT OF CARBON DIOXIDE—

Carbon dioxide should have no effect on the pH indicated, provided that the latter is below 5, for the same reason that it does not affect conventional indicators that change colour at pH values below 5. The results in Table VIII, for pyrogallol red complexes, show that this is generally true. At higher pH values the free metal-ion concentration could be reduced by complex formation with hydrogen carbonate, carbonate or hydroxide ions, thus giving a positive shift in pK_{MI} as exemplified in Table VIII.

DETERMINATION OF THE COMPONENTS OF THE INDICATOR COMPLEX—

Fig. 4 shows the pH - titration curve for xylenol orange titrated with a zinc solution. The end-point obtained by extrapolation is within 1 per cent. of the equivalence point.

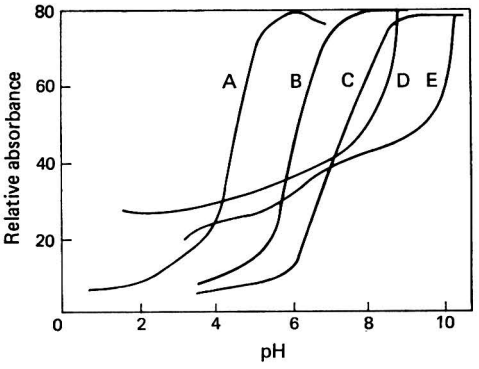

Fig. 3. Absorbance *versus* pH curves for A, copper - pyrogallol red (pyrogallol red concentration $0{\cdot}98 \times 10^{-5}$ M, $r = 3{\cdot}2$); B, nickel - pyrogallol red (pyrogallol red concentration 5×10^{-5} M, $r = 3{\cdot}8$); C, nickel - pyrogallol red (pyrogallol red concentration $0{\cdot}98 \times 10^{-5}$ M, $r = 3{\cdot}2$); D, free pyrogallol red, concentration $0{\cdot}98 \times 10^{-5}$ M; and E, free pyrogallol red, concentration 5×10^{-5} M

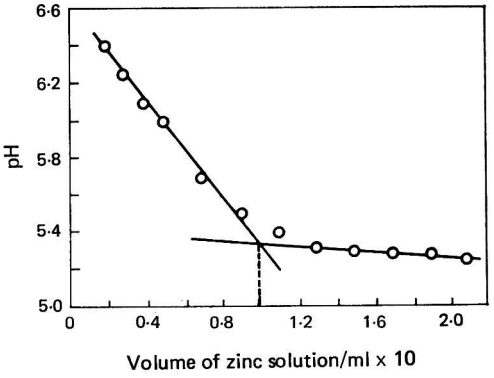

Fig. 4. Titration of xylenol orange with zinc: 2 ml of xylenol orange of concentration 2×10^{-4} M with 4×10^{-3} M zinc sulphate solution

Table VII

Effect of C_{MI} on pK_{MI} of pyrogallol red

Metal	$C_M/10^{-5}$ M	$C_I/10^{-5}$ M	$C_{MI}/10^{-5}$ M	pK_{MI}
Nickel	6·2	5	5·0	6·4, 6·4
	4·13	3·92	3·92	6·77, 6·71
	3·10	2·94	2·94	7·05, 7·01
	2·48	2·0	2·0	7·12, 7·10
	2·07	1·96	1·96	7·17, 7·25
	1·24	1·0	1·0	7·63, 7·50, 7·57
Copper	5·27	4·90	4·90	4·16, 4·06
	4·22	3·92	3·92	4·24, 4·24
	3·16	2·94	2·94	4·32, 4·36
	2·11	1·96	1·96	4·51, 4·47
	1·05	0·98	0·98	4·69, 4·73, 4·69

Table VIII

Effect of carbon dioxide on metal - pyrogallol red complexes

Metal	Carbon dioxide present pH		Carbon dioxide boiled out pH	
	Orange	Pink	Orange	Pink
Nickel	5·5	5·95	5·0	5·4
Calcium	4·2	4·6	4·1	4·5
Bismuth	1·7	2·0	1·8	2·1
Lead	5·0	5·35	5·05	5·35
Zinc	5·7	6·1	5·5	5·8
Cadmium	5·9	6·1	5·3	5·8
Iron	3·8	4·3	4·1	4·5
Copper	4·5	4·83	4·1	4·4
Aluminium	3·5	3·9	3·5	4·0

EFFECT OF IONIC STRENGTH—

In the theoretical part of this paper the effect of ionic strength on pK_{MI} was not considered, but from inspection of equation (2) it appears likely that the influence of change of ionic strength on the activities of the various species involved would to a large extent be self-compensating.

[Apply the Debye-Hückel approximation to equation (2) for $h = n$; the effects on $\{M^{n+}\}$ and $\{H^+\}$ will largely cancel out, and so will those on the activities of the two forms of the indicator.] Tests were made with the ionic strength varied by addition of potassium chloride, and for the zinc - xylenol orange complex the value of pK_{MI} was 4·67, 4·75, 4·82 and 4·83 for added salt concentrations of 0, 0·1, 0·5 and 0·85 M, respectively, thus confirming the predictions made.

DETERMINATION OF STABILITY CONSTANTS—

If we write

$$M^{2+} + H_xI \rightleftharpoons MH_{(x-2)}I + 2H^+ \quad .. \quad .. \quad .. \quad (16)$$

then

$$K_{eq} = \frac{[MH_{(x-2)}I]\,[H^+]^2}{[M^{2+}][H_xI]} \quad .. \quad .. \quad .. \quad .. \quad (17)$$

The stability constant of the complex is given by

$$K_M^{MH_{(x-2)}I} = \frac{[MH_{(x-2)}I]}{[M^{2+}][H_{(x-2)}I^{2-}]} \quad .. \quad .. \quad .. \quad .. \quad (18)$$

and because for the species $[H_{(x-2)}I^{2-}]$ we can write

$$K_{H_{(x-2)}I}^{H_xI} = \frac{[H_xI]}{[H_{(x-2)}I^{2-}][H^+]^2} \quad .. \quad .. \quad .. \quad .. \quad (19)$$

then

$$K_M^{MH_{(x-2)}I} = K_{eq} \cdot K_{H_{(x-2)}I}^{H_xI} \quad .. \quad .. \quad .. \quad .. \quad (20)$$

As the value of $K_{H_{(x-2)}}^{H_xI}$ can usually be found in the literature and K_{eq} can be calculated experimentally from equations (6) and (15), the stability constant for the metal - metallochromic indicator complex can be obtained. As examples, the stability constants for the lead and zinc complexes of xylenol orange were determined. For xylenol orange in the pH range of these indicator complexes, $\log K_{H_{(x-2)}I}^{H_xI}$ is given[4] as 9·6. The results obtained are given in Table IX.

TABLE IX

DETERMINATION OF STABILITY CONSTANTS

Metal	r	pK_{MI}	$[M^{2+}]$ at pK_{MI}, 10^{-5} M	$\log K_{eq}$	$\log K_M^{MH_{(x-2)}I}$
Zinc	2·27	4·45	1·77	−4·14	5·46
	11·35	4·07	10·85	−4·18	5·43
Lead	2·09	3·70	1·59	−2·60	7·00
	10·45	3·30	9·95	−2·60	7·00

SINGLY AND DOUBLY SUBSTITUTED INDICATORS—

It is known that in the preparation of such indicators as xylenol orange and methylthymol blue condensation of two iminodiacetic acid groups on to the parent acid - base indicator may not have occurred, and the singly substituted products, known as "semi-xylenol orange," *etc.*, can still act as metallochromic indicators but without the facility to form binuclear complexes of the type M_2L. For the present purposes it is immaterial whether there is contamination with the "semi" product, provided the doubly substituted product gives stepwise formation of ML and ML_2, as there will be the same number of protons released per molecule of L in each step and in the "semi" reaction. If the stability constants for

the "semi" and "full" complexes of the metal are fairly similar, there will be no overall effect on the pK_{MI} of the complex, and if they are different then the more stable of the two will determine the pH of colour change in a titration from low to high pH and the less stable will do so for titration in the reverse direction, provided that the molar absorptivities of the complexes are similar and there is at least about 20 to 30 per cent. of the minor component present in the mixture.

We thank Dr. I. L. Marr for helpful discussion throughout the work.

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The Influence of the Formation of Metal-Indicator Complexes of the M_2I Species on the Accuracy of Complexometric Micro Titrations with Photometric End-point Determination*

By J. KRAGTEN

(*Natuurkundig Laboratorium, University of Amsterdam, The Netherlands*)

When micromolar amounts of tervalent and quadrivalent metals such as bismuth, thorium and iron are bound to triphenylmethane dyes and titrated complexometrically, large systematic deviations are found.

There are reasons to suppose that M_2I type complexes are formed. It is shown that if M_2I predominates in solution, the large systematic deviations can be accounted for theoretically. The use of azo-type indicators, such as PAR, TAR and TAN, gives more accurate results.

In a previous paper[1] the complexometric micro titration of metal ions in the presence of an approximately equivalent amount of indicator is described for the situation in which a 1:1 complex is formed between the metal M and the indicator I.

In practice azo dyes and triphenylmethane dyes are regularly used. These indicators form MI and MI_2 complexes, although M_2I complexes are also known.

Kotrlý[2] has already considered the stepwise formation of metal - indicator complexes of the type MI_m (m = 1, 2 and 3) and the influence of a system of these three simultaneously occurring species on the shape of the titration curve.

If MI_m predominates in solution, the combination of Kotrlý's consideration[2] and the theoretical considerations in our previous paper[1] leads to the conclusion that the systematic error can be kept below 0·5 per cent. if the following titration conditions are satisfied—

$$\log Z_{MI_m} = \log C_I^m K_{MI_m} > 1 \quad .. \quad .. \quad .. \quad .. \quad (1)$$

and

$$\log \frac{Z_M}{Z_{MI_m}} = \log \left(\frac{C_M K_{ML}}{Z_{MI_m}} \right) > 3{\cdot}5 \quad .. \quad .. \quad .. \quad (2)$$

When both MI and MI_2 occur in solution, the systematic error can be kept below 0·5 per cent. if the above conditions are satisfied for the complex that predominates, because immediately before the equivalence point the concentration of both MI and MI_2, and therefore the absorbance of the solution, will be linearly dependent on the volume of added titrant.

In a few instances the accuracy was unexpectedly poor, namely when triphenylmethane dyes, *e.g.*, xylenol orange (XO), pyrocatechol violet (PCV) and chromazurol S (CAS), were used in combination with tervalent and quadrivalent metals. It is known that the combinations iron - CAS, thorium - XO, bismuth - XO, thorium - PCV and bismuth - PCV[3,4,5] form M_2I complexes. For this reason the influence of the formation of M_2I complexes on the shape of the titration curve has been investigated. It will be shown that the systematic deviations found in practice can be explained theoretically.

Terminology and symbols used follow present practice in this field of investigation.[3] It is assumed that M_2I is the only complex formed between M and I. C_M, C_L and C_I are the total concentrations of M, L and I, respectively, present in any form; $m_2i = \frac{[M_2I]}{C_M}$ equals

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"reduced" concentration; the absorbance is a linear function of m_2i. The reduced concentrations, mi, mi_2, m, i, l and ml, are defined similarly. The relative amount of indicator, β, is equal to $\left(\frac{C_I}{C_M}\right)$. The mathematical treatment is simplified by using reduced concentrations and by using the dimensionless conditional constants Z instead of the conditional constants K.

Now,

$$Z_M = C_M K_{ML} = \left(\frac{ml}{m \times l}\right) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

and

$$Z_{M_2I} = C_I^2 K_{M_2I} = \beta^2 \left(\frac{m_2i}{m^2 \times i}\right) \quad .. \quad .. \quad .. \quad (4)$$

The following mass balances hold—

$$l = m + ml + 2m_2i \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

$$\beta = i + m_2i \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$f = l + ml = \left(1 + \frac{1}{m} \times \frac{1}{Z_M}\right) ml \quad .. \quad .. \quad .. \quad (7)$$

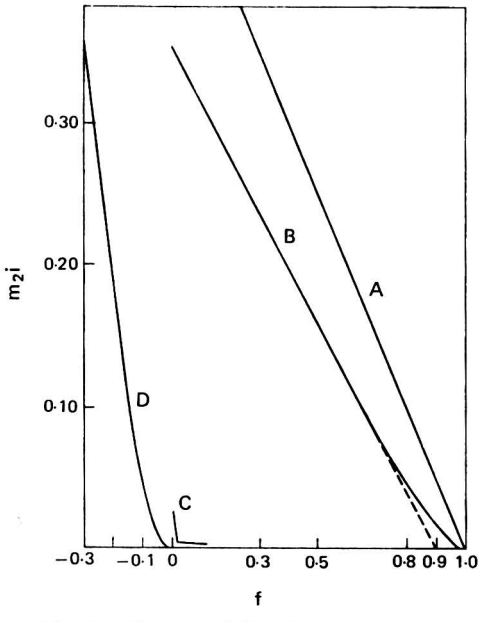

Fig. 1. Theoretical titration curve constructed from $\beta = C_I/C_M = 2$, log $Z_M = 8$ and $Z_{M_2I} = 10$. The term f_3 is represented ten times enlarged. f is found by adding f_1, f_2 and f_3 in the "horizontal" direction: A, $f_1 = (1 - 2m_2i)$; B, $f = f_1 + f_2 + f_3$; C, $f_3 \approx 1$; and D, $f_2 = (-m)$

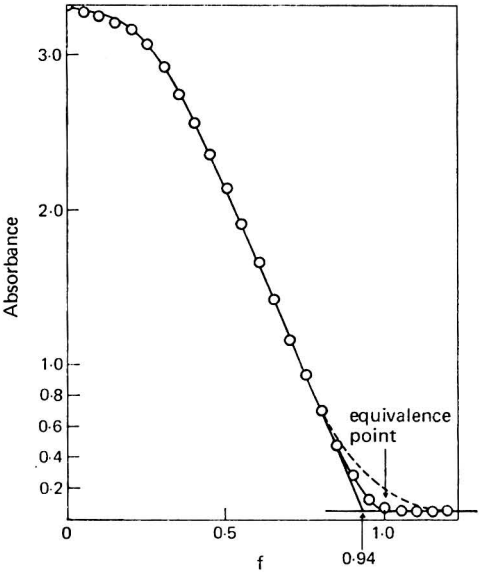

Fig. 2. Practical titration graph of thorium with EDTA. The thorium solution has been standardised microgravimetrically and by back titration with lead and cerium (xylenol orange indicator). The dotted line is the curve found with commercial quality xylenol orange (85 per cent. pure). The drawn line has been found with chromatographically purified xylenol orange. The difference is presumably caused by the presence of semi-xylenol orange (SXO) in the commercial quality XO. SXO may be assumed to form only a 1:1 complex with thorium. For convenience the absorbance is plotted *versus* f. Thorium taken = 0·507 μmole; β = 0·5; pH = 2·5. The pH adjustments have been made with monochloroacetic acid to prevent the formation of thorium hydroxide, which dissolves slowly

From these equations the titration parameter f is found as a function of m_2i—

$$f = (1 - 2m_2i) - \left\{ \frac{m_2i}{\left(\frac{Z_{M_2I}}{\beta}\right)\left(1 - \frac{m_2i}{\beta}\right)} \right\}^{\frac{1}{2}} + \left(\frac{1}{m_2i}\right)^{\frac{1}{2}} \frac{(Z_{M_2I}/\beta)^{\frac{1}{2}}}{Z_M} \quad \ldots \quad (8)$$

$$= f_1 + f_2 + f_3.$$

Comparison of the analogous relationships for the cases in which solely 1 : 1 or 1 : 2 complexes are formed ($Z_{MI} = C_I K_{MI}$ and $Z_{MI_2} = C_I^2 K_{MI_2}$)—

for 1 : 1 complexes $$f = (1 - mi) - \frac{mi}{Z_{MI}(1 - mi/\beta)} + \frac{1}{mi}\left(\frac{Z_{MI}}{Z_M}\right) \quad \ldots \quad (9)$$

and for 1 : 2 complexes

$$f = (1 - mi_2) - \frac{mi_2}{Z_{MI_2}\left(1 - \frac{2mi_2}{\beta}\right)^2} + \frac{1}{mi_2}\left(\frac{Z_{MI_2}}{Z_M}\right) \quad \ldots \quad (10)$$

shows that a marked difference in the corresponding f_2 terms exists. The importance of this for the shape of the titration curve and for the accuracy of the determination is shown for the titration of iron(III) with EDTA, with CAS as indicator.

If C_{Fe} is approximately 10^{-4} (1 μmole in a 10-ml cell) and $\log Z_{Fe-EDTA} = 10$ (pH = 3; $\log K = 14$), from the pH dependence of the colour formed between iron and CAS it can be assumed that at pH 3 the Z value of the metal - indicator complex is between 10 and 50 regardless of whether M_2I, MI or MI_2 is formed in the solution.

When Fe - CAS or Fe - CAS_2 is formed in the solution, substitution of the data shows that equations (1) and (2) are satisfied. The systematic deviation should be negligible ($\simeq 10^{-7}$) and the end-point is easily found from the intersection of the linear portions of the titration graph [see equations (9) and (10)].

In practice, however, the change in the slope of the titration graph is not very abrupt, and the systematic deviations appear to be between 2 and 4 per cent. This can be explained by assuming that Fe_2 - CAS has been formed. Substitution of the above Z values in equation (8) shows that the term f_3 can be neglected up to the equivalence point ($m_2i > 10^{-6}$). The dissociation of ML, therefore, makes no contribution to the curvature of the graph near the equivalence point. f_2 does not depend linearly upon m_2i near the equivalence point. It makes a negative parabolic contribution to the m_2i - f curve for small values of m_2i (see Fig. 1).

In practical titration graphs the curvature near the equivalence point cannot be distinguished from a possible curvature originating from f_3. Therefore, the curvature will normally be attributed to the dissociation of ML or to the interference of impurities from the commercial quality indicators (see broken line in Fig. 2).

If the end-point is determined by extrapolation, or if the intersection of a tangent with the f-axis is taken as the end-point, a systematic deviation will occur (see Figs. 1 and 2).

To give an idea of the magnitude of this systematic deviation the tangent procedure has been adopted for the determination of the end-point. A value for Δf_e can be calculated from equation (8), and from the equation for the tangent at the point $[(m_2i)_o; f_o]$,

$$f_e = f_o - (m_2i)_o \left(\frac{df}{d(m_2i)}\right)_o \quad \ldots \quad (11)$$

we get

$$\Delta f_e = -\frac{1}{2}\left\{\frac{(m_2i)_o}{Z_{M_2I}/\beta}\right\}^{\frac{1}{2}} + \frac{1}{2Z_M}\left\{\frac{Z_{M_2I}/\beta}{(m_2i)_o}\right\}^{\frac{1}{2}} \quad \ldots \quad (12)$$

In general the tangent is drawn at $m_2i = 0{\cdot}1$.[1] Furthermore, the amount of indicator is approximately equal to the amount of metal, so $\beta \simeq 1$. Substitution together with $\log Z_M = 10$ and $Z_{M_2I} = 30$ gives the theoretical result $\Delta f_e = -3$ per cent. for the Fe_2 - CAS - EDTA titration.

In practice systematic deviations of this magnitude are found for other combinations also, *e.g.*, bismuth and thorium with XO and PCV (Table I). For comparison, also, some practical results are given for titrations with azo dyes.

TABLE I

Metal	Indicator	Mole taken	β	pH	Δf_e, per cent.
	Triphenylmethane dyes				
Iron	CAS	1·0	1·0	2·8	− 2
		0·5	1·0	3·0	− 4
Thorium	XO	0·2	1·0	2·5	− 5
		0·5	0·5	2·5	− 6
		0·5	0·5	2·5	− 8
		0·5	1·0	4·0	− 1
		0·5	1·0	4·0	− 1·5
Bismuth	XO	0·5	2·0	2·6	− 3
		0·5	1·0	2·6	−10
Thorium	PCV	0·5	1·0	4·0	− 3
		0·5	1·0	4·0	− 3
	Azo dyes				
Bismuth	PAR	1·0	1·0	1·6	+ 0·5
		1·0	1·0	1·6	+ 0·4
		2·0	0·5	1·8	− 0·1
	TAN	0·5	1·0	1·6	− 0·9
		1·0	3·0	1·5	+ 0·1
		2·0	3·0	1·6	+ 0·5
		2·0	3·0	1·8	+ 0·1
Thorium	PAR	0·5	1·0	3·5	+ 0·8
		0·5	2·0	3·5	+ 0·3
		0·5	1·5	3·5	− 0·7

A detailed comparison with the theory is impossible because of a lack of knowledge of accurate values of K_{M_2I}.

Some transition-point values have been determined experimentally for xylenol orange.[3] The value of Z_{M_2I} can be estimated from these results but the accuracy is too poor for our purposes. From preliminary investigations under titration conditions $Z_{M_2I} = 10 \pm 30$ per cent. was found at pH 2·5 for $(\text{thorium})_2$ - XO; this value largely accounts for the experimentally found systematic deviations.

It can be noted that according to the theory the systematic deviation will be negligible when (Z_{M_2I}/β) is greater than 500. This condition, however, could not be satisfied by increasing the pH, probably because of a simultaneous increase in the side-reaction coefficient $\alpha_{M(OH)}$, which prevents a large increase in K_{M_2I} and Z_{M_1I}.

The purpose of this paper was to seek a theoretical explanation for the large systematic deviations found in practice from the formation of M_2I alone. In practice, mixtures of M_2I, MI and MI_2 can be expected. The absorbance - titrant volume curve depends upon the concentrations of these compounds and their molar absorptivities. It may be expected that when M_2I predominates, large deviations will occur; therefore, the dominant formation of M_2I should be avoided in micro titrations.

The formation of M_2I can be expected for combinations of tervalent and quadrivalent metals with indicators such as PCV, XO, CAS and, presumably, methylthymol blue; these indicators have two distinct groups with which metal bonds can be formed. The use of azo-type indicators, *e.g.*, PAR, TAR and TAN, is preferable in these cases.

I thank Dr. K. Pypers for the chromatographically purified xylenol orange, and Dr. G. den Boef for critically reading the manuscript.

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The Use of Partial-pressure Mass Spectrometry in the Study of the Thermal Desorption and Oxidation of Carbon and Graphite*

BY F. E. AUSTIN, J. G. BROWN, J. DOLLIMORE, C. M. FREEDMAN AND B. H. HARRISON

(*Department of Pure and Applied Physics, University of Salford, Salford 5, Lancashire*)

A partial-pressure mass-spectrometer system is described for measuring thermally desorbed species from solid surfaces. The system evaluates not only the ratio of masses present in the gaseous phase but also relates the specific mass (mg g^{-1} of solid) desorbed or decomposed during thermal treatment. The extension of the method to include oxidation studies is also described.

A study has been made of the initial evolution of gases from graphitic and non-graphitic carbons. These range in properties from a ground graphite of specific surface 103 $m^2 g^{-1}$ to a nuclear-type graphite of 0·6 $m^2 g^{-1}$. A study of a non-graphitic carbon, saran charcoal, of molecular-sieve type is also included.

The formation of surface oxide on a clean surface at low pressures is evaluated together with the resultant thermal decomposition of the surface oxide. The results from this paper together with other published work on graphites are reviewed and used to illustrate the application of the results of thermal desorption to oxidation studies on carbons and graphites.

THERMOGRAVIMETRIC methods have been developed, with quartz springs and vacuum microbalances, to investigate the kinetics of decomposition. In terms of orders of magnitude, however, this is the least sensitive parameter. Measurements of evolved gas pressure provide a sensitivity of at least an order of magnitude greater than that obtained from conventional weight-loss methods. Methods involving total pressure change have produced results of kinetic interest when one gas is evolved.[1,2] The logical extension of this approach is to use a partial-pressure mass spectrometer when multiple evolution occurs and to calibrate the expansion volumes so that conventional weight loss results can still be obtained. The authors have shown that an exact correlation can be established (Table I)[3] between conventional microbalance results and the weight loss calculated from such a calibrated mass spectrometer system.

TABLE I

CUMULATIVE WEIGHT LOSS*; A COMPARISON OF SIMULTANEOUS MASS SPECTROMETER AND MICROBALANCE DATA FOR AN ACHESON GRAPHITE OF 103 $m^2 g^{-1}$ SURFACE AREA

Temperature range	400 °C to 500 °C	500 °C to 600 °C	600 °C to 650 °C	650 °C to 700 °C	700 °C to 750 °C	750 °C to 800 °C	800 °C to 900 °C	900 °C to 950 °C
Carbon monoxide/mg	0·063	0·199	0·335	0·473	0·663	0·823	1·010	1·085
Carbon dioxide/mg	0·052	0·094	0·111	0·121	0·128	0·128	0·128	0·128
Cumulative total/mg g^{-1} (MS10 mass spectrometer)	0·432	1·100	1·675	2·231	2·971	3·573	4·275	4·557
Cumulative total/mg g^{-1} (Cahn RG vacuum microbalance) ..	0·451	1·146	1·705	2·261	3·043	3·610	4·320	4·624

* From sample of weight 0·266 2 g. The oxygen complex was adsorbed on the clean surface at 250 °C and 2 torr oxygen pressure.

* This paper is based on a lecture given by J .Dollimore to the Thermal Analysis Group on November 13th, 1969.

An example of a complex evolved-gas analysis is the thermal oxidation and desorption of carbons and graphites when conventional thermobalance methods often give unreliable results because of the small weight losses involved, so pressure-rise systems have to measure separately both the evolved carbon monoxide and dioxide if the correct value for the amount of carbon burn-off is to be obtained.

The above problem was solved by Walker, Laine and Vastola,[4] who placed a partial-pressure mass spectrometer in a constant-volume reactor system. Their work showed that two reactions occurred, *viz.*, thermal decomposition and subsequent desorption of stable oxygen complex from the surface in which the fractional coverage (θ) of the active surface is reduced with increased temperature; and an oxidation reaction with carbon burn-off from ($1 - \theta$) of the active surface (A_s).

The equation

$$\frac{dP}{dt} = kP\,(1 - \theta)\,A_s \qquad .. \quad .. \quad .. \quad .. \quad (1)$$

has been shown to describe the oxidation kinetics of Graphon at low pressures. An initial transient period occurs with variable θ (surface complex formation) followed by a logarithmic oxidation law with θ constant.

The carbon - oxygen system is of great interest for two reasons. In the first place many aspects of the mechanism are still in doubt,[5] and secondly, the desorption of the surface oxygen complex is a "thermal decomposition" experiment that presents difficulties in interpretation when conventional thermal-analysis systems are used. This paper is concerned with a review of the results of the authors' work on the carbon - oxygen system with the mass-spectrometer method, together with some previously unpublished data on the carbon monoxide and dioxide evolution. The carbons studied are an Acheson graphite (surface area 103 $m^2\,g^{-1}$), a nuclear reactor graphite (surface area 0·60 $m^2\,g^{-1}$) and poly(vinylidene chloride) char (surface area 1000 $m^2\,g^{-1}$). The wide range of surface area and gas evolution involved leads to a consideration of the sensitivity, utility and difficulties in the application of this type of thermal analysis.

Apparatus

The apparatus consisted of a constant-volume reactor system with the gas phase monitored by an A.E.I. MS10 mass spectrometer and has been described in detail elsewhere.[3] The volumes and ion currents were calibrated to enable the conversion of partial pressures to specific mass. The mass spectrometer was connected to the reactor volume by a variable leak valve. This gave a convenient working pressure range of 2 000 mtorr down to 10^{-5} mtorr.

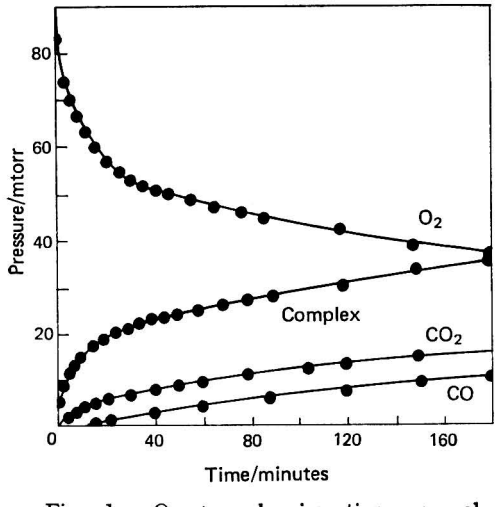

Fig. 1. Oxygen chemisorption on poly-(vinylidene chloride) char at 300 °C

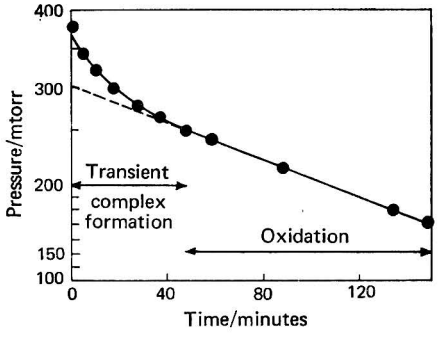

Fig. 2. Oxygen chemisorption on Acheson graphite plotted as log pressure *versus* time at 400 °C

PROCEDURE

Consideration was given to the relationship between the mass of sample and the volume of the reactor in order that the gas evolution would be detectable over the residual pressure. Thermal-desorption studies from carbons require stringent vacuum conditions over a wide range of temperatures, ensuring that any carbon gasification is due solely to decomposition of the surface oxygen complex and not to the presence of residual oxygen in the system. Fortunately an independent check is possible. Fig. 1 shows the pressure - time graph of an oxygen chemisorption on a clean carbon surface (poly(vinylidene chloride) char). The surface oxygen complex as an equivalent (O_2) pressure can be calculated with an oxygen mass balance. If this is followed by a desorption to 950 °C, then the surface oxygen complex can be calculated independently from the total evolution. In the course of a large number of experiments we have come to expect an agreement of approximately 5 per cent. between these values. This is rather an exacting condition and if it is not achieved because of a small leak or, more probably, by de-gassing of the system components, this does not entirely invalidate the results. Oxidation experiments in which the system pressure is of the order of 200 mtorr may not require such careful technique. In thermal desorption, however, residual oxygen is rapidly taken up as additional surface oxygen complex, with the carbon acting as a scavenger in the system. Provided this effect is small the desorption results can still be analysed for mechanisms. The authors consider, however, that this precludes a direct comparison with the preceding chemisorption experiment in which the surface complex was formed.

