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Some Practical Aspects of the Determination of Chlorinated Pesticides by Electron-capture Gas Chromatography

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Some problems in the determination of residues of chlorinated pesticides by electron-capture gas chromatography and ways of overcoming them are discussed. Special consideration is given to injection technique, decomposition of pesticides, contamination of columns, the behaviour of detectors and the interpretation of chromatograms.

A simple and sensitive electron-capture detector is described.

GAS chromatography of chlorinated pesticides with electron-capture detection was introduced as a method of residue determination in 1961,[1] and several papers have appeared subsequently[2,3,4,5] establishing it as a versatile and sensitive technique. Several practical difficulties may be encountered when the method is applied, and in the interpretation of chromatograms. The purpose of this Paper is to deal with some of the relevant problems that have not been treated adequately in the literature.

The speed with which the sample is injected into the gas-chromatographic column is not normally regarded as important, but the work described here shows that under certain conditions it can be critical. Goodwin, Goulden and Reynolds[1] showed that the addition of Epikote resin to the stationary phase overcomes the decomposition of pesticides inside metal columns. However, if the design of the injection port is such that the injected sample impinges on hot metal, partial and variable decomposition of chlorinated pesticides may occur. Contamination of the inlet port and the top of the column by non-volatile components of extracts, which, for some fatty tissues, remain even after exhaustive clean-up, causes progressive deterioration in the performance of the column. Experimental work reported in this Paper shows how these difficulties can be overcome by careful preparation of columns and by the insertion of a replaceable glass liner in the injection chamber.

A simple, reliable and extremely sensitive electron-capture detector is described, and the response of detectors is discussed with special reference to the range that can be used for quantitative work. Finally, a quick, simple method of interpreting chromatograms based on measurement of the peak height is discussed. The application of this method to partially overlapping peaks, which can be a serious complication in residue analysis, is also described in detail.

PREPARATION OF COLUMNS

Several small improvements to the published method[1] were used. To avoid uneven coating of the Celite support, it was found necessary to dissolve the stationary phase completely. For silicone, which is difficult to dissolve, extreme care is needed, since undissolved material is not easily seen. Slow addition of the stationary-phase material to the Celite gave better results than the reverse procedure, and it was found advisable to have an excess of supernatant liquor present before the evaporation stage. Efficient mixing during the evaporation is essential and should be done carefully to prevent fragmentation of the Celite particles. Mechanical rotary evaporation or careful manual stirring gave satisfactory results.

The principal stationary phases used were—

(*a*) 2·5 per cent. w/w silicone elastomer E 301 *plus* 0·25 per cent. w/w Epikote resin 1001 and

(*b*) 3 per cent. w/w Apiezon L grease *plus* 0·3 per cent. w/w Epikote resin 1001.

Both phases were supported on Celite 545, graded to 100 to 120 mesh after coating. Columns were 2-foot, U-shaped tubes made from 20-s.w.g. copper and had a $\frac{1}{4}$-inch outside diameter.

Difficulty was experienced in packing the column in a consistent manner. Apiezon columns were more difficult to pack than silicone columns because of the relatively open and sticky nature of the coated support. Neither mechanical vibration nor various methods of manual tapping were wholly satisfactory with either type of column, the best results being obtained by the inertial-packing method described below. Small amounts (about 0·2 g) of the prepared packing material are introduced slowly into each limb of the column, and the column allowed to fall freely in a vertical position several times from a height of 1 to 2 cm on to a wooden block. Further packing is added to each limb alternately, repeating the impact treatment between additions until both limbs are filled to within 5 mm of the top and no further settlement of the filling occurs with repeated impaction. From 200 to 300 impactions are normally required.

After packing, the ends of the columns are plugged with asbestos or glass wool sufficiently firmly to prevent the plug being blown out by the carrier gas, but without increasing the resistance to the flow of gas. Excessively firm plugging of the ends of the column has been observed to affect the reproducibility of results obtained by using certain columns. Columns can be stored between periods of use without harmful effect, provided they are cooled in a flow of nitrogen and then firmly corked; when re-used they required a conditioning period similar to that for a new column before regaining their full efficiency. Columns prepared in this way had a useful life of up to three months. Towards the end of this time, resolution deteriorated, and some pesticides decomposed on the column. This decomposition is readily observed with *pp'*-DDT, which serves as a useful indicator of its onset.

The causes of decomposition of chlorinated pesticides on some columns are not fully understood. Cassil[6] and Beckman and Bevenue[7] found that the use of column materials other than copper can prevent this decomposition. Goodwin, Goulden and Reynolds[1] found that the addition of Epikote resin 1001 to the stationary phase also prevented decomposition and eliminated the need to condition columns by heavy injections of chlorinated pesticides before reliable results could be obtained. The fact that these authors found no improvement when glass beads were used as the supporting medium instead of kieselguhr, suggests that a partial explanation of the effect of Epikote is that it forms a thin film on the inner surface of the metal tube. Another possible cause of decomposition, which can be avoided by careful handling of the prepared Celite, is the exposure of active sites by fragmentation of the particles.

The inertial method of packing described should be compared with the recent work of Bayer, Hupe and Mack,[8] who also found that it is essential, if reproducible results are to be obtained, to allow the column to fall freely and to vary both the distance dropped and the surface on to which the column is dropped, according to the particle size of the supporting medium and the material of the column.

Injection technique

The gas chromatograph used in the work described below was the Shandon Universal; the instrument was fitted with a liner insert in the injection port except where indicated.

The optimum injection speed for a particular column was found to be a function of the packing density and mesh size of the Celite support. The efficiency of some columns was relatively unaffected by the injection speed over an appreciable range (from less than 0·1 to 5·0 seconds); but speed was found to be a critical feature for other columns that behaved in a reproducible manner only with a slow-injection technique.

The quantitative behaviour of Apiezon columns prepared by inefficient packing techniques was found to be less satisfactory than that of silicone columns. In the former instance, an improved response was obtained by varying the injection speed according to the particle size of the Celite support; normal fast injections (less than 0·1 second) were satisfactory only for 100 to 120 mesh Celite, a long injection time (up to 5 seconds) being necessary for Celite passing 120 mesh, and intermediate times (1 to 2 seconds) for mixed material passing 100 mesh. This effect, illustrated in Fig. 1, was found to be more marked for loosely packed columns, but it also occurred with overpacked columns. The retention times for fine-mesh columns were found to be reduced by as much as 20 per cent. when the injection time was increased to 5 seconds.

For inefficient columns, which give a response that varies with the speed of injection, the effects of altering the depth and angle of the syringe needle have also been investigated. It was sometimes found that reproducible results could be obtained, provided that the syringe needle did not penetrate below a certain depth. Beyond this depth a marked variation in response occurred. Altering the injection angle also sometimes produced a marked improvement, a similar effect being obtained in the presence of glass wool in the injection chamber.

Typical loosely packed columns, sensitive to injection speed, were obtained by using a massage vibrator and contained about 10 per cent. less filling than a correctly packed column, owing to a tendency of the vibrator to fluidise the filling during the packing operation.

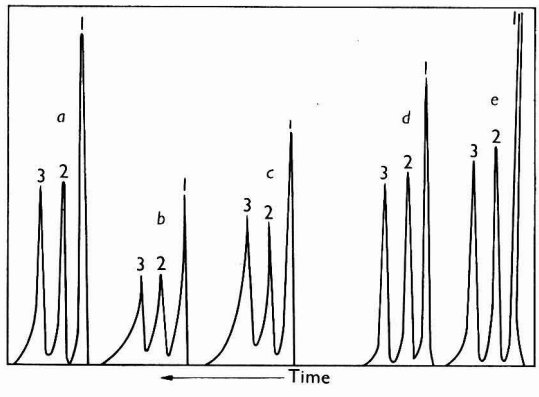

Fig. 1. Effect of variation of injection speed on differently packed columns. Peaks 1, 2·5 nanograms of lindane; peaks 2, 2·5 nanograms of heptachlor epoxide; peaks 3, 4 nanograms of HEOD. Group (*a*), fast injection of sample mixture on a 100- to 120-mesh column; group (*b*), fast injection of sample mixture on a 120-mesh column; group (*c*), fast injection of sample mixture on a mixed 60 per cent. 120-mesh - 40 per cent. 100-mesh column; group (*d*), slow injection (5 seconds) of sample mixture on same column as group (*b*); group (*e*), slow injection (1 second) of sample mixture on same column as group (*c*)

There is some published work on the speed of injection.[9 to 13] It is normally assumed that rapid injection is desirable for the greatest column efficiency.[14] The work reported in this Paper shows that the injection characteristics of a column can in fact be used as a convenient means of distinguishing correctly packed and inefficient columns. These characteristics can be explained as follows. As the packing density increases, increased resistance to the rise in pressure that occurs when the sample solution is injected may tend to cause the vaporised sample to surge momentarily away from the column towards the carrier-gas entry port. For a given sample volume this effect will be most marked for the more rapid injections. Difficulties in obtaining reproducible results can be explained by failure of the sample to vaporise efficiently in the injection head, or "blow-back" of a portion of the sample along the carrier-gas line. These effects depend on uniform packing density rather than on uniformity of particle size, and can be eliminated without reducing the overall efficiency of the column by using slower injections, provided that the period of injection is not too long in relation to the retention times of the pesticides.

The ideal time taken by an injection for quantitative response is as short as possible compatible with the minimum disturbance of the carrier-gas flow, which may be gauged by direct observation of the flow-meter during the period of injection. In general, columns prepared from finer packing materials will have a greater disturbing effect on the flow-rate of the carrier gas than those prepared from coarse materials; and a rapid injection, accompanied by rapid expansion of the solvent, will have a greater effect than a slow injection. Variations in the angle to the vertical and the depth of penetration of the syringe needle may similarly effect the flow-meter during the injection process, particularly if from the geometry of the

injection head these features have a critical bearing on the jet or spray character of the issuing sample solution. By direct observation it was found that rapid operation of the syringe produces a spray whereas a slightly slower operation produces a jet. The evaporation of a solvent jet impinging on hot metal has a more abrupt effect on the carrier, gas flow-rate than evaporation of a spray in the gas stream. Which of these two types of evaporation predominates for the same syringe depends, when used with a given injection head and column, on the orientation and depth of penetration of the needle. The fact that loosely

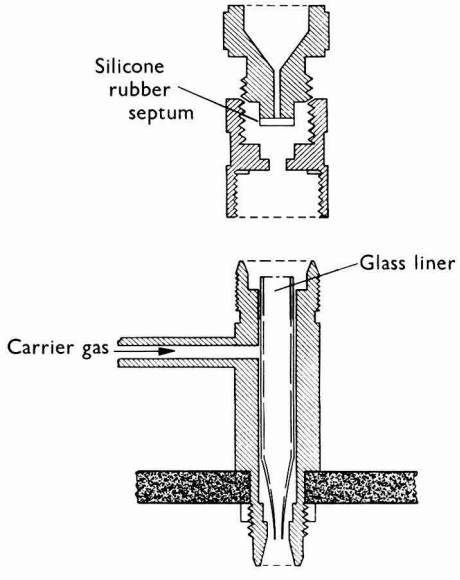

Fig. 2. Diagram of glass liner

packed columns are more sensitive to injection speed than correctly packed columns may be caused by a sudden but momentary compression suffered by the former type when the sample (including the solvent vapour) is injected: the more densely packed column is able to withstand the impact of the surge of solvent vapour without substantial physical compression.

Use of a liner

The response of some columns to chlorinated pesticides was so poor and erratic that the results could not be used for quantitative work. In an attempt to improve such columns, the replaceable glass liner shown in Fig. 2 was designed. The use of this liner in the injection chamber gave a great improvement in reproducibility obtained on inefficient columns and

Table I

Comparison of different injection techniques

		Coefficient of variation of peak heights, per cent.	
	Analyst	Slow injection	Fast injection
Liner absent	A	7·5	2·5
	B	4·5	6·5
	C	6·5	2·0
Liner present	A	2·0	1·0
	B	1·5	1·0
	C	2·0	1·0

a substantial improvement for good columns. Table I gives a comparison of the peak heights obtained by three individuals using an Apiezon column, with slow and fast injections and both with and without a glass liner in the injection head. Each coefficient of variation is calculated from five successive injections of γ-BHC. The coefficients of variation of peak height for an inefficient column with a glass liner and fast injection did not exceed 2 per cent. for the three analysts (14 injections per analyst). On the inefficient column without a liner, a different mean was obtained by each analyst; the coefficients of variation were greater than 20 per cent., with slight changes in the retention times.

Similar improvements were sometimes obtained with a glass-wool plug in the injection chamber, but occasionally, *e.g.*, for inefficient columns, glass-wool plugs had a detrimental effect. Because of this unpredictability, and difficulties in removing glass wool completely from the injection chamber, the glass liner was used in all subsequent work. Fig. 3A illustrates the improved resolution with the liner; results obtained with glass wool are similar, and Fig. 3B illustrates the reduction in interference from non-volatile material.

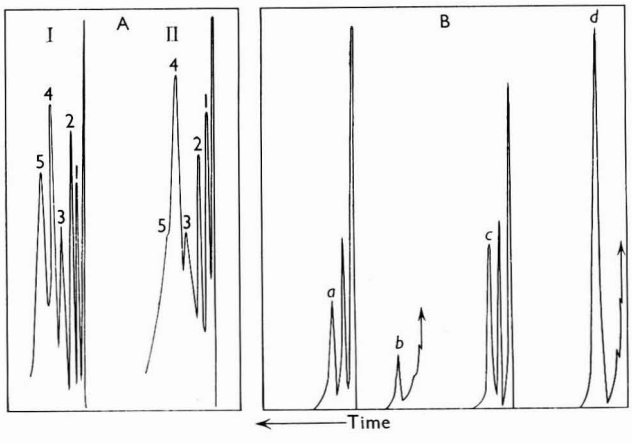

Fig. 3A. Diagram showing the effect of the glass liner on the resolution of an inefficient column: peak 1, zinaphos; peak 2, fenchlorphos; peak 3, parathion; peak 4, dieldrin; peak 5, ethion. Group I, with liner; group II, without liner

Fig. 3B. Diagram showing the effect of packing in the injection head: peak *a*, 4 nanograms of HEOD, peak height 18·5; peak *b*, fat *plus* 10 nanograms of HEOD, peak height 8 (calculated 4), retention volume = 480 ml; peak *c*, HEOD, peak height 29; peak *d*, peak height 72·5 (calculated 72·5), retention volume = 480 ml. Peaks *a* and *b* obtained without glass wool, and peaks *c* and *d* with glass wool

The glass liner appears to perform three separate functions. Firstly, it streamlines the gas flow, eliminating eddy currents in the injection port so that the injected sample is immediately swept on to the column. The tendency to "blow-back," discussed in the previous section, is completely eliminated, with a consequent improvement in the performance of poorly packed columns. Secondly, the glass liner reduces decomposition by preventing the sample from coming into direct contact with the hot metal surface in the injection chamber. Thirdly, the liner reduces the amount of non-volatile contaminating material reaching the column and when replaced regularly prolongs its life. A certain amount of non-volatile material remains in extracts even after efficient clean-up, particularly in the extracts of fatty or waxy materials. This can accumulate in the injection chamber and "bleed" on to the top of the column, causing progressive deterioration of performance, shown by poor resolution of peaks, and leading eventually to erratic response. When the liner is used, nearly all the interfering non-volatile matter is deposited on it, and can be removed by replacing the liner regularly.

Apart from the effect on subsequent injections, non-volatile components of an extract may affect the behaviour of pesticides in the same extract by holding them back, resulting

in poorer resolution with flatter peaks and longer retention times. To obtain accuracy approaching that achieved with pesticides in pure solution, an effective clean-up procedure must be used, and the injected solution should be sufficiently dilute.

The use of a quartz liner as an extra clean-up stage for plant extracts has recently been reported by Cassil,[6] and some manufacturers now supply glass liners for their instruments.

DETECTORS

The response of a number of electron-capture detectors was investigated by injecting a range of concentrations of chlorinated pesticides. Typical results for γ-BHC are given in Table II. When the deflections exceeded full scale, the injections were repeated with a lower amplifier-gain setting, and the peak heights corrected to the higher gain. Maximum

TABLE II

DETECTOR RESPONSE

	Full-scale deflection, per cent.		
	Shandon detector*		Electron-capture detector (see Fig. 5)†
Pesticide injected, ng (10^{-9}g)	Amplifier No. 1 (peak height corrected to gain × 50)	Amplifier No. 2 (peak height corrected to gain × 50)	Amplifier No. 1 (peak height corrected to gain × 20)
γ-BHC—			
0·1	2	2	2
0·25	4	5·5	4
0·5	8	11	9
1·0	16	22·5	16
1·25	19	27	20
1·5	23	33	24
2·0	28	45·5	30
2·5	35	54	—
5	60	100	64
6	67	110	—
10	92	136	101
20	118	—	—
25	125	169	153
50	138	181	182
125	149	188	201
500	149	191	206
Dieldrin—			
500	149	187	204
Hexachlorobenzene—			
500	149	—	206

* 2·5 per cent. w/w silicone column. Average response for 2·5 nanograms of γ-BHC is 70 to 80 divisons on amplifier No. 2.

† 10 per cent. w/w silicone column at 188° C.

deflections for each detector, which were obtained with massive amounts of some pesticides, are given in the Table. The shapes of the response curves for different detectors obtained by plotting peak height against amount of pesticide injected show similar features. The peak height approaches a maximum value, R_0, that, for each detector, is independent of the pesticide used; it is a characteristic of the particular detector related to the maximum electron flux, in turn controlled by the applied potential, the degree of contamination and the nature and the flow-rate of the carrier gas. The response curve is at first virtually linear; it then changes slowly to an approximately logarithmic form and finally reaches a maximum value.

The electron-absorption characteristics of the detector are analogous to the absorption of light in spectrometry.[15] The agreement with a relationship analogous to Beer's law, expressed as—

$$\log\left(\frac{R_0}{R_0 - R}\right) = kQ$$

where k is a constant and Q is the amount of pesticide injected, is illustrated in Fig. 4. This relationship can be assumed to apply up to about 80 per cent. of the maximum response. In practice the response of the detector can be considered linear up to one-third of the maximum

response. It may, with suitable calibration, be used up to two-thirds of the maximum value. Other workers have also noted the breakdown in linearity beyond 30 per cent. of the working range.[16,17,18]

Two types of electron-capture detector were used; an ionisation detector supplied with the apparatus, and, for work requiring greater sensitivity, the detector illustrated in Fig. 5. The basic materials for the latter are 14-mm bore, 25-mm external diameter polytetrafluoroethylene tube, B, brass rod, tube and gauze, A, A′, D, and a radioactive source, C, cut from a 1 cm × 3 cm tritium-loaded strip source (200 to 300 millicuries, Radiochemical Centre catalogue No. TRT3): the source is sprung into the brass component A. With regular cleaning, a typical sensitivity obtained with this detector on an efficient silicone or Apiezon column, at an amplifier gain of ×5, is 10 per cent. full-scale deflection per nanogram (10^{-9} g) of HEOD injected; the sensitivity with inefficient columns is often less than half this value.

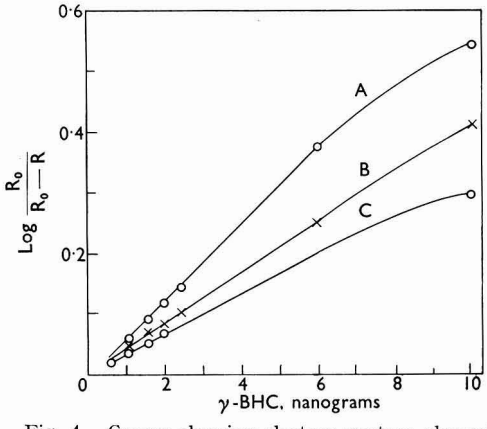

Fig. 4. Curves showing electron-capture absorption characteristics. Curve A, obtained by using amplifier No. 2 with the Shandon detector; curve B, obtained by using amplifier No. 1 with the Shandon detector; curve C, obtained by using amplifier No. 1 with home-made detector (see Table II)

The response of each individual detector varies with time owing to contamination and loss of the radioactive isotope, but it can be improved somewhat by periodical cleaning or by adjustment of the applied voltage. Tritium is lost by radioactive decay, desorption and isotope exchange. Radioactive decay of a typical 1 cm × 3 cm tritium source accounts for a loss of about 40 microcuries per day, but five times this loss occurs by desorption if the detector is heated to a column temperature of 188° C.[19] Even if a total loss of approximately 250 microcuries per day continued without reduction for 500 days, such a detector would still retain one-half of its original sensitivity. At 250° C, 1 per cent. of the tritium would be lost per day by desorption; for this reason it is not advisable to use the detector above 200° C. Washing the detector with solvent is the largest single cause of loss of activity of tritium detectors. Solvents containing labile hydrogen cannot safely be used, so that alcohols should not be used and acetone or ethyl acetate, if used, should not be left in contact with the source for prolonged periods. Benzene is a suitable solvent, having a low tritium exchange rate.[19] A quick wash is usually adequate, but if a simple solvent wash is ineffective, treatment with warm alcoholic potash is necessary.[6] Gentle abrasive cleaning of the anode gauze with a cloth soaked in solvent[1] is periodically necessary: an ultrasonic method[17] of cleaning the detector has also been described.

Interpretation of Chromatograms

Measurement of peak heights—

Measurements of peak area should, ideally, be used for the quantitative interpretation of chromatograms, but accurate methods of area measurement are difficult and time consuming.[20] A quicker method often used is based on the assumption that the peaks are Gaussian in form

and that the area under them can, to a first approximation, be represented by a suitably drawn triangle. Provided the column is not overloaded, the true base of such a triangle should be virtually independent of the height, which if the detector response is linear would then be proportional to the amount of pesticide present. If follows that the peak height is proportional to the height of the triangle and measurement of the peak height should be not only a quicker way of determining the area, but also a more accurate one, since it largely eliminates the error inherent in the difficulty of locating the inflection points and drawing the correct slopes. Work described earlier in this Paper showed that good reproducibility can be obtained by measuring the peak height by a suitable experimental technique, and that peak heights exhibit an approximately linear relationship to the amount of pesticide for small deflections. When linearity of the detector response breaks down, both peak-area and peak-height measurement are less reliable, but the latter lends itself more easily to calibration.

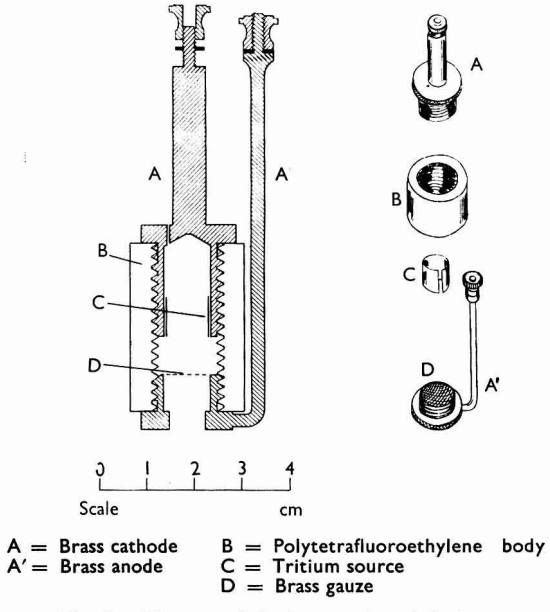

Fig. 5. Diagram of electron-capture detector

External calibration is easier and quicker by the peak-height method, and is especially useful when applied in conjunction with the procedure for interpreting overlapping peaks described later. An apparent disadvantage of peak-height measurement over peak-area measurement is the need to avoid injecting extracts that contain sufficient non-volatile lipophilic impurity to increase appreciably the retention times of pesticides. However, when such lengthening occurs, the resolution is always adversely affected with the consequent greater difficulty in measuring area. Injection of such extracts without further dilution or clean-up is inadvisable, since the column is slowly poisoned, resulting in erroneous results being obtained by either peak-height or peak-area measurement.

PARTIALLY OVERLAPPING PEAKS—

It is, in general, desirable for quantitative work to measure only peaks that are clearly resolved. Where peaks overlap, an attempt should first be made to effect complete resolution with a different column before making any quantitative interpretation of the results. If the overlapping peaks are obtained for pesticides that in pure solution are completely resolved, there is interference due to the presence of co-extracted matter, and improved resolution may be obtained on re-injecting the extract at a higher dilution. However, it is not always possible

to achieve complete resolution in practice, particularly if several different pesticides are present. If there is some resolution, it may be possible to estimate the amounts of the components. As in the case of completely resolved peaks, calculations may be based on peak height rather than on peak area. The measurement is thereby simplified and the interpretation of partially overlapping peaks by peak height as described below is normally less subject to error than when the peak-area method is used.