The actual experimental technique used for the desorption analysis was an isothermal incremental method with the gases removed after each 100 °C step to reduce the possibility of any secondary gas phase and gas - solid reactions.

ACHESON GRAPHITE—

Acheson graphite can be regarded as a plate-like graphite with additional edges produced by grinding. Before cleaning in a high vacuum to 950 °C it would have been expected to permit enhanced carbon dioxide evolution because of ground-in defects in the basal plane.[6] The gas evolution from the original surface (0 to 950 °C) had been shown to be a coverage of 10 $m^2 g^{-1}$ for carbon dioxide and 14·8 $m^2 g^{-1}$ for carbon monoxide. Subsequent oxygen chemisorption on this clean surface at 250 mtorr and 300 °C followed by thermal desorption confirmed the reduction in carbon dioxide evolution, as the carbon dioxide coverage was 1·3 $m^2 g^{-1}$ and the carbon monoxide 36·9 $m^2 g^{-1}$. The above calculations were based on a carbon atom occupying an area of 0·83 nm^2 on the edge plane.[4]

It is interesting to compare this result with that obtained by an independent method. A simple model of graphite is to associate the active sites with an edge-plane area.[4,7] An investigation of the X-ray line profiles by using the 110 and 004 diffraction lines gives values of l_a and l_c, the dimensions of the crystallites parallel and perpendicular to the layers, respectively. These can be converted to an edge-plane surface of 46 $m^2 g^{-1}$ and a basal-plane surface of 54 $m^2 g^{-1}$. Neither the X-ray diffraction photographs nor the nitrogen BET surface are sensitive to thermal treatment of the surface. Although the mass spectrometer method can detect both small and large changes in the active surface area it is not yet possible to put this on an absolute basis as the coverage also varies with the initial temperature and pressure of chemisorption. Saturation coverage, or total active surface, has been taken arbitrarily for Graphon as that occurring at 300 °C and 500 mtorr. The theoretical concepts are not understood and at the moment saturation coverage for a carbon can only be determined by amassing sufficient experimental results around these temperatures and pressures.

The final problem concerning the interpretation of the Acheson thermal analysis results is one that is common to a number of carbons and graphites, including poly(vinylidene chloride) char. The chemisorption or depletion of oxygen follows a graph similar to Fig. 1 in that, when it is plotted as the log of the pressure *versus* time, there is a linear oxidation region in accordance with equation (1) (Fig. 2). The desorption results from the same chemisorptions, however, give linear (Elovich) $\log\left(\frac{t + t_0}{t_0}\right)$ *versus* pressure graphs.[8] This presents

the mechanistic problem that the theory of chemisorption assumes a constant activation energy while the desorption shows a variation of activation energy with coverage. There is some evidence that this variation is not even a simple linear function as has been suggested for Graphon[9] and poly(vinylidene chloride) char.[10]

To obtain such graphs of activation energy against coverage, the isothermal pressure data are used to fit an *n*th degree Chebebysev[8] polynominal equation to give continuous pressure *versus* time kinetic curves. Suitable curves were found by using variable values of *n* on a computer programme. Usually *n* was in the range 6 to 12. The same programme is used to provide the rate data, which are plotted as log rate *versus* accumulative desorption. A small extrapolation of the often linear curves permits the application of the Arrhenius equation at one value of coverage (θ). The use of a number of temperature intervals then makes the plotting of a graph of activation energy *versus* coverage possible. At the moment the temperature intervals of 100 °C are not entirely satisfactory for this calculation and a linear temperature rise may offer a more complete solution. An already difficult problem is complicated by the fact that both the carbon monoxide and carbon dioxide desorptions independently follow the Elovich equation. For Acheson graphite the activation energy *versus* coverage functions are not identical; the carbon dioxide function is linear while the carbon monoxide function is clearly non-linear.[8]

NUCLEAR-REACTOR GRAPHITE—

The problem with thermal analysis data on this material is that the gas evolution from a surface of 0·6 $m^2 g^{-1}$ is so small that it is on the limit of the most sensitive vacuum microbalances. For the mass spectrometer method this means a conversion to high vacuum baking techniques to reduce the residual gas pressure to less than 1 μtorr. Table II shows the cumulative desorption from a nuclear graphite in $\mu g\ g^{-1}$. The number of significant figures is as yet uncertain and it will require many more results to estimate an error for the smallest

TABLE II

CUMULATIVE THERMAL DESORPTION FROM ORIGINAL NUCLEAR GRAPHITE

	Quantities per gram of sample/μg					
Temperature range/°C	Hydrogen	Carbon monoxide	Carbon dioxide	Water	Nitrogen	Hydrocarbons
0 to 200	0·04	0·57	5·66	1·08	1·58	0·31
0 to 400	0·10	6·75	14·98	2·45	5·33	1·12
0 to 600	0·55	8·25	48·69	2·45	7·62	2·01
0 to 800	1·27	14·74	54·19	2·45	8·09	2·01
0 to 900	1·78	20·12	56·34	2·45	8·66	2·01

gas evolutions. Three general points are of interest. The proportion of active surface is high, possibly as much as 30 per cent. of the total surface. This means that these graphites are of low reactivity because of the reduced specific surface and its limited accessibility rather than of the perfection of the crystalline graphite. This is confirmed by an X-ray analysis, which gives an apparent edgeplane surface of the order of 50 $m^2 g^{-1}$. It follows that most of this must be completely inaccessible to the gas phase. The graphs of oxygen chemisorption at low temperatures are no longer logarithmic, but can be plotted as linear pressure *versus* $\sqrt{}$time. The reactivity has apparently moved out of the region of control by surface chemical reactivity. The most likely rate-controlling step is a diffusion mechanism.[11,12]

POLY(VINYLIDENE CHLORIDE) CHAR—

By analysis methods similar to those already described, the authors have been able to establish some relationship between the mechanisms of adsorption and desorption for this material.[13] The very large surface of 1 000 $m^2 g^{-1}$ makes detailed calculations possible. The transient formation of a surface oxygen complex during a chemisorption has been shown to obey Elovich's adsorption equation. The thermal desorption products carbon monoxide and carbon dioxide have been found individually to obey the Elovich equation, and a linear graph of increasing activation energy of desorption of carbon monoxide *versus* decreasing coverage (θ) of the active sites was obtained. The log oxygen pressure *versus* time graphs still retain the linear oxidation region but equation (1) has to be modified to account for the formation of a surface complex conforming to Elovich's equation.

The data above are all in the region of extensive coverage, *i.e.*, that corresponding to 300 °C and 200 mtorr pressure. A large amount of data concerning the carbon monoxide - carbon dioxide ratio has been collected generally for the carbon - oxygen system and more resulted from this particular investigation, the mass-spectrometer method facilitating its collection. Even so, the rôle of the different carbon monoxide and carbon dioxide evolutions, both in oxidation and desorption, is still not clearly understood in relation to carbon - oxygen kinetics.

For oxidation it has been suggested[14] that the carbon monoxide - carbon dioxide ratio follows an exponential law

$$\text{carbon monoxide/carbon dioxide} = 10^{3.4}\, e^{-12\,400/RT} \quad .. \quad .. \quad .. \quad (2)$$

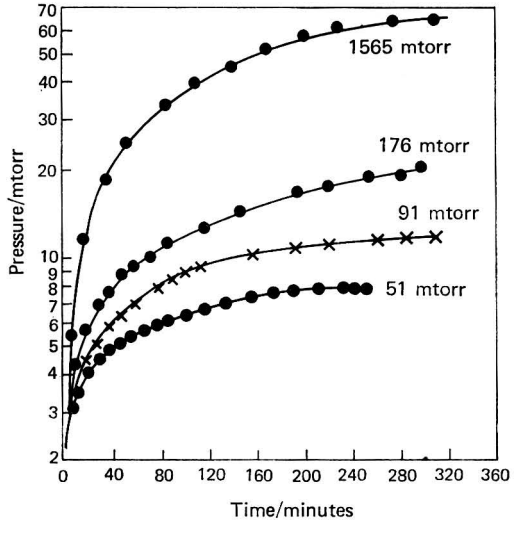

Fig. 3. Graphs of carbon dioxide evolution during the oxidation of poly(vinylidene chloride) char at 300 °C

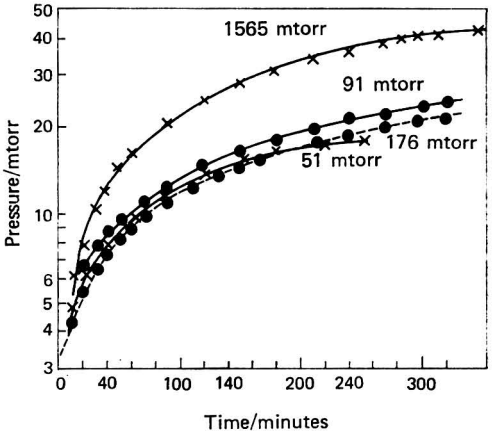

Fig. 4. Graphs of carbon monoxide evolution during the oxidation of poly(vinylidene chloride) char at 300 °C

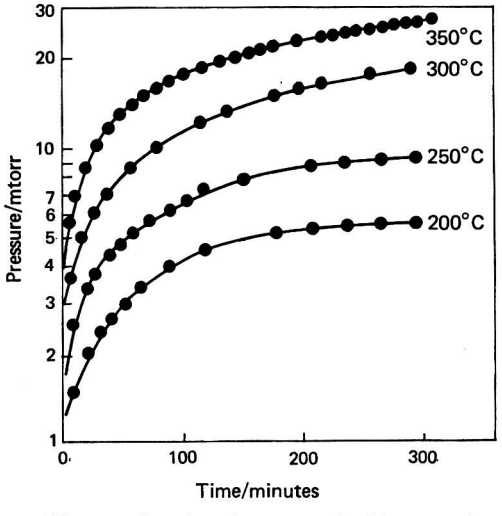

Fig. 5. Graphs of carbon dioxide evolution during the oxidation of poly(vinylidene chloride) char at 172 mtorr initial oxygen pressure

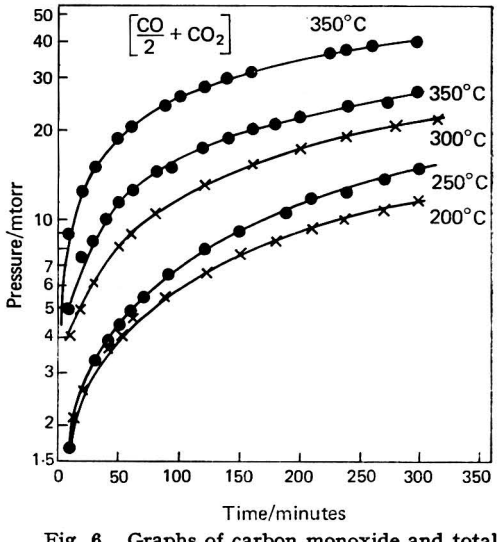

Fig. 6. Graphs of carbon monoxide and total oxygen evolution during the oxidation of poly-(vinylidene chloride) char at 172 mtorr oxygen pressure

At the low pressures used in the present and similar mass-spectrometer[4] experiments the secondary reaction $C + CO_2 \rightarrow 2CO$ can be reduced, such that it is of minor importance. In this event the meaning of the carbon - carbon dioxide ratio as an equilibrium constant is not clear. It may be related to the distribution of carbon monoxide and carbon dioxide active sites. Equation (2) can only be a general trend, as an inspection of Figs. 3 and 4 shows that the oxygen pressure has only a small effect on the carbon monoxide evolution but alters the carbon dioxide evolution markedly. These figures show the carbon dioxide and carbon monoxide evolution during chemisorption at 300 °C for various pressures. The reason for the log pressure *versus* time graph is that the chemisorption at 176, 91 and 51 mtorr all gave linear log pressure *versus* time graphs for oxidation. Equation (1) can be used to strictly define two regions; complex formation *plus* oxidation, followed by oxidation at constant θ. A mass conservation of oxygen must then apply for oxygen depletion and carbon monoxide and dioxide evolution. The simplest solution to this condition would be a linear logarithmic evolution law but Figs. 3 and 4 show that this could only apply approximately and after excessive time. The same difficulty occurs with graphs plotted at different temperatures as shown in Figs. 5 and 6. These are at a pressure of 172 $\pm$ 4 mtorr. For completeness, a graph of total oxygen $\left(\frac{CO}{2} + CO_2\right)$ is included in Fig. 6. The above result indicates some of the difficulties in the current theories for the carbon - oxygen reaction. It also shows that gravimetric work depending on carbon burn-off will give a different solution from that derived from oxygen depletion results. The authors think that this may be due to the complexity of the mathematics of concurrent gas depletion and evolution equations in a closed volume, and that this problem requires further clarification.

Conclusion

Because current oxidation theories are not entirely satisfactory there is room for speculation with regard to this problem. The formation of the complex has been shown to follow the Elovich[15] adsorption equation and Fig. 7 shows an attempt to describe the concurrent carbon monoxide evolution in a similar way. The initial period may well be in agreement with Elovich's findings. The second linear region and others at greater time are rather speculative as it is very easy to construct apparent linear portions on a curve of decreasing slope.

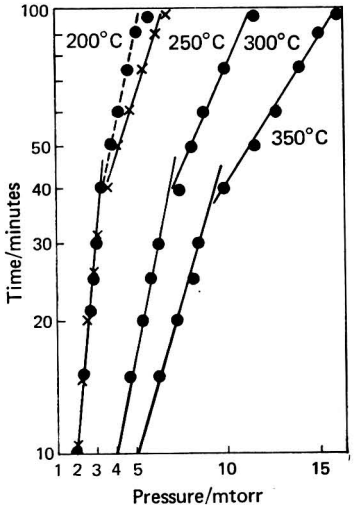

Fig. 7. Elovich log time *versus* pressure graphs for carbon monoxide evolution during the oxidation of poly(vinylidene chloride) char at 172 mtorr initial oxygen pressure

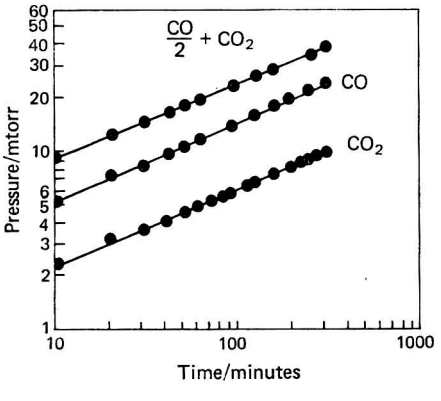

Fig. 8. Log pressure *versus* log time graphs for chemisorption at 350 °C and 172 mtorr initial oxygen pressure (poly-(vinylidene chloride) char)

The curves in Figs. 4 to 6 can be made linear by plotting log pressure *versus* log time. This is shown in Fig. 8 for the 350 °C system kinetics, the slopes of which are approximately 0·5, indicating that $p \propto t^{\frac{1}{2}}$. This was the criterion associated with diffusion for the nuclear graphite. It is difficult, however, to see for poly(vinylidene chloride) char how complex formation and its subsequent thermal desorption conform to the Elovich equation while the carbon monoxide and carbon dioxide evolutions during oxidation are diffusion controlled.

The use of the constant volume pressure fall method has been criticised in the past. It has been suggested that the kinetics are a function of the system. The correct analysis, however, should account for this and such systems have led to many advances in the understanding of heterogeneous catalysis.[16] Chemisorption at an initial oxygen pressure of 1 565 mtorr, as shown in Fig. 3, is an interesting example. In this case the pressure is so high that oxygen depletion is too small to be measured. However, the gradual decrease in the rate of carbon monoxide and carbon dioxide evolution shows that saturation is being achieved and that, despite the abundance of oxygen present, the oxidation reaction is decaying rapidly. The decay rate is greater than the usual logarithmic decay associated with what is often called "Gaedes equation" for pumping speed in a constant volume, where the gas is removed at a constant volume rate. This is analogous to Langmuir adsorption models where $\frac{1}{p} \cdot \frac{dp}{dt}$ is constant.

The intention of this paper is to illustrate the data that can be obtained on a complex reaction system by the use of the partial-pressure mass-spectrometer method. The fact that the carbon - oxygen system is an as yet unresolved problem adds further point to the investigation.

B. H. Harrison acknowledges the financial support of a research studentship from the Gas Council and J. Dollimore acknowledges the opportunity and invitation to present this paper to the Thermal Analysis Group of the Society for Analytical Chemistry on November 13th, 1969. As this was also the occasion of Dr. D. Dollimore's becoming the Chairman of the Thermal Analysis Group, J. Dollimore also acknowledges the interest shown and advice given by his brother on this subject over many years.

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The Determination of Fluorine in Rock Materials by γ-Activation and Radiochemical Separation

BY J. S. HISLOP, A. G. PRATCHETT AND D. R. WILLIAMS
(*Analytical Sciences Division, Atomic Energy Research Establishment, Harwell, Berks.*)

A technique is described for the determination of fluorine in rock materials involving irradiation in a source of high energy γ-photons to induce the ^{19}F (γ,n) ^{18}F reaction. Fluorine-18 is then separated from the radioactive matrix by distillation and its activity measured either in the distillate or as precipitated calcium fluoride and compared with that of irradiated calcium fluoride standards. The technique has been applied to the analysis of standard rock materials G1 (638 p.p.m.), W1 (221 p.p.m.), T1 (476 p.p.m.) and to Apollo 11 lunar fines (76 p.p.m.). Precautions are taken to eliminate interferences. The results obtained for the standard rocks are in good agreement with those of conventional methods but disagree with other activation results. The limit of detection of the method is 0·002 μg.

SEVERAL methods have been developed for the determination of fluorine in rocks. These may be conveniently classified into chemical and activation techniques. Chemical methods, particularly those involving the spectrophotometric measurement of the coloured complex of fluorine with zirconium - Eriochrome cyanine R[1,2] can be extremely sensitive but are susceptible to contamination errors unless rigorous precautions are taken. Activation methods on the other hand are much less susceptible to contamination errors and are independent of reagent blanks. Radiometric methods for the determination of fluorine have been reviewed by Foreman.[3]

Jeffery and Bakes[4] have proposed a non-destructive technique involving fast-neutron activation in which the ^{19}F (n,α) ^{16}N reaction is used, but because of the high limit of detection, *viz.*, 0·5 per cent. of calcium fluoride, this technique is only applicable to fluorine ores and concentrates. Thermal-neutron activation to give fluorine-20 has also been used by Mapper at A.E.R.E., Harwell, but the short half-life (11·2 s) of this nuclide and interference from the ^{28}Si (n,p) ^{28}Al reaction restricts its application. On the other hand, activation by γ-photons permits the determination of fluorine to be made via the ^{19}F (γ,n) ^{18}F reaction. Fluorine-18 is a pure β^+-emitter and its non-destructive determination in a rock matrix, except in a few exceptional cases, for example, in the absence of titanium-45 (β^+, half-life 3·1 hours), is likely to be difficult. This technique, by using a Betatron, has recently been reported by Kosta and Siunecko[5] for the non-destructive analysis for fluorine in a variety of other matrices. Carpenter[6] claims that this technique is applicable to samples of minerals from marine and terrestrial sediments. Fluorine-18 produced by the ^{19}F (n,2n) ^{18}F reaction has been used to determine fluorine non-destructively in fluoro-organic materials.[7]

The half-life of fluorine-18 (110 minutes) is sufficiently long to enable it to be separated chemically from the irradiated matrix. Wilkniss, Skinner and Cheek have used this technique following γ-activation for the determination of fluorine in synthetic rain water[8] by distillation of fluorine-18, and in sea water[9] by adsorption of fluorine-18 on calcium sulphate. The distillation technique has also been reported by Reed[10] and Reed and Jovanovic[11] for the determination of fluorine in meteorites and rock material. This technique has, however, produced significantly higher results for the analysis of standard rock materials than those reported by more conventional methods. The work reported in this paper is based on radiochemical separation of fluorine-18 from γ-irradiated rock matrices by using a modification of Willard and Winter's distillation technique, and efforts have been made to eliminate several possible sources of error in the technique reported by Reed,[10] particularly with regard to interferences and standards.

INTERFERENCES—

Fluorine-18 is not produced solely from fluorine and it is necessary to consider alternative reactions by which it can be formed. Several reactions may occur during γ-activation, mainly ^{20}Ne (γ,d) and (γ,np), ^{21}Ne (γ,t) and (γ,nd), ^{22}Ne (γ,tn) and ^{23}Na (γ,nα), together with a considerable number involving charged-particle activation, which include ^{16}O (α,d) and (^{3}He,p) and ^{18}O (p,n). Because of the extremely low concentration of neon present in rock material, interference from this element is likely to be small. Similarly the flux of charged particles during γ-irradiation is also likely to be low. Nevertheless, complete dismissal of charged-particle interference may not be justified. Wilkniss[12] has investigated the production of low levels of fluorine-18 activity from γ-irradiation of pure water and has shown that it may be produced by the ^{18}O (p,n) ^{18}F reaction, the protons resulting from the ^{16}O (γ,p) ^{15}N reaction. As rock matrices are complex this or similar reactions may occur, but their effect would be expected to be reduced the lower the energy of γ-radiation used.

Similarly fluorine-18 may be produced by both the ^{19}F (γ,n) ^{18}F and ^{19}F (n,2n) ^{18}F reactions, and thus the possibility of interference exists should a higher fast-neutron flux be present in the sample than in the standard resulting from the presence of an element with high γ,n cross-section in the former but not in the latter. The relative specific activities of the two reactions under the experimental conditions used, however, suggest that this interference will be small.

The most significant source of fluorine-18 interference in rock materials is that from ^{23}Na (γ,nα), bearing in mind the high sodium concentration in many rocks. There are two methods of overcoming this interference: (*a*) irradiation can be carried out with γ-radiation with maximum energy below 20·9 MeV, the threshold for the ^{23}Na (γ,nα) reaction, and above 10·4 MeV, the threshold for the ^{19}F (γ,n) reaction; (*b*) alternatively, irradiation can be performed at an energy higher than the threshold energy for the ^{23}Na (γ,nα) reaction, but with the inclusion of sodium standards from which a correction can be made for sodium interference when the sodium concentration of the sample is known.

With the Harwell 45-MeV electron linear accelerator it is extremely difficult to operate reliably at an energy as low as 20 MeV for long periods of time, consequently a conveniently low energy (23 to 25 MeV) was used and the sodium correction procedure adopted. Certain samples, particularly those with high sodium content, were irradiated by using the 17-MeV electron accelerator (Linac) at Harwell's Wantage Research Laboratory, which eliminated sodium interference in these analyses.

EXPERIMENTAL

IRRADIATION—

About 50 to 100 mg of material were packaged in an aluminium sample container in double aluminium foil cups, 6 mm in diameter, which were sandwiched between two standards of Optran pure grade calcium fluoride and two standards of AnalaR sodium sulphate. The total volume occupied by the standards and sample was a right cylinder 6 mm in diameter and 0·5 cm long. The samples were irradiated for 30 minutes in the brehmsstrahlung produced by bombarding an air-cooled $\frac{1}{8}$-inch thick tungsten target with electrons of maximum energy of about 23 to 25 MeV by using the Harwell Linac, or electrons of maximum energy 17 MeV by using the Wantage Linac. The Harwell irradiation facility has been described elsewhere.[13] The Wantage Linac, manufactured by Vickers, is a double-section, single klystron machine with energy variable between 5 and 17 MeV. Unlike that of the Harwell Linac the γ-irradiation facility is not a permanent feature of the machine and the tungsten converter and sample container were placed in the dead-ahead electron beam position prior to each irradiation. To ensure homogeneous distribution of flux across the diameter of the samples the sample container was rotated about the axis through the centres of the samples during irradiation. Beam currents, measured on the tungsten converter, of 5 to 10 μA were obtained with the Harwell Linac and 50 to 60 μA with the Wantage Linac. Samples were cooled by air in both instances. Specific activities of fluorine-18 were comparable with the two machines, the lower energy of the Wantage machine, and hence lower activation cross-section, being compensated for by the higher beam currents available.

CHEMICAL SEPARATION—

After irradiation the sample was weighed into a platinum basin, 50 mg of Optran calcium fluoride carrier were added and mixed thoroughly with the sample, and the mixture fused for 2 minutes with 0·7 g of sodium hydroxide. The melt was cooled and extracted with 14 ml of distilled water. The solution was neutralised with 3 ml of 35 per cent. perchloric acid and transferred with 13 ml of water to a single-necked 50-ml distillation flask containing 25 ml of 35 per cent. perchloric acid, 1 ml of silver perchlorate (prepared as in reference 14) and some glass beads. Distillation of fluorine as fluorosilicic acid (H_2SiF_6) was then carried out by using a modification of Kubota's method.[15] This technique, which involves the distillation of the fluorosilicic acid in an apparatus containing a heated (145° C) outer jacket, in which the dilute acid is used as the source of steam, permits high, reproducible recoveries of fluorine in a small volume of distillate to be achieved. Perchloric acid was used in preference to sulphuric or phosphoric acids to simplify yield determinations. A 25-ml volume of distillate was collected in about 1 hour, which was either counted directly in a 50 mm diameter polycarbonate container or the fluoride precipitated as calcium fluoride. Yield determination was carried out in one of two ways: (*i*) if fluorine was counted in the 25 ml of distillate the yield was determined when the activity had decayed, by using the method of Popov and Knudson,[16] which involved the precipitation of fluorine with excess of lanthanum nitrate and determination of the excess of lanthanum with cupferron; and (*ii*) initially, problems were encountered in obtaining a reliable method of precipitating fluorine in a form that could be used directly for counting and for yield determination. That eventually used consisted of co-precipitation of calcium fluoride with calcium carbonate by a method similar to that used by Berzelius.[17] The pH of the 25 ml of distillate was adjusted to 8 by addition of 2 N sodium carbonate and 1 ml added in excess. To the heated solution were then added 10 ml of 0·5 N calcium chloride and, on further heating for 2 to 3 minutes, calcium fluoride - calcium carbonate was precipitated. The precipitate was cooled, centrifuged and washed with 10 ml of hot water. It was then transferred to a platinum basin with ethanol and ignited in an oven at 700° C for 2 minutes. The residue was crushed and calcium carbonate decomposed with 10 ml of 10 per cent. acetic acid. After evaporating the solution to dryness the residue was re-heated on a hot-plate for 15 minutes. The residue was transferred to a filter tube with 10 ml of water and washed with a further 20 ml of water. The filter-paper and contents were then ignited at dull red heat and the residue transferred to a weighed counting tray. The total time required for distillation and preparation of precipitated calcium fluoride sources was about 3 hours.

Experiments with radioactive calcium fluoride showed that yields from distillation were in excess of 85 per cent. and, for distillation *plus* precipitation, in excess of 70 per cent. A similar tracer experiment resulted in no significant loss of fluorine-18 during fusion.

A further tracer experiment also showed that yields obtained by the lanthanum fluoride - cupferron technique were in excellent agreement with those for the radiochemical technique.

SOURCE PREPARATION—

Calcium fluoride standards used when the fluorine-18 from the samples was counted as 25 ml of distillate were dissolved in boric acid - nitric acid solution, a suitable dilution being made up in 25 ml with water and counted in similar geometry to the sample. When samples were counted as precipitated calcium fluoride sources the standards consisted of weighed aliquots (about 250 mg) of a solution of fluorine-18 prepared from the calcium fluoride standards evaporated to dryness on a counting tray with an infrared lamp. To provide maximum β^+-annihilation, and to obtain a reproducible source geometry, both fluorine standards and the sample, precipitated as calcium fluoride, were counted on aluminium trays sandwiched between two discs of copper sheet (100 mg cm^{-2}). Amounts of about 30 mg of sodium sulphate irradiated to monitor ^{23}Na (γ,nα) ^{18}F interference were either diluted and counted in 25 ml of water or counted directly on a counting tray sandwiched between copper sheets as described.

DETECTION OF ACTIVITY—

All distillates or samples precipitated as calcium fluoride were examined with a Laben 512-channel 3 × 3-inch NaI(Tl) γ-ray spectrometer to determine whether activities other than β^+-annihilation radiation were present; in no instance was this found to be the case. The activity of all samples was then followed for at least four half-lives of fluorine-18 by

using a 100-channel 3 × 3-inch NaI(Tl) γ-ray spectrometer incorporating a multi-position sample changer. The area under the 0·51-MeV annihilation full energy peak was then calculated by Covell's method,[18] and decay curves obtained to verify the purity of the calcium fluoride sources.