With a satisfactory column and detector working within the linear range, the heights of partially overlapping peaks are strictly additive. This is illustrated in Fig. 6 (*a*), showing the recorder tracings for a mixture of 15 nanograms of *pp'*-TDE and 20 nanograms of *pp'*-DDT on a silicone column. Each tracing runs from right to left so that in the diagram the first peak, *pp'*-TDE, is on the right. The resolution, calculated as the ratio of twice the difference of the retention volumes to the sum of the peak volumes (peak widths), is about 0·9. Three possible simple methods for measuring the height of the *pp'*-DDT peak are shown in Figs. 6 (*b*), (*c*) and (*d*). In Fig. 6 (*b*), interference by the *pp'*-TDE peak is ignored, the vertical distance from the *pp'*-DDT peak to the base-line being used; for columns affording good, although incomplete, resolution this is a justifiable approximation for the height of the second of two peaks, provided that the peak is reasonably large. In Fig. 6 (*c*), the supposed base-line is constructed as in spectrophotometry; the peak height obtained is a poorer approximation to the true value than that obtained in Fig. 6 (*b*), where the resolution is good, but the converse tends to be true if the resolution is poor. The true interference is illustrated in Fig. 6 (*d*), in which the tail of the *pp'*-TDE peak is constructed by reference to a standard peak, shown in Fig. 6 (*e*), of approximately the same height. The value of this method can be demonstrated practically: thus in the example shown, the difference between Figs. 6 (*b*) and 6 (*d*) is less than 1 scale division in 20, whereas the difference between Figs. 6 (*c*) and 6 (*d*) is 5 divisions. The *pp'*-TDE reference peak may be traced and superimposed on the chart record of the mixture.

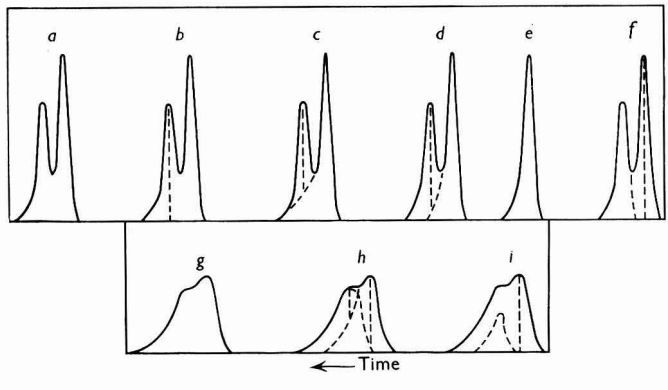

Fig. 6. Diagram showing additivity of partially overlapping peaks. Peak *a*, mixture of 15 nanograms of *pp'*-TDE and 20 nanograms of *pp'*-DDT; peaks *b*, *c* and *d* show methods for measuring the height of the *pp'*-DDT peak; peak *e* shows the tail of the *pp'*-TDE peak; peak *f* shows a method for correcting the *pp'*-TDE peak height. Peak *g* shows the resolution of the same mixture when it was separated on a less efficient column; peak *h* shows a method for correcting the peak heights; peak *i* shows the true shape and size of the *pp'*-DDT peak

A similar procedure may be used to correct the height of the *pp'*-TDE peak as shown in Fig. 6 (*f*); there is no correction in this instance. The same mixture of pesticides on a much less efficient column is shown in Fig. 6 (*g*), where the resolution is of the order of 0·3. The construction for correcting the peak heights is shown in Fig. 6 (*h*), from which the corrected *pp'*-DDT peak height is found to be 7 scale divisions compared with 7·5 for a standard solution. The true size and shape of the second (*pp'*-DDT) peak in this mixture is shown more clearly in Fig. 6 (*i*), from which it is seen that there is in fact no interference with the first (*pp'*-TDE) peak height as measured from the normal base-line. When the second peak is

considerably larger than the first peak, the constructions shown in Figs. 6 (*f*) and 6 (*h*) still give accurate results for the height of the first peak. The greatest error arises in locating the intersection of the perpendicular from the first peak with the projected leading edge of the second peak; in practice, good results can be obtained by visual assessment with the aid of a transparent rule.

A series of traces for dieldrin (HEOD) and *pp'*-DDE mixtures is shown in Fig. 7, which illustrates the application of the constructions set out above. In no instance is there any interference by the *pp'*-DDE peak on the HEOD peak. A tracing of a separate HEOD peak can be superimposed on the corresponding peak in the mixture. The method can be used for estimating the approximate height of relatively small shoulders on major peaks as shown in Fig. 7 (*g*). For more accurate results it is necessary to use either a better column or a different stationary phase capable of resolving the mixture. In Fig. 7 (*g*), the true height of the

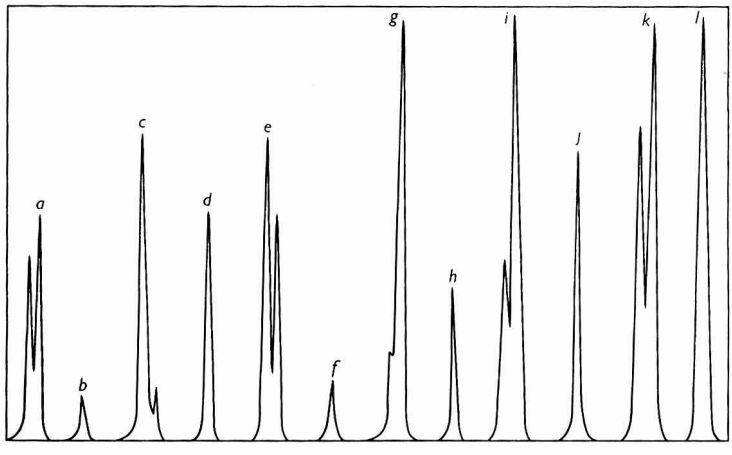

Fig. 7. Chromatograms obtained for mixtures of dieldrin (HEOD) and *pp'*-DDE; peaks *a*, 5 nanograms of HEOD and 5 nanograms of *pp'*-DDE; peak *b*, 1 nanogram of HEOD; peaks *c*, 1 nanogram of HEOD and 10 nanograms of *pp'*-DDE; peak *d*, 5 nanograms of HEOD; peaks *e*, 5 nanograms of HEOD and 10 nanograms of *pp'*-DDE; peak *f*, 2 nanograms of *pp'*-DDE; peak *g*, 10 nanograms of HEOD and 2 nanograms of *pp'*-DDE; peak *h*, 5 nanograms of *pp'*-DDE; peaks *i*, 10 nanograms of HEOD and 5 nanograms of *pp'*-DDE; peak *j*, 10 nanograms of *pp'*-DDE; peaks *k*, 10 nanograms of HEOD and 10 nanograms of *pp'*-DDE; peak *l*, 10 nanograms of HEOD

pp'-DDE peak is 10·5 scale divisions, from Fig. 7 (*f*), the height estimated by using the method illustrated in Fig. 6 (*b*) is 13 scale divisions as compared with 4 scale divisions calculated by using the method shown in Fig. 6 (*c*). The recommended method gives 10·5 scale divisions. The Fig. 7 (*g*) results confirm that the peak height of minor peaks or shoulders from the base-line is not a suitable measurement of the concentration, but the height from the extrapolated base-line is described by Pecsok[21] as suitable for area measurement.

Conclusion

Two further Papers in this issue of *The Analyst* describe the clean-up of animal fats and certain dairy products for residue analysis by the general techniques set out above[22]; and the extension of the same approach to the problem of the analysis of organo-phosphorus residues by electron-capture gas chromatography.[23]

It is important to realise the limitations of the electron-capture method when it is used for determining pesticides. It is not an absolute means of establishing the identity of otherwise unknown residues. For pesticide residues it is desirable wherever possible to use an independent confirmatory method such as infrared spectroscopy or paper chromatography or, where only one or two compounds are present, the multi-column spectrochromatographic method described by Goulden, Goodwin and Davies.[24] Gas chromatography with an

electron-capture detector is the most sensitive method available for the detection of certain pesticides: when it is used near to the limit of detection there is no other method available with which to seek confirmation of the identity of the substances found. Such low results should, therefore, be interpreted with caution. Attention is drawn to some possible difficulties in the interpretation of peaks apparently due to known pesticides. The present knowledge of the metabolism of some chlorinated pesticides in animals is incomplete. Unknown peaks with long retention times are found in some samples and appear to be associated with paper-chromatographic spots with a low R_F value and these may be caused by unknown metabolic products; their presence raises the suggestion that small amounts of further unknown metabolites could influence the heights of peaks ascribed to known compounds. Impurities in technical materials may behave similarly.

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Clean-up of Animal Fats and Dairy Products for the Analysis of Chlorinated Pesticide Residues

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A simple dimethylformamide partition method is described for the clean-up of animal fat samples and of certain dairy products for the analysis of chlorinated pesticide residues. Supplementary column chromatography is necessary for some samples. The purified extracts can be examined quantitatively by gas chromatography with an electron-capture detector. The extracts are often suitable for qualitative examination by paper chromatography as further evidence of the identity of individual residues. Practical methods are given for the clean-up of mutton, beef and human fats; muscle tissue, liver, kidney and brain; milk, butter, eggs; and fleece and flour.

THE acetone - hexane partition clean-up method described by Goodwin, Goulden and Reynolds for plant tissue extracts[1] and extended by Taylor to extracts of certain animal tissues[2] is not in general suitable for the examination of animal fats. Goodwin, Goulden and Reynolds,[3] have also described the application of a similar technique to pig fat and blood. However, acetone is a poor solvent for animal fats, and its use sets a limit to the range of animal tissue samples that can be handled successfully. The original partition technique of Jones and Riddick[4] used acetonitrile (methyl cyanide) as the polar organic solvent. In their method, fat containing the residue of a chlorinated pesticide is dissolved in hexane (or a suitable light-petroleum fraction), the solution is shaken with acetonitrile, and the acetonitrile phase is separated and shaken with water; the resultant one-phase system is then extracted with hexane, and the aqueous acetonitrile phase is discarded. Most of the original fat has been removed in the initial hexane phase, and the pesticide residue is contained in the second hexane phase. If this method is used, it is important to ascertain that the partition coefficients are favourable for each of the pesticides and to adjust the volumes of the various solvents accordingly; departure from the appropriate conditions must be avoided. The disadvantages of the process are the toxic nature of the solvent, its relatively high cost, the interference that it causes in the operation of the electron-capture detector in gas chromatography and, for certain residues, a relatively poor efficiency of the separation process. Burchfield and Storrs[5] showed, for γ-BHC and *pp'*-DDT, that dimethylformamide (DMF) can be used as an alternative to acetonitrile in the partition clean-up process. Other workers have used dimethyl-sulphoxide[6]; both this and sulpholane (tetrahydrothiophen-1,1-dioxide[7]), which has been briefly investigated in the present work, are regarded as too expensive for routine use and are, in any event, unpleasant to use. Sulpholane (m.p. 27° to 28° C) gives considerable emulsion trouble in the extraction process, and, when used without purification, gives an unacceptable degree of interference with the gas-chromatographic responses. Haenni, Howard and Joe[6] compared the partitioning of polynuclear hydrocarbons from waxes, between hexane and the four separate polar solvents acetonitrile, nitromethane, DMF and dimethylsulphoxide; they concluded that the latter two were the most efficient for this separation process and showed that dimethylsulphoxide may be preferable to acetonitrile for work on pesticide residues.

EXPERIMENTAL

The partition characteristics of several common chlorinated pesticides between hexane and DMF were first examined on the milligram scale. Two hundred milligrams of each individual pesticide were weighed into separate, small tubes and each dissolved in 10·00 $\pm$ 0·05 ml of hexane saturated with DMF and extracted with three successive 5-ml portions of DMF.

TABLE I

PARTITION OF SOME CHLORINATED PESTICIDES BETWEEN HEXANE AND SUCCESSIVE DIMETHYLFORMAMIDE (DMF) EXTRACTS

Pesticide, 100 mg	Percentage recovered in DMF extracts			Total recovery	Percentage in final hexane extract	Overall recovery
	1st extract	2nd extract	3rd extract			
HHDN (aldrin) ..	55·5	26·5	10	92	0	92
HEOD (dieldrin) ..	83	10	0	93	9·5	102·5
pp'-TDE	93	3·5	1	97·5	4	101·5
pp'-DDT	89·5	6·5	0	96	3·5	99·5
γ-BHC	94·5	1·5	0	96	0	96
Heptachlor	70	17·5	3	90·5	6	96·5

The results are shown in Table I. The average efficiency of extraction with three successive partitions for all of the pesticides examined is 94 per cent., with a mean deviation of 2·5 per cent.

DIMETHYLFORMAMIDE PARTITION PROCESS FOR MUTTON FAT—

A series of recovery experiments was conducted with hexane solutions of mutton fat, to which microgram amounts of pesticide had been added, by using the gas-chromatographic method described by de Faubert Maunder, Egan and Roburn.[8] A preliminary freezing-out technique, similar to, though much simpler than, that used by Anglin and McKinley[9] for acetone extracts of plant tissue, was found to be advantageous, in that it reduced the initial concentration of fat in the hexane extract without materially altering the concentration of the pesticide residue. Three successive extractions of the initial hexane solution with 10-ml portions of DMF were used, and the combined DMF phases were washed with 10 ml of hexane to reduce the concentration of fat further; the hexane was then itself washed with another 10 ml of DMF to reduce any loss of residue, and the combined 40 ml of DMF used for further work. The efficiency of the final back-titration of the residue from aqueous DMF into hexane was improved by using 2 per cent. aqueous sodium sulphate solution instead of water. Results obtained for the recovery of various chlorinated pesticides added to mutton fat are given in Table II.

TABLE II

RECOVERY OF CHLORINATED PESTICIDES ADDED TO MUTTON FAT BY THE DIMETHYLFORMAMIDE PARTITION PROCESS

Pesticide	Level of addition, p.p.m.	Recovery, per cent.
γ-BHC	0·25	100, 99, 98, 101
HEOD (dieldrin)	0·5	96, 87, 93, 92
pp'-DDE	1·0	78, 73, 80, 82
pp'-DDT	2·0	92, 93, 96, 89
Endrin	1·0	94, 97, 97, 98
Heptachlor	0·5	77, 76, 79, 78
Heptachlor epoxide	0·5	97, 96, 91, 95
pp'-TDE	2·0	111, 112, 113, 113
Dehydrochlorinated *pp'*-TDE ..	1·0	89, 93, 92, 94
Telodrin	0·5	97, 96, 96, 98

A similar partition process, in which dimethylsulphoxide was used instead of DMF, gave considerable separation difficulties owing to the formation of an emulsion. Comparative results (Table III) show that DMF gives better overall residue recoveries than dimethylsulphoxide, so that DMF is considered to be preferable.

Several commercial samples of DMF have been examined. Preliminary distillation is necessary in most instances to reduce the amount of impurities that interfere with detection by electron-capture in gas chromatography.*

* DMF from the Badische Anilin- und Soda-Fabrik AG was found suitable for use without further treatment.

TABLE III

RELATIVE EFFICIENCIES OF DIMETHYLFORMAMIDE (DMF) AND DIMETHYLSULPHOXIDE (DMSO) PARTITION CLEAN-UP

Pesticide	Level of addition, p.p.m.	Recovery, per cent. DMF	Recovery, per cent. DMSO
γ-BHC	0·25	99	93
pp'-DDT	1·0	92	64
HEOD (dieldrin)	0·8	90	69
Heptachlor epoxide	0·25	94	75

APPLICATION OF THE DIMETHYLFORMAMIDE PROCESS TO OTHER SAMPLES—

The DMF partition clean-up process for mutton fat can be applied, with suitable modifications, to extracts of several other fats including dairy products, in preparation for gas-chromatographic examination. In some instances an additional columnar clean-up is also necessary, particularly where the residues encountered are low.

(*i*) *Other animal fats*—The mutton-fat process has also been applied to beef suet and, by omitting the preliminary freezing-out stage and adding a chromatographic step on an alumina column, to human fat. Recovery values for these are given in Table IV.

(*ii*) *Dairy produce*—Milk is extracted with an acetone - hexane mixture, the extract clarified by centrifugation, separated, the hexane phase dried, its volume adjusted and then treated by the DMF partition process. The final extract requires treatment with an activated alumina column and is then suitable for either gas- or paper-chromatographic examination. The same method is applicable to human milk.

Butter is first clarified and the filtered fat dissolved in warm hexane. Extraction with DMF, and then treatment with an alumina column, is suitable for gas chromatography, but is usually inadeqaute for paper chromatography, for which technique some further column treatment is desirable.

TABLE IV

RECOVERY OF CHLORINATED PESTICIDES ADDED TO HUMAN FAT BY THE DIMETHYLFORMAMIDE PARTITION PROCESS

Pesticide	Level of addition, p.p.m.	Recovery, per cent.
γ-BHC	0·25	98
HEOD (dieldrin)	0·8	75
pp'-DDE	1·0	116
pp'-DDT	1·0	114

(*iii*) *Eggs*—For fresh eggs, the contents of the shell are ground together with anhydrous sodium sulphate and extracted with an acetone - hexane mixture; the volume of the extract is reduced and the acetone simultaneously removed by evaporation. Partition with DMF and treatment with an alumina column is necessary.

(*iv*) *Other animal products*—Lean tissue of animals such as mammalian and avian muscle and kidney normally do not require a DMF partition clean-up for gas-chromatographic work, but such a clean-up is advisable for muscle tissue containing an appreciable proportion of interstitial or other fat and for brain and liver tissue. Treatment of the extract with an alumina column is beneficial in all instances, permitting a higher sensitivity to be obtained; it is essential for liver extracts, which must be eluted with additional hexane because some samples (particularly those of carnivores) contain substances that cause the residues to be retained more strongly by the alumina. This effect is less pronounced after a DMF clean-up.

A clean-up with DMF is usually unnecessary for fleece extracts, unless they are to be examined by paper chromatography.

(*v*) *Other samples*—The DMF method has proved useful for examining some extracts of wheaten flour, but for most samples it is unnecessary, *e.g.*, for clay soil, water and extracts of scrubland herbage for gas-chromatographic examination.

PRACTICAL CLEAN-UP METHODS

APPARATUS—

Separating funnels—100-ml, 250-ml and 350-ml capacity.
Calibrated flasks—100-ml capacity.
Graduated cylinders—10-ml capacity.
Soxhlet extraction apparatus.
Filter funnel—A 4-inch, short-stem funnel (for butter).
Centrifuge with 250-ml tubes—For milk.
Evaporators—Kuderna - Danish type, 500-ml capacity.
High-speed food mixer.
Chromatographic columns—12-mm diameter × 300 mm.

REAGENTS—

Hexane—Boiling-point 68° to 70° C; examine for gas-chromatographic purity under working column conditions; distil from sodium hydroxide if necessary.

Acetone—General-purpose reagent.

Dimethylformamide—Examine a hexane extract for interference peaks by using a gas-chromatograph and re-distil the solvent if necessary.

Hexane saturated with dimethylformamide.

Dimethylformamide saturated with hexane.

Sand, acid washed.

Alumina, activated—Heat aluminium hydroxide to 800° C for 4 hours, cool, add carefully 5 per cent. v/w of water and mix the solid thoroughly in a closed vessel. Keep the vessel well stoppered and use the alumina within 10 days.

Distilled water—Examine a hexane extract for interfering peaks by using a gas chromatograph.

EVAPORATION OF EXTRACTS—

Dry the extracts, if necessary, by passing them through a column of anhydrous, granular sodium sulphate, and reduce them from a large volume to a few millilitres in a 500-ml Kuderna - Danish evaporator[10] fitted with a splash head or a short Snyder column, on a steam-bath or a warm-water bath. (This is quick and efficient and causes little or no loss of residue.) Keep the concentrated solution at 35° C in a warm-water bath, and reduce the volume further with a current of dry air. (If the final volume required is 1 ml or less, the last 0·5 ml is best removed at room temperature with a blow-ball.) A vigorous air stream can cause losses by splashing or losses by volatilisation from the walls of the vessel, and must be avoided. Rubber and certain synthetic plastic tubing materials are liable to contain traces of volatile electron-capturing substances that will contaminate the air stream and give rise to serious interference with the gas-chromatographic estimation of residues. Silicone rubber is generally free of these interfering substances. Such contamination, which is occasionally present in samples as received, can be removed or substantially reduced by treatment with an activated-alumina column.

DIMETHYLFORMAMIDE PARTITION PROCESS—

Extract 25 ml of the solution of fat in hexane with 10 ml of DMF saturated with hexane. Set the mixture aside for 2 to 3 minutes and run the clear DMF phase into a 100-ml separating funnel, retaining any interfacial emulsion in the first separating funnel. Repeat the extraction of the hexane solution with two further 10-ml portions of DMF. Combine the DMF extracts, and wash them with 10-ml of hexane saturated with DMF to remove any traces of fat. Separate the 10 ml of hexane, wash them with a further 10 ml of DMF, add this and the original 30 ml of DMF extract to a 350-ml separating funnel and shake the extracts briskly for 2 minutes with 200 ml of 2 per cent. aqueous sodium sulphate solution. Set the mixture aside for 20 minutes; hexane, previously held in solution, will separate. Gather the hexane phase by gentle swirling, run the aqueous layer to waste, dry the stem of the separating funnel with filter-paper, and run the hexane into a 10-ml graduated cylinder. Wash the side of the separating funnel with small amounts of hexane, and add these to the cylinder. Record the total volume of hexane, or adjust it to a suitable volume.

TREATMENT WITH AN ACTIVATED-ALUMINA COLUMN—

Pour a slurry of 10 g of activated alumina in hexane into a chromatographic column containing a of pledget solvent-washed cotton wool as a support. Allow the slurry to settle

keeping the surface of the alumina covered with hexane. Add more hexane, cover the alumina with a 5-cm layer of anhydrous sodium sulphate and allow the hexane level to fall to the level of the upper sodium sulphate surface. Run on the solution to be treated, which should be concentrated to about 2 ml, and wash it into the column with three successive 2-ml portions of hexane. Elute the sample with 90 ml of hexane, and collect a similar volume of eluate.

PROCEDURES—

(*a*) *Mutton fat and beef suet*—Samples containing only a small proportion of connective tissue, such as kidney fat, can conveniently be dealt with by a simple solution method. Grind 20 g of prepared, minced sample in small portions with an equal weight of sharp sand, add 50 ml of hexane and warm the mixture gently on a steam-bath to dissolve the fat completely. Decant the solution into a 100-ml calibrated flask, together with additional small amounts of hexane used as washings, cool the solution to 20° C and dilute it to 100 ml. Cool the flask and contents to 3° C and maintain them at this temperature for 1 hour to precipitate a proportion of the fat. While it is still cold, remove a 25-ml portion of the solution, and subject it to the DMF partition process; measure and record the volume of the final hexane solution, and examine it by gas chromatography.[8] Samples containing high residues may be examined in solution without cooling by dissolving only 5 g of sample in 100 ml of hexane.

(*b*) *Human fat*—Grind 10 g of chopped, mixed sample with 20 g of sand and sufficient sodium sulphate in a stout, glass beaker with a heavy glass rod to give a uniform, dry, granular mass. Warm the ground material successively with 50-, 20-, and then 20-ml portions of hexane on a steam-bath until it is boiling gently, stirring carefully; decant the solution through a Whatman No. 1 filter-paper into a 100-ml calibrated flask and carefully transfer the solid to the filter-paper. Wash the beaker, filter-paper and its contents with a further 10 ml of warm hexane, cool the flask and its contents to 20° C and dilute the solution to the mark. Treat a 25-ml portion of this solution by the DMF partition process described above for mutton fat, omitting the preliminary freezing-out stage, and treat the extract by passing it through an activated-alumina column, adjusting the final volume of the eluate as necessary.

(*c*) *Milk*—Transfer 40 ml of well mixed cow's milk (or human milk), 80 ml of acetone and 80 ml of hexane in that order to a 250-ml vortex beaker and macerate the mixture for three minutes. Transfer it immediately to a 250-ml centrifuge tube, washing the macerator blades with 10 ml of hexane, then with 5 ml of water, and add the washings to the tube. Spin the tube in a centrifuge of radius 12·5 cm at 2500 r.p.m. for 5 minutes, separate the hexane solvent layer and pass it through a short column of anhydrous sodium sulphate. Wash the contents of the tube with two successive 25-ml portions of hexane and run the washings through the column. Reduce the combined extracts to about 15 ml in an evaporator, transfer the solution to a 100-ml separating funnel calibrated at 25 ml, adjust the volume to 25 ml and treat by the DMF partition process. Treat the extract by passing it through an activated-alumina column. Evaporate the eluate in a Kuderna - Danish evaporator, fitted for preference with a small calibrated tube, and adjust the volume to 1·0 ml by careful evaporation in a current of dry air.

(*d*) *Butter*—Dissolve 5 g of clarified butter fat in 10 ml of hexane and transfer the solution to a 100-ml separating funnel with a further three successive 5-ml portions of hexane. Carry out the DMF partition process. Concentrate the final extract to 2 ml, and run it through a prepared activated-alumina column. Reduce the final extract in an evaporator fitted with a 5-ml calibrated tube, cool the extract and dilute it to 5 ml. Gas-chromatographic traces should be made for long enough to clear the peaks associated with residues of DDT.