The peak area values were also analysed by a least squares fitting technique, which enabled accurate determinations of fluorine-18 activity at the end of irradiation, and hence the fluorine concentration of the samples, to be made. In addition, the errors resulting from counting statistics of the samples and standards were calculated.

SENSITIVITY—

Specific activities of fluorine-18 (at end of irradiation) obtained from irradiation of calcium fluoride standards were of the order of 10 to 100 counts s^{-1} μg^{-1} of fluorine with the irradiation and counting conditions already described. The background count on the detector used was about 5 counts s^{-1} in the region of 0·5 MeV. Assuming that samples can be counted for a 2-hour period, 2 hours after irradiation the detection limit for fluorine (giving a fluorine count equivalent to 3σ of the background) is 0·02 to 0·002 μg under the present conditions.

RESULTS

The calculated fluorine concentrations together with the calculated counting errors for individual analyses of three standard rocks and Apollo 11 lunar fines are given in Table I. G1, a granite, and W1, a diabase, are samples of standard rock material issued by the U.S. Geological Survey. T1 is a tonalite issued by the Geological Survey of Tanganyika. A summary of the results calculated for each material is included.

TABLE I

FLUORINE CONCENTRATIONS OF ROCK MATERIALS

Irradiation, MeV	Sample	Calculated interference from sodium, per cent.*	Corrected fluorine concentration, p.p.m. (with counting errors)
23 to 25	W1	13·7	214 ± 9†
23 to 25	W1	16·1	227 ± 9†
23 to 25	W1	3	233 ± 3†
23 to 25	G1	9·5	651 ± 14†
17	G1	—	620 ± 7‡
17	G1	—	617 ± 14‡
17	G1	—	648 ± 10‡
17	G1	—	655 ± 13‡
17	T1	—	496 ± 18‡
17	T1	—	477 ± 5‡
17	T1	—	488 ± 9‡
17	T1	—	474 ± 8‡
17	W1	—	227 ± 2‡
17	W1	—	203 ± 5‡
23 to 25	Lunar fines	1·1	77 ± 1†
23 to 25	Lunar fines	Nil	75 ± 2‡

Summary—

Sample	Mean chlorine concentration	δ	Number of determinations	Coefficient of variation, per cent.
G1	638	18	5	3
W1	221	12	5	6
T1	484	10	4	2
Apollo 11 fines	76	—	2	–

* Based on fluorine-18 activity induced in sodium sulphate and sodium concentration obtained from literature.

† Counted as 25 ml of distillate.

‡ Counted as calcium fluoride.

DISCUSSION

Certain aspects of this work require further comment.

The results obtained at higher irradiation energies and corrected for sodium interference are not significantly different from those obtained at lower energy in the absence of interference, and confirm the validity of the interference correction. This lack of dependence on irradiation energy also indicates that charged-particle reaction interferences are not significant.

TABLE II

SUMMARY OF LITERATURE RESULTS FOR DETERMINATION OF FLUORINE IN STANDARD ROCKS[24,25]

Author	G1	W1	T1	Techniques
Huang and Johns[2] ..	705 ± 92 (3)	208 ± 5 (4)	390 ± 10 (2)	Spectrophotometric
Evans and Sergeant[1] ..	622 ± 8 (5)	228 ± 8 (6)	455 ± 5 (2)	Spectrophotometric
Shapiro[19]	700	200	—	Spectrophotometric
Ingamells[20]	720 (3)	305 (2)	—	Chemical
Jeffery[21]	629 (4)	290 (4)	—	Photometric
Jeffery[21]	627 (4)	290 (4)	—	Titrimetric
Shima[22]	720 ± 70	190 ± 30	—	Colorimetric
Peek and Smith[23] ..	600 (3)	220 (3)	—	Spectrophotometric
Reed and Jovanovic[11] ..	831 ± 66	279 ± 28	—	γ-Activation
		329		
Reed[10]	935 ± 509	426 ± 35	—	γ-Activation
	1153 ± 90	552 ± 24		
	1080 ± 116	—		
	1138 ± 52	—		
	1023	—		
	1050	—		
Present work	638 ± 18(5)	221 ± 12(5)	484 ± 10(4)	γ-Activation

Numbers in brackets indicate number of determinations.

The precision of each determination calculated on the basis of counting statistics alone is significantly less than the standard deviation calculated from several replicate analyses, which is in agreement with our experience of γ-activation analysis and may largely be attributed to uncertainties in the technique used for the calculation of the relative γ-photon flux in the irradiation position of the sample compared with that of the standards. Because of this uncertainty, the accuracy of individual analyses is estimated to be not better than ±10 per cent.

A summary of the values recorded in the literature for the fluorine concentrations of standard rocks is given in Table II. Several features can be observed: the present results are in general agreement with most of the published values, and are in particularly good agreement with those of Evans and Sergeant[1] and Peek and Smith[23]; and there are significant differences between the results of the present work and those of Reed[10] and Reed and Jovanovic[11] who also used a γ-activation technique. Reed and Jovanovic[11] have commented on the differences between the two sets of results that they reported for G1 and W1 and attribute them to the fact that two different aliquots were used. This heterogeneous sampling may also be the reason why their results are higher than those of other workers. The fact that the results of the present work are in general agreement with those obtained with a range of other techniques, in which, presumably, a range of aliquots was used, indicates that γ-activation does not necessarily produce high results for fluorine concentration in rock materials.

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Pyridine-2,3-diol as Metal Indicator in the Chelatometric Determination of Iron(III) with EDTA

BY D. P. GOEL* AND R. P. SINGH
(*Department of Chemistry, University of Delhi, Delhi-7, India*)

Pyridine-2,3-diol can be satisfactorily used as an indicator in the chelatometric determination of iron(III) over the pH range of 1 to 4, the end-point being sharp and distinct. The indicator is effective in the presence of common bivalent metal ions. Interferences from some quadrivalent, tervalent and bivalent metal ions have been prevented by using masking agents, but oxalate and thiocyanate seriously interfere. Borate, tartrate and citrate do not interfere. However, the presence of a large excess of acetate ions must be avoided.

AMONG the indicators[1,2,3,4] that have been used with varying degrees of success for the titrimetric determination of iron(III) are tiron, salicylic acid, thiosalicylic acid, pyrocatechol violet, kojic acid, thiocyanate ions and triphenylmethane dyes. Recently 2- and 6-hydroxy-*m*-toluic acids,[5] 5-hydroxy-*m*-toluic acid,[6] 1-hydroxy-2-naphthoic acid[7] and 3-hydroxy-2-naphthoic acid[8] have also been recommended for the purpose.

During the course of investigations on the spectrophotometric determination of iron,[9] it was observed that the intense colour of the complex of iron with pyridine-2,3-diol (2,3-dihydroxypyridine) is discharged by the addition of EDTA, which indicated that pyridine-2,3-diol might be capable of use as a metal indicator in the complexometric determination of iron. The present investigations were carried out to ascertain its usefulness for this purpose.

EXPERIMENTAL

REAGENTS AND APPARATUS—

Standard iron(III) chloride solution—Prepare a 0·05 M standard solution of iron(III) chloride by dissolving freshly precipitated iron(III) hydroxide in AnalaR concentrated hydrochloric acid and diluting to 1 litre with doubly distilled water. The iron solution was standardised gravimetrically.

Titrant—Prepare a 0·05 M stock solution of analytical-reagent grade EDTA, disodium salt, by dissolving it in doubly distilled water.

Buffer solutions—Prepare 1·0 M stock solutions of hydrochloric acid and anhydrous sodium acetate and use for preparing buffers as required. These chemicals were of AnalaR grade.

Pyridine-2,3-diol indicator—A 0·5 per cent. solution of pyridine-2,3-diol (Aldrich Chemicals Co., U.S.A.) in ethanol.

All other solutions were prepared with analytical-reagent grade chemicals by using doubly distilled water.

A Metrohm pH meter, E-350, was used for pH measurements.

CONDITIONS FOR CHELATOMETRIC TITRATION—

The colour of the iron(III) - pyridine-2,3-diol complex varies from sky blue to red, depending on the degree of acidity, and is sky blue up to a pH of 2·0, above which it changes to purple - red. Thus if titration is carried out at pH below 2·0, the colour change is from blue to colourless (yellowish with large amounts of iron). The colour change at the end-point between pH 2·0 and 4·5 is from purple - red to colourless or yellow, depending on the concentration of iron.

* Present address: St. Stephen's College, Delhi-7, India.

EFFECT OF pH—

Titrations of iron(III) against EDTA were carried out at various pH values. The metal and indicator concentrations were kept constant and the pH was adjusted with sodium acetate - hydrochloric acid buffers of different pH. The results are shown in Table I.

TABLE I

TITRATION OF IRON(III) AT DIFFERENT pH VALUES

Amount of iron(III) taken, 5·585 mg (≡2·0 ml of 0·05 M EDTA); 5 to 10 drops of 0·5 per cent. indicator solution

pH	0·7	1·0	1·5	2·0	2·4	2·8	3·2	3·5	4·0	4·3	4·6
Volume of 0·05 M EDTA required/ml	2·08	2·01	2·00	2·00	2·00	2·00	2·00	2·00	2·01	2·03	2·06

It is evident that satisfactory results are obtained in the pH range of 1·0 to 4·0. Below pH 1·0 the amount of EDTA required is more than that indicated by theory. Higher results are also obtained above pH 4·0 and appear to be caused by the complexing behaviour of acetate ions. In the presence of larger amounts of acetate ions even higher results were obtained. However, the use of acetate cannot be avoided as its use is necessary to prevent the precipitation of iron(III) hydroxide.

CONCENTRATION OF IRON—

Titrations with different amounts of iron were carried out at pH 2·0, with acetate buffer, at which pH the colour intensity is appreciable and most of the bivalent metals do not interfere. Moreover, the effect of acetate ions is negligible.

Results of these titrations showed that when iron is present in high concentrations (higher than 70 mg per 100 ml), detection of the end-point becomes difficult as the iron(III) - EDTA complex imparts a more or less pronounced yellow colour to the solutions. Up to about 70 mg of iron(III) in a total volume of about 100 ml could be fairly accurately determined. With larger amounts of iron (up to 150 mg) the end-point could still be detected to within 0·05 to 0·10 ml of standard 0·05 M EDTA solution, and in such instances is indicated by the disappearance of the last trace of greenish shade, the solution becoming yellow.

AMOUNT OF INDICATOR—

The titrimetric determination of iron(III) was carried out by adding different volumes of 0·5 per cent. indicator solution, and it was found that 5 to 10 drops were sufficient at all pH values between 1·0 and 4·0. Increased amounts of indicator were required in the presence of larger amounts of complexing anions such as tartrate or citrate.

EFFECT OF TEMPERATURE—

It was observed that the titrations could be performed within a fairly wide range of temperatures (10 to 60 °C). At temperatures below 25 °C the titration must be carried out slowly as the formation of the iron(III) - EDTA complex is slow. At room temperature (30° C) the titration can be carried out fairly accurately without much delay (not more than 1 minute). Above 60 °C, the iron(III) - pyridine-2,3-diol complex dissociated to an appreciable extent and the end-point was reached a little sooner, thus giving low results.

RECOMMENDED PROCEDURE—

To an acidic solution containing 3·0 to 70·0 mg of iron, add 5 to 10 drops of 0·5 per cent. solution of indicator. Adjust the pH to about 2·0, with pH indicator paper, by adding dilute ammonia solution, then add 10 ml of buffer solution of pH 2·0. Dilute the solution with doubly distilled water to nearly 100 ml and add, from a microburette, standard 0·02 to 0·05 M EDTA solution until the blue colour completely disappears. The colour change is from blue to yellow with larger concentrations of iron(III). Results obtained by using the procedure outlined above for some of the titrations are recorded in Table II, which shows that the method can be used to determine amounts of iron(III) ranging from 3·3 to 70 mg, with an error not exceeding ±0·5 per cent.

TABLE II

TITRATION OF IRON WITH EDTA

1·0 ml of 0·05 M EDTA ≡ 2·793 mg of iron; pH 2·0

Iron(III) taken/mg	Iron(III) found/mg	Error, per cent.
0·14	0·14	0·0
0·28	0·279	−0·35
0·56	0·558	−0·36
1·12	1·11	−0·90
3·35	3·36	+0·30
7·82	7·84	+0·25
15·64	15·67	+0·19
31·28	31·36	+0·25
54·74	54·90	+0·29
78·20	78·50	+0·39

INTERFERENCES—

Interferences caused by various cations and anions in the determination of iron have been investigated in the pH range of 1·5 to 2·0.

The anions oxalate and thiocyanate interfere seriously. No interference is caused by SO_4^{2-}, Br^-, I^- (at pH values above 2·5), borate, tartrate and citrate when present in 100-fold excess. The tolerated amounts of the anions PO_4^{3-}, $S_2O_3^{2-}$, F^- and IO_3^- are given in Table III.

The cations vanadium(V), bismuth(III) and nickel(II) interfere seriously even in the presence of the usual masking agents. Most of the common bivalent metals, *viz.*, zinc(II), magnesium(II), cadmium(II), manganese(II), barium(II), strontium(II), cadmium(II), beryllium(II) and UO_2^{2+} do not interfere at the pH used for the titrations, even when present in considerable excess. Interferences caused by some quadrivalent, tervalent and bivalent metal ions have been prevented by using masking agents (Table III).

A solution of iron(III) perchlorate was used with lead(II), silver(I) and thallium(I) ions.

TABLE III

EFFECT OF FOREIGN IONS

Amount of iron(III), 5·58 mg (≡5 ml of 0·02 M EDTA)

Foreign ion added	Amount of foreign ion/mg	Volume of 0·02 M EDTA solution used/ml	Masking agent
Copper(II), pH 3·0	25·0	4·98	Thiourea in presence of NaF
Cobalt(II)	10·0	5·01	—
Lead(II)	50·0	5·02	—
Aluminium(III)	50·0	4·98	Sodium citrate
Antimony(III)	25·0	4·97	Sodium potassium tartrate
Zirconium(IV), pH 3·0	20·0	4·97	Sodium potassium tartrate and NaF
Thorium(IV)	60·0	4·98	Sodium citrate
Titanium(IV), pH 3·0	20·0	4·97	Sodium potassium tartrate and NaF
Molybdenum(VI)	100·0	5·01	Sodium potassium tartrate
Tungsten(VI)	100·0	5·01	Sodium potassium tartrate
PO_4^{3-}, pH > 2·5	100·0	4·99	—
IO_3^-, pH 2·5	50·0	4·99	—
$S_2O_3^{2-}$, pH 3·5	50·0	4·98	—
F^-, pH 3·0	50·0	4·98	—

DISCUSSION

Of the indicators made use of in the chelatometric determination of iron with EDTA,[1,2,3,4] salicylic acid, thiosalicylic acid and tiron have been widely used, including the determination of iron(III) in ores, soils and cements. Unfortunately, with these indicators titanium(IV), zirconium(IV), thorium(IV), antimony(III) and bismuth(III) interfere. The pH range for accurate determination is 2·0 to 3·0.

Results with thiocyanate are dependent on the concentration of the indicator, and the pH must also be controlled strictly in the range of 2·0 to 2·4. To obtain good results the use of 50 per cent. acetone medium has been recommended by some workers.

Kojic acid is used within the narrow pH range 2·0 to 3·0. The advantage with this indicator is that it can be used successfully within the wide temperature range of 40 to 100 °C. The ions copper(II), nickel(II), mercury(II), bismuth(III), thorium(IV), PO_4^{3-}, F^-, oxalate and tartrate interfere in the determination. Pyrocatechol violet has also been used successfully, but thorium(IV) and aluminium(III) interfere.

Triphenylmethane dyes can be used successfully within a narrow pH range. The pH range for 2-, 5- and 6-hydroxy-*m*-toluic acids is narrow in comparison with that for pyridine-2,3-diol and the same is true for the hydroxynaphthoic acids.

Compared with the above indicators for use in the chelatometric determination of iron(III) with EDTA, pyridine-2,3-diol is superior as it can be used over a wider pH range (1·0 to 4·0) and has a sharp and distinct end-point. The value of this indicator has been enhanced by using tartrate, citrate and fluoride as masking agents for the cations that normally interfere in the determination of iron with EDTA when using other indicators.

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Spectrophotometric Determination of Vanadium with *N*-Benzoyl-*o*-tolylhydroxylamine

BY A. K. MAJUMDAR AND S. K. BHOWAL

(Department of Inorganic and Analytical Chemistry, Jadavpur University, Calcutta-32, India)

The method prescribed by Majumdar and Das for the spectrophotometric determination of vanadium(V) with *N*-benzoyl-*o*-tolylhydroxylamine has been re-examined. The validity of the method originally reported is supported by the further results presented.

MAJUMDAR and Das introduced *N*-benzoyl-*o*-tolylhydroxylamine as a specific reagent for the spectrophotometric determination of vanadium[1,2] in the quinquivalent state and recommended the use of sodium fluoride to prevent interference by titanium. In their method they prescribed the extraction of vanadium(V) from 4 to 8 N hydrochloric acid solution with an ethanol-free chloroform solution of the reagent and measurement of the optical density of the extract at 510 nm. Jeffery and Kerr, while using this reagent for the determination of vanadium in rocks and minerals,[3] preferred the use of carbon tetrachloride to chloroform as the extractant because of the possible presence of ethanol in the latter solvent. Moreover, during the preparation of calibration graphs they observed that somewhat lower optical densities were obtained unless the standard vanadate solution was re-oxidised prior to reaction with the reagent. They found, however, that the vanadium complex in chloroform solution gave calibration graphs similar to those obtained with solutions in carbon tetrachloride, isobutyl methyl ketone and toluene.

These observations led the present authors to re-evaluate the method of Majumdar and Das. Thus ethanol-free chloroform was found to be a more satisfactory extractant for the vanadium - benzoyl-*o*-tolylhydroxylamine complex than carbon tetrachloride. Both the reagent and its vanadium complex are highly soluble in chloroform. While only two extractions with chloroform are sufficient to extract the vanadium complex completely, at least four extractions with carbon tetrachloride are needed for its complete removal. Moreover, the vanadium complex has a higher optical density in ethanol-free chloroform than in carbon tetrachloride, the spectrophotometric sensitivities ($\log I_0/I = 0{\cdot}001$) being 0·010 8 and 0·012 3 μg cm^{-2} of vanadium, respectively. The optical densities remain the same whether or not the aliquots of standard vanadate solution are oxidised before treatment with the reagent solution.

However, in the procedure followed by Jeffery and Kerr for the determination of vanadium in silicate minerals, oxidation of vanadium was obviously necessary because after the decomposition of the mineral vanadium was present in a lower state of oxidation. For the preparation of the calibration graph from a standard metavanadate solution, re-oxidation of the vanadium seems to be unnecessary.

The presence of a very small proportion of vanadium(IV) should not affect the accuracy of the result because the present study has shown that when a solution of vanadium(IV) in 4 to 8 N hydrochloric acid is maintained in contact with a solution of the reagent in chloroform for some time and the mixture shaken, the chloroform layer slowly assumes the red - violet colour characteristic of the vanadium(V) complex with the same maximum absorption region at 510 nm.

Moreover, the solid complex isolated from a 4 to 8 N hydrochloric acid solution of vanadium(IV) (A. K. Majumdar and B. C. Bhattacharyya, unpublished work) has been found to be identical with that isolated when the vanadium is in the quinquivalent state.[4] The compounds obtained with both vanadium(IV) and (V) melt with decomposition at 133° C

and are diamagnetic. Thus it is reasonable to assume that as vanadium(V) in the presence of the reagent forms a more stable complex, the reduction potential of the vanadium(IV) - vanadium(V) system increases so that vanadium(IV) becomes rapidly oxidised by air to vanadium(V) to furnish the complex.

Two measuring instruments of different sensitivities have been used in this investigation for comparison of optical densities. Two procedures, one being that suggested by Majumdar and Das and the other in which permanganate was used as oxidant, with carbon tetrachloride or chloroform as the extractant, are described.

Method

Apparatus, reagents and solutions—

Two spectrophotometers, a Unicam SP600 and a Hilger Uvispek, were used for optical density measurements.

A standard solution of vanadium was prepared, as described by Majumdar and Das,[1] by dissolving ammonium metavanadate in water rendered ammoniacal.

The chloroform used for extraction was freed of ethanol by washing it successively with dilute sulphuric acid followed by dilute ammonia solution and water, then dried over fused calcium chloride and distilled.

A 0·5 per cent. solution of *N*-benzoyl-*o*-tolylhydroxylamine in carbon tetrachloride or purified ethanol-free chloroform was used.

All chemicals used were of analytical-reagent grade.

Procedure for extraction after re-oxidation—

Acidify an aliquot of the standard vanadate solution (4 ml containing 200 μg of vanadium) in a 50-ml separating funnel with 4 or 5 drops of dilute sulphuric acid. Add dropwise dilute potassium permanganate solution until the pink colour of the solution persists for more than 5 minutes. Add 2 ml of 0·05 M sulphamic acid solution followed by 10 ml of 8 M hydrochloric acid, then add 4 ml of a 0·5 per cent. solution of the reagent in purified chloroform or carbon tetrachloride. After the addition of another 4-ml portion of chloroform or carbon tetrachloride, shake the mixture for 1 to 2 minutes and transfer the organic layer to a 25-ml calibrated flask. Extract the aqueous layer once with a 5-ml portion of chloroform or three times with 5-ml portions of carbon tetrachloride. Make the volume of the combined extracts up to 25 ml with the respective solvents and measure the extinctions at 510 nm against the pure solvents as reference (see Table I).

Table I

Variation of optical density with solvent

Solvent	Optical density (Unicam SP600)	Optical density (Hilger Uvispek)
Chloroform	0·750	0·800
Chloroform	0·745	0·797
Carbon tetrachloride	0·645	0·706
Carbon tetrachloride	0·645	0·710

Procedure for extraction without re-oxidation—

The extraction procedure followed was that suggested by Majumdar and Das, the same amount of vanadium(V) being used as that given for the above procedure. The results obtained are shown below.

Solvent	Optical density (Unicam SP600)	Optical density (Hilger Uvispek)
Chloroform	0·745	0·797
Carbon tetrachloride	0·650	0·710

The molar extinction coefficients of the vanadium complex calculated for ethanol-free chloroform and carbon tetrachloride solutions were 4 776 and 4 202, respectively, with the Unicam SP600, and 5 095 and 4 496, respectively, with the Hilger Uvispek.

PREPARATION OF THE VANADIUM(IV) COMPLEX—

To a calculated amount of vanadyl dichloride ($VOCl_2$) solution diluted to 30 ml, add 40 ml of concentrated hydrochloric acid. Pass sulphur dioxide gas through the solution to ensure that the vanadium is present in the quadrivalent state, and boil off the excess of sulphur dioxide. Add 0·01 mole of the reagent in 140 ml of acetone, when the violet complex almost immediately begins to separate out. After cooling the solution for about 3 hours, filter off the complex, wash and dry it in a vacuum desiccator. The complex melts, with decomposition, at 133 °C and is diamagnetic.

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The Analysis of Tin Stabilisers Used in Poly(vinyl chloride) Compositions*

By J. UDRIS

(*Research Department, Imperial Chemical Industries Limited, Plastics Division, Welwyn Garden City, Herts.*)

Schemes are described for the analysis of commercial tin stabilisers commonly used in poly(vinyl chloride) compositions. Methods are given for the chemical breakdown of a sample and subsequent separation and identification of the breakdown products, which may include dialkyltin oxides, alcohols, thiols, carboxylic acids and thioacids. Separations are carried out by precipitation, solvent extraction and thin-layer chromatographic techniques, and the components identified by gas-chromatographic, infrared, nuclear magnetic resonance and mass-spectroscopic methods.

Manual and potentiometric titration procedures for quantitative examination together with recommended methods for the determination of tin are either described in this paper or in the literature cited, and methods for identification of additives and procedures for identification of tin stabilisers in the presence of excess of plasticiser, by means of column chromatography and ion-exchange procedures, are also included.

Organotin compounds are widely used in the plastics industry as stabilisers for poly(vinyl chloride) compositions. The most important compounds used for this purpose are based on dialkyltin groups $\begin{matrix}R\\R\end{matrix}\!\!>\!Sn\!<$, especially where R = butyl or octyl, and this paper is confined to the analysis of these two classes of compounds.

For analytical considerations it is convenient to divide tin stabilisers into those containing sulphur and those without sulphur. To the first group belong compounds like dialkyltin mercaptides, mercapto-esters and mercapto carboxylates, to the second, dialkyltin carboxylates and their esters. The identification of acids and alcohols present in tin stabilisers containing no sulphur is usually straightforward and the identification of alcohols from stabilisers containing mercapto-esters also presents little or no difficulty. However, the identification of the thioacid and of the alkyl groups attached directly to tin can prove more difficult, especially if long-chain acids form part of the compound or if the stabiliser is not pure, *e.g.*, if it contains plasticisers. The thioacid may also tend to decompose during hydrolysis procedures.

QUALITATIVE ANALYSIS OF STABILISERS

Dialkyltin thiocompounds

Tin stabilisers belonging to this group have characteristic sulphurous odours, but the presence of sulphur should be confirmed by a specific chemical test or X-ray fluorescence examination prior to the commencement of chemical analysis. Compounds belonging to this group include dialkyltin dialkylthioglycollates, $\begin{matrix}R\\R\end{matrix}\!\!>\!Sn\!<\!\begin{matrix}SCH_2COOR'\\SCH_2COOR''\end{matrix}$, dialkyltin dilaurylmercaptides, $\begin{matrix}R\\R\end{matrix}\!\!>\!Sn\!<\!\begin{matrix}SCH_2(CH_2)_{10}CH_3\\SCH_2(CH_2)_{10}CH_3\end{matrix}$, and stabilisers in which the dialkyltin group is combined with both thiol and carboxyl groups. R, R′ and R″ denote alkyl groups; they can be the same or different. The scheme of analysis in Fig. 1 illustrates the chemical procedures involved in the characterisation of the structural groups present.

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Determine the infrared spectrum of the sample. This will show the presence of ester groups, metal carboxylates, *etc.*, and will indicate whether or not the sample is a mixture and in need of separation (see sections on the detection of impurities and of stabiliser in the presence of excess of plasticiser).

Place about 1 g of the sample into a 150-cm³ flat-bottomed stoppered flask and add 100 cm³ of acetone. Swirl to dissolve, then add dropwise, with swirling, 2 cm³ of a 50 per cent. solution of silver nitrate in distilled water. Shake the flask well and allow it to stand

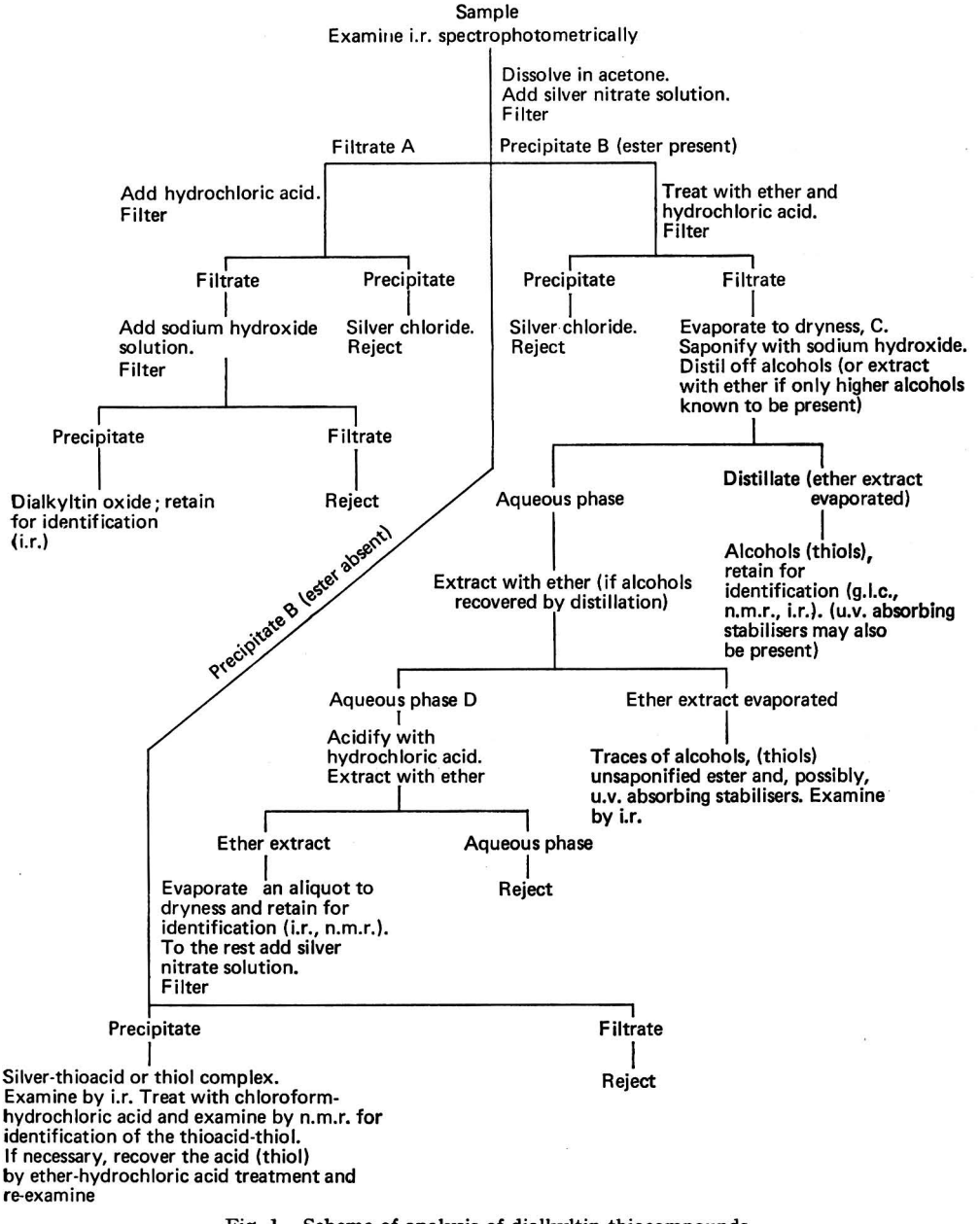


Fig. 1. Scheme of analysis of dialkyltin thiocompounds

until the precipitate has settled out. Filter the precipitate through a Whatman No. 541 filter-paper, wash it with a small amount of distilled water, then with acetone and finally with a small amount of diethyl ether. Retain the filtrate and washings (filtrate A, Fig. 1). Dry the precipitate (precipitate B) in a desiccator and determine its infrared spectrum by using the nujol emulsion technique.