(*e*) *Eggs*—If on inspection the contents of the shell prove fluid, incorporate sufficient anhydrous sodium sulphate to give a granular mass. If the contents are dehydrated and solid or plastic, grind them with sand and sodium sulphate, incorporating the shell if necessary. Transfer the granular mass to a suitable extraction thimble and extract it for 2 hours in a Soxhlet extractor with an acetone - hexane mixture (1 + 2). Cool the extract and filter it through a short column of anhydrous sodium sulphate into an evaporator fitted with a 25-ml flask; wash the column with a further 25 ml of hexane in small portions. Reduce the combined extracts to about 20 ml, cool and dilute them to 25 ml. Treat the solution by DMF partition, and then pass it through an activated-alumina column. Adjust the final volume of the hexane extract as required, and examine the sample on a gas chromatograph.

(*f*) *Muscle tissue; liver, kidney, brain*—For lean mammalian and avian tissues, including liver, brain, kidney and muscle tissue containing only a small proportion of interstitial fat, weigh 10 g (or the whole sample, if less) into a glass mortar, and grind with an approximately equal weight of sharp sand and sufficient anhydrous sodium sulphate to a uniform, dry powder. Transfer the ground material to a 150-ml beaker, simmer it for about 2 minutes with 50-, 20-, 20- and 20-ml successive portions of hexane (use these to wash the mortar), stirring carefully. Allow each solution to cool for a few minutes before decanting them into 100-ml calibrated flasks. Dilute the solutions to the mark and treat 25-ml portions by the DMF partition process and then by passage through an activated-alumina column; liver extracts are treated similarly, except that the elution and collection of hexane from the activated-alumina column is continued until the eluate is substantially free from residues, as indicated by gas-chromatographic examination.

When the sample is small or contains little fat, the DMF partition process may be omitted.

(*g*) *Wheaten flour*—Place 25 g of a representative sample in an extraction thimble (30 mm × 80 mm), plug it lightly with solvent-washed cotton wool and extract the sample with 150 ml of hexane for 2½ hours in a Soxhlet extractor. Cool the extract and pass the hexane solution through a column of anhydrous sodium sulphate into an evaporator; wash it through the column with further portions of hexane, and adjust the final volume as necessary. Treatment of the concentrated extract with both DMF and an activated-alumina column may be omitted for most samples to be examined on a gas chromatograph.

(*h*) *Fleece*—Prepare a sample by cutting at least 200 g and taking at least 10 g for each extraction. Press 10 g of fleece into a thimble (35 mm × 23 mm) and extract the sample for 8 hours in a Soxhlet extractor with hexane. Cool the extract, and, if necessary, pass it through a short column of anhydrous sodium sulphate and a fine filter-paper (Whatman No. 42) into a suitable calibrated flask; adjust the volume. This extract is usually suitable for gas-chromatographic examination without further clean-up.

PAPER CHROMATOGRAPHY—

Extracts prepared by a method including treatment with an activated-alumina column for gas-chromatographic examination can normally be used for Evans' paper-chromatographic method,[11] for determining chlorinated insecticides. However, some extracts still contain troublesome traces of oil, wax or pigment, even after treatment by DMF partition and with an activated-alumina column, with consequent streaking on paper chromatograms; further clean-up is necessary in such instances. If there is a large amount of residue, a smaller volume of extract can be taken for examination, but butter and oil extracts cannot normally be examined by this method without further clean-up.

TABLE V

RECOVERIES OF CHLORINATED PESTICIDES ADDED TO VARIOUS DAIRY PRODUCTS AND HUMAN MILK

Sample	Pesticide	Level of addition, p.p.m.	Average recovery, per cent.
Milk	γ-BHC	0·2	84
	HEOD (dieldrin)	0·2	88
	pp'-DDT	0·2	74
	pp'-DDE	0·2	78
	Heptachlor epoxide	0·12	88
Butter fat	γ-BHC	0·2	94
	HEOD (dieldrin)	0·8	82
	pp'-DDT	2·0	76
	pp'-DDE	1·0	73
	Heptachlor epoxide	0·2	87
Eggs	γ-BHC	0·1	81
	HEOD (dieldrin)	0·1	80
	pp'-DDT	1·0	75
	pp'-DDE	1·0	80
	Heptachlor epoxide	0·2	100
Human milk	γ-BHC	0·25	96
	HEOD (dieldrin)	0·8	75
	pp'-DDT	1·0	114
	pp'-DDE	1·0	116

RECOVERY EXPERIMENTS—

It is obviously desirable to check the validity of the combined extraction, clean-up and subsequent analytical stages of a method for analysing residues, for gross interference and loss of residue by conducting a recovery test. This should be performed whenever any type of sample is being examined for the first time, with those pesticides whose presence is confirmed or suspected. Unless the sample is liquid or melts at a low temperature, it is impossible to add pesticide to it in a manner completely simulating its occurrence in practice. Small measured volumes of standard pesticide solution, normally prepared in the same solvent as that to be used for the subsequent extraction, are added to the liquid or comminuted solid sample material, mixed in a vessel closed in such a manner as to avoid the possibility of absorption of the residue by liner or gasket materials, and brought to a state of equilibrium for several hours before analysis. Flasks with sprung stoppers (500-ml or 1-litre flasks fitted with B34 stoppers) are particularly suitable and may be "tumbled" in a suitable rotating frame periodically at about 60 revolutions per minute with an end-over-end motion. In some instances, *e.g.*, soils, the standard pesticide solution does not penetrate the sample efficiently, and it is preferable to make the addition in another solvent such as acetone in place of a simple standard hexane solution.

Results for the recovery of various residues, added to different commodities at the levels indicated and determined on a gas chromatograph after the extraction and clean-up processes described above, are given in Table V.

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The Analysis of Organo-phosphorus Pesticide Residues by Gas Chromatography

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An accurate method is described for the gas-chromatographic separation and estimation of organo-phosphorus pesticides in sub-microgram amounts. A general method for extracting several petroleum-soluble organo-phosphorus residues is developed and applied to these residues on several plant materials.

The use of the Lovelock electron-capture ionisation detector[1,2] for the gas-chromatographic examination of chlorinated insecticide residues in plant extracts described by Goodwin, Goulden and Reynolds[3] and extended by Watts and Klein[4] to residues in dairy produce, has been a notable contribution to the subject of residue analysis, offering a combination of high sensitivity with a reasonable degree of specificity. The method is exceptionally sensitive for chlorinated compounds owing to their highly "electrophoric" nature,[5] by virtue of which, halogen and certain other constituents show a high affinity for free, low energy-electrons. The experimental gas-chromatographic conditions used by Goodwin, Goulden and Reynolds[3] were found to be unsuitable for separating organo-phosphorus compounds, although parathion, which has a highly electrophoric nitro group, was found to be chromatographed with reasonable sensitivity. This Paper describes the practical conditions found to be suitable for the general application of electron-capture gas chromatography to organo-phosphorus residue work.

One problem in the analysis of pesticide residues is the significance of results obtained with pure (or technical) pesticide materials in relation to residues arising in field and storage practice and which may include one or more products. Only the extraction and separation of pesticidal compounds themselves is described here. Kinetic studies of the pyrolysis of these and of other breakdown products under varying column conditions are in progress.

Experimental

The Shandon "Universal" gas chromatograph was used throughout, with 2-foot, U-shaped columns, 5/32 inch in internal diameter, the electron-capture detector being made from brass and thick polytetrafluoroethylene tubing (14-mm internal diameter, 25-mm external diameter). A tritium-loaded target strip (1 cm × 3 cm) source* as described by de Faubert Maunder, Egan and Roburn[6] was also used. Celite (100 to 120 mesh) was used as column supporting-phase, with various stationary phases. Of the latter, 3 per cent. w/w Apiezon L or 3 per cent. w/w Apiezon N, each with 0·3 per cent. w/w Epikote 1001, were found to be useful for estimating some organo-phosphorus compounds, especially parathion and malathion, although such columns require extremely careful preparation: 10 to 20 per cent. w/w silicone elastometer E 301 with 2·5 per cent. w/w Epikote 1001 is generally preferred for most other compounds. Column temperatures of 160° C and 188° C were used, the injection head and detector both being operated at the column temperature. The column and injection-head temperatures are both important; above 220° C considerable decomposition of certain organo-phosphorus pesticides may occur. Pyrolysis leads to low recoveries, and quantitative work is impossible. At temperatures below 140° C, many organo-phosphorus compounds are insufficiently volatile for successful chromatography, again making quantitative work impossible.

Detector response—

Fig. 1 shows the detector response to four organo-phosphorus pesticides with a 10 per cent. w/w silicone column at 188° C, an applied detector potential of 40 V, a flow-rate of 100 ml per minute of oxygen-free nitrogen carrier-gas and an amplifier gain of ×20. Under these conditions the detector responds in a linear fashion up to a peak height of between 35 to

* 200 to 300 millicuries: Radiochemical Centre catalogue No. TRT3.

40 per cent. full-scale deflection, so that by using a gain of $\times 50$ the relation between the peak-height response and the amount of the organo-pesticide injected is virtually linear to full-scale deflection.

Relative retention results and an indication of the general sensitivity for a number of organo-phosphorus compounds are given in Table I for both copper and stainless-steel columns.

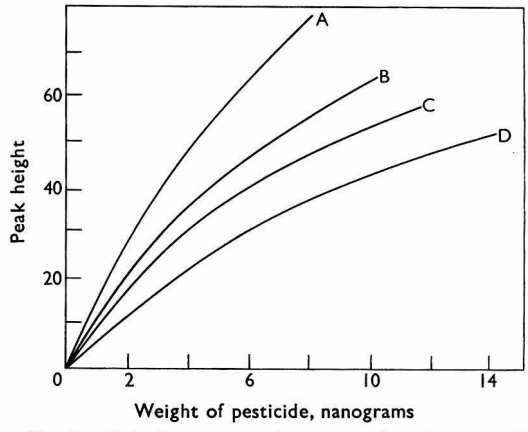

Fig. 1. Detector response to organo-phosphorus pesticides: curve A, fenchlorphos; curve B, chlorthion (weight scale $\times$ 5); curve C, parathion (weight scale $\times$ 5); curve D, malathion (weight scale $\times$ 10)

Beckman and Bevenue[7] have shown that the recovery of some chlorinated pesticides is lower for copper than for stainless steel and Crosby and Laws[8] have observed a similar effect with certain organo-phosphorus compounds on the microgram scale. Table I gives general support

TABLE I

RESULTS OF RELATIVE RETENTION AND SENSITIVITY DETERMINATIONS FOR ORGANO-PHOSPHORUS PESTICIDES

HEOD = 100

Pesticide	Sensitivity, nanograms (10^{-9} g)		Relative retention volume	
	(*a*)*	(*b*)*	Silicone, 188° C	Apiezon, 188° C
Petroleum-soluble—				
Chlorthion	5	1	60	42
Delnav	—	50	27	—
Diazinon	5	5	78	70
Disulphoton	50	10	26	20
Ethion	50	5	141	108
Fenchlorphos	1	0·5	40	33
Malathion	10	5	47	28
Parathion	5	2·5	49	40
Parathion-methyl	5	2·5	38	29
Phenkapton	5	4	60, 325	14, 430
Phorate	50	10	18	18
Thiometon	50	15	19	13
Trithion	—	4	175	—
Zinaphos	5	0·5	14	13
Water-soluble—				
Dimethoate	50	10	25	29
Morphothion	50	50	108	225
Phosdrin	5	2	6, 11	6
Phosphamidon	25	10	6, 28, 37	4, 27
Schradan	500	—	6	36

* Upper limit of range, which, at gain $\times 50$ on a silicone column, gave a peak height of at least 10 units (1 inch) with (*a*) a copper and (*b*) a stainless-steel chromatographic column.

to these results and shows that greater sensitivity for organo-phosphorus pesticides can be obtained by using the stainless-steel column. The separation of six individual organo-phosphorus pesticides on a stainless-steel column with a stationary phase of 10 per cent. w/w silicone elastomer and 0·5 per cent. w/w Epikote resin is shown in Fig. 2.

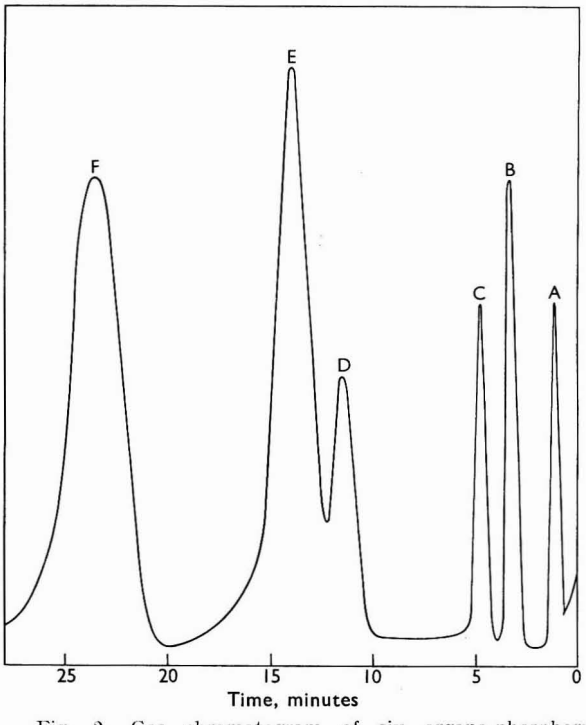

Fig. 2. Gas chromatogram of six organo-phosphorus pesticides: curve A, zinaphos (2·5 nanograms); curve B, fenchlorphos (2·0 ng); curve C, parathion (10·0 ng); curve D, ethion (20·0 ng); curve E, trithion (25·0 ng); curve F, phenkapton (25·0 ng)

Method

The method for the extraction and clean-up of organo-phosphorus pesticide residues for a number of plant tissues is given below. The method has been applied to chlorthion, ethion, fenchlorphos, malathion, parathion, phenkapton and trithion. Table II gives the recoveries obtained.

Apparatus—

Shandon "Universal" gas chromatograph—With accessories for electron-capture detection.
Separating funnels.
Filter funnel.
Centrifuge—Capable of holding 250-ml tubes.
Evaporator—Kuderna - Danish type.
High-speed food mixer.
Chromatographic columns—15 mm in diameter.

Reagents—

Reagents should be of analytical-reagent grade whenever possible.
Hexane—Boiling-point 68° to 70° C.
Ethyl methyl ketone—Boiling-point 78·5° to 80·5° C.
Sodium sulphate—Use granular anhydrous material.

Sodium sulphate solution, 5 per cent. w/v, aqueous.

Magnesium oxide, heavy.

Aluminium oxide, activated—Heat aluminium hydroxide to 800° C for 4 hours, cool, add carefully 5 per cent. v/w of water and mix the slurry thoroughly in a closed vessel. Keep the vessel well stoppered and use the alumina within 10 days.

TABLE II

EXTRACTION OF ORGANO-PHOSPHORUS PESTICIDES WITH ETHYL METHYL KETONE - HEXANE AND ESTIMATION BY GAS - LIQUID CHROMATOGRAPHY

Organo-phosphorus pesticide	Source	Added pesticide concentration, p.p.m.	Column clean-up	Recovery, per cent.
Chlorthion	Lettuce	0·08	Magnesia	84
Ethion	Onions	0·15	Alumina	86
	Sugar beet	0·10	Alumina	91
	Apples	0·20	Alumina	80
	Lettuce	0·50	Magnesia	80
Fenchlorphos	Lettuce	0·08	Magnesia	73
Malathion	Barley	0·20	*	80
Parathion	Apples	0·20	Alumina	86
	Sugar beet	0·10	Alumina	91
	Brussels sprouts	0·20	Alumina	75
	Lettuce	0·30	Magnesia	80
Phenkapton	Apples	0·10	Alumina	82
Trithion	Apples	0·20	Magnesia	84

* No clean-up required.

EXTRACTION AND CLEAN-UP—

Macerate 50 g of chopped sample with 85 ml of ethyl methyl ketone - hexane mixture (in the ratio of 3 to 2) for four minutes in a high-speed macerator, allow the mixture to separate and decant the non-aqueous phase through a short column of anhydrous sodium sulphate into a Kuderna - Danish evaporator fitted with a 50-ml round-bottomed flask. Repeat the process with two further 85-ml portions of mixed solvent. Shake the aqueous residue with 100 ml of hexane, spin the mixture in a centrifuge, add the separated hexane phase to the non-aqueous phase in the evaporator and reduce the volume to about 20 ml. Transfer the extract to a large separating funnel with a further 20-ml portion of hexane and shake the solution for two minutes with 500 ml of sodium sulphate solution. Separate the hexane phase, re-extract the aqueous phase with a further 20-ml portion of hexane, separate off the latter, stir the combined hexane phases with 30 g of anhydrous sodium sulphate and filter the extract through a Whatman No. 1 filter-paper into an evaporator fitted with a 20-ml flask. Wash the sodium sulphate with three successive 20-ml portions of hexane, add the washings to the evaporator and reduce the volume to about 5 ml. Pour a slurry of 10 g of activated alumina with hexane into a column and introduce the concentrated extract. Elute the extract with a total of 250 ml of hexane. Reduce the volume of the eluate as necessary, and inject portions on to the gas-chromatographic column.

DISCUSSION

Some difficulty is encountered in eluting malathion from an alumina column, and a column containing 30 g of magnesium oxide has been used in place of the alumina column. However, reasonable recovery from barley is possible with the above method without column clean-up. The magnesia column has also been successfully used for the clean-up of several other organo-phosphorus pesticides.

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Measurement of Elution Peaks in Gas-Liquid Chromatography

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Replication tests have been carried out by ten laboratories to determine the precision of three of the commonly used methods for the measurement of peak areas. Two of the methods depend on the assumption that the peak area is proportional to the product of the peak height and either the peak width at any specified fraction of the peak height or the distance between the intersections of the tangents to the points of inflexion with the base-line. The third method involves the use of commercial planimeters. The tests have proved conclusively that the method in which the peak height and the width at half the peak height is used, is the most precise; even with the preferred method, the precision obtained in dependent on the location of the base-line. The procedure is described in detail together with results obtained.

GAS - LIQUID chromatography has become an important technique for analysing volatile industrial products and, in many laboratories, it has replaced the older methods for routine analysis. The chief reason for this is the versatility and greater speed of the technique in comparison with other methods and its development has therefore been rapid. The accuracy of the method has, however, been open to doubt, and it is evident that insufficient attention has been devoted to this aspect in the past. Thus wide variations are often noticed when results obtained by different laboratories for any analysis are compared. Replicate analyses performed by a single laboratory often give a satisfactory precision, and the results are frequently accepted even though the average value of the analysis may differ considerably from the true value. These variations almost certainly arise from the use of different apparatus, techniques and conditions and consequently, different orders of prominence are attributable to various sources of error. Therefore, unless these sources of error are fully investigated and minimised by the application of standardised procedures, significant differences are bound to persist in the results from even the most careful inter-laboratory testing. Complete and rigid standardisation of the procedure would, however, be impracticable since many types of apparatus are in common use. Moreover, chromatographic columns cannot be exactly duplicated and may necessitate different operative conditions. On the other hand, the main sources of error will almost certainly lie in the methods used for the calibration of the apparatus and the interpretation of the chromatograms. It is within this context that standard procedures could be devised.

In order to investigate these problems thoroughly the Standardization of Tar Products Tests Committee (STPTC) appointed a Panel whose aim was to provide recommendations for the use of gas - liquid chromatography as a standard method for testing coal-tar products. Many of the results that have been obtained, however, are of general interest and the present Paper is a report of the work undertaken by the Panel on the methods of measuring peak areas. The methods that have been considered are those that are most commonly used in practice and all involve a degree of judgment by the operator. Obviously, the use of fully automatic integration methods would eliminate the personal errors involved but suitable devices are still undergoing development, and are therefore considered to be beyond the scope of the present work.

Methods commonly used for the measurement of peak areas

General method—

Peak area is proportional to the product of the peak height and the peak width at a constant fraction of the peak height.

This method is strictly valid for Gaussian curves, and hence its application depends on how nearly this condition is realised in practice. In instances of gross asymmetry, the product

of the two linear measurements involved does not bear a constant relationship to the true peak area, and an error is introduced. The appearance of grossly asymmetrical peaks is, however, indicative of unfavourable working conditions and these can generally be adjusted such that satisfactory peak shapes are more readily realised.

The only geometrical construction involved in this method is the extension of the base line beneath the peak. The accuracy with which this can be accomplished depends on the stability of the existing base-line and hence, as with all methods of peak-area measurement, the zero stability of the equipment should be of a high quality. The peak width at half the peak height was chosen for the purposes of the present tests as being a convenient point for the measurement.

TRIANGULATION METHOD—

Peak area is proportional to the product of the peak height and the distance between the intersection of the tangents to the points of inflexion of the curve with the base-line.

This method involves the careful construction of the necessary tangents in addition to the base-line and therefore creates a further source of operator error. A modification of the method is sometimes used in which the vertical height of the apex of the triangle formed by the tangents is measured rather than the actual peak height. This modification was excluded from the present series of tests after serious errors were found to arise in the measurement of narrow peaks. With such peaks, the near-parallel condition of the tangents to the points of inflexion resulted in an extremely small angle at their point of intersection, which was consequently of an indefinite nature. The same symmetry condition applies to this method as to the general method.

PLANIMETRIC METHOD—

This method is used fairly extensively, and some study of its precision in comparison with the geometric methods previously discussed was thought to be necessary.

OTHER METHODS—

Various other methods are used for measuring the areas of elution peak, *viz.*—

(*a*) Peak area $\propto$ retention time, or distance $\times$ peak height. This method is theoretically incorrect and cannot be seriously considered for accurate analysis.

(*b*) Peak area $\propto$ weight of paper contained within the boundary of the peak. This method has the advantage that the actual peak is obtained and the condition of symmetry does not apply. The method, however, involves cutting out the peaks from the chromatogram and weighing the paper, and this was not considered since the effect of variations in the paper could not be assessed in a manner that would include all grades of paper in common use. Further, the necessary destruction of the chromatogram that the method entails was thought to be an undesirable feature.

REPLICATION TESTS

Before replication tests could be commenced on the measurement of peak areas, a method had to be evolved for the accurate replication of chromatograms for circulation to the participating laboratories. The method finally adopted was as given below.

A transparency was first prepared from the original chromatogram on Ozarapid film and this was then printed through a normal printing machine on Oza film (a polyester-base material having a thickness of 0·003 inches).

An initial replication test was carried out in which each member of the Panel measured the areas of peaks on five replicate copies of a single chromatogram produced by the above method. Apart from specifying that each of the three main methods for the measurement of peak areas should be used, no control was exercised on the methods of base-line construction or the actual points on the trace to which measurements should be made. The results were inconclusive, and a rigid control over these factors was obviously necessary. The question of possible "wheel slip" also arose in the planimeter measurements, and the effect of the nature of the paper surface in this connection was tested. The area of a standard circle was measured by several members of the Panel on five different grades of paper. The results indicated that the effect of "wheel slip" was not significant and the grade of paper used was therefore unimportant.

The standardised procedure given below was then devised, based on recommendations provided by the Statistical Panel of the STPTC.

(*i*) Two replicate chromatograms were sent to each laboratory. Each chromatogram consisted of five peaks of approximately equal areas, but having increasing retention times so that the peak heights decreased and the peak widths increased. Measurements were made first on one chromatogram and the results reported before the second chromatogram was circulated. This spacing of the measurements was designed to eliminate, as far as possible any "memory effects." The peak areas were measured by each of the three chosen methods, *viz.*, peak height times peak width at half the peak height, peak height times distance between the intercepts of the tangents to the points of inflexion with the base-line and thirdly the planimetric method.

(*ii*) All linear measurements were made with a standard precision wooden rule having ivorine edges. The scale was marked in centimetres subdivided into millimetres. Readings were estimated to the nearest 0·1 mm.

(*iii*) The base-line was constructed with a fine pencil from the beginning of one peak to the beginning of the subsequent peak. For the last peak, the base-line was constructed from the beginning of that peak to the end of the trace.

(*iv*) For the general method, the peak width was measured at 0·5 times the peak height measured from the base-line. The horizontal distance between the inside of the ascending trace and the outside of the descending trace at this point, was taken as the peak width.

(*v*) Peak heights were measured from the point on the base-line vertically below the peak maximum to the absolute extremity of the peak.

(*vi*) Each method, including the planimeter method, was applied once only to each peak.

Results

Ten laboratories participated in the scheme and their results are given in Tables I, II and III.