IDENTIFICATION OF ALCOHOLS—

If the infrared spectrum of precipitate B (Fig. 1) shows the presence of ester bands transfer the precipitate to a 150-cm³ flat-bottomed flask and shake it with 50 cm³ of diethyl ether. Add 1 cm³ of concentrated hydrochloric acid and shake the flask vigorously for 1 minute to precipitate the silver as silver chloride. Allow it to stand for 30 minutes, then decant into a 150-cm³ flat-bottomed extraction flask through a Whatman No. 541 filter-paper, washing the flask and the precipitate with 20 cm³ of diethyl ether. Reject the precipitate but evaporate the ether extract to dryness on a water-bath. This yields the residue C (see Fig. 1). Determine the infrared spectrum of the residue. This spectrum should show the presence of ester bands and may already suggest the nature of the alcohol involved.

Next, add 20 cm³ of 10 per cent. aqueous sodium hydroxide solution and saponify the ester by refluxing on a hot-plate. Normally a refluxing time of 1½ hours is sufficient. Wash the condenser with a small amount of distilled water and allow to cool, then transfer the reaction mixture to a distillation apparatus. Distil, separate and identify the alcohols by gas - liquid chromatographic methods. If the gas - liquid chromatography retention times alone are considered unsatisfactory for complete identification, use the separated fractions for infrared and for nuclear magnetic resonance spectroscopic examination. Sometimes the gas - liquid chromatographic examination can provide information as to the approximate ratios of the alcohols present.

Extract the aqueous phase by shaking it with diethyl ether. Evaporate the ether extract to dryness and examine by infrared spectrophotometry. Retain the aqueous phase D (Fig. 1) for examination for thioacids.

If the infrared spectrum of precipitate B suggests the presence of only a mercaptide or carboxylate or mercapto-carboxylate with no ester bands proceed as outlined under the identification of thioacids and thiols, *i.e.*, omitting the saponification step.

NOTE—

In the case of the less volatile alcohols, such as 2-ethylhexanol and higher alcohols, the alcohol can be recovered by extraction of the alkaline saponification mixture with diethyl ether, followed by careful evaporation of the solvent. Subsequent infrared (or nuclear magnetic resonance) examination of the residue usually suffices for the identification of the alcohol. Of course, if more than one alcohol is present, the gas - liquid chromatographic examination is called for.

IDENTIFICATION OF THIOACIDS AND THIOLS—

Acidify the aqueous phase D, remaining after the removal of alcohols (see above), with concentrated hydrochloric acid and extract the liberated thioacid with diethyl ether. Liquid - liquid extraction for 4 hours produces more material, but shaking with some 50 cm³ of diethyl ether for about 1 minute normally gives enough extract for identification. Because of partial decomposition of the thioacid during the foregoing saponification, sulphides are produced

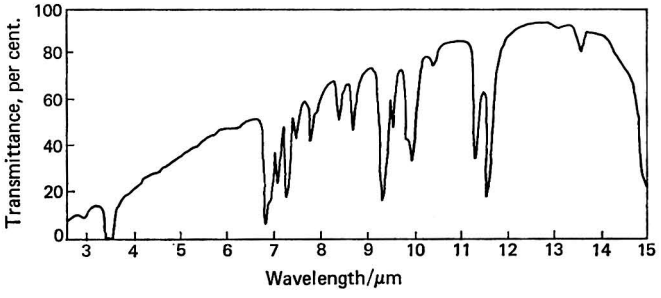


Fig. 2. Infrared spectrum of dibutyltin oxide

and the above acidification and extraction should be carried out in a fume cupboard. The ether extract can be evaporated to dryness on a water-bath and examined by infrared or nuclear magnetic resonance spectroscopy or by both techniques, but, because of the mentioned partial decomposition, a better way of identification, at least for the thioacid, is as follows.

Evaporate to dryness and examine only an aliquot of the ether extract. To the rest, prior to evaporation, add a few drops of 50 per cent. silver nitrate solution, dropwise, with shaking. Shake well and allow the precipitate to settle, then filter through a Whatman No. 541 filter-paper, washing well with ether. Dry in a desiccator and examine by infrared spectrophotometry and the nujol emulsion technique. Also, suspend an aliquot of the precipitate in chloroform, acidify with a little hydrochloric acid, shake and carry out nuclear magnetic resonance spectroscopic examination of the chloroform extract. This should suffice for the identification of the thioacid. The dark colour of the silver complex is probably due to the presence of silver sulphide.

If precipitate B contains no ester groups [see Fig. 1, precipitate B (ester absent)], the hydrolysis with sodium hydroxide should be omitted. In this event, examine the precipitate by the above infrared and nuclear magnetic resonance technique for identification of the thioacid or the thiol. Although this particular nuclear magnetic resonance technique was not used for the identification of thiols it could prove equally effective.

If required, the free acid, usually thioglycollic, or thiol can be obtained in a purer state by acidifying the silver complex with hydrochloric acid and extracting the liberated acid or thiol with diethyl ether. The infrared and nuclear magnetic resonance examinations can then be repeated on the thioacid or thiol itself.

Mass spectrometric examination is also a useful test for the identification of thioacids, for example, thioglycollic acid gives the characteristic ion at $m/e = 91$.

IDENTIFICATION OF THE ALKYL GROUPS ATTACHED DIRECTLY TO TIN—

Precipitation with sodium hydroxide and infrared examination—To the filtrate A add a slight excess of concentrated hydrochloric acid (about 1 cm³) to precipitate the excess of silver ions as silver chloride. Shake the mixture well, allow to settle and filter through a Whatman No. 541 filter-paper. Make the filtrate alkaline by the addition of 25 cm³ of 10 per cent. sodium hydroxide solution and again shake and filter through a Whatman No. 541 filter-paper, this time washing well with acetone. Dry at 105° C for 30 minutes. This gives a crude sample of the dialkyltin oxide, but this is sufficiently pure for identification by infrared spectrophotometric examination (potassium bromide).

Good samples of dibutyl and dioctyltin oxides have also been obtained by dispersing about 0·8 g of the stabiliser in 25 cm³ of methanol and refluxing for 3 hours with 20 cm³ of N sodium hydroxide solution. The white powder, isolated after filtering through a G3 sintered-glass crucible, washing with distilled water followed by methanol and drying at 105° C, usually gives a characteristic infrared spectrum of the dialkyltin oxide, believed to be in polymeric form (see Figs. 2 and 3).

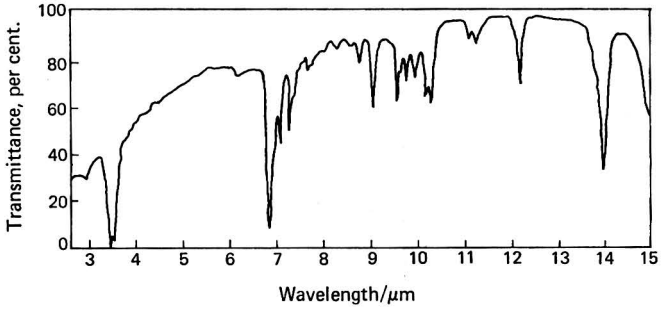
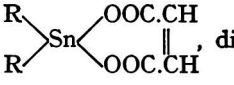


Fig. 3. Infrared spectrum of dioctyltin oxide

Thin-layer chromatography—In addition to the above, the thin-layer chromatographic method described by Belpaire[1] with hexane - glacial acetic acid (12 + 1) as eluting agent has been found satisfactory. A small amount of the original tin stabiliser is dissolved in the elution solvent, applied to a thin-layer chromatographic plate coated with a 0·25-mm thick layer of Kieselgel G as the stationary phase, and eluted with the hexane - glacial acetic acid mixture. After drying, the stationary phase is sprayed with a 0·1 per cent. solution of catechol violet in 95 per cent. ethanol, blue spots appearing where tin compounds are present. For an illustration see Fig. 4. The approximate R_F values are 0·17 for dibutyltin (wide band) and 0·27 for dioctyltin.

If mixed tin stabilisers are believed to be present, for example, a thio-compound and a dialkyltin dialkylcarboxylate, it is advantageous to acidify the acetone filtrate after removal of the dialkyltin oxides with hydrochloric acid, evaporate most of the acetone on a water-bath under an air-jet, cool and extract by shaking with diethyl ether. The ether extract then contains hemiesters, acids, *etc.*, liberated from the other types of tin stabilisers originally present and can be examined further. Although reaction products produced by the action of sodium hydroxide on acetone are often present, esters of maleic and benzoic acids have been identified in this way.

Dialkyltin carboxylates and hemiesters

To this group belong stabilisers like dialkyltin maleates,  , dialkyltin dialkylmaleates, $\begin{matrix}R\\R\end{matrix}\!\!>\!Sn\!<\!\begin{matrix}OOC.CH:CH.COOR'\\OOC.CH:CH.COOR''\end{matrix}$, and dialkyltin dilaurates, $\begin{matrix}R\\R\end{matrix}\!\!>\!Sn\!<\!\begin{matrix}OOC(CH_2)_{10}CH_3\\OOC(CH_2)_{10}CH_3\end{matrix}$, where R, R′ and R″ are alkyl groups that can be the same or different. The scheme in Fig. 5 illustrates the analytical procedures involved in the characterisation of the structural groups present.

Examine the infrared spectrum of the sample and deduce its general nature from the absence or presence of ester groups, free carboxyl groups, plasticisers or significant amounts of other constituents. More detailed methods of examination for the presence of impurities and additives, or both, and techniques of separation are described later.

Identification of alcohols—

Place about 1 g of the sample containing ester groups into a 150-cm³ extraction flask and saponify by refluxing with 50 cm³ of 10 per cent. aqueous sodium hydroxide solution

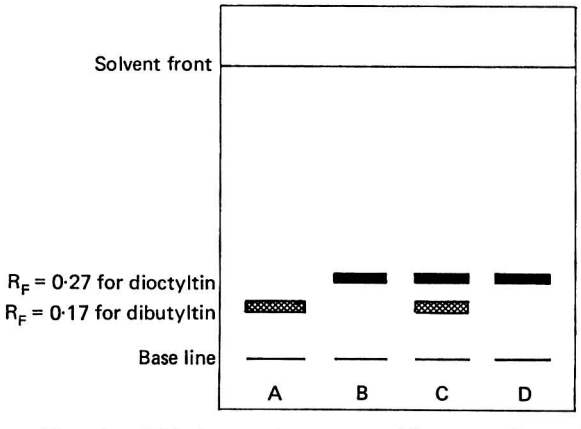


Fig. 4. Thin-layer chromatographic separation of dialkyltin compounds: A, dibutyltin maleate; B, dioctyltin laurate; C, A *plus* B; and D, dioctyltin dioctylthioglycollate

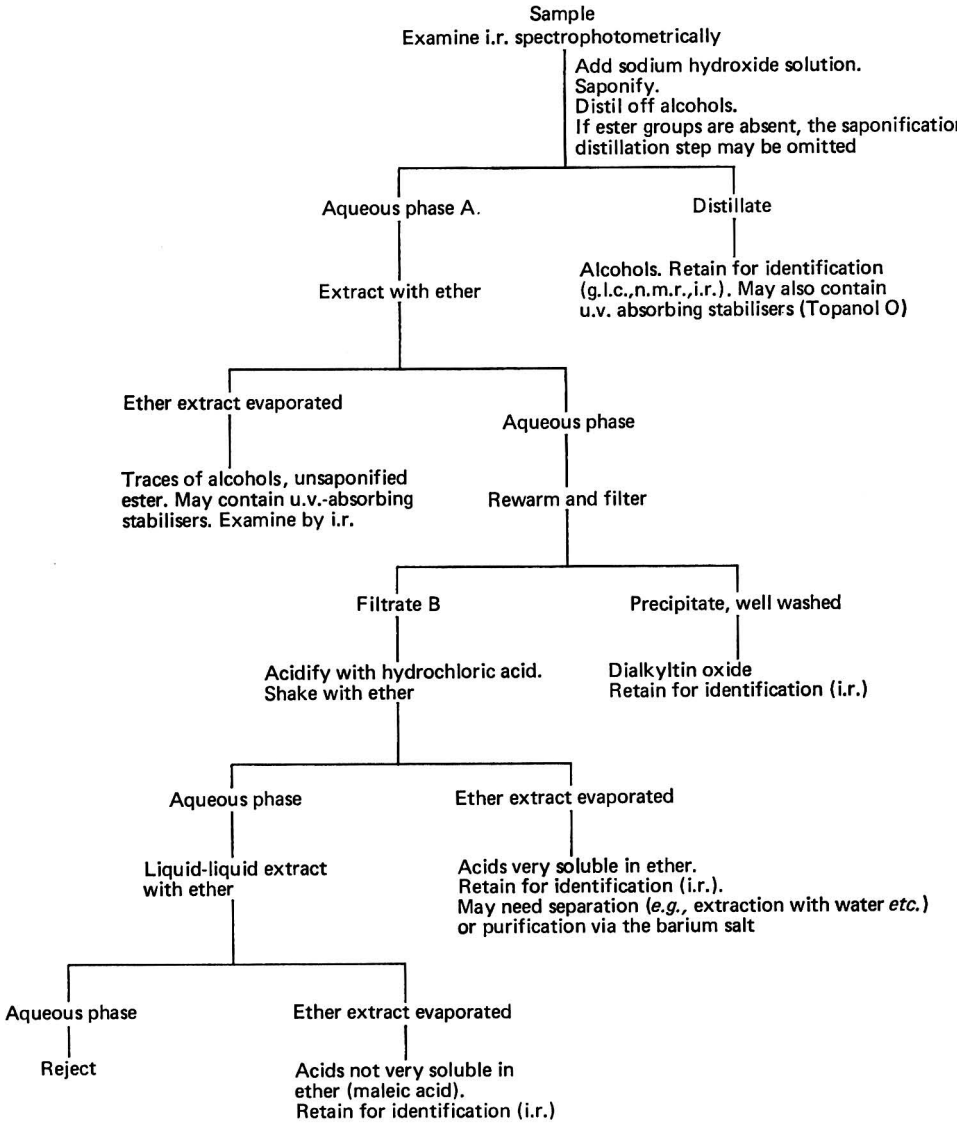


Fig. 5. Scheme of analysis of dialkyltin carboxylates and hemiesters

for 4 hours. Wash the condenser with distilled water, cool the flask, transfer it to a distillation apparatus and distil off the alcohols. Identify the alcohols and, if possible, determine their approximate ratios by gas - liquid chromatographic methods. Retain the alkaline solution (see aqueous phase A, Fig. 5). See also the previous section on identification of alcohols. If no esters are present, the above saponification step may be omitted.

IDENTIFICATION OF THE ALKYL GROUPS ATTACHED TO TIN—

Transfer the alkaline solution, aqueous phase A, to a 150-cm^3 separating funnel, adding more water if necessary, and extract by carefully shaking with 50 cm^3 of diethyl ether. This is best accomplished by repeated inversion of the separating funnel because if long-chain acids are present emulsification tends to occur. Filter the aqueous phase, diluting with

distilled water or warming if necessary, through a Whatman No. 541 filter-paper and wash the precipitate well with hot distilled water, especially if a long-chain acid salt is present. This filtration is a tedious procedure, but it is not usually necessary to filter all of the suspension. Dry the precipitate at 105 °C and examine by infrared spectrophotometry to identify the dialkyltin group. Retain the filtrate, filtrate B (Fig. 5), but evaporate the ether to dryness and examine the residue by infrared spectrophotometry. This may contain traces of alcohols, unsaponified esters, *etc.*

If the only concern is the identification of the dialkyl groups attached directly to tin, then the method with methanolic sodium hydroxide solution described earlier is recommended (Figs. 2 and 3). The thin-layer chromatographic method described in the same place is also suitable (Fig. 4). For pure samples of dialkyltin carboxylates with no ester present, the time of reflux with alkali can usually be much reduced, the recovery and identification of the dialkyltin oxide, believed to be in polymeric form, still being quite satisfactory. In addition, maleic acid has been recovered from a sample of dibutyltin maleate after decomposition by this simplified method.

IDENTIFICATION OF ACIDS—

Reduce the volume of filtrate B (Fig. 5) to about 150 cm^3 by boiling, acidify it with hydrochloric acid and extract by shaking it with 50 cm^3 of diethyl ether. Evaporate the solvent on a water-bath, dry the residue at 105 °C for 20 minutes and determine its infrared spectrum to identify the acid readily soluble in ether.

Extract the aqueous phase with diethyl ether for 4 hours by liquid - liquid extraction, evaporate the solvent, dry the residue at 105 °C and again examine it by infrared spectrophotometry, this time to identify the acid less soluble in ether, usually maleic acid.

If the infrared spectrum of the first ether extract, *i.e.*, the one obtained by shaking, indicates a mixture of a long-chain and another acid, triturate the residue several times with small amounts of distilled water. Recover the fraction soluble in water by evaporation and dry both fractions at 105° C. Examine both the water-soluble and the water-insoluble fractions by infrared spectrophotometry. If necessary, prepare and examine the barium salt of the long-chain acid. A mixture of phthalic and lauric acids has been separated and the acids identified by this method.

NOTE—

The long-chain acids obtained by this method are usually contaminated by metal salts and a dialkyltin dichloride, the latter originating from the presence of residual dialkyltin groups. For positive identification of the acid by infrared spectroscopic examination, the preparation of the barium salt has been found helpful. Alternatively, the acid can be identified by mass spectrometric examination.

QUANTITATIVE ANALYSIS OF STABILISERS

The tin content is usually determined. Accurate results have been obtained by wet oxidation of the sample followed by precipitation of tin with cupferron. When an accuracy of $\pm 0{\cdot}2$ per cent. absolute is sufficient the sulphated ash procedure may be combined with spectrographic examination to check that other metals are absent. Total sulphur may also be determined by standard methods. A number of titrimetric methods, potentiometric and manual, have been tried, and some of these are outlined below.

POTENTIOMETRIC TITRATION

SULPHUR ABSENT—

For the non-sulphurous stabilisers we agree with Groagova and Přibyl[2] that titration of organotin compounds with alkali in methanolic medium is unsatisfactory. These authors have stated that, because of the formation of hydrolytic intermediates, the reaction does not go to completion. Their method of titrating the $RCOO^-$ groups with sodium methoxide in pyridine by using antimony and calomel electrodes has been tried and found satisfactory. The end-point roughly coincides with the change of colour when thymolphthalein is used as indicator. The titrant, *i.e.*, sodium methoxide, is standardised against benzoic acid and the titration carried out under nitrogen as a precautionary measure. The antimony electrode, however, should be well cleaned before each series of determinations or poor end-points will result.

SULPHUR PRESENT—

We have titrated dialkyltin thio-compounds, especially dialkyltin mercaptides, with a solution of silver nitrate in isopropanol - water (about 1 + 1). The sample is dissolved in a solvent consisting of a 1 + 1 mixture of benzene - methanol, containing sodium acetate trihydrate (13·7 g l^{-1}). The indicator electrode is a length of silver wire coated with sulphide. It is prepared by immersion of a silver wire into an alkaline solution of sodium sulphide followed by addition of a silver nitrate solution (0·1 N) and stirring. The electrode is wiped clean and polished with a clean cloth and is then ready for use. The reference electrode consists of a copper wire immersed in a pool of mercury covered with a solution of 0·1 N sodium acetate. Contact with the test solution is made through an agar-gel bridge. More than one end-point is sometimes produced. However, titration of reference samples under identical conditions helps in the interpretation of the results.

The determination of sulphur in organometallic mercaptides by potentiometric titration with aqueous potassium iodate in glacial acetic acid and with platinum and silver electrodes has been described by Stapfer and Dworkin.[3] In our experience this method gives very good end-points, but the results are somewhat lower than those obtained by the titration with silver nitrate outlined above.

MANUAL TITRATION

The manual titration method for sulphur-free compounds with sodium methoxide in pyridine[2] has been tried and found reasonably satisfactory.

For samples containing sulphur as a thiol, the method described by Stapfer and Dworkin[3] is used. The sample, about 1 g, is dissolved in benzene, a small excess of 0·1 N iodine solution in benzene is added, and, after vigorous shaking, the reaction mixture is diluted with distilled water and back-titrated with 0·1 N sodium thiosulphate with starch as indicator.

The method is simple and, in experienced hands, satisfactory for titration of sulphur as a thiol in tin stabilisers. However, the end-point is weak and back-titration with sodium thiosulphate is essential.

DETECTION OF IMPURITIES AND ADDITIVES

Tin stabilisers often contain phenol derivatives; metal carboxylates and small amounts of phthalate esters may also be present.

Determine the infrared spectrum of the original sample. This may show the presence of major impurities and additives, but the minor ones are likely to be missed. These may be separated and identified by thin-layer chromatographic separation followed by infrared spectrophotometric examination. Also discussed below is ultraviolet spectrophotometric examination.

Metal salts present in tin stabilisers have been identified by diluting the sample with diethyl ether, centrifuging (or filtering) and examining the precipitate by infrared spectrophotometry and by emission spectrography (for metals).

THIN-LAYER CHROMATOGRAPHIC SEPARATION

The stationary phase for this separation is a 1-mm layer of Kieselgel G (E. Merck, A.G.), incorporating Fluolite C180 as a fluorescing agent to facilitate band detection under ultraviolet light. The eluting agent is chloroform. Although the silica gel acts as an ion-exchange resin and makes it difficult to isolate the actual tin stabiliser, the additives and impurities can be recovered readily by extraction of the separated bands with chloroform. If diethyl ether is used for this extraction more of the actual tin compound is recovered, but mainly in an acidic form, for example, dibutyltin dioctyl maleate produces octyl maleate hemiester; the dialkyltin group appears to be retained by the silica gel. A small amount of di-2-ethyl hexyl phthalate has been separated in this way and then identified by infrared spectrophotometric examination.

Ultraviolet spectrophotometric examination

Tin stabiliser compositions often contain substituted phenols added at concentrations of about 10 per cent., and substituted phenols at concentrations down to about 3 per cent. can usually be detected from their ultraviolet spectra.

The sample is dissolved in absolute ethanol, suitable for spectrophotometric work, and its ultraviolet absorption spectrum obtained over the range of wavelengths 230 to 350 nm in 1-cm cells with ethanol in the reference cell. The amount of sample necessary depends on: the absorption characteristics of the additive, *i.e.*, wavelength and intensity, the amount of the additive present and background absorption by the tin stabiliser. Usually, 0·05 to 0·1 per cent. sample solutions in ethanol should produce reasonable ultraviolet absorption curves.

The change in absorption on addition of alkali can be used to help in the identification if the sample solution is made alkaline by the addition of alcoholic potassium hydroxide and the spectral trace recorded again. The approximate concentration of the additive can be deduced by reference to an appropriate calibration graph.

Separation and identification of tin stabilisers in the presence of excess of plasticiser

Methods have been examined for the recovery and identification of tin stabilisers in excess plasticiser (90 to 95 per cent.). The plasticisers included diisooctyl phthalate and mixtures of diisooctyl phthalate and tritolyl phosphate in various proportions.

The quantities of sample (2 to 3 g) carried through the procedure were such as to enable stabiliser to be isolated, if necessary by combining two or more of the separated fractions of the stabiliser, for identification by saponification, *etc.* (see the foregoing sections).

These methods have been useful for the particular mixtures investigated. For other combinations they should be treated as recommendations only and may require modification.

Column-chromatographic separation

Determine the infrared spectrum of the mixture before attempting the separation.

Pack a chromatographic column, 1·5 cm in diameter, with about 10 g of a 1 + 1 mixture of Celite 545 and silica gel, 200 to 300 mesh. Wash the column with carbon tetrachloride. Transfer 2 to 3 g of the sample to the top of the column packing and elute with carbon tetrachloride until all the plasticiser is just washed out (usually about 200 cm³ of carbon tetrachloride suffice). Then wash the column with 200 cm³ of diethyl ether. Evaporate the ether on a water-bath to recover the tin stabiliser. Dioctyltin dinonylmaleate has been separated from diisooctyl phthalate (90 per cent.) by the above method.

Note—

Here again silica gel acts on tin stabilisers as an ion-exchange resin, hence avoid using freshly activated silica gel. The separated stabiliser, however, usually contains free carboxyl groups.

Ion-exchange separation

For this investigation Permutit ion-exchange resins were found to be suitable.

Isolation of the acidic hemiester with the anion-exchange resin De-Acidite FF (SRA69) 14 to 52 mesh, methanol as solvent and methanolic hydrochloric acid as the recovery liquid failed, but the dialkyltin group was successfully separated and identified by the following method. Disperse about 10 g of the cation-exchange resin Zeo-Karb 225 (H form), 14 to 52 mesh, in methanol and transfer to a column of 1-cm diameter, washing the column with methanol. Dissolve 2 to 3 g of the sample in 50 cm³ of methanol and slowly run the solution down the column. Wash the column with methanol until all the plasticiser has been removed (about 250 cm³ of methanol should suffice). Then wash the column with a mixture of 40 cm³ of methanol and 10 cm³ of concentrated hydrochloric acid, followed by another 50 cm³ of methanol. Evaporate the methanol off on a water-bath under an air-jet. Allow the residual acid phase to cool, transfer it to a small separating funnel and extract with two consecutive 20-cm³ portions of diethyl ether, shaking the funnel well during each extraction. Combine the ether extracts and evaporate them to dryness on a water-bath. Dry at 105 °C for 20 minutes and examine by infrared spectrophotometry. Dioctyltin and dibutyltin dichlorides have characteristic infrared spectra and are easily identified even if not pure (see Figs. 6 and 7).

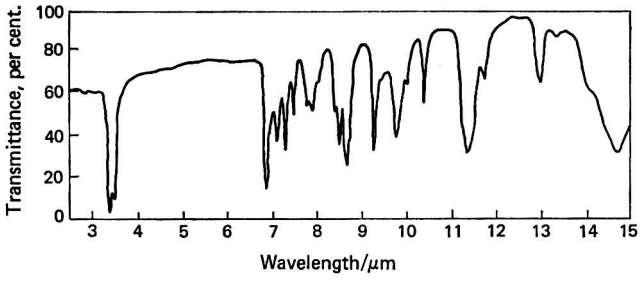


Fig. 6. Infrared spectrum of dibutyltin dichloride

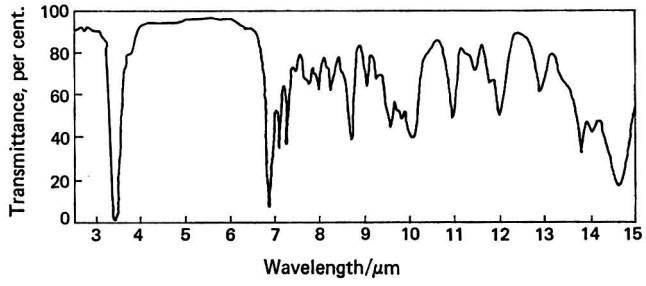


Fig. 7. Infrared spectrum of dioctyltin dichloride

Good samples of dialkyltin dichlorides have been isolated by the above method from mixtures of tin stabilisers with diisooctyl phthalate and tritolyl phosphate; the dialkyltin groups have been identified in dibutyltin dinonylmaleate, dioctyltin dilaurate, dibutyltin dinonylthioglycollate and dioctyltin thioglycollate.

If a separating column is not at hand, prolonged shaking of the sample solution in methanol with the ion-exchange resin and allowing to stand for 1 hour with occasional shaking will also effect the desired ion-exchange.

Although the above methods have been designed for the examination of tin stabilisers as such, they can be readily adapted for the analysis of tin stabilisers recovered from actual samples of plastic materials, provided enough sample is available.