Table I

Peak-area measurement by the general method

Peak height × peak width at 0·5 peak height

Laboratory	Peak area, sq. cm × 0·94				
	1	2	3	4	5
A	3·58	3·87	3·94	2·72	2·75
	3·22	3·76	3·94	2·69	2·88
B	3·94	3·97	3·98	2·71	2·84
	3·22	4·09	4·13	2·84	3·07
C	3·58	3·99	3·98	2·66	2·89
	3·59	3·99	3·91	2·62	2·90
D	3·76	3·97	3·92	2·63	2·78
	3·58	4·08	4·06	2·75	2·88
E	3·77	3·99	4·07	2·71	2·98
	3·59	4·09	4·12	2·68	2·97
F	3·40	4·09	3·99	2·74	2·87
	3·40	4·08	4·10	2·75	2·84
G	3·77	4·09	4·06	2·76	2·91
	3·58	3·88	4·01	2·73	2·91
H	3·58	4·08	4·09	2·74	2·84
	3·76	3·96	4·03	2·77	2·80
J	3·58	3·88	4·00	2·66	2·84
	3·58	4·10	3·98	2·64	2·81
K	3·58	4·09	4·00	2·71	2·86
	3·58	3·99	4·00	2·73	2·86

The statistical analysis was based on the assumptions (*a*) that the chromatograms circulated to the participating laboratories were identical and (*b*) that the variance of each method was homogeneous for the different peaks.

A standard two-factor analysis, with replication, was performed, and the results obtained gave estimates of inter-laboratory, inter-sample, interaction and residual variances. Before

the results were analysed, however, they were checked for outliers by Dixon's "Q" test.[1] One pair of results with the general method, laboratory A and peak 2, and with the triangulation method, the first result of laboratory H, peak 4, were found to be outliers. Replacement values for these results were calculated by using Snedecor's formula.[2] In the planimetric method, laboratory J had one value low for each of the samples 2 and 3 and the total low for sample 4. As this requires four replacements the complete range of results from laboratory J were excluded from the statistical analysis. The precision figures obtained are given in Tables IV and V.

TABLE II

PEAK-AREA MEASUREMENT BY THE TRIANGULATION METHOD

Peak height × base

	Peak area, sq. cm × 1·61				
Laboratory	1	2	3	4	5
A	5·73	6·67	6·65	4·49	4·70
	6·26	6·88	6·53	4·54	4·81
B	7·53	7·30	6·89	4·62	4·84
	6·44	7·32	6·89	4·76	5·29
C	6·45	6·68	6·48	4·43	4·72
	6·46	7·01	6·69	4·56	4·86
D	6·80	7·30	6·84	4·62	4·72
	6·62	7·08	7·00	4·82	4·86
E	7·72	6·90	7·12	4·40	5·02
	8·06	7·10	6·96	4·78	4·92
F	6·98	7·31	6·76	4·61	4·80
	6·44	7·09	6·81	4·55	4·83
G	7·00	7·32	7·05	4·75	4·98
	7·35	7·55	6·89	4·68	4·90
H	7·16	7·19	6·80	6·78	4·81
	6·44	6·52	6·78	4·61	4·88
J	6·45	7·41	6·93	4·65	4·88
	6·80	6·91	6·69	4·59	4·80
K	6·09	6·99	6·72	4·55	4·81
	6·45	6·68	6·77	4·56	4·86

TABLE III

PEAK-AREA MEASUREMENT BY THE PLANIMETRIC METHOD

	Peak area, sq. cm				
Laboratory	1	2	3	4	5
A	3·7	4·1	4·4	2·9	3·0
	3·6	4·1	4·0	3·2	3·1
B	3·4	4·6	4·5	3·0	3·5
	3·3	4·4	4·2	3·1	3·6
C	4·1	4·5	4·3	2·9	3·0
	4·1	4·3	4·5	2·9	3·2
D	—	—	—	—	—
E	3·7	4·2	4·4	2·8	3·0
	3·7	4·3	4·4	2·8	3·7
F	—	—	—	—	—
G	3·81	4·06	4·06	2·97	3·23
	4·00	4·19	4·13	2·97	3·10
H	3·80	4·19	4·71	2·90	2·97
	3·03	3·87	4·32	3·23	3·10
J	3·80	3·80	4·10	2·50	3·10
	3·50	3·10	3·20	2·10	2·80
K	4·0	4·2	4·2	3·0	3·1
	4·1	4·4	4·1	3·0	3·1

The definitions of *Repeatability* and *Reproducibility* used in Tables IV and V are—

Repeatability is a measure of the precision of the test results obtained by one operator using one set of apparatus. In this paper, repeatability is the difference between duplicate measurements on copies of the same chromatogram that would be equalled or exceeded, in the long run, in only one instance in twenty.

Reproducibility is a measure of the precision of the test results obtained by different operators using different sets of the specified apparatus. In this paper, reproducibility is the difference between a single measurement made by one operator at one laboratory, and a single measurement on a copy of the same chromatogram by another operator in another laboratory, that would be equalled or exceeded, in the long run, in only one instance in twenty.

The degrees of freedom were calculated using Welch's formula for the degrees of freedom in linear combinations of several variances.[3]

TABLE IV

PRECISION OF PEAK-AREA MEASUREMENTS

Method of measurement	Repeatability		Reproducibility	
	95 per cent. limits, sq. cm	Degrees of freedom	95 per cent. limits, sq. cm	Degrees of freedom
General	0·29	93	0·29	93
Triangulation	0·37	49	0·55	52
Planimetric	0·65	65	0·65	65

TABLE V

PRECISION OF PEAK-HEIGHT AND PEAK-WIDTH MEASUREMENTS

	Repeatability		Reproducibility	
	95 per cent. limits, mm	Degrees of freedom	95 per cent. limits, mm	Degrees of freedom
Peak height	0·06	50	0·15	48
Peak width	0·04	95	0·04	95

In the analysis of the results for the general and planimetric methods and peak widths, the mean squares obtained for the inter-laboratory and interaction effects were found to be less than or not significantly greater than the residual variance. The results were therefore pooled to give a better estimate of the residual variance.

DISCUSSION

It is clear from the results that the general method is the most precise. To investigate the sources of error in this method, the precision of the peak-height and peak-width measurements was analysed separately. The results are given in Table V. These results show that the error associated with measuring peak heights is greater than that for peak widths. However, as the peak widths are generally much smaller than the height, the errors contribute more or less equally to the error of the area.

The analysis also indicated that the repeatability of peak-width measurements increased almost linearly with peak width, but that the repeatability of peak-height measurements fell with increasing peak height. It was expected that in general the precision of peak heights would be lower than that for peak widths, since the heights depend on the location of the base-line by the operator. On sharp, narrow peaks this lower precision has only a small effect on the accuracy of the peak-width measurements, as both sides of the peak are almost parallel. On later peaks, however, the measured width at 0·5 times the peak height becomes more critically dependent on the measured peak height and explains the decreasing precision of the peak-width measurements. These effects demonstrate clearly the importance of maintaining stable operating conditions to ensure the maximum base-line stability, and also the care that must be exercised in fixing the base-line to each peak. The relative importance of the distances measured must also be realised. Thus, although the precision of peak-width

measurements is greater than that of peak-height measurements, the ultimate precision of the peak areas is dependent on both the size and shape of the peak being measured. Thus the area of a tall, narrow peak may suffer more from the small error associated with the measurement of the peak width than the larger error associated with the measurement of the considerably larger peak height.

Such comment must also be made concerning the validity of the results for the planimetric method. The planimeters used for the tests were of varying types, and it is possible that the use of a standard instrument of high precision would give better results. Tests carried out with the existing instruments showed, however, that the largest part of the error arises from the operator. The extent of this error would depend on the experience of the operator in handling the instrument and hence the variance estimate of the measurements would depend largely on this factor. In general, however, the errors associated with the planimeter measurements arise from slight deviations of the planimeter tracing-arm about the trace and are, therefore, of a random nature. From a theoretical viewpoint, the sum of such random variations would be greater than the errors arising from the geometric methods, and hence the conclusions of the Panel relating to this method are valid.

Conclusions

Replication tests carried out by the Gas - Liquid Chromatography Panel have shown that the general method (peak area $\propto$ peak height $\times$ peak width at half-peak height) is the most precise of the three methods examined. However, it is emphasised that considerable care must be exercised in the construction of the base-line to each peak, and in the measurement procedure. To minimise the errors involved, the actual peak areas and the peak widths should be of reasonable magnitude. Increasing the chart speed may be one method of ensuring this condition, but this has not yet been verified.

The above conclusions apply only to the measurement of the areas of symmetrical peaks of the shape normally associated with gas - liquid chromatography. In practice, gross distortion sometimes occurs owing to adsorption effects within the system or overloading of the column or detector. The application of any single geometric method is then invalid, and the use of a planimeter may be preferred.

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The Anion-exchange Separation of Titanium, Zirconium, Niobium, Tantalum, Molybdenum and Tungsten, with Particular Reference to the Analysis of Alloys

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A detailed study has been made of the adsorption of titanium, zirconium, niobium, tantalum, molybdenum and tungsten on the anion-exchange resin, De-Acidite FF, from solutions containing mixtures of two or more of hydrofluoric acid, hydrochloric acid, ammonium fluoride and ammonium chloride, with a view to obtaining a separation scheme for these elements that could be completed within 5 hours. A completely satisfactory separation scheme was achieved, after determining the weight-distribution coefficients of these elements between aqueous solutions of the above reagents and the resin, and after investigating numerous elution curves for these elements from columns of the resin.

Titanium^{IV}, zirconium, niobium^{V}, tantalum, molybdenum^{VI} and tungsten^{VI} were retained on the resin and separated from aluminium, vanadium^{IV}, chromium^{III}, manganese^{II}, iron^{III}, cobalt^{II}, nickel and copper^{II}, by passing a solution of these elements in M hydrofluoric acid, followed by a wash solution of M hydrofluoric acid, through a column of the resin, of 100- to 200-mesh size, in the chloride form. Titanium *plus* zirconium, tungsten, niobium, molybdenum and tantalum were then quantitatively eluted from the resin with 0·01 M hydrofluoric acid - 9 M hydrochloric acid, 3 M hydrofluoric acid - 10 M hydrochloric acid, 0·2 M hydrofluoric acid - 7M hydrochloric acid, 3 M hydrofluoric acid - 3 M hydrochloric acid and M ammonium fluoride - 4 M ammonium chloride mixtures, respectively.

Good results were obtained when the scheme was applied to the separation of these elements in synthetic mixtures and alloys, in which instances spectrophotometric or volumetric methods were used for quantitatively determining the separated elements.

As a result of the determination of the distribution coefficient of metallic ions between an anion-exchange resin and hydrofluoric acid - hydrochloric acid solutions, it was concluded in a previous paper[1] that titanium^{IV}, zirconium, niobium^{V}, tantalum, molybdenum^{VI} and tungsten^{VI} would be readily separated from aluminium, vanadium^{IV}, chromium^{III}, manganese^{II}, iron^{III}, cobalt^{II}, nickel and copper^{II}, by passing these elements, dissolved in M hydrofluoric acid containing less than 0·1 M hydrochloric acid, through a column of De-Acidite FF resin in the chloride form. The first six elements would be adsorbed at the top of the resin column, while the remaining elements would be quickly eluted with M hydrofluoric acid.

The problem now was to elute these six elements successively from the column of anion-exchange resin, before quantitatively determining them. The previous study[1] had indicated that it should be possible to elute titanium^{IV} and zirconium rapidly with a M hydrofluoric acid - 6 M hydrochloric acid mixture, and then to separate the four remaining elements into niobium^{V} *plus* tungsten^{VI}, molybdenum^{VI} and tantalum (left on the column) by eluting with more M hydrofluoric acid - 6 M hydrochloric acid mixture, and then with M hydrofluoric acid - 2 M hydrochloric acid mixture. However, it was felt that a more satisfactory separation scheme would be possible after more distribution coefficients had been determined by using solutions containing ammonium fluoride and chloride besides hydrofluoric and hydrochloric acid.

This further work, complemented by elution studies on columns, which finally led to a completely satisfactory separation within five hours, is described in this Paper.

* This paper reports part of the work carried out by E. J. Dixon for his Ph.D. degree (1963).

EXPERIMENTAL

APPARATUS—

The polythene-ware, shaker, spectrophotometer and pH meter were the same as used in the previous study.[1]

POLYTHENE COLUMNS FOR ANION-EXCHANGE SEPARATIONS—

Columns were designed to permit continuous flow by gravity feed. All joints were of a tight push-fit. Fig. 1 shows the cross-sectional view of a column of dimensions 13 cm × 0·19 sq. cm. The body of the column was a piece of polythene tubing 14 cm long having in internal diameter of 0·50 cm and an external diameter of 0·70 cm. Two discs of diameter 0·60 cm were cut out from polythene sheet of thickness 0·30 cm, and were drilled with about seven holes of diameter about 0·05 cm. One disc was then forced into the bottom of the column until it was about 1·0 cm up the tube, the plane of the disc being at right-angles to the axis of the tube. A few polythene drillings were then packed fairly tightly in this gap and the second disc inserted about 0·3 cm up the tube. This arrangement was sufficient to support the column of resin. A short piece (about 3 cm) of flexible poly(vinyl chloride) tubing of internal diameter about 0·5 cm was pushed over the bottom of the column and fitted with a screw clamp.