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Revision of a Field Method for the Determination of Total Airborne Lead

BY D. M. GROFFMAN AND R. WOOD

(*Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.*1)

An improved field method is described for determining airborne lead at concentrations up to 0·8 mg m^{-3} of lead. After collection on a filter the lead is dissolved in acid and complexed with dithizone. The lead dithizonate is extracted into 1,1,1-trichloroethane and the colour intensity of the complex is compared visually with standards. The apparatus used is simple to operate and the time required for a complete determination is about 15 minutes.

THE field method for the determination of total airborne lead devised by Dixon and Metson[1] was later adopted by H.M. Factory Inspectorate Committee on Tests for Toxic Substances in Air and published by H.M. Factory Inspectorate in their booklet series[2] "Methods for the Detection of Toxic Substances in Air." It is the policy of this Committee to request that the Laboratory of the Government Chemist carry out periodic revisions of the field methods published in this booklet series.[3] This allows account to be taken of any changes in the threshold limit value of the particular toxic substance or even in its use within industry. Also, any procedural disadvantages in the test which have been shown up since it was first developed can be investigated and, if these are overwhelming, a new test can be devised. The lead test had been in existence for eight years and some experimental disadvantages had emerged in that time. This paper describes the modifications made to the test to obviate these.

EXPERIMENTAL

The original test[2] involved four stages: the collection of the airborne lead (dust or fume); the dissolution of the collected sample; the formation and extraction into carbon tetrachloride of the coloured lead dithizonate complex from a buffered (pH 11) aqueous solution; and, finally, the visual comparison of the colour obtained with a range of standard colours. Each stage was examined in this present revision.

COLLECTION OF AIRBORNE LEAD—

Millipore type AA membrane filters were selected. These have been recommended[4] as suitable for the collection of industrial fume samples and their use would bring the lead test into line with field tests for the determination of copper, iron oxide and zinc oxide fumes and dusts in industrial atmospheres, which are also being devised in this laboratory and which are to be published. There appeared to be no particular advantage in the use of either Whatman No. 2 or Munktell No. 00 filter-papers for collection of the sample as in the original method.[2]

A lead fume was generated by atomising aqueous lead acetate solutions and igniting the resultant aerosols formed in an apparatus that is to be described elsewhere.[5] It was found that the collection efficiency of the Millipore filters was constant at least over a range of 1 to 5 l $minute^{-1}$ sampling rate. There was no reason to expect any variation from this when larger sized particles such as dusts were being collected.

DISSOLUTION OF COLLECTED LEAD SAMPLE—

It was found possible to dissolve the collected lead quantitatively from Millipore filters by soaking the filters for 5 minutes in the nitric acid - hydrogen peroxide solution originally used.[2] There was therefore no need, as in the original method, to decompose the filter to achieve dissolution.

COLORIMETRIC DETERMINATION OF LEAD—

The original test[2] based on the use of the reagent dithizone was considered, on the basis of eight years' experience, to be sufficiently sensitive and specific for the determination of lead at its threshold limit value[6] of 0·2 mg m^{-3} when a 15-litre atmosphere sample was taken. However, certain disadvantages had become apparent. These were the use in the test of the highly toxic solvent carbon tetrachloride (threshold limit value 65 mg m^{-3}),[6] the poor and variable keeping properties (only a few hours in some cases) of the carbon tetrachloride solutions of dithizone, the variable reagent blanks and responses to a standard amount of lead of different batches of dithizone, and the absence of a one-half threshold limit value colour standard.

Choice of organic solvent—Of the other chlorinated hydrocarbons that could be used, only 1,1,1-trichloroethane possessed a threshold limit value high enough (1 900 mg m^{-3})[6] to make the change from carbon tetrachloride worthwhile. In 1,1,1-trichloroethane solution it was found that the spectrophotometric response of lead dithizonate was increased by a factor of 1·2 compared with that in carbon tetrachloride under similar test conditions, the wavelength of maximum absorption being 520 nm. A slight disadvantage in the use of 1,1,1-trichloroethane was that it separated less clearly than carbon tetrachloride from the aqueous phase. However, this was easily overcome by running the organic phase through a strip of Whatman No. 1 chromatographic paper (20 × 80 mm) previously rolled up and inserted into the stem of the separating funnel. The inhibitors present in some of the commercially available brands of 1,1,1-trichloroethane tested, apparently comprising a number of compounds, appeared either to react with the dithizone producing yellow background colours or to decompose the dithizone so that insufficient reagent remained to complex the lead completely. The use of uninhibited solvent, which is available from some chemical suppliers, is therefore essential.

Dithizone reagent—While the original dithizone solution[2] (40 mg dissolved in 1 litre of carbon tetrachloride) was reasonably stable if it was shaken with a reducing solution, such as sulphur dioxide in water, and thereafter kept in a refrigerator, this was inconvenient for field use. At room temperature (20 °C) this solution was found to deteriorate markedly in a few hours. A more concentrated 1,1,1-trichloroethane solution of the reagent, 40 mg per 100 ml, was found to be considerably more stable at room temperature, the rate of deterioration being such that solutions up to 7 days' old could be used. In the field this stock solution required to be diluted ten times prior to use in a test. However, even by using this procedure the dithizone reagent blank, shown as a yellow colour, gradually increased with time such that the visual colour differentiations at the various concentrations of lead became difficult. Tests appeared to show that the production of the yellow blank was a temperature effect rather than a photochemical one. Removal of this yellow background colour from the 5 ml of 1,1,1-trichloroethane solution of dithizone to be used in a test, together with any coloured metal dithizonate complex blank that may have been present in the reagents, was found to be possible simply by extracting the dithizone solution in a separating funnel with the aliquot of buffer solution to be used in the test. The lower, organic, layer containing the coloured interferences was separated and discarded.

The buffer solution containing the dithizone reagent was retained in the separating funnel and to this was added the dissolved lead sample, followed by 5 ml of fresh 1,1,1-trichloroethane. (Since dithizone appeared to have a limited stability of a few hours in the alkaline buffer solution it was found desirable to pre-extract each aliquot of dithizone working solution just prior to its use). The lead dithizonate was then extracted into the 1,1,1-trichloroethane layer, which was run off, and its colour was compared with standards. The colours produced from 0 to 15 μg of lead were found to be stable for at least 3 to 4 days provided that the organic solutions were tightly stoppered. The dilute dithizone solution was usable for at least 8 hours after preparation provided that each aliquot was treated as above before being used in a test.

A survey of the different commercially available dithizone reagents indicated that several gave reduced colour responses with lead. Stock 1,1,1-trichloroethane solutions of such dithizone reagents also decomposed rapidly, usually producing a red instead of green colour. In view of this a simple sorting test was devised to preclude the use of unsatisfactory dithizone reagent in this method for lead. Details of the sorting test are given later in the method.

Buffer solution—With the introduction, as indicated above, of a preliminary extraction designed to remove any lead reagent blank, involving the aliquots of dithizone and buffer solutions that were to be used in the test, it was no longer found necessary to prepare singly the constituents of the buffer solution. The revised buffer solution (see section on Reagents) had a pH of 11.

Colour standards—The original test[2] had visual colour standards representing 0, 3, 6, 12 and 15 μg of lead, which corresponded respectively to 0, 1, 2, 4 and 5 times the threshold limit value when a 15-litre sample of atmosphere was taken. The disadvantage of this set of standards was the absence of a half threshold limit value. With the increased colour response obtained through the use of 1,1,1-trichloroethane instead of carbon tetrachloride, colour differentiation between the two highest levels became more difficult and it was decided in this revision to omit the original top level. It is now recommended that if test atmospheres are suspected to contain greater than four times the threshold limit value of lead a smaller sample than the normal 15 litres should be taken and the appropriate correction factor should be applied to the standards. With the increased colour response and lowering of reagent blanks observed with this revision, it was found possible to include a half threshold limit value standard. Details of the preparation of the colour standards are given below.

INTERFERENCES—

Although not departing from the main feature of the original method,[2] *i.e.*, the use of dithizone reagent for the determination of lead, it was considered prudent to re-examine the possible interference effects of other metals that might occur with lead in industrial atmospheres on this revised method. This was done by adding known amounts of these metals to solutions containing 1·5 μg of lead (equivalent to a 15-litre sample of an atmosphere containing half the present threshold limit value of lead) prior to the determination of the latter. Under the test conditions up to 3 μg of copper, 15 μg of antimony(III) or 6 μg of cadmium did not interfere. These amounts were respectively equivalent to those which would be present in a 15-litre sample of an atmosphere containing twice the present threshold limit value of the interfering metal. On a similar basis iron(III) and zinc interfered but the levels that were tolerated, 50 μg in each case, were considered to be sufficiently high to allow lead to be determined by the field test in all but the most exceptional circumstances without interference from iron or zinc. Tin(II) ions in solution at a concentration as low as 15 μg interfered strongly with the test. However, it was considered that tin trapped on a filter would not be extracted in sufficient quantity by the nitric acid - hydrogen peroxide solution to cause interference. This was confirmed when 60 μg of tin(II) and 1·5 μg of lead were spotted on to a filter-paper that was allowed to dry. An extraction was carried out, and the lead was determined, 1·45 μg of lead being recovered.

METHOD

APPARATUS—

Filter-paper holder—A holder that will take filter-papers of 25 mm diameter.

Filter-paper—Millipore type AA (0·8 μm), 25 mm in diameter, and strips of Whatman No. 1 chromatographic paper, 20 × 80 mm.

Sampling pump—A pump capable of drawing air through the filter-paper at a fixed rate of between 3 and 5 l minute^{-1}.

Separating funnel—This was 100 ml in capacity, having a PTFE stop-cock and a glass stopper.

Glass tubes for colour comparison—These were 13·5 mm in i.d. and had a capacity of 10 ml. (Tintometer Ltd., Salisbury, supply pairs of tubes suitable for use in conjunction with the Lovibond "1000" Comparator.)

REAGENTS—

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

Nitric acid - hydrogen peroxide solution—Dilute 5 ml of lead-free concentrated nitric acid (sp.gr. 1·42) to 100 ml with water and add 0·2 ml of 30 per cent. w/v hydrogen peroxide.

Buffer solution—Dissolve 3 g of potassium cyanide, 6 g of sodium metabisulphite and 5 g of ammonium citrate in about 200 ml of water, add 325 ml of ammonia solution (sp.gr. 0·88) and dilute to 1 litre with water. (This solution is stable for several months if stored in a polythene screw-topped bottle.)

Standard lead solution—Dissolve 192 mg of lead nitrate in 1 litre of 0·1 N nitric acid to give a solution containing 120 μg ml^{-1} of lead. Dilute 5 ml to 200 ml with 0·1 N nitric acid to give a solution containing 3 μg ml^{-1} of lead.

1,1,1-*Trichloroethane, containing no inhibitor.*

(Solvents supplied by Hopkin and Williams Ltd. or Fisons Scientific Apparatus Ltd. were found to be suitable for use.)

Dithizone stock solution—Dissolve 40 mg of dithizone in 100 ml of 1,1,1-trichloroethane. Renew this solution after 7 days.

Dithizone working solution—Dilute 5 ml of the dithizone stock solution to 50 ml with 1,1,1-trichloroethane. Renew this solution after 8 hours. (The suitability or otherwise of any batch of dithizone reagent must be assessed prior to the use for test purposes of a working solution prepared from that dithizone. A suitable sorting test for dithizone reagent is given below.)

PROCEDURE—

Place a Millipore type AA filter-paper in the holder, attach the assembly to the pump and draw a 15-litre sample of air through the paper at a rate of 3 to 5 l minute^{-1}. Disconnect the holder from the pump, remove the paper and place it in a small beaker not less than 25 mm in diameter. Add 2·5 ml of the nitric acid - hydrogen peroxide solution and leave it to stand for 5 minutes.

Add 15 ml of the buffer solution to the separating funnel followed by 5 ml of the dithizone working solution. Stopper the funnel and shake it vigorously for about 15 s. Allow the mixture to stand until the layers separate, then run off the lower layer and discard it. Dry the inside of the stem of the separating funnel with a rolled-up piece of filter-paper and then insert into the stem a rolled-up strip of Whatman No. 1 chromatographic paper, 20 × 80 mm in size. Transfer the acidic solution from the beaker to the funnel, wash the beaker with two 1-ml aliquots of water and add the washings to the funnel. Add 5 ml of 1,1,1-trichloroethane to the mixture, stopper the funnel and shake it vigorously for about 15 s. Allow the mixture to stand until the layers separate, run the lower layer into a colour comparison tube and compare the colour, preferably in daylight, with each of the lead colour standards contained in similar tubes. View the respective liquids against a white paper background.

SORTING TEST FOR DITHIZONE REAGENT—

Carry out the procedure (as above) from the stage at which 15 ml of buffer solution is added to the separating funnel up to, and including, the insertion of the rolled-up strip of paper into the stem of the funnel. Then add 2 ml of the dilute standard lead solution (3 μg of lead ml^{-1}) to the funnel. Add 5 ml of 1,1,1-trichloroethane to the mixture, stopper the funnel and shake it vigorously for about 15 s. Allow the mixture to stand until the layers separate, run the lower layer into a colour comparison tube and compare the colour, preferably in daylight, with the 0·4 mg m^{-3} of lead colour standard (see Table I) contained in a similar tube. Again, view the liquids against a white paper background. Unless a good match in both colour and intensity is obtained, the dithizone working and stock solutions should be rejected and fresh solutions should be prepared with a new batch of dithizone reagent. (If a spectrophotometer is available, an optical density of 0·36 $\pm$ 0·02 should be obtained in the sorting test by using a 10-mm cell and reading at 520 nm against a 1,1,1-trichloroethane blank.)

TABLE I

VOLUMES OF COBALT SULPHATE, COPPER SULPHATE AND POTASSIUM DICHROMATE SOLUTIONS PER 25 ml TO PRODUCE AIRBORNE LEAD FIELD TEST COLOUR STANDARDS

Lead standard/mg m^{-3}	0	0·1	0·2	0·4	0·8
Yellow component/ml	0·70	0·20	0·25	0·20	0·20
Blue component/ml	0·00	0·05	0·10	0·60	0·50
Red component/ml	0·20	1·00	2·80	5·50	10·20

PREPARATION OF COLOUR STANDARDS—

The various components were added as follows: red component, dissolve 10 g of cobalt sulphate heptahydrate ($CoSO_4.7H_2O$) in 85 ml of water; yellow component, dissolve 0·1 g of potassium dichromate in water and make up to 100 ml; and blue component, dissolve 10 g of copper sulphate pentahydrate ($CuSO_4.5H_2O$) in water, add 1 ml of concentrated hydrochloric acid and make up to 100 ml with water. Prepare the colour standards by mixing these solutions in the proportions shown in Table I, diluting each to 25 ml with water and mixing throroughly.

As an alternative to the above colour standards, a series of permanent glass standards on a comparator disc was prepared in collaboration with, and is available from, Tintometer Ltd., Salisbury.

DISCUSSION AND APPLICATION OF THE METHOD—

Although designed specifically as a field test and not intended for the accurate determination of lead in air, this method can be used to determine lead accurately if a spectrophotometer is used. The required calibration graph can be prepared by using standard lead solutions and reading the intensities of the lead dithizonate colours in a 10-mm cell at 520 nm against a reagent blank.

The sampling technique in this revised method, apart from a change in the filter used, had not been materially changed from the adequately tested original.[2] Consequently, a detailed evaluation of sampling procedure was not considered necessary for this present work. However, by using samples of lead dust collected in an industrial atmosphere, trials were carried out primarily to check on the efficiency of dissolution of dust samples (compared with laboratory-generated and collected fume samples) collected on Millipore type AA filters. A wide range of sample weights was deliberately obtained by sampling volumes of the atmosphere considerably in excess of the normal 15 litres taken in the proposed field test. After carrying out the usual field test extraction the filters were decomposed by wet combustion and any lead present was determined. Table II shows that over a range of weights of lead, 2·2 to 62 μg in the dusts collected, the percentage extraction of the metal was independent of the weight and also entirely satisfactory for field test purposes. Table II also indicates that with a spectrophotometric finish the scope of this test can be extended to cope with larger samples of lead than can be determined using the visual colorimetric finish.

This work was carried out on behalf of the Department of Employment and Productivity Committee on Tests for Toxic Substances in Air. We thank the Government Chemist for permission to publish this paper and H.M. Factory Inspectorate for arranging facilities for the field tests.

TABLE II

EFFICIENCY OF EXTRACTION OF LEAD DUST SAMPLES FROM FILTERS BY USING THE PROPOSED FIELD TEST PROCEDURE

Sample	Lead found*/μg: By extraction† of filters	Lead found*/μg: By wet combustion‡ of extracted filters	Efficiency of extraction, per cent.
1	2·2	0	100
2	2·3	0·35	87
3	4·0	0·40	91
4	4·9	0·15	97
5	16·0	0	100
6	18·6	0·9	95
7	19·0	0	100
8	52·0	0·25	100
9	53·6	0·25	100
10	62·0	0·8	99

* By spectrophotometric version of field test procedure.
† With nitric acid - hydrogen peroxide solution.
‡ With fuming nitric acid - perchloric acid mixture.

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The Spectrophotometric Determination of the Purity of Commercial Dithizone and the Purification of Small Amounts of the Reagent by Chromatography

BY H. G. C. KING AND G. PRUDEN

(Rothamsted Experimental Station, Harpenden, Herts.)

The purity of commercial dithizone is determined quantitatively by comparing the optical density of a solution at wavelength 620 nm with that of a pure sample. Qualitatively, the purity is estimated from the pattern obtained by chromatographing the crude dithizone on Whatman SG81 (silica-impregnated) paper.

Small amounts of the pure reagent are separated by chromatography on large sheets of Whatman SG81 paper or on a column consisting of a mixture of equal parts of acid-washed silica gel (Kieselgel N) and Celite 545, with benzene as eluting agent.

DITHIZONE (diphenylthiocarbazone) is widely used as a colorimetric reagent for metals, but variations in the purity of samples may necessitate purification of the reagent before making a determination.

The impurities associated with dithizone are oxidation products, which are usually retained in the organic phase after extracting the dithizone into aqueous ammonia solution from carbon tetrachloride or chloroform. The ammoniacal solution is then acidified and the pure dithizone is extracted into carbon tetrachloride or chloroform. The procedure may be lengthy and often requires the use of large volumes of the organic solvent.

Sandell[1] suggests that purification is not always necessary, the presence of small amounts of yellow oxidised impurities being significant only when traces of lead or cadmium are to be determined after extraction from a basic solution.

We found that the purity of a dithizone sample can be determined by chromatography on silica-impregnated paper (Whatman SG81), with benzene as solvent. Oxidation products, the mobilities of which are greater than that of dithizone, are easily distinguished visually and by their R_F values.

The purity of the reagent can be determined quantitatively by spectrophotometry. Pure dithizone has absorption maxima at wavelengths 446 nm and 620 nm in carbon tetrachloride, the ratio of the optical density of the lower-wavelength peak to that of the higher-wavelength peak being 0·627 : 1. A solution of 1 mg of pure dithizone in 100 ml of carbon tetrachloride has an optical density of 1·290 at wavelength 620 nm. The wavelength of maximum optical density of this solution, when oxidised completely by sunlight to a yellow colour, is 390 nm ($E_{max} = 0·254$). Although the absorbance of the impurities at wavelength 620 nm is negligible, their presence decreases the optical density at this wavelength by a dilution effect that is proportional to the amount of impurities present. Measurement at 620 nm gives the purity of the sample by reference to the value at the corresponding peak in a pure sample.

The following two methods of purification have proved satisfactory.

(*i*) *Large-scale chromatography on Whatman SG81 paper*—The chromatogram is developed with benzene and pure dithizone is eluted from the chromatogram with acetone, which is subsequently removed by distillation *in vacuo*. Fifty milligrams of crude dithizone can be chromatographed in this way.

(*ii*) *Column chromatography*—Dithizone can also be purified on a column of silica gel, with benzene as eluting agent. The most satisfactory column packing consists of a mixture of equal parts of acid-washed silica gel (Kieselgel N) and acid-washed Celite 545; use of the latter increases the mobility of the separated components. Benzene is the preferred eluting agent for

column chromatography, but the amount of dithizone that can be purified at one time is limited by the relatively low solubility of dithizone in this solvent. Iwantscheff[2] gives the solubility of dithizone in benzene as 1·64 g l^{-1}; we found it to be 1·43 g l^{-1} for a sample containing 83·3 per cent. of pure dithizone when determined spectrophotometrically.

For colorimetric work 100 mg of dithizone are usually dissolved in 1 litre of carbon tetrachloride, which is sufficient for about 100 determinations. A column containing 20 g of Kieselgel N - Celite 545 cannot accept more than 5 ml of solution without excessive spreading of the separated bands. The preparation of 100 mg of pure dithizone from starting material of reasonable quality thus requires a column containing a bed weight of 280 g of silica gel - Celite. As in the paper-chromatographic separation, the oxidised impurities run ahead of the pure dithizone band. The benzene is removed from the eluate by evaporation and the residue is dissolved in carbon tetrachloride as required.

Experimental

Because of the toxicity of benzene, the necessary precautions should be taken when handling the solvent or solutions containing it.

Spectrophotometric determination of the purity of commercial dithizone samples—

Weighed samples of commercial dithizone were dissolved in carbon tetrachloride to give a concentration of about 1 mg in 100 ml of the solvent. The optical density of each solution was measured at 620 nm, the wavelength of the main spectral maximum, and the purity of the sample was determined by reference to the corresponding optical density of a pure sample of the reagent. Table I gives optical densities at wavelength 620 nm of several commercial samples of dithizone.

Table I

Purity of commercial dithizone samples, determined spectrophotometrically

Description of sample	$E_{620\ nm}$ (1 mg per 100 ml of carbon tetrachloride)	Purity, per cent.
Dithizone, purified by column chromatography	1·290	100
1. Laboratory-reagent grade	0·485	37·6
2. Laboratory-reagent grade	0·519	40·2
3. Analytical-reagent grade	0·761	58·5
4. Analytical-reagent grade	1·069	82·8
5. Organic reagent for metals	1·079	83·3
6. Analytical-reagent grade	1·123	86·5
7. Organic reagent for metals	1·128	87·5
8. Metal indicator	1·218	94·0

The results show the variable quality of commercial samples; even analytical-reagent grades of the reagent may contain appreciable amounts of impurity. The purest sample, No. 8, is not specifically described as suitable for quantitative colorimetric work.

Chromatography of dithizone on Whatman SG81 paper—

Samples of commercial dithizone show the presence of several components when chromatographed on Whatman SG81 paper. Table II shows the colours and R_F values of the components of the most impure sample (No. 1, Table I). The solvent travelled 33 cm in 7 hours.

Table II

Paper-chromatographic separation of a low-grade dithizone sample

Colour of separated spot	R_F value
Dull green	0·0
Bright green (dithizone)	0·49
Yellow - green	0·55
Pink	0·67
Yellow	0·72

PREPARATIVE CHROMATOGRAPHY OF DITHIZONE ON WHATMAN SG81 PAPER—

A sheet of Whatman SG81 paper, 50 cm long and 45 cm wide, is folded 2 cm from one end of the longer direction, and the outer edge of the fold is dipped into a solution of crude dithizone (50 mg dissolved in 5 ml of chloroform) contained in a chromatographic trough 45 cm long. When all of the solution has been absorbed by the paper, the chloroform is allowed to evaporate and the chromatogram developed downwards with benzene.

Initially, the strip of absorbed dithizone is usually about 5 cm deep with straight leading and rear edges. After developing the chromatogram the strip is about three times its original depth. Oxidised components run ahead of the dithizone band. Pure dithizone is isolated by tearing off the band and dissolving the dithizone from the paper with acetone, which is removed by vacuum distillation, weighing the residue and dissolving it in carbon tetrachloride to give a solution containing 1 mg of dithizone in 10 ml of solvent. With the simultaneous development of several chromatograms the amount of pure dithizone isolated can be increased as required.

PREPARATION OF PURE DITHIZONE BY COLUMN CHROMATOGRAPHY—

Ten grams of silica gel (Kieselgel N) and 10 g of Celite 545, both acid washed and dried at 110 °C, are stirred thoroughly with benzene and the slurry is poured into a glass-chromatographic column, 20 × 2 cm in diameter. After washing with benzene, the column bed is about 17 cm deep when settled. A filtered solution of crude dithizone, containing 7 mg in 5 ml of benzene, is applied to the top of the column. The chromatogram is developed with benzene, the band of pure dithizone is isolated and the benzene is removed by distillation *in vacuo*.

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The Determination of Dimethylpolysiloxane in Beer and Yeast

BY A. SINCLAIR AND T. R. HALLAM

(*Allied Breweries Process Research Department, Burton upon Trent, Staffordshire*)

An infrared method that provides an estimate of the silicone content of beer and yeast has been developed. Because of the increased sensitivity over other reported methods it is possible to use smaller samples, which could be an advantage with biological material. The recommended working concentration range is 0·2 to 2 mg l^{-1}.

THE control of foams, which is a common problem in the food industry, can be achieved by the addition of antifoam agents. Dimethylpolysiloxane ("silicone") is commonly used as a foam-control agent and is recognised as a permitted additive in foodstuffs subject to a recommended limit of 10 p.p.m.[1] in the final product. Analytical control is therefore necessary to ensure that this level is not exceeded.

Two methods are commonly used for the assay of silicones in toiletries and foodstuffs. The first is a colorimetric method,[2,3] which is dependent on the conversion of silicones into silicates and the subsequent formation of a coloured heteropolyacid. While this method has satisfactory sensitivity for trace analysis, it is subject to interference if other forms of silicon are present in the original sample.

In the second method[3,4] the silicone is extracted from the sample and determined specifically and quantitatively by infrared spectroscopy, by using the Si–CH_3 band at 7·95 μm. However, it is insufficiently sensitive for trace analysis.

A recent method for assaying silicones in foodstuffs and paper coatings is by atomic absorption,[5,6] which is reported to be both highly sensitive and free from interference by silicates. As this method was not available to the present authors, the infrared method was chosen for further study.

In the method described by Horner, Weiler and Angelotti,[2] silicone was determined in the range 2 to 20 p.p.m. in pineapple juice by azeotropic distillation of the sample with benzene. The extracted silicone was then assayed in carbon disulphide solution by infrared spectroscopy at wavelength 7·95 μm. Application of this method to the analysis of beer and yeast gave low and variable results (0 to 5 per cent. recovery within the concentration range 2 to 20 p.p.m. of silicone). Investigations showed that the following factors affected the efficiency of the method: the extraction of silicones was reduced by the presence of beer components; terpenoid components in hop oil interfered with the infrared spectra; and finally the Si–CH_3 absorbance peak was too small for satisfactory measurement.

These problems have been overcome by altering the solvent-extraction conditions, removing interfering components and increasing the sensitivity of the method by using potassium bromide discs instead of liquid solutions for the infrared measurement. By using this method recovery of silicone, although low, is constant for a given medium, and the enhanced sensitivity permits the specific determination of dimethylpolysiloxane within the range 0·2 to 2·00 p.p.m.

EXPERIMENTAL

REAGENTS—

All reagents, unless otherwise stated, are of analytical-reagent grade and solutions are prepared with distilled water.

Dimethylpolysiloxane—As supplied by Hopkin and Williams Ltd.

Potassium bromide—Infrared spectroscopic grade; Merck Ltd.

Silicic acid, 100 mesh—As supplied by Mallinckrodt Chemical Co., U.S.A.

Sodium hydroxide solution, 0·1 M.
Benzene—Analytical-reagent grade, re-distilled.
Acetone—Analytical-reagent grade, re-distilled.
Chloroform—Analytical-reagent grade, re-distilled.
Aqueous chloroform—Shake 20 ml of re-distilled chloroform vigorously with 20 ml of water and use the lower layer.

APPARATUS—

Infrared spectrophotometer—Perkin-Elmer, Model No. 257, grating range 4000 to 625 cm^{-1}.
Hydraulic press—Research and Industrial Instrument Co., Model No. 0025, 0 to 25 tons.
Die press, 13-mm diameter—For preparation of potassium bromide discs.
Care should be taken to ensure that all apparatus is free from silicone grease.

PROCEDURE—

Preparation of calibration samples—Add, with an Agla microburette, 250-µl aliquots of standard 0·2, 0·4, 0·6, 1·2 and 2·0 mg ml^{-1} solutions of dimethylpolysiloxane in chloroform to individual samples of 250 ml of the beer or suspensions of 5 g of the yeast in 100 ml of water. Analyse these samples by using the described technique.

Extraction—Add the sample, consisting of 250 ml of beer or 5 g of yeast suspended in 100 ml of water, to a 1-litre round-bottomed flask and evaporate it to 40 ml by using a rotary evaporator and water-bath (80 °C). Transfer the residue with 10 ml of distilled water to a separating funnel, add 75 ml of acetone and rinse the flask with 50 ml of benzene, adding the rinsings to the separating funnel. Shake the mixture for 1 minute, allow it to separate and run off the lower aqueous layer. Add 100 ml of acetone - benzene mixture (1 + 1) to the aqueous layer and carry out two more extractions.

Combine the solvent fractions, shake them gently for 5 s with two 25-ml volumes of 0·1 M sodium hydroxide solution and finally with two 25-ml volumes of water to remove residual sodium hydroxide. Transfer the upper solvent layer to a 250-ml round-bottomed flask and evaporate it to dryness on a rotary evaporator at 50 °C. Dissolve the residue in 1 ml of aqueous chloroform and transfer it to a column containing silicic acid (with bed size 50 × 5 mm), which has been previously washed with aqueous chloroform. Rinse the flask with two 1-ml portions of aqueous chloroform and use the rinsings to elute the column. Run the eluate from the column on to powdered potassium bromide (0·3 g), contained in an agate mortar, and remove the excess of solvent with a current of warm air.

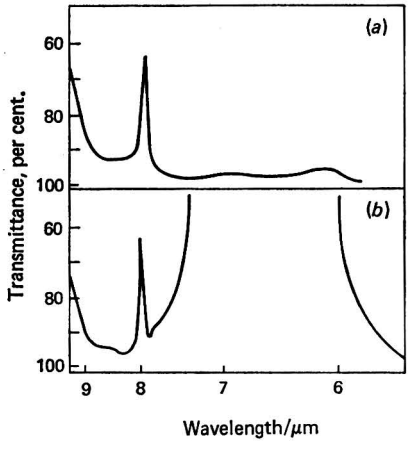

Fig. 1. Comparison of infrared spectra for: (*a*), 0·05 mg of dimethylpolysiloxane in potassium bromide; and (*b*), 2·0 mg of dimethylpolysiloxane in carbon disulphide solution

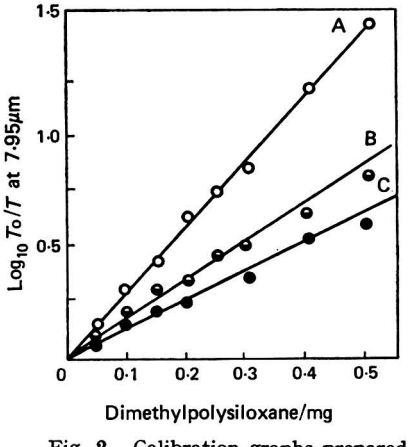

Fig. 2. Calibration graphs prepared by adding known amounts of dimethylpolysiloxane to potassium bromide, yeast and beer: A, potassium bromide; B, yeast; and C, beer

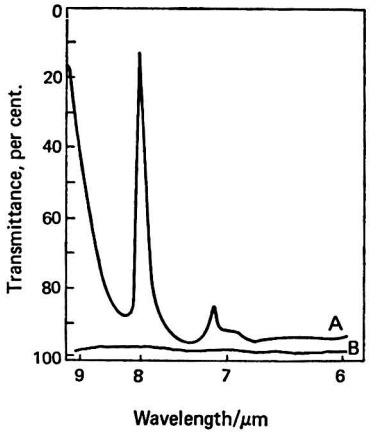

Fig. 3. Base-line transmittance of silicone standard and potassium bromide reference disc: A, 0·5 mg of dimethylpolysiloxane in potassium bromide; and B, potassium bromide reference

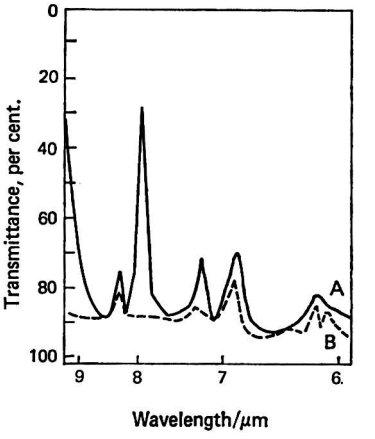

Fig. 4. Base-line transmittance of beer sample containing no silicone (A) and 0·5 mg of silicone (B)

Infrared spectroscopy—Dry the potassium bromide at 105 °C for 5 minutes under an infrared lamp and transfer it to a die press. Remove any silicone adhering to the mortar by grinding a further 0·2-g portion of powdered potassium bromide in the mortar, then add it to the die and mix the two portions thoroughly.

Apply pressure (15 tons inch^{-2}) and vacuum to the die for 2 minutes. Scan the resulting disc from 6 to 9 μm in the spectrophotometer with a blank disc prepared from potassium bromide in the reference beam. A scan speed of 50 cm^{-1} minute^{-1} has been found satisfactory for measurements of transmission at wavelength 7·95 μm, which is converted into absorbance (A) in the usual way.

Prepare a calibration graph by plotting absorbance (A) *versus* milligrams of silicone for the samples containing known added amounts of silicone, then determine the silicone content of the sample by extrapolation.

Results and discussion

Sensitivity of the infrared measurement—

Previous workers[2,4] have determined silicones by measurement of the Si–CH_3 absorbance at wavelength 7·95 μm in a suitable solvent, *viz.*, carbon disulphide. By incorporating the extracted silicone in a potassium bromide disc 13 mm in diameter increased sensitivity was achieved while the large solvent absorbance adjacent to the Si–CH_3 peak was eliminated. This effect is demonstrated by comparison of the spectra obtained from 0·05 mg of silicone in potassium bromide and 2·0 mg of silicone in carbon disulphide (Fig. 1).

Linearity of response—

Calibration graphs were prepared either by adding known amounts of silicone to potassium bromide and preparing discs, or extracting silicone from aqueous solution prior to disc preparation. It was found that a linear response was obtained between 0·05 and 0·5 mg of silicone per disc. Silicone contents greater than this are not recommended because of fogging of the discs, but higher silicone contents can easily be determined by adjusting the sample size to ensure that the silicone content of the disc lies within the quoted range. Fig. 2 demonstrates typical calibrations obtained by extracting added silicone from samples of beer and yeast and by addition to potassium bromide discs.

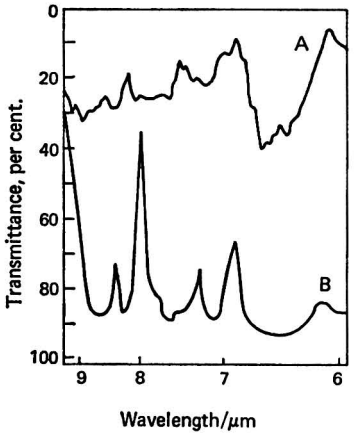

Fig. 5. Removal of interfering material from extract: A, spectrum before removal; and B, spectrum after removal

BASE-LINE CORRECTION—

For silicone standards prepared from aqueous solutions the base-line transmittance was taken as 100 per cent. (Fig. 3). However, for samples the base-lines were obtained by drawing a straight line across the base of the Si–CH_3 peak. That this technique is acceptable is shown in Fig. 4, which illustrates the close proximity of the Si–CH_3 base-line and the true base-line of the same sample that contained no added silicone.

EXTRACTION OF SILICONE—

A major problem that arises in the determination of silicone in beer is its effective extraction from the aqueous sample. When the extraction method of Horner, Weiler and Angelotti was used in the assay of beer containing silicone low recoveries (about 5 per cent.) were obtained. Attempts to improve the extraction by the study of model systems showed that whereas silicone could be quantitatively extracted from water or water containing 3 per cent. w/w of ethanol, the presence of carbohydrates in beer reduced the recoveries obtained (Table I).

TABLE I

EXTRACTION OF SILICONE FROM MODEL SYSTEMS AND BEER

Sample	Silicone added/mg	Recovery of silicone added, per cent., by: Extraction method of Horner *et al.*	Recovery of silicone added, per cent., by: Proposed extraction procedure
Water	0·1	100	50
	0·4	87	50
Water containing 3 per cent. w/w of ethanol	0·1	100	50
	0·4	100	62
Starch solution (1 per cent. w/v)	0·1	<10	60
	0·4	<10	—
Carbohydrate solution*	0·1	30	60
	0·4	—	75
Beer	0·05	<10	60
	0·10	—	50
	0·20	—	40
	0·30	—	50
Yeast	0·10	—	70
	0·20	—	60

* Carbohydrate solution: mixture of glucose, sucrose, maltose and dextrins all at 1 per cent. w/v in water containing 3 per cent. w/w of ethanol.

The extraction method, which has been found most useful in the assay of beer and yeast, involves dilution of the aqueous concentrate with a semi-polar water-miscible solvent, *viz.*, acetone, prior to extraction with benzene. Although a quantitative extraction of silicone cannot be achieved (Table I), this disadvantage can be overcome by appropriate calibration.

If a calibration graph, obtained by extraction of silicone from water, is used it is necessary to derive an extraction recovery factor for the sample being analysed. As this factor can vary with the sample composition an alternative, more convenient procedure is to prepare a calibration graph by adding known amounts of silicone to the sample. When the samples are of a similar type, *e.g.*, beers of similar specific gravity, then one calibration graph may suffice for the analysis of several samples.

Removal of interfering beer constituents—

When samples of beer containing silicone were extracted with benzene, it was found that the absorbance due to the Si–CH_3 peak could not be observed because of irrelevant absorption (Fig. 5, A). It was assumed at this stage that hop components present in the beer, *viz.*, isohumulone derivatives, essential oils, *etc.*, were responsible. When the solvent extract was washed with 0·1 N sodium hydroxide and water, most of the interference was removed and a green pigment of unknown constitution was left. After an investigation of various chromatographic adsorbents, silicic acid was found preferentially to adsorb this material when aqueous chloroform was used to dissolve the residue obtained after evaporation of the benzene extract (Fig. 5, B).

Recovery of silicone from beer and yeast—

Samples of beer and yeast were treated with known amounts of silicone to give concentrations of 0·2 to 2·0 mg l^{-1}. These samples were then assayed for silicone content by the described method. The results obtained (Table II) demonstrate that the method gives good recoveries of silicone within the concentration range chosen. Samples of beer and yeast containing unknown amounts of silicone were analysed in replicate, and the results obtained are summarised in Table III.

Table II

Recovery of silicone added to beer and yeast

	Beer			Yeast		
Silicone added/mg	Silicone found/mg	Average	Error	Silicone found/mg	Average	Error
0·05	0·06, 0·03	0·045	−0·005	0·06, 0·08	0·07	+0·02
0·10	0·08, 0·09	0·085	−0·015	0·10, 0·09	0·095	−0·005
0·15	0·13, 0·13	0·13	−0·02	0·12, 0·18	0·15	0·00
0·20	0·20, 0·20	0·20	0·00	0·24, 0·26	0·25	+0·05
0·25	0·28, 0·28	0·28	+0·03	0·25, 0·28	0·265	+0·015
0·30	0·26, 0·31	0·285	−0·015	0·30, 0·30	0·30	0·00
0·40	0·35, 0·43	0·39	−0·01	0·38, 0·43	0·405	+0·005
0·50	0·46, 0·50	0·48	−0·02	0·55, 0·57	0·56	+0·06
Mean error			0·014			0·013
Standard deviation	0·027			0·023		

Table III

Silicone content of miscellaneous samples

Sample	Silicone content/mg l^{-1}
Beer	0·90 0·88
	0·56 0·59
	0·50 0·44
	1·20 1·10
Centrifuge sludge	14·0 —
	34 35
Yeast	21·0 —

We thank the Directors of Allied Breweries Ltd. for permission to publish this paper, and Mrs. A. P. Harrison for skilful technical assistance.

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Determination of Cyanogen Chloride in Activated Niacin Test Strips

By MORTON S. LEFAR,* ALVIN MASLANSKY, WILLIAM D. YOUNG AND DONALD P. KRONISH

(Departments of Analytical/Physical Chemistry and Diagnostics Research, Warner-Lambert Research Institute, Morris Plains, New Jersey 07950, *U.S.A.)*

A simple paper-strip method for the determination of niacin in cultures of human tubercle bacteria based on the reaction with cyanogen chloride is described. Although a yield of only about 5 per cent. of cyanogen chloride is obtained, it represents an excess of from 5 to 50 times that needed to react with the 5×10^{-7} to 5×10^{-8} mole of niacin expected in samples. The chemistry of the system, the determination of cyanogen chloride and an evaluation of the procedure are presented.

Recently, a reagent-impregnated paper strip test† for the determination of niacin has been developed.[1,2] The niacin test is used for the differential identification of mycobacteria as human tubercle bacilli produce more niacin than other mycobacteria.[3,4,5,6] The strip has been tested with 378 clinical isolates of mycobacteria and saline solutions of niacin; it is sensitive to about 3 μg of niacin in 0·6 ml of saline.

The chemistry of the niacin detection test strip involves the reaction between cyanogen chloride, which is formed when the reagents on the strip are moistened, and a test solution containing niacin in the presence of 4-aminobenzoic acid.

Because of the potentially toxic nature of large amounts of cyanogen chloride, it was desirable to limit the amount of gas generated without affecting the performance of the test strip. It was necessary, therefore, to develop a procedure for the determination of cyanogen chloride generated by the test strip.

Several methods are known for the determination of cyanogen chloride.[7 to 12] However, they are rather cumbersome and would not be appropriate for use with the test strip. Malatesta and Dubini[13] have shown that it can be determined colorimetrically by using the procedure proposed by Aldridge[8] and modified by other workers.[9,10,11,12] Malatesta and Dubini allowed cyanogen chloride, which is formed by the reaction of hydrocyanic acid with an aqueous solution of chloramine T, to react with an excess of pyridine to give glutaconic aldehyde. The reaction of this aldehyde with compounds containing active methylene groups yields additional products the colours of which varied from orange - red to violet - red. The presence in diethyl acetonedicarboxylate of two active methylene groups made this compound particularly sensitive to glutaconic aldehyde. The reddish violet colour that developed has a maximum absorption at 525 nm and Beer's law was followed between 0·8 and 2·2 μg of sodium cyanide. The colour developed in the cold (8 minutes) and was stable for 16 minutes.

In this paper we describe the determination of cyanogen chloride formed in the activated niacin test strip.

* Present address: Rhodia Inc., P.O. Box 111, New Brunswick, N.J. 08903, U.S.A.

† Pathotec-Niacin, made by the General Diagnostics Division, Warner-Lambert Pharmaceutical Co., Morris Plains, New Jersey.

METHODS AND MATERIALS

The composition of the niacin test strip is shown in Fig. 1. On dipping area 1 of the strip into the solution to be tested, contained in a test-tube, the potassium thiocyanate reacts with the chloramine T to form cyanogen chloride. A positive test for niacin is indicated by the development of an orange to yellow colour in the solution. The presence of citric acid affords an optimum pH of 4 to 7.

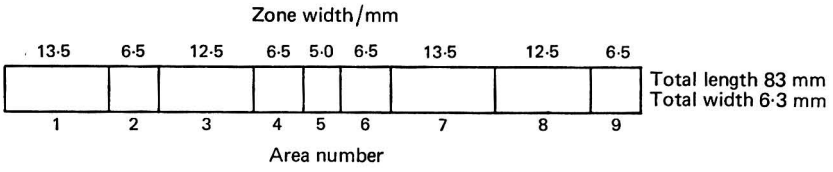

Fig. 1. Composition of the niacin test strip. Areas: 1, 4-aminobenzoic acid, sodium salt; 2, 4 and 6, untreated; 3, potassium thiocyanate; 5, citric acid; 7, chloramine T; 8, waterproof acrylic barrier; and 9, identification band

REAGENTS—

Pyridine - ester reagent—To 100 ml of distilled water in a 200-ml calibrated flask, add 20 ml of analytical-reagent grade pyridine and 0·2 ml of diethyl acetonedicarboxylate, n_D^{20} 1·439 0 (Aldrich Chemical Co., Milwaukee, Wisconsin, used without purification).

Chloramine T—Dissolve 1 g of chloramine T in 100 ml of distilled water.

DETERMINATION OF CYANOGEN CHLORIDE IN PAPER STRIPS—

Method 1—Place the paper strip into a 1 × 10-cm test-tube containing 0·6 ml of distilled water and then rapidly stopper the tube. Allow the tube to remain at room temperature for 20 minutes, occasionally shaking it. Remove a 10 to 50-μl sample with a syringe and transfer it to a 10-ml calibrated flask containing 1 ml of distilled water. Add 6 ml of the pyridine - ester reagent, shake the flask thoroughly and allow 7 minutes for colour development at room temperature. Determine the absorbance at 525 nm with a Beckman DU spectrophotometer (with covered cuvettes) against a sample of distilled water in the reference cell. The light reddish violet colour is stable for 12 minutes after full development.

Method 2—Place the paper strip (with or without areas 1 and 5) into a 10-ml calibrated flask and add 10 ml of distilled water. Allow the flask to remain at room temperature for 20 minutes, occasionally shaking it, and then remove a 0·10 to 0·30-ml sample and make it up to 1 ml with distilled water. Add the pyridine - ester reagent and determine the concentration of cyanogen chloride as previously described.

Method 3—This method is identical with method 2 except that a 2-μl sample of solution was removed and transferred to a 10-ml calibrated flask containing 1 ml of water. Chloramine T solution (0·2 ml) was then added.

TABLE I

DETERMINATION OF CYANOGEN CHLORIDE BY METHOD 1

Batch*	Aliquot/μl	A_{525}	Concentration of CNCl/μmole	Average concentration of CNCl/mg × 10^{-2}
M	25	0·331	0·86	5·3
M	25	0·325	0·84	
B	25	0·345	0·89	5·8
B	25	0·392	1·00	

* Two batches were examined: 9646–D128 (M) and 9646–D128 (B).

CALCULATIONS—

$$\text{mmol of cyanogen chloride} = \frac{A_u}{A_s} \times C_s \times \frac{V_u}{V_a}$$

(mg of cyanogen chloride = mmol of cyanogen chloride × 61·471)

where A_u is the absorbance of unknown at 525 nm; A_s the absorbance of standard; C_s the concentration in mmole ml^{-1} of sodium cyanide corresponding to A_s; V_u the volume of distilled water (0·6 or 10 ml); and V_a the volume of aliquot removed from V_u, ml.

Results and discussion

Each strip contained about 6×10^{-5} mole each of potassium thiocyanate and chloramine T, which would afford 6×10^{-5} mole of cyanogen chloride provided the reaction was taken to completion. With method 1, which most closely resembles the manner in which the test strip will be used, $0{\cdot}8$ to $1{\cdot}0 \times 10^{-6}$ mole of cyanogen chloride was detected (Table I). This is adequate for the performance of the niacin test as the expected range of niacin concentrations is from 6 to 60 μg for 0·6-ml samples or about 5×10^{-7} to 5×10^{-8} mole. The approximately 5 per cent. yield of cyanogen chloride obtained in this laboratory, therefore, represents an excess of from 5 to 50 times that needed to react with the expected amount of niacin sample. Three reasons for the small amounts of cyanogen chloride formed upon strip activations were suspected: sodium 4-aminobenzoate or citric acid might interfere with the detection or formation of cyanogen chloride, or the concentration of chloramine T on the strip might be too small to allow a complete reaction. In the method of Malatesta and Dubini, the concentration of chloramine T was about 1 000 times that of cyanide. The loss of cyanogen chloride into the air above the test solution could also introduce an error. This would be important if the generation of cyanogen chloride were rapid.

Table II

Determination of cyanogen chloride by method 2

Strip area removed	Batch*	Aliquot/ml	A_{525}	Concentration of CNCl/μmole	Average concentration of CNCl/mg $\times 10^{-2}$
—	M	0·15	0·162	1·19	7·32
—	M	0·25	0·254	1·19	
—	B	0·30	0·235	0·921	5·14
—	B	0·15	0·096	0·753	
Area 1	M	0·10	0·281	3·03	18·8
	M	0·10	0·288	3·11	
Area 1	B	0·15	0·284	2·04	13·2
	B	0·15	0·314	2·26	
Areas 1 and 5 ..	M	0·15	0·360	2·56	14·8
	M	0·15	0·315	2·26	
Areas 1 and 5 ..	B	0·15	0·230	1·80	11·1
	B	0·15	0·257	1·85	
Area 1 (excess of chloramine T added) by method 3	B	0·002	0·125	69·1	424

* Two batches were examined: 9646–D128 (M) and 9646–D128 (B).

To examine the first two possibilities, first the sodium 4-aminobenzoate and then both the aminobenzoate and citric acid areas were removed from several strips and then activated according to method 2 (*cf.* Table II). This afforded $1{\cdot}2$ to $3{\cdot}1 \times 10^{-6}$ mole of cyanogen chloride per strip. As the air space above the solution contained in the calibrated flask was small, any error caused by loss of cyanogen chloride into the air space above the solution should also be small.

Removal of both citric acid and sodium 4-aminobenzoate gave about the same yield of liberated cyanogen chloride compared with removal of the aminobenzoate alone.

Removal of the sodium 4-aminobenzoate from several strips and addition of about a 1000-fold excess of chloramine T, followed by analysis as described above, showed the presence of $6{\cdot}9 \times 10^{-5}$ mole of cyanogen chloride per strip.

Conclusion

A method has been developed for the determination of cyanogen chloride when the niacin test strip is activated. Analysis in our laboratory shows a maximum of 0·19 mg (about 5 per cent. yield) of cyanogen chloride formed per strip. A large excess of chloramine T is apparently needed to afford the theoretical amount of 4 mg of cyanogen chloride per strip.

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The Use of *NN*-Dimethylcasein in the Determination of Proteolytic Enzymes in Washing Products and Airborne Dust Samples

BY E. DUNN AND R. BROTHERTON

(*Procter & Gamble Limited, Newcastle Technical Centre, Newcastle upon Tyne*)

NN-Dimethylcasein is used as substrate in an automatic method for the determination of proteolytic enzymes in washing products and airborne dust samples. Amino-acids formed by reaction with the enzyme are caused to react with 2,4,6-trinitrobenzenesulphonic acid to form stable, coloured Meisenheimer complexes. As *NN*-dimethylcasein does not react with trinitrobenzenesulphonic acid there is no need to remove excess of substrate before colour development, and the enzyme digestion and colour reactions can be conducted simultaneously. This leads to high sensitivity, which is of particular value in dust analysis and allows the use of a simple trouble-free manifold.

THE methods usually used for the determination of proteolytic enzymes are not directly applicable to washing products. Modifications have to be made to overcome the effects of the large excesses of surfactants, alkaline builders and oxidising agents such as sodium perborate. A widely used general approach is to allow the enzyme to digest a proteinaceous substrate, such as gelatin,[1] casein[1] or haemoglobin,[2] under controlled conditions for a given period and, after removal of undigested substrate, to determine the formed amino-acids by ultraviolet spectrophotometry,[1] titration[1,2,3,4] or colorimetry.[2,5] During removal of excess of substrate, many of these amino-acids are also removed from the system.

Recent reports[6,7] on the uses of *NN*-dimethylated proteins as substrates for proteolytic enzymes have led us to the development of a method with considerable advantages of simplicity and sensitivity over those in which unmodified proteins are used. Basically, the method involves digestion of *NN*-dimethylcasein, and determination of the formed amino-acids by their reaction with trinitrobenzenesulphonic acid to give stable, coloured Meisenheimer complexes.[8]

In dimethylcasein essentially all of the amino groups have been methylated. Methylation does not alter many of the properties of the protein, but prevents reaction with trinitrobenzenesulphonic acid. Consequently, excess of the substrate does not have to be removed and all of the amino-acids formed during digestion are available for this reaction. The digestion and colour development reactions can be carried out simultaneously. Over-all, this leads to a simplified and more accurate method with greater sensitivity.

Recently, several papers[9,10,11] have been published on the health hazards associated with the manufacture of enzyme-containing products. As part of an extensive programme of work designed to eliminate this risk it has been necessary to measure the enzyme content of the atmosphere in the factories concerned. Sample collection is achieved with the aid of an air sampling device. In this machine, air is drawn through a 15-cm diameter glass-fibre filter-paper (Whatman GF/C) at a rate of about 50 m^3 $hour^{-1}$. The high sensitivity of the analytical method described in this paper is of particular value in measuring the small amounts of enzyme collected over periods of about 1 hour.

EXPERIMENTAL

PREPARATION OF *NN*-DIMETHYLCASEIN—

A scaled-up version of the method of Lin, Means and Feeney[6] was used as follows.

REAGENTS—

Borate buffer, pH 9—To 5·12 g of sodium hydroxide *plus* 18 g of boric acid add 6 litres of water.

Casein—Hammarsten type as supplied by Merck.

Sodium borohydride.

Formaldehyde solution, 40 per cent.

Glacial acetic acid.

Octan-2-ol.

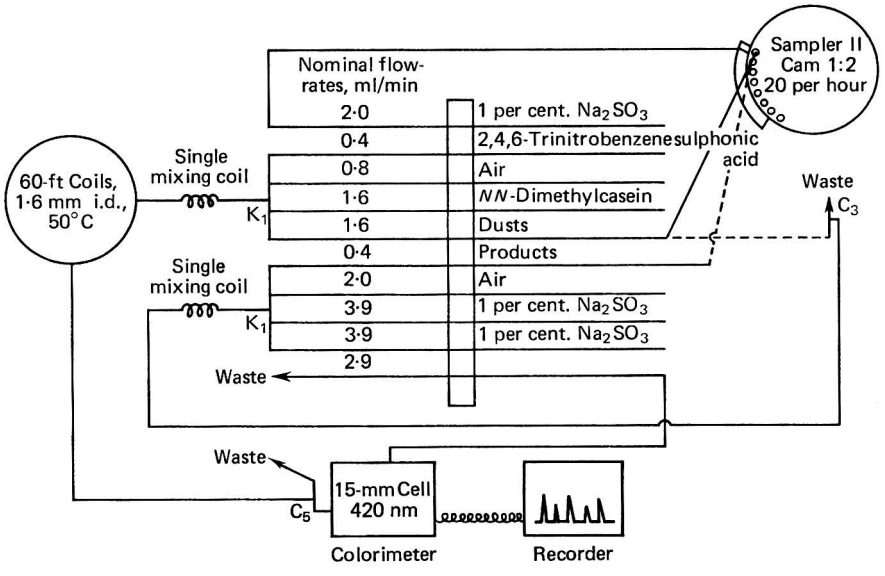

Fig. 1. Manifold for the determination of proteolytic enzymes

PROCEDURE—

Warm all of the borate buffer solution to between 40 and 50 °C and slowly add 180 g of casein, with rapid stirring. When all the casein has dissolved, cool the solution in a brine - dry-ice bath to 0 °C.

Add, from a total of 10 ml, 4 to 5 ml of octan-2-ol (as suds depressant) and add 36 g of sodium borohydride during at least 30 minutes, with rapid stirring. The remaining octan-2-ol can be added during the borohydride addition to reduce foaming.

Add 360 ml of formaldehyde solution from a burette at a steady rate of 1 ml every 5 s and then 50 to 55 ml of glacial acetic acid until the solution has a pH of 6. The solution is not dialysed as it was shown that dialysed material did not have any advantages over un-dialysed material. The solution is then lyophilised in an Edwards EF2 freeze-drying unit fitted with electrically heated trays, the coil temperature being −40 °C. Adjust the vacuum to boil the sample without excessive frothing until it becomes frozen. When the temperature falls to between −5 and −10 °C, apply full vacuum, then heat the trays. When the sample is dry, the temperature rises to +20 °C. The dried material, after grinding, consists of a fluffy white powder containing about 50 per cent. of dimethylcasein. Store it in a refrigerator.

ANALYTICAL METHOD

REAGENTS—

Sodium sulphite solution, 1 per cent. w/v—Dissolve 50 ± 0·1 g of anhydrous sodium sulphite or 100 ± 0·1 g of the hydrated salt, $Na_2SO_3.7H_2O$, in 5 litres of distilled water. Add 5 ml of Brij 35 solution.

Brij 35 solution—As supplied by Technicon.

NN-Dimethylcasein—Prepared as above. Store in a refrigerator.

NN-Dimethylcasein solution—Dissolve 64·8 ± 0·1 g of analytical-reagent grade sodium tetraborate, $Na_2B_4O_7.10H_2O$, and 37·6 ± 0·1 g of analytical-reagent grade sodium dihydrogen orthophosphate, $NaH_2PO_4.2H_2O$, in about 1 500 ml of distilled water in a 2-litre beaker. Boil 200 ml of distilled water in a 400-ml beaker, then remove it from the source of heat. Add slowly 8 g of *NN*-dimethylcasein and stir vigorously for 5 minutes. Transfer both solutions to a 2-litre calibrated flask, add 2 ml of Brij 35 solution and make up to volume with distilled water. Filter through a Whatman No. 54 filter-paper. The pH of this solution should be 8 ± 0·05. When not in use store the solution in a refrigerator and re-filter it if necessary; solutions stored in a refrigerator can be used for up to 5 days.

2,4,6-*Trinitrobenzenesulphonic acid solution*—Dissolve 1·00 ± 0·01 g of trinitrobenzenesulphonic acid tetrahydrate (0·80 ± 0·01 g of the anhydrous acid) in 500 ml of distilled water. Store the solution in an amber bottle and renew every 2 days.

Standard enzymes—A sample of enzyme as supplied by the manufacturer. The activity is expressed in Anson units (A.U.). The activities of all samples are determined by comparison with the standard and expressed as percentages of that standard.

APPARATUS—

A Technicon AutoAnalyzer comprising the following modules, arranged as shown schematically in Fig. 1, was used.

Sampler II—Fitted with a cam to give twenty samples per hour, 1-minute sampling and 2-minute wash time. Use 4-ml sample cups.

Proportioning pump.

Heating bath—Maintained at a temperature of 50 °C and fitted with a 40-foot and a 20-foot coil, 1·6 mm i.d., joined in series.

Colorimeter—A 15-mm tubular flow cell with 420-nm filters.

Recorder—Optical density chart paper.

The manifold can be used for the determination of enzyme activity in enzyme receipts and in all kinds of soap and detergent products or for the assay of airborne dust samples.

The extra dilution stage (see dotted line from sampler to pump) is omitted for dust samples. The dilution stage can also be omitted for products and enzyme receipts if an extra manual 20-fold dilution of the sample solutions is made before feeding them to the sampler.

PREPARATION OF STANDARDS FOR THE ANALYSIS OF ENZYME RECEIPTS AND PRODUCTS—

Weigh an amount in grams of the standard equal to about

$$\frac{16}{\text{Activity of standard in Anson units}}$$

to an accuracy of 0·000 2 g. Dissolve it, transfer the solution to a 1-litre calibrated flask and make up to volume with distilled water. Fill a burette with the solution and dispense 2, 4, 6, 8 and 10-ml portions into five 1-litre calibrated flasks. Add 10 ± 0·1 g of sodium sulphite (anhydrous) to each flask, then add about 500 ml of distilled water and shake the flasks vigorously until the sulphite dissolves. Make up to volume with distilled water. Solutions should be renewed after 24 hours.

PREPARATION OF STANDARDS FOR THE ANALYSIS OF AIRBORNE DUST SAMPLES—

With a pipette, introduce 50-ml portions of each of the standards prepared as above into 1-litre flasks. Add 10 ± 0·1 g of sodium sulphite (anhydrous) and shake the flasks vigorously until the sulphite dissolves. Make each solution up to volume with distilled water. Solutions should be renewed after 24 hours.

PREPARATION OF SAMPLES—

Enzyme receipts—Proceed as for the standards but use only a 5-ml portion to prepare the final solution.

Finished detergent products—Weigh a representative sample of about 20 g to an accuracy of 0·01 g. Dissolve it and transfer the solution to a 2-litre calibrated flask with about 1 litre

of distilled water. Add 20 ± 0·1 g of sodium sulphite (anhydrous) and shake the flask vigorously until the sulphite dissolves. Make up to volume with distilled water.

Airborne dust samples—Airborne dust samples are collected on pre-weighed 15-cm Whatman GF/C glass-fibre filter-papers by using an air sampling device.

Carefully remove the filter-paper from the sampler. Fold it in upon the dust and re-weigh it. Place the glass-fibre filter-paper in a 250-ml beaker and, with a pipette, introduce 50 ml of a 1 per cent. sodium sulphite solution. Pulp the pad with the flattened end of a glass rod. By using the glass rod to compress the pulp, squeeze out as much liquid as possible and filter it through a glass-fibre filter-paper into a 50-ml conical flask. If colloidal carbon is present it is necessary to re-filter it through a nylon Millipore filter of about 7-μm pore size. Fill a sampler cup with this solution and analyse it. If the optical density of the solution falls within the range of the calibration graph, denature the rest of the solution as described below. If the optical density is too high, introduce by pipette a 5-ml portion into a calibrated flask of suitable size and dilute to volume with 1 per cent. sodium sulphite solution.

DENATURING OF SAMPLES—

Pour about 10 ml of the final sample solution into a 50-ml Erlenmeyer flask and add a few anti-bumping granules. Place the flask on a hot-plate and bring the contents to the boil. As soon as the entire surface of the solution is bubbling, remove the flask from the hot-plate. (Do not prolong the boiling.) Cool the solution to room temperature, stopper the flask and invert it to mix the contents.

If batches of the same product are being run, it is not necessary to denature each sample. Two or three samples taken at random should be sufficient to give an average value with which to correct the calibration graph for that batch of samples.

All airborne dust samples must be denatured and run individually.

DISCUSSION

Interference from oxidising agents is overcome by dissolving samples in sodium sulphite solution, which reagent also enhances the colour development. The proportions of buffer recommended in the method are sufficient to hold the pH value of all samples at 8, which is favourable for both digestion and colour development.

Simultaneous digestion and colour development enables a simple manifold to be used. The throughput time is short (14 minutes) and there is very little cross-contamination of samples. For a small decrease in accuracy the rate of sampling could be increased to 30 samples per hour, but the time taken for the preparation of sample solutions would be a limitation.

Ideally, samples should always be analysed against a calibration graph prepared by adding known amounts of enzyme to a non-active sample of the same product. However, a large number of finished product formulations have been examined and the matrix effects have been shown to be very small. One calibration graph can be used for several types of product, a typical graph being given in Fig. 2.

The exact composition of dust samples is not known, but the method described has been proved to be adequate for overcoming matrix effects. One portion of the sample solution is analysed directly, and another de-activated and analysed. The difference in colour between the two solutions is a measure of the enzyme activity. Complete elimination of protease activity is achieved by bringing the solution to the boil. As the boiling is not prolonged, little concentration of the solution occurs and, consequently, the amount of colour developed by reaction of impurities in the sample with the colorimetric reagent (2,4,6-trinitrobenzene-sulphonic acid) is the same in both the original and de-activated solutions. In addition, it has been demonstrated that ammonium compounds, which give a colour with this reagent, are not lost by volatilisation.

Some problems have been encountered with the analysis of dust samples collected in damp foggy weather in areas where there is much atmospheric pollution. When the sulphite solution is added to such a sample, an extremely fine suspension of carbon, which will pass through the glass-fibre filter-paper, can be obtained. The colloidal carbon causes light scattering in the colorimeter cell, thus leading to high absorption; this effect can be overcome by substituting a nylon Millipore filter for the glass-fibre filter-paper.

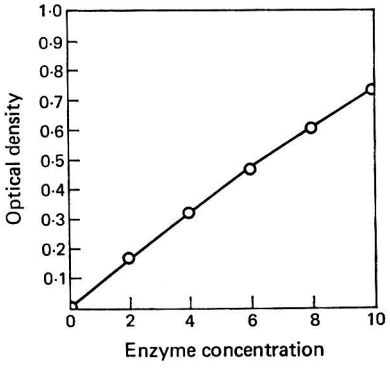

Fig. 2. Typical calibration graph. Values for enzyme concentration correspond to the portions taken in "Preparation of standards for the analysis of enzyme receipts and products"

Results

The accuracy and precision of the method were determined by using soap and soap-synthetic powders to which known additions of enzyme had been made. The results reported below in Table I included variations resulting from sampling as well as from the analytical method. Ten results were obtained for each sample.

Table I

Recoveries of enzyme from washing products

Product type	Perborate	Alcalase 1·5 A.U., per cent. Theory	Found	Recovery, per cent.	Coefficient of variation
Soap/synthetic 1 ..	Present	0·580	0·593	102·2	1·71
Soap	Present	0·785	0·777	99·0	2·88
Soap/synthetic 2 ..	Absent	0·798	0·798	100·0	1·74
Soap/synthetic 3 ..	Present	0·602	0·612	101·7	2·66

The accuracy of the method as applied to dusts was more difficult to assess as it was virtually impossible to simulate samples with accurately known amounts of activity of the order encountered in practice and with realistic dust matrices. Twelve samples containing amounts of enzyme ranging from 100 to 200 μg of 1·5 Anson unit activity were prepared by weighing on to glass-fibre filter-papers the appropriate amounts of a low concentration protease sample. The mean recovery was 104 per cent. with a coefficient of variation of 9·5 per cent. Sampling would account for many of the errors involved in this work. Dust samples collected in areas believed to be entirely free from enzyme gave results ranging from -5 to $+5$ μg of 1·5 Anson unit activity protease.

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A Simple Cutting, Holding and Back-flushing Arrangement for Dual Flame-ionisation Chromatographs

By NORMAN MELLOR

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

A simple cutting, holding and back-flushing arrangement, involving only on - off gas valves, is described, which can be fitted to commercial dual flame-ionisation gas chromatographs and can be operated from a single pressure-controlled carrier-gas supply. It does not interfere with the normal independent operation of either column. Any peak or portion of a peak emerging from the first column can be transferred either directly or via an interceptive trap to the second column for further examination (cutting operation). Simultaneously, by equilibrating the pressure across the first column, material remaining on this column can be held (holding operation) for subsequent elution or examination on the second column or, by releasing the pressure at the inlet to the first column, can be eluted in a reverse direction (back-flushing operation). The arrangement has proved particularly useful for determining trace impurities that are not completely separated from the tail of a major component.

Cutting and back-flushing are well established techniques in gas chromatography, and many systems have been developed in which more than one separating column is used. They have been particularly useful when it is necessary to re-examine a peak containing unresolved or partially resolved components on a different column packing without interference from adjacent peaks, and also for reducing the analysis time and preventing column contamination by reversed elution of unwanted higher boiling material.

Many of the earlier examples involved the use of fairly complex multi-path taps or valves which are often difficult to construct, expensive and are not always commercially available. The valves that are available usually have a large dead volume and not infrequently develop leaks or produce line contamination when operated at higher temperatures or used with corrosive compounds. The simple and elegant technique devised by Deans,[1] in which hot valves are replaced with externally operated pressure controllers, has largely overcome these difficulties and has opened the way to more complex multi-column analysers. However, in many of the problems which require the determination of a trace impurity, often high boiling, that appears on the tail of a major component, some operational difficulties were experienced with Dean's system when attempts were made to monitor the effluent from both columns on flame-ionisation detectors and also when trying to introduce an interceptive trap between the columns to concentrate the cut material.

The procedure described by Simmons and Snyder[2] is particularly attractive because of its use of simple on - off valves and needle valves. Their system, however, was limited to cutting, and had no facilities for holding, back-flushing or trapping, and the valves were not suitable for high temperature gas chromatography. However, it served to initiate the development of more suitable on - off valves described by Davenport,[3] and to the work described below, which represents a further simplification and extension of their work. The method described below, in which only on - off valves and a single pressure-regulated carrier-gas supply are used for its operation, allows the effluent from the first column to be monitored up to the time of cutting.

Experimental

Design and construction of apparatus—

The chromatograph used for this work was a dual-column flame-ionisation temperature-programmed Pye, Model 24, 104 Series Chromatograph modified as shown in Fig. 1. Two stainless-steel on - off gas valves, V_1 and V_2, as described by Davenport,[3] were fitted through the Marinite oven roof close to the flame-ionisation detectors so that the control knobs just protruded above the oven top. Any on - off valve capable of working at the required temperature and pressure with a small dead volume can be used. The exits from the valves were connected to the appropriate detectors with 1/16 inch o.d. stainless-steel tubing, and similar tubing is used for all other connections (shown as single lines in Fig. 1). The thermometer hole was used to bring the back-flushing line from the oven, and a similar hole was bored in such a way as to allow the trap, when required, to protrude. Three brass on - off toggle valves (Hoke International Ltd.), first tested for leaks at 50 p.s.i. and mounted on an aluminium plate, were screwed to the metal surround on top of the oven with the bolt fittings provided on the apparatus. Two of these valves, T_1 and T_2, were connected on one side to a pressure-controlled nitrogen carrier-gas supply and on the other side to the appropriate injection head assembly. The other toggle valve, T_3, was connected to the back-flushing line.

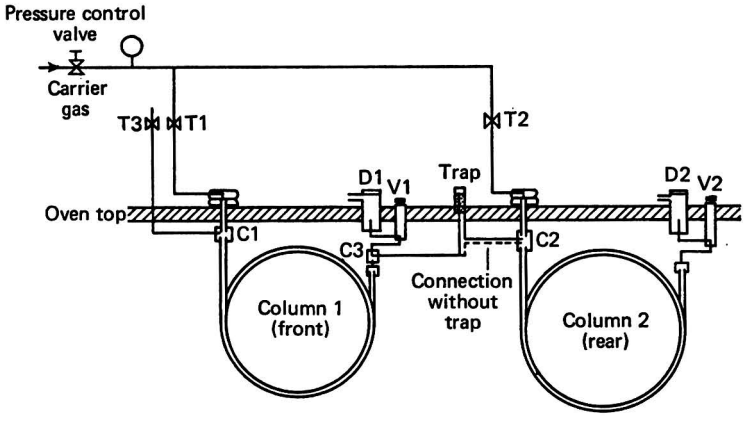

Fig. 1. Schematic flow diagram of modified chromatogram (lettered parts of the apparatus are referred to in the text)

Column modifications—

The columns were standard 5 foot long, ¼ inch o.d. as supplied by Pye Ltd. (both stainless steel and glass have been used), and these were cut 3½ inches below the top of the inlet arm and re-connected with straight ¼-inch metal couplings, in the centre of which 12 inches of 1/16 inch o.d. stainless-steel tubing had been silver-soldered to form a T-piece (C_1 and C_2). For the stainless-steel columns the T-piece connections were made from ¼-inch stainless-steel Swagelok couplings, and for the glass columns from ¼-inch brass Drallim captive seal coupling (Drallim Tube Coupling Ltd.) in which the rubber rings were replaced with Viton-rings, provided as standard column fittings with the apparatus. The T-piece connection, C_3, linking the exit of column 1 with valve, V_1, and either the trap or the inlet, C_2, to column 2, was made from a 1/16-inch straight steel coupling (Pye Ltd.) fitted with 1/16 inch o.d. stainless-steel tubing, silver-soldered into the hexagonal centre of the coupling.

Trap—This was made from a stainless-steel tube (1½ inches long, ¼ inch o.d.), with two 6-inch lengths of 1/16 inch o.d. tubing, silver-soldered at one end to give an inlet and outlet, and internally threaded at the other end to take a gas-tight end stop (Fig. 2). In the work described below the trap was packed with 10 per cent. Silicone Elastomer E301 on 100 to 120-mesh acid-washed Celite.

Desorption heater—This was a copper tube (1¼ inches × 7 mm bore), insulated with asbestos tape, wound with 18 inches of resistance wire (24 Ω yard^{-1}) and lagged with asbestos tape. When in use it was connected to the pre-heater power supply set at maximum, and could be slipped over, or off, the protruding trap as required.

Gas supplies, hydrogen and air—The flow-rate of hydrogen to the detector was set a little higher than normal, *i.e.*, 65 ml minute^{-1}, to reduce the tendency of the flame in detector 1 to be extinguished when changing from holding to normal elution on column 1. The flame was still occasionally extinguished, and it was advantageous to reduce the air flow to a level that permitted immediate ignition of the flame with the ignite switch. This resulted in the detector operating below its optimum sensitivity.

Nitrogen carrier gas—The mass flow controllers fitted on the instrument were not used. A single pressure-controlled nitrogen supply was attached to the inlets of toggle valves, T_1 and T_2.

Procedure—

When the columns, valves and connections have been assembled, as in Fig. 1, and checked for leaks, the main operations are as follows.

Operation number	Valve settings: closed	Valve settings: open	Operation
A1	V_2 T_2 T_3	V_1 T_1	Normal elution through column 1
A2	V_1 T_1 T_3	V_2 T_2	Normal elution through column 2
B	V_1 T_2 T_3	V_2 T_1	Cutting from column 1 to column 2 or trap Also for eluting desorbed material from trap to column 2
C	V_1 T_3	V_2 T_1 T_2	Holding column 1 and eluting column 2
D	V_1 T_1	V_2 T_2 T_3	Back-flushing column 1 and eluting column 2

Initially a chromatogram of the mixture run in the normal way on column 1 is examined and a decision is made on the cutting or back-flushing, or both, required. Operation A1 is repeated until the component of interest just begins to show. By closing valve V_1 and opening valve V_2 simultaneously, to give operation B, the component is either passed directly on to column 2 or is collected on the external trap. If the component has been passed directly on to column 2 it is only necessary to open T_2 to change to operation C, which equilibrates the pressure across column 1 to prevent further elution, and also continues the elution of the cut component on column 2. If the component has been collected on the trap it is first necessary to heat the trapped material by turning on the electrical heater after opening T_2 (operation C) and then switching to operation B, allowing 1 minute for the desorbed material to be swept on to column 2 before reverting to operation C. After examination of the cut material on column 2 it is usual to return to normal elution through column 1 (operation A1) and either continue the chromatogram with further cutting and holding if necessary, or commence another test. Alternatively, after the cut material has been transferred to column 2 any material remaining on column 1 can be back-flushed out of the column by simultaneously closing T_1 and opening T_3 to give operation D.

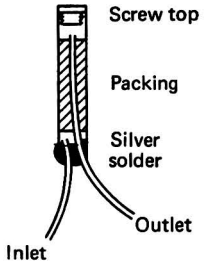

Fig. 2. Details of the trap

Results and discussion

Preliminary tests on several synthetic mixtures are shown in Figs. 3, 4 and 5. These mixtures were chosen for convenience in studying the various factors affecting the behaviour of the apparatus, not because they presented any difficulty in separation; they can all be separated on one column with a suitable packing. Column 1 was packed with 10 per cent. Silicone Elastomer E301 on acid-washed, 100 to 120-mesh Celite, and column 2 with 10 per cent. poly(ethylene glycol adipate) on acid-washed, 100 to 120-mesh Celite.

Effect of cutting time and the use of an interceptive trap—

In the arrangement described the effluent from column 1 is monitored on detector 1 until the peak of interest just appears, and the cutting on to column 2 or the trap is then controlled by time. The time needed to transfer the peak is not only the time recorded on the preliminary test on column 1, because by introducing the additional resistance of column 2 the cutting operation occurs at reduced gas velocity. The time required can be calculated approximately if it is assumed that equilibrium of the new column-pressure system is reached rapidly—

$$\text{Cutting time, minutes} = t_1 \frac{F_1}{F_2}\left[\frac{P_1^2}{P_0^2} + x\left(1 - \frac{P_1^2}{P_0^2}\right)\right]^{\frac{1}{2}} \quad \text{(ref. 4)}$$

where t_1 = time of elution from column 1, minutes;
F_1 = flow-rate at exits of column 1, ml minute^{-1};
F_2 = flow-rate at exit of combined columns 1 and 2, ml minute^{-1};
x = length of column 1/length of column 2;
P_1 = inlet pressure, p.s.i.; and
P_0 = outlet pressure, p.s.i.

If it is assumed that the pneumatic resistances of the two columns are identical this can be simplified to—

$$\text{cutting time, minutes} = 2\,t_1\left[\frac{P_1^2}{P_0^2} + \tfrac{1}{2}\left(1 - \frac{P_1^2}{P_0^2}\right)\right]^{\frac{1}{2}}$$

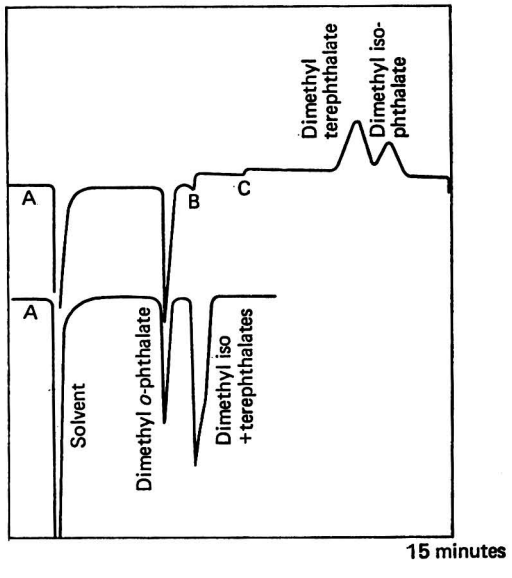

Fig. 3. Separation of dimethyl isophthalate and dimethyl terephthalate after cutting without trap

With an inlet pressure of 7 p.s.i. the cutting time was calculated in this way to be about 2·5 times the elution time from column 1. The time can also be established from preliminary trials. For example, Fig. 3 shows the effect of cutting, without trapping, a peak of unresolved dimethyl iso and terephthalates, which took 2 minutes to be eluted from column 1. The experiment was repeated several times, starting with a 2-minute cut and increasing this time by 1 minute in each successive test. In this way it was found that about 97 per cent. of the peak was cut after 5 minutes.

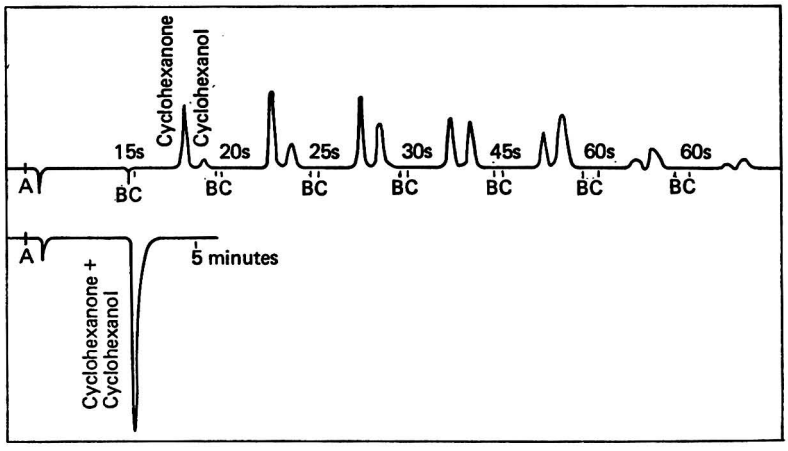

Fig. 4. Effect of repeatedly cutting and holding an unresolved peak of cyclohexanone and cyclohexanol

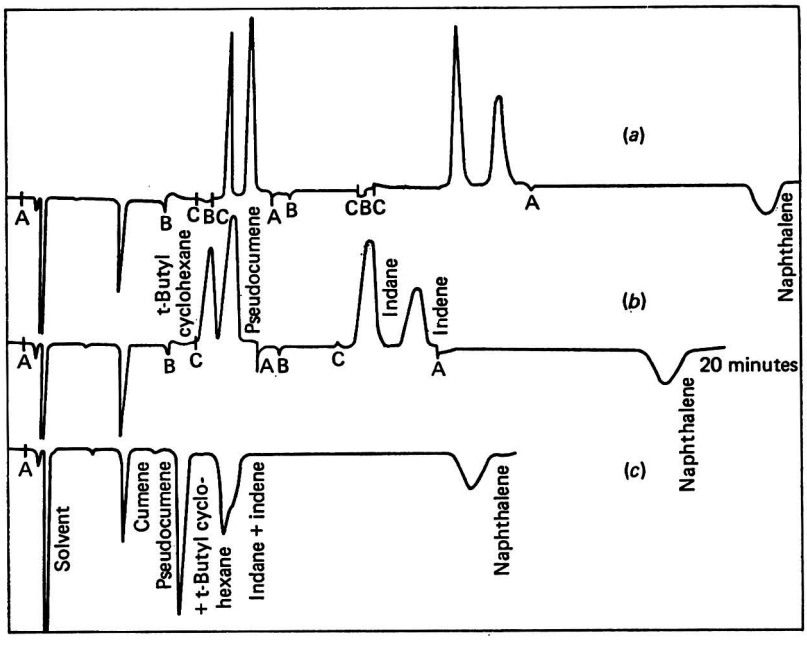

Fig. 5. Cutting: *a*, with trap; *b*, without trap; and *c*, column 1 alone

This was interesting because it showed a gradual change in the isomer ratio with increasing cutting time, resulting from the isomers being partially resolved on column 1; and by careful timing of the cut, *i.e.*, 2 minutes 40 seconds, and it was possible to isolate most of the dimethyl terephthalate in a comparatively pure state. The material held on column 1 when subsequently cut on to column 2 was shown to be mainly dimethyl isophthalate. Similar behaviour is shown in Fig. 4 with a mixture of cyclohexanone and cyclohexanol, which although apparently not resolved on the non-polar packing in column 1 is shown to be partially resolved by repeatedly cutting without the trap and examining each fraction on column 2.

In these examples cutting has been carried out successfully without the use of a trap. However, peak broadening occurs with increasing cutting time, which is particularly noticeable when the retention times on column 2 are short (Fig. 4, B), and can lead to peak distortions which may give rise to errors in peak measurement. This effect is caused by the transfer volume being large relative to the retention volume of the component on column 2, and is reduced by selecting a packing that gives an increased retention volume. It may be completely obviated by introducing a trap between the exit of column 1 and the start of column 2 that will collect the cut material and then allow it to be desorbed in a smaller volume on to column 2. For the trap to be effective the volume of the cut being collected should be less than the breakthrough volume of the material on the trap packing. As the breakthrough volume is dependent upon the trap temperature and the nature of the packing it may be necessary either to cool the protruding trap or to choose a more retentive packing. The breakthrough time of a component can be determined by temporarily connecting the exit lead from the trap to the inlet of valve V_2. This allows the component of interest, when detected at D_1, to be passed into the trap and the elution continued until the component is detected on D_2. If the time between cutting and detection on D_2 is greater than the normal elution time from column 1 on D_1 the component will be completely trapped.

The relative improvement in peak sharpness that can be effected by trapping is shown in Figs. 5 and 6. However, with the arrangement shown in Fig. 1, trapping can only safely be applied to those peaks that are not contiguous with a following peak because the desorbed material in the trap is swept on to column 2 with effluent from column 1, and might be contaminated with part of a following peak. This can be avoided by introducing another gas line from the pressure-controlled supply to a point after the exit of column 1 and before the trap; our work so far has not required this, and the arrangement has not been tried.

Holding—

The holding operation is achieved by equilibrating the pressure across column 1 from the single pressure-controlled carrier-gas supply. This operation, which normally follows cutting, produces some reversal in flow on column 1 because of the increase in pressure that occurs at point C_3. The resulting back-flow will be small, however, because the gas volumes in the connecting tubes and the column are relatively small and may be advantageous as it will have the effect of sweeping back into the packing any material in the exit line t hat might otherwise diffuse into column 2. It was possible to hold material on column 1 for fairly long periods without serious diffusion. Fig. 5 shows that no significant broadening of the naphthalene peak occurred after being held for 10 minutes. Tests on anthracene showed only a small decline in plate value after being held on column 1 for 60 minutes. Fig. 4, which shows the effect of repeatedly cutting slices from an apparently unresolved cyclohexanone - cyclohexanol peak, was repeated with a 1 hour hold between the second and third cuts with no significant effect on the subsequent cuts.

Back-flushing—

This involves a reversal in the pressure drop across column 1 and, therefore, the time required to back-flush a component that has just passed to the exit of the column will be the same as the time taken from injection. For any other component less forwardly eluted, the time required for back-flushing should be slightly less than the forward elution time because of the effects of the pressure changes across the column.[4] However, normal peak broadening will increase the time, and in practice the time required for complete back-flushing was found to be about the same as for the forward elution. The only problem experienced

with this operation was a tendency for the exit line from the oven to become blocked with condensing materials, and it would probably be advantageous to introduce a replaceable trap filled with silica gel or molecular sieve 5A in the line immediately outside the oven roof.

COLUMN PACKINGS—

Usually the packing in column 2 is different from the first column to effect a further separation, but with trace impurities that appear on the tail of a major component both column packings will normally be the same unless more than one impurity is present in a peak.

COLUMNS WITH UNEQUAL PNEUMATIC RESISTANCE—

All of the work described has been carried out on standard 5 foot long columns of about the same pneumatic resistance, but it should be possible to use the arrangement with different column resistances. The effect of unequal column resistances will be to produce a higher flow-rate in the column with the least resistance, but provided the different flow-rates are acceptable and the pressure regulator will maintain the required pressure, the apparatus could be used as described. If the differences in flow-rates are unacceptable the column resistances need to be balanced by introducing a suitable length of capillary tubing at the end of the shortest column and, if this were column 1, a similar length of capillary may also be necessary in the back-flushing exit line to obtain similar back-flushing times.

TRACE IMPURITIES AFTER MAJOR COMPONENTS—

The appearance of a trace impurity on the tail of a major component is not infrequently experienced in gas-chromatographic work and the quantitative determination of such impurities may be difficult. Even when the separation factors are comparatively large, *e.g.*, 1·2 for α- and β-naphthylamines on polyethyleneimine, it becomes difficult to effect a satisfactory determination of a minor component due to peak broadening of the major component peak caused by overloading the column to obtain the necessary detection sensitivity. Under the best conditions previously found on one column it was not possible to determine β-naphthylamine content satisfactorily much below 500 p.p.m. because below this level the impurity showed only as an inflection on the α-naphthylamine tail, and below 250 p.p.m. the inflection could not be detected. Fig. 6 shows the effects of cutting the tail containing the β impurity on to a second column containing the same packing (10 per cent. of polyethyleneimine on 100 to 120-mesh acid-washed Celite) both directly and through the trap. The improvement derived from trapping is clearly shown and makes it possible to determine 50 p.p.m. of

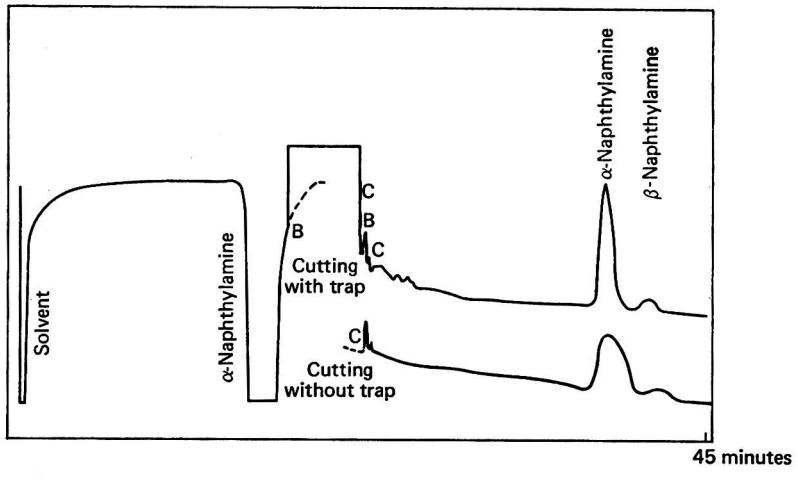

Fig. 6. Effect of cutting with and without trap on trace amounts (50 p.p.m.) of β-naphthylamine in α-naphthylamine

β-naphthylamine with no difficulty. A similar improvement was also obtained in the determination of traces of β-naphthol in α-naphthol. In these tests the residue time on column 1 was reduced to about half of that found to be most suitable with a one-column technique (by increasing column temperature) because it was no longer necessary to effect the best separation on column 1. Consequently the over-all time of analysis for this product was only slightly longer than the previous best and the separation and detection limit were greatly improved. Because the impurity concentration, when small, could not be detected on the tail of the major zone emerging from column 1, cutting was made when the recorder pen had fallen to a pre-determined position on the tail, which was previously established with samples or synthetic mixtures containing larger amounts of the impurity. It was convenient to set the detector at a lower sensitivity until after cutting. With high column temperatures or detector-sensitivity settings, appreciable base-line disturbance may occur during and immediately after cutting. Fig. 6 shows an off-scale deflection that occurs on cutting (operation B). This was not adjusted until after the cutting was completed when attenuation changes were made to give the increased sensitivity required; the backing-off control was then adjusted to set the recorder pen at the opposite side of chart to record the cut material.

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Book Reviews

LASER RAMAN SPECTROSCOPY. By T. R. GILSON and P. J. HENDRA. Pp. xiv + 266. London, New York, Sydney and Toronto: Wiley-Interscience, a division of John Wiley & Sons Ltd. 1970. Price 90s.

The successive and successful introduction to the analytical laboratory of u.v., i.r., mass and n.m.r. spectroscopy has convinced analysts and instrument manufacturers that there are other fields of spectroscopy ripe for exploitation. This book is concerned with a currently well backed runner—laser Raman.

The book is written conventionally—introduction, theory, experimental and instrument descriptions are followed by chapters on applications of the method. It is the work of experimentalists rather than chair-borne scientists and, as such, has an engaging freshness of style. It is perhaps not surprising that it gives the impression of enthusiastic rather than considered construction. Thus on p. 182 we learn that Koenig and Boerio have successfully examined polyvinyl fluoride and also that "among the failures of laser Raman is polyvinyl fluoride."

The analyst will no doubt read the four chapters on applications before bothering with the theory. I am doubtful whether these chapters do justice either to the authors or their subject; there is a strong emphasis on academic applications, which I feel will be of limited interest only to the general reader.

In view of the first-class and first-hand information which the book contains, the enthusiastic analyst will not begrudge the price asked. This book is fun to read, but unfortunately is unlikely to provide the analyst in industry with a case strong enough to convince his manager of the importance of making laser Raman their first priority in the purchase of new equipment for the laboratory.

HARRY WILLIS

ATOMIC ABSORPTION SPECTROSCOPY. APPLICATIONS IN AGRICULTURE, BIOLOGY, AND MEDICINE. By GARY D. CHRISTIAN and FREDERIC J. FELDMAN. Pp. x + 490. New York, London, Sydney and Toronto: Wiley-Interscience, a division of John Wiley & Sons. 1970. Price 155s.

Atomic-absorption spectroscopy has generated a steady flow of books by noted workers in the field. That there is a place for yet another book reflects the continuing development of the subject and the skill and industry of Drs. Christian and Feldman.

The book consists of 23 chapters divided into two parts: "Principles and Theory" and "Applications"; the latter section comprises two-thirds of the book. Part I considers all aspects of atomic-absorption spectroscopy and relates theoretical considerations to instrument design and experimental observation. The treatment is detailed and comprehensive. There is also a short chapter outlining the principles of atomic-emission and fluorescence spectroscopy. In this chapter attention is drawn to the complementary nature of these techniques with respect to atomic absorption.

Part II includes introductory chapters presenting the general function of metals in the body and methods of sample preparation. The emphasis in this section is on the analysis of samples from man and animal. Analytical procedures for 55 elements are described. In addition, for each element there is a useful summary of its biological function.

The book is well documented (1350 references), adequately indexed and as up-to-date as possible (mid-1969). The presentation is clear and free from significant errors. Any worker who uses atomic absorption will profit from reading Part I, while the whole book can be unhesitatingly recommended to all analysts concerned with the measurement of metals in biological materials.

J. B. DAWSON

APPLIED SPECTROSCOPY REVIEWS. VOLUME 3. Edited by EDWARD G. BRAME, jun. Pp. xii + 345. New York: Marcel Dekker Inc. 1970. Price $17.50; £8 7s.

Volume 3 of this series contains seven reviews, four of which are of direct concern to the analytical chemist. These are (*i*) Recent advances in analytical emission spectrometry (A. M. Yoakum); (*ii*) Application of X-ray spectroscopy to clinical analyses and biological research (S. Natelson); (*iii*) Quantitative analysis by infrared spectrophotometry (J. A. Perry); and (*iv*) The combination of gas chromatography with mass spectrometry (C. Merrit, jun.). Each of these

reviews is important and valuable and is written excellently. Emission spectroscopy is one of the oldest of physical methods of analysis, but it still evokes considerable study and new methods and applications widening its scope are abundant. This review covers the years 1964 to 1967, but there are 300 references mainly to original work in this period. For a text of only 34 pages, there are obvious disadvantages in writing critically about so many references, but the author draws attention to the main advances, such as new sources, determination of minor and trace amounts and use of computers. X-ray spectrometry provides rapid results for samples that are limited in size and in which the concentration of the element sought is low. The short review (10 pages, 47 references) by Natelson adequately covers its application to clinical and biochemical problems and shows the unique value of the technique. The review (31 pages, 13 references) on quantitative infrared analysis is outstanding and a timely reminder that quantitative infrared spectrometry can be precise and accurate. It is of particular value in cases in which gas chromatography is inapplicable or higher precision is sought. Merrit provides a very comprehensive review (59 pages, 97 references up to 1969) of methods for coupling the techniques of gas chromatography and mass spectrometry to give the analyst one of his most powerful tools. The other three reviews: Application of infrared spectroscopy to structural studies of nucleic acids (M. Tsuboi), Infrared studies of hydrogen - deuterium exchange in biological molecules (F. S. Parker and K. R. Bhasker) and Electronic spectra of radical ions (C. N. R. Rao, V. Kalyanaraman and M. V. George), are of special interest to the theoretical spectroscopist.

The aims and standards set by the Editor are fully maintained, and production of the new volume is generally good. An index of authors cited in the many references is useful, but the corresponding subject index is too short to have much value. It is hoped that the high cost of the volume will not deter the analyst from studying the four reviews which are of vital interest in analytical chemistry.

W. CULE DAVIES

ANNUAL REPORTS ON NMR SPECTROSCOPY. Edited by E. F. MOONEY. VOLUME 3. Pp. xii + 505. London and New York: Academic Press. 1970. Price 140s.

With Volume 3 of this series it has been found desirable to change the title from "Annual Review" to "Annual Reports"; this is to reduce confusion with the Annual Review series of texts of another publisher. No change of style is implied, although editorial decision has imposed the δ scale for n.m.r. chemical shifts on all authors. This has the reference compound for each nucleus with $\delta = 0{\cdot}00$ p.p.m. and a low field or high frequency shift therefrom implies a positive value of δ.

The type of volume is indicated by saying that there are over 1800 references and 78 pages of index. Sentences such as "cyclobutene has been re-examined by PMR spectroscopy and the vicinal couplings in the CH_2–CH_2 fragment found to be $+4{\cdot}65$ Hz (*cis*) and $+1{\cdot}75$ Hz (*trans*)[300]" or "many bufadienolides have been examined and the structure of cinobufaginol assigned[317]" are the bread and butter of the volume; the jam is thin. However, such information is an essential guide to the literature for those really concerned, and such volumes are an important aid to efficient modern research. The chapter on paramagnetic species, including many metal complexes, is perhaps of greatest interest to the general reader.

Your reviewer can do little but say that all is competently undertaken and quote the authors and chapter headings. Items II and V extend articles by the same principal authors in Volume 1. I, General Review of Proton Magnetic Resonance by T. N. Huckerby; II, NMR Spectroscopy as an aid in Conformational Analysis by W. A. Thomas; III, NMR Spectra of Steroids by J. E. Page; IV, NMR Spectroscopy of Paramagnetic Species by G. A. Webb; and V, Fluorine-19 Magnetic Resonance Spectroscopy by K. Jones and E. F. Mooney.

D. H. WHIFFEN

THE NMR OF POLYMERS. By I. YA. SLONIM and A. N. LYUBIMOV. Translated by G. NIGEL TURTON and TATIANA I. TURTON. Pp. x + 365. New York: Plenum Press. 1970. Price 185s, $19.50; DM 78.

The title of this book and its length lead the reader to expect cover-to-cover information on this subject. It is surprising to find that of a total of 309 pages of text, the first 145 pages are devoted to the theoretical aspects of n.m.r. and details of the type of instrument with which to attempt the n.m.r. experiment. As there are many excellent texts that deal exclusively with the theory of n.m.r. it is a pity that so much space is devoted to it, rather than taking the opportunity to write at length and in detail about one practical use of this technique.

The authors of this book have decided on a presentation that deals in turn with the wider aspects of the subject such as "n.m.r. in oriented polymers" or "molecular motion in polymers." Although this approach is useful in giving the reader a broad view of the field, it creates difficulties in finding information about particular polymers. However, this information is to be found if the reader perseveres, especially as so many references are given.

A great deal of attention is paid to wide-line n.m.r., and this is well reported with considerable detail. It is to be regretted that the high-resolution n.m.r. of polymers is not also dealt with in similar detail. This most important aspect is neglected and what information there is is scattered throughout the text. One of the most important features of high-resolution n.m.r. in the polymer field is its ability to determine composition in an absolute manner. The authors see fit to deal with this in about 1 page of text, although many references are given.

The original publication date was 1966, so the more recent and up-to-date information is missing from the text. Some attempt has been made to overcome the considerable time lag between the original publication and the 1970 English translation by the addition of an appendix of more up-to-date references. Nevertheless, the latest of these is still some 3 years old.

It is proper to congratulate the translators of this book as it now appears in clear and concise English. Generally, graphical results are well presented, but the spectra that are shown are very poor and appear to be hand drawn.

To sum up, this is a disappointing book, which is largely a review of the literature when it deals with its subject "The N.M.R. of Polymers." The cost seems excessive in view of its content and the length of time it has taken to publish in the English language. M. E. A. CUDBY

ANALYTICAL CHEMISTRY OF NICKEL. By CLYDE L. LEWIS and WELLAND L. OTT. Pp. x + 233. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 110s.; $14.85.

If the range of metallic elements determined in all analytical laboratories throughout the world could be assessed, it would be likely to reveal that few attract more regular attention than nickel.

One explanation for this is that nickel is a major or minor constituent of many universally important alloys in both the ferrous and non-ferrous metallurgical industries. Small amounts of nickel are widely distributed in plants and animals, and compounds of the metal are also invariably present in coal, petroleum, asphalt, *etc.* Thus determinations of this ubiquitous element may range from a few p.p.m. in granites and atmospheres containing nickel carbonyl to 90 per cent. in Chromels and Nimonics.

The book covers the qualitative and quantitative aspects of this particular determination as applied to the wide range of materials in which the metal is present, and is of interest.

This latest addition to this international series of monographs is not to be confused with an earlier publication in the same series, in which Lewis, Ott and Sine deal with the *analysis* of nickel. In contrast, this book by two of these authors deals precisely with what its title implies. Much of the information has been taken from published work; nevertheless, the extensive combined practical experience of the writers is apparent, especially in relation to the analysis of materials such as rocks, ores and intermediate products associated with the concentration and refining of nickel and its use as an alloying constituent.

The reader will find a comprehensive coverage of the subject with many complete procedures and over 1100 supporting references.

One of the fifteen chapters deals with the Preparation of Samples for Analysis, and more than half of the book is devoted to instrumental methods for determining nickel, *e.g.*, by X-ray fluorescence spectrometry, atomic-absorption spectrophotometry, neutron-activation analysis and mass spectrometry. The earlier sections cover the more conventional chemical aspects of the analytical chemistry of nickel, and all chapters begin with a brief, though adequate introduction.

The paper, printing, layout, binding and technical content of the book are all up to the usual high standard of the series. W. T. ELWELL

THE DETERMINATION OF HYDRAZINO-HYDRAZIDE GROUPS. By HUGH E. MALONE. Pp. xvi + 393. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1970. Price 140s.; $18.75.

This book is the fifth volume in a series of monographs on organic functional group analysis; earlier volumes deal with the determination of epoxides, diols (and other groups oxidisable by

periodate), carboxylic acids and organic peroxides. The choice of hydrazino-hydrazide groups as the subject of the present volume may require some explanation. It turns out that the title covers compounds as diverse as hydrazine and isonicotinic acid hydrazide; the former typifies the current interests of rocket-propellant chemists and fuel-cell technologists, while the latter is an important ingredient of pharmaceutical preparations. Sections of the book describing the determination of these and similar compounds in biological fluids further extend its potential usefulness.

The author's declared intention is "to summarise all the analytical methods for the analysis of hydrazino, hydrazide, hydrazine, substituted hydrazines, and hydrazine derivatives . . . to provide a complete digest with experimental details." This is a fair description of the book. It is a comprehensive and well organised compilation based on a large number of original papers, each of which is duly cited, with author and subject indices.

Classical oxidation and gasometric analytical methods based on the oxidation of hydrazine to nitrogen and water, which are arranged according to reagents, are described in the first two chapters, these accounting for more than a third of the book. The various papers pertaining to a given reagent are usually presented in chronological order, and care is taken to emphasise limitations and subsequent improvements.

In view of the toxicity of hydrazine and its derivatives and the pharmaceutical applications of various hydrazides it is not surprising that a great deal of work has been done to develop sensitive methods of trace analysis. These depend largely on colorimetric, spectrophotometric and chromatographic techniques, and two chapters of the book record the considerable progress which has been made. Shorter chapters cover coulometric, polarographic, and acid - base methods based on titration in aqueous and non-aqueous media.

There is a long chapter on the analysis of mixtures, particularly the mixed-amine rocket fuel hydrazine - dimethylhydrazine, which includes numerous examples drawn from the author's own research at the U.S. Air Force Rocket Propulsion Laboratory in California. A final chapter deals with the use of hydrazines as analytical reagents, *e.g.*, in the determination of carbonyl compounds.

The author has wisely made no attempt to arrange the techniques or methods in any order of preference. It is left to the reader to make his own choice by taking into account the nature of the particular analytical problem and the instrumental facilities available to the laboratory. The task of making such a choice is considerably simplified by the publication of this book.

I. DUNSTAN

MULTICOMPONENT CHROMATOGRAPHY: THEORY OF INTERFERENCE. By FRIEDRICH HELFFERICH and GERHARD KLEIN. Pp. viii + 419. New York: Marcel Dekker Inc. 1970. Price $24.50; £11 13s.

Chromatographic methods of separation are undoubtedly amongst the most widespread and successful of the array of separation techniques available to the modern chemist. Perhaps to some extent because of the success of the techniques, the background physicochemical theories of the processes involved have not developed at the same rate as have the points of technique and the range of applications of chromatographic methods. Most of the classical theories of chromatography are based on the concept of an ideal distribution state in which the various solute species are present in such low concentrations that classical thermodynamics or statistical methods may be used to treat any multi-component system as a series of mutually independent single-component systems.

It may well be that there are cases in trace analysis for which this assumption is tenable, although when one calculates the probable thickness of the effective mobile and stationary phases and the subsequent volumes of phases in which a majority of the separated solutes will be, then one may ask if these systems are ideal and whether the assumptions that are inherent in many of the classical theories are valid in practice.

If one still further increases the loading of the solute, then it soon becomes apparent that there may be a limited number of sorption sites available and that the relative affinities of each of the solutes for the material of the stationary phase become of great importance in such situations. Each multi-component system in preparative chromatography may need to be so considered when one attempts to explain the order and magnitude of the separations; the growing use of chromatography for large-scale industrial separations that involve kilogram quantities makes it both necessary and timely that the basic theories are reviewed and reassessed and, if necessary, that new theories are evolved.

Although chromatography is intrinsically a dynamic system of distribution of solutes under equilibrium conditions, there is no doubt that as the conditions are varied, the systems can become non-equilibrium ones, in which, under the practical limitations imposed, steady-state conditions exist at any one time, but in which, as the chromatography progresses, the actual conditions change. For example, if one considers that the number of solutes competing for the available sorption sites or present in the mobile phase diminishes by one each time a peak is resolved or a "spot" is separated, then it can be seen that the amount of possible mutual interference decreases as the chromatography proceeds.

The treatment of such systems envisaged by these authors does not involve new premises or hypotheses, but does involve some rather unconventional mathematical techniques and treatment, and for these reasons this is perhaps not the most readable of books.

However, by following the book in the order written one can appreciate the ideas behind the work and the conclusions drawn by the authors. The main advance is perhaps the manner in which the concept of coherence of molecules is applied to chromatography, and the linking of theories of chromatography with ones from other areas of physical sciences makes the approach to a general theory for separation techniques a much more feasible proposition.

The book is not solely concerned with theoretical principles: various examples of particular types of operation are analysed in considerable detail and are used to illustrate the application of the general principles, and it is indeed possible to see how some of the problems that have not been discussed will be further elucidated by application of the ideas found in this book.

One point in the book is worthy of especial note—in the chapter dealing with the mathematical aspects of the work, the equations, conditions and rules are stated without proof. These proofs are given in a separate appendix. One is thus saved the trouble of trying to re-form the thread of the arguments necessary to develop the theory after having had it broken by a mathematical proof not immediately pertinent to the argument.

It should not be thought that the advent of this book means a casting away of established theories; indeed, many aspects of existing theories are given a new relevance by this work. This is a highly original piece of work and will be of use to many different types of scientist and technologist. It will probably become necessary reading for all concerned with the design of systems for large-scale separations, and also for those who wish to examine the theories of chromatography.

L. S. BARK

DISSOCIATION CONSTANTS OF INORGANIC ACIDS AND BASES IN AQUEOUS SOLUTION. International Union of Pure and Applied Chemistry. Analytical Chemistry Division Commission on Electroanalytical Chemistry. By D. D. PERRIN. Pp. vi + 133–236. London: Butterworths. 1970. Price 44s.

This compilation was prepared at the request of the International Union of Pure and Applied Chemistry as part of the work of the Commission on Electrochemical Data. It is a companion volume to the two other books on dissociation constants also prepared for I.U.P.A.C. on "Dissociation Constants of Organic Acids in Aqueous Solution" by G. Kortum, W. Vogel and K. Anderson, and "Dissociation Constants of Organic Bases in Aqueous Solution," by D. D. Perrin. All provide extremely valuable and comprehensive collections of data. In the present volume the tables are well laid out and the information is readily accessible. There are few errors and the literature is covered up to 1967 with one or two additional references to papers published in 1968. The compilation includes all reactions that involve the gain or loss of a proton or hydroxyl ion and besides conventional acids and bases also contains the reactions of hydrated metal ions and free radicals such as the hydroxyl radical.

J. M. OTTAWAY

APPOINTMENTS VACANT

PUBLIC SERVICE OF TASMANIA, AUSTRALIA

CHEMIST — MINES DEPARTMENT

Applications are invited for appointment to a position of Chemist in the Chemical/Metallurgical Division of the Department of Mines, Tasmania.

Location: Launceston

Salary Range: $A3768–$A7749 per annum
Commencing salary will be according to qualifications and experience, and will be payable from date of embarkation for Australia.

Duties: Chemical research and analyses of ores and metallurgical and mineral products and rocks using chemical and instrumental methods, particularly AAS and XRF.

Qualifications: Degree or Diploma in Chemistry with acceptable experience.
Passages from the United Kingdom are provided under the Commonwealth Migration—Assisted Passage—Scheme, arranged through the Agent General for Tasmania. In addition, further assistance with passages and freight charges, etc., is available.

Recreation leave, sick leave and long service leave are provided.

Superannuation is compulsory for permanent officers.
Enquiries for Application Forms and publications should be addressed to the Agent General for Tasmania, 458/9 Strand, London, W.C.2.

LECTURES AND COURSES

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

MAY AND BAKER STUDENTSHIP IN PHARMACEUTICAL ANALYSIS

Applications are invited from Honours graduates in Pharmacy or Chemistry, and from undergraduates now in their final year for the May & Baker Studentship, which is available for award to students enrolled on the course leading to the University's M.Sc. Degree in Pharmaceutical Analysis, commencing in October, 1971. The course which includes instruction in a wide range of modern instrumental methods and a project in pharmaceutical analysis is specifically orientated to the requirements of the pharmaceutical industry for specialists in analytical research and quality control of pharmaceutical products used in human and veterinary medicine.

The Studentship covers University fees and a maintenance award of £550 per annum.

Applications should be addressed to Professor J. B. Stenlake, Department of Pharmaceutical Chemistry, University of Strathclyde Royal College Building, George Street, Glasgow, C.1., from whom further particulars of the course can also be obtained.

Revision of a Field Method for the Determination of Total Airborne Lead

An improved field method is described for determining airborne lead at concentrations up to 0·8 mg m^{-3} of lead. After collection on a filter the lead is dissolved in acid and complexed with dithizone. The lead dithizonate is extracted into 1,1,1-trichloroethane and the colour intensity of the complex is compared visually with standards. The apparatus used is simple to operate and the time required for a complete determination is about 15 minutes.

D. M. GROFFMAN and R. WOOD

Department of Trade and Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1.

Analyst, 1971, **96**, 140–145.

The Spectrophotometric Determination of the Purity of Commercial Dithizone and the Purification of Small Amounts of the Reagent by Chromatography

The purity of commercial dithizone is determined quantitatively by comparing the optical density of a solution at wavelength 620 nm with that of a pure sample. Qualitatively, the purity is estimated from the pattern obtained by chromatographing the crude dithizone on Whatman SG81 (silica-impregnated) paper.

Small amounts of the pure reagent are separated by chromatography on large sheets of Whatman SG81 paper or on a column consisting of a mixture of equal parts of acid-washed silica gel (Kieselgel N) and Celite 545, with benzene as eluting agent.

H. G. C. KING and G. PRUDEN

Rothamsted Experimental Station, Harpenden, Herts.

Analyst, 1971, **96**, 146–148.

The Determination of Dimethylpolysiloxane in Beer and Yeast

An infrared method that provides an estimate of the silicone content of beer and yeast has been developed. Because of the increased sensitivity over other reported methods it is possible to use smaller samples, which could be an advantage with biological material. The recommended working concentration range is 0·2 to 2 mg l^{-1}.

A. SINCLAIR and T. R. HALLAM

Allied Breweries Process Research Department, Burton upon Trent, Staffordshire.

Analyst, 1971, **96**, 149–154.

Determination of Cyanogen Chloride in Activated Niacin Test Strips

A simple paper-strip method for the determination of niacin in cultures of human tubercle bacteria based on the reaction with cyanogen chloride is described. Although a yield of only about 5 per cent. of cyanogen chloride is obtained, it represents an excess of from 5 to 50 times that needed to react with the 5×10^{-7} to 5×10^{-8} mole of niacin expected in samples. The chemistry of the system, the determination of cyanogen chloride and an evaluation of the procedure are presented.

MORTON S. LEFAR, ALVIN MASLANSKY, WILLIAM D. YOUNG and DONALD P. KRONISH

Departments of Analytical/Physical Chemistry and Diagnostics Research, Warner-Lambert Research Institute, Morris Plains, New Jersey 07950, U.S.A.

Analyst, 1971, **96**, 155–158.

The Use of *NN*-Dimethylcasein in the Determination of Proteolytic Enzymes in Washing Products and Airborne Dust Samples

NN-Dimethylcasein is used as substrate in an automatic method for the determination of proteolytic enzymes in washing products and airborne dust samples. Amino-acids formed by reaction with the enzyme are caused to react with 2,4,6-trinitrobenzenesulphonic acid to form stable, coloured Meisenheimer complexes. As *NN*-dimethylcasein does not react with trinitrobenzenesulphonic acid there is no need to remove excess of substrate before colour development, and the enzyme digestion and colour reactions can be conducted simultaneously. This leads to high sensitivity, which is of particular value in dust analysis and allows the use of a simple trouble-free manifold.

E. DUNN and R. BROTHERTON

Procter & Gamble Limited, Newcastle Technical Centre, Newcastle upon Tyne.

Analyst, **1971**, **96**, 159–163.

A Simple Cutting, Holding and Back-flushing Arrangement for Dual Flame-ionisation Chromatographs

A simple cutting, holding and back-flushing arrangement, involving only on - off gas valves, is described, which can be fitted to commercial dual flame-ionisation gas chromatographs and can be operated from a single pressure-controlled carrier-gas supply. It does not interfere with the normal independent operation of either column. Any peak or portion of a peak emerging from the first column can be transferred either directly or via an interceptive trap to the second column for further examination (cutting operation). Simultaneously, by equilibrating the pressure across the first column, material remaining on this column can be held (holding operation) for subsequent elution or examination on the second column or, by releasing the pressure at the inlet to the first column, can be eluted in a reverse direction (back-flushing operation). The arrangement has proved particularly useful for determining trace impurities that are not completely separated from the tail of a major component.

NORMAN MELLOR

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

Analyst, **1971**, **96**, 164–171.

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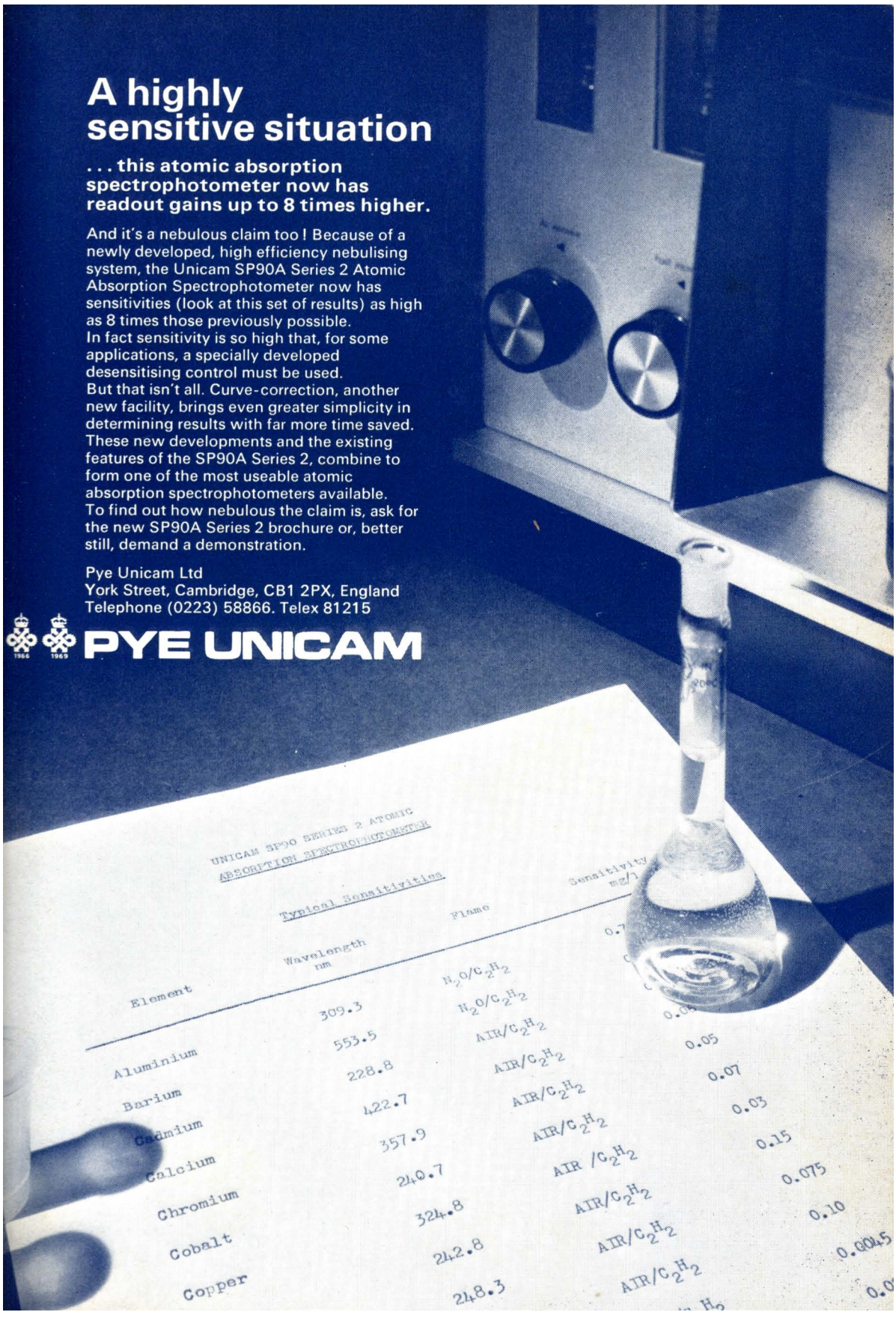

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