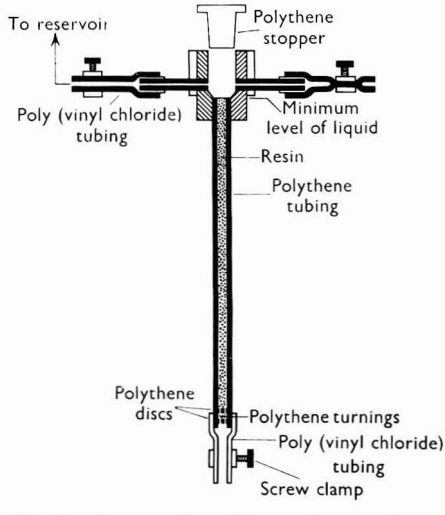

Fig. 1. Cross-section of ion-exchange column

For the fitting at the top of the column, a piece of polythene rod of diameter 1·90 cm and length 3·0 cm with a 0·635-cm diameter hole drilled along its axis was used. The upper end of the column was then forced 1·0 cm into this hole, the remainder of which was then enlarged to a diameter of 0·95 cm and to such a depth that the upper edge of the column tube was neatly chamfered. The upper end of the rod was forced into a 2·0 cm length of polythene tube of 1·90 cm bore and 0·32 cm wall. The top surface was then machined smooth. The purpose of this was to strengthen the fitting.

Six holes of diameter 0·32 cm were then drilled at angles of 60° to each other through the top of the column about 0·5 cm above the top of the column tube. The side arms were made from narrow bore (0·05 cm) polythene tubing, external diameter 0·40 cm, and were forced into each of the six holes until flush with the inside of the column top.

A length of about 2 cm was allowed to project outside the column, and to this was fitted a length of flexible poly(vinyl chloride) tubing, the other end of which was fitted to an outlet tube of polythene welded into the bottom of a 500-ml polythene bottle.

A screw-clamp was fitted near to the lower end of the poly(vinyl chloride) tubing. The head of solution normally applied to the column was about 20 cm. The ribs of a polythene

stopper that is usually fitted to a 100-ml calibrated flask were machined off; the stopper was then coated with a small amount of silicone grease and gently, but firmly, inserted into the top of the column. This formed an airtight seal at the top of the column.

ANION-EXCHANGE RESINS—

De-Acidite FF(Cl), types SRA 70 and SRA 71, were used. The former were used for all determinations of weight-distribution coefficients and early column work. The resins were both 7 to 9 per cent. cross-linked with divinylbenzene, and were identical in all respects except that SRA 70 was 52- to 100-mesh size and SRA 71 was 100- to 200-mesh size. The resin was prepared for use by introducing it into a large glass column as a slurry with water, and back-washing it to remove the finest particles. It was then washed with about four column-volumes of 5 M hydrochloric acid and then with water until the effluent was free from chloride.

For determining distribution coefficients, the resin was dried over anhydrone at 60° C, under reduced pressure.

REAGENTS—

Standard solutions of metals—Standard solutions of iron^{III}, nickel, cobalt^{II}, titanium^{IV} zirconium, niobium^{V}, tantalum, molybdenum^{VI}, and tungsten^{VI}, were prepared as in the previous study.[1]

Other reagents—Hydrofluoric, hydrochloric, nitric and sulphuric acids, and ammonium fluoride and chloride were of analytical-reagent grade.

Reagents and solutions for colorimetric determinations—These were of the purest grade available.

DETERMINATION OF WEIGHT-DISTRIBUTION COEFFICIENTS—

The weight-distribution coefficients were determined by using the same procedure as that adopted in the previous paper.[1]

TREATMENT OF SOLUTIONS BEFORE COLORIMETRIC DETERMINATION—

As hydrofluoric acid readily attacks glass, it was necessary to remove this acid from solutions before performing an analysis, except in the determination of tantalum. If it was known that the colorimetric method was not subject to interference from ions present in Pyrex glass, 1 to 2 ml of concentrated sulphuric acid were added to about 1 g (accurately weighed) of the solution to be analysed in a tall 100-ml Pyrex beaker, and the mixture evaporated until fumes of sulphur trioxide were evolved. (When solutions containing titanium or zirconium are being treated in this manner, it is advisable to pass a flame over the upper half of the beaker, to remove the last traces of hydrofluoric acid.) The solution was cooled, 2 or 3 drops of concentrated nitric acid added to it and the solution strongly boiled until fumes had been evolved for about a minute, to remove as much as possible of the excess of nitric acid. This procedure destroyed the charred products caused by the reaction between fuming sulphuric acid and traces of products from the resin that resulted when strong acids were used in the aqueous phase.

When solutions containing trace amounts of iron, titanium and niobum were analysed, the evaporations were performed in tall 50-ml silica beakers. The sulphuric acid solution was cooled and usually diluted with water, heated until any salts had dissolved and transferred to a graduated flask or analysed in bulk. Solutions of niobium were diluted with 1 per cent. tartaric acid solution, and solutions of tungsten were diluted with concentrated hydrochloric acid. In both instances, warming for a few minutes was necessary to ensure complete dissolution of the element.

This treatment of the solutions before colorimetric determinations was the same for the aqueous phase, which had to be analysed for determinations of weight-distribution coefficients, and for column eluates.

COLORIMETRIC DETERMINATION OF METALS—

Iron^{III}, cobalt^{II}, nickel, molybdenum^{VI}, and tungsten^{VI} were determined in the fluoride-free solutions by the methods outlined in the previous paper.[1] Titanium^{IV}, zirconium and niobium^{V} in fluoride-free solutions and tantalum in fluoride solution were determined by the methods given below.

DETERMINATION OF TITANIUM—

Titanium was determined by using chromotropic acid in an adaptation of the method of Brandt and Preiser.[2] Considerable care was necessary to remove all traces of fluoride from the titanium solution before analysis, because fluoride seriously interferes with the formation of the coloured complex. While fumes of sulphur trioxide are being evolved from the beaker, it is advisable to pass a flame over the upper half of the inside of the beaker to remove the last traces of hydrofluoric acid.

Transfer up to 25 μg of titanium in not more than 4 ml of solution to a 10-ml graduated flask. Add 1 ml of 0·10 per cent. w/v aqueous chromotropic acid and then 3 M sodium acetate until the pH is about 4·5 (*i.e.*, until the red-brown colour first produced is just converted to yellow-brown). Add 2·0 ml of pH 4·5 buffer solution (prepared by dissolving 13·6 g of sodium acetate trihydrate and 8·8 ml of glacial acetic acid in water, and diluting the solution to 250 ml) and dilute to the mark. Measure the optical density at 470 mμ against a reagent blank solution.

Up to 88 μg of zirconium has no effect on this method for the colorimetric determination of titanium, provided that the volume of aqueous chromotropic acid added is increased to 3 ml.

DETERMINATION OF ZIRCONIUM—

Transfer a portion of the solution, containing not more than 60 μg of zirconium, to a 25-ml calibrated flask and add 5 N sulphuric acid or N sodium hydroxide to make the free acid present equivalent to 1 ml of 5 N sulphuric acid. Add 2 ml of 0·10 per cent. w/v xylenol orange,[3] dissolved in 0·01 N sodium hydroxide, from a pipette down the side of the flask (to prevent frothing) and dilute to 25 ml with water. Measure the optical density at 540 mμ against a reagent blank solution.

Up to 10 μg of titanium has no effect on the blank value, or on the optical density due to 44 μg of zirconium.

DETERMINATION OF NIOBIUM—

Niobium was determined by using the method of Freund and Levitt.[4]

By means of pipettes add 5 ml of concentrated hydrochloric acid, 1 ml of freshly prepared 22·5 per cent. stannous chloride solution in concentrated hydrochloric acid, 2 ml of water and 5 ml of acetone to a 25-ml calibrated flask, mixing well after each addition. Add up to 5 ml of niobium solution, 1 per cent. w/v in tartaric acid and 4 per cent. v/v in sulphuric acid, and containing not more than 100 μg of niobium. Add 1 per cent. w/v aqueous tartaric acid solution, containing 4 per cent. v/v sulphuric acid, to give a total volume of 5·0 ml of this solution. Add 5 ml of a freshly prepared 29 per cent. aqueous potassium thiocyanate solution. Dilute to the mark with water and measure the optical density at 385 mμ against a reagent blank solution.

DETERMINATION OF TANTALUM—

In the previous study,[1] tantalum was determined spectrophotometrically at 365 mμ as a complex with pyrogallol,[5] but the sensitivity of the reagent was rather low, and a search was made for a better one. An adaptation of the method of Lauer and Poluektov,[6] in which methyl violet was used, was finally used for determining the distribution coefficients and elution curves.

To a 30-ml polythene bottle, add x ($\leqslant$3·0) g of tantalum solution M in ammonium fluoride, 0·5 x ml of 2 N hydrochloric acid, (3 — 0·5 x) ml of water, (3 — x) ml of M hydrofluoric acid, 2·0 ml of 0·2 per cent. aqueous crystal violet solution and 2·0 ml of pH 2·0 glycine buffer (prepared by dissolving 18·8 g of glycine and 10·7 ml of 11·7 M hydrochloric acid in water and diluting the solution to 250 ml). Add 5 ml of analytical-reagent grade benzene, and shake for two minutes. Set aside for 25 minutes, decant as much as possible of the benzene layer into a 10-ml polythene centrifuge tube, and spin it for about 1 minute. Decant the benzene layer into a 1-cm cell and measure the optical density at 605 mμ against water. Subtract the value of the reagent blank solution.

The optical-density value of 5 μg of tantalum in a 1-cm cell was 0·56.

Although this method was reasonably satisfactory for determining distribution coefficients and elution curves, where errors of up to 20 per cent. can be tolerated, it had its disadvantages for strictly quantitative determinations, since the optical density of the organic phase decreased almost linearly with increasing ammonium chloride concentration in the aqueous

phase, which could contain varying amounts of ammonium chloride. It was found that tantalum could be eluted satisfactorily from a column of anion-exchange resin by using only M ammonium fluoride - 4 M ammonium chloride mixture. Quantitative determinations of tantalum were therefore required in solutions of composition approximately M ammonium fluoride - 4 M ammonium chloride. The method given below was therefore used for quantitative determinations.

Add M ammonium fluoride, 2 M hydrochloric acid, 4 M ammonium chloride and water to a portion of the tantalum solution, so that the total volume is 8 ml and the concentrations of the constituents are equivalent to 3 ml of 4 M ammonium chloride, 3 ml of M ammonium fluoride, 0·25 ml of 2 M hydrochloric acid and 1·75 ml of water. Then add 2 ml of 0·2 per cent. aqueous crystal violet solution, extract the tantalum complex with 5 ml of benzene, and measure the optical density at 605 mμ after 30 minutes.

The optical-density value of 5 μg of tantalum in a 1-cm cell was about 0·38.

The results obtained by using this method were reasonable, giving a precision of about $\pm$0·02 optical density units, which is about $\pm$5 per cent. for 5 μg of tantalum. However, the method has its limitations, since the colour of the solution was still fading slowly even after 30 minutes.

Further work is required on this method before it becomes generally acceptable.

Theory

It is possible to predict quite accurately the volume of eluant needed for obtaining the maximum concentration of an eluted element in the effluent, from a knowledge of its weight-distribution coefficient in that eluant. This is given by the equation below, for low column loadings[1]—

$$v_{max.} = \rho D + i \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where $v_{max.}$ is the volume, in bed-volumes, eluted when the maximum concentration of element is observed in the effluent,

ρ is the density of the resin bed in g per ml,

D is the weight-distribution coefficient of the element between the resin and the solution and

i, in bed-volumes, is the volume of solution in the column, *i.e.*, the volume of solution in, and between, the resin beads and particles, and above and below the resin bed.

The value of D is found by batch-equilibration experiments and is defined by the expression—

$$D = \frac{\text{amount of element on resin per gram of resin}}{\text{amount of element in solution per millilitre of solution}}$$

If w is the weight, in g, of dried resin in the column, v_1 is the volume, in ml, eluted when the maximum concentration of element is observed in the effluent, i_1 is the volume, in ml, of solution in the column and v_b is the volume, in ml, of the resin bed, then—

$$v_{max.} = \frac{v_1}{v_b},\ \rho = \frac{w}{v_b} \text{ and } i = \frac{i_1}{v_b}$$

Therefore equation (1) may be rewritten as—

$$v_1 = wD + i_1$$

In all work with columns, the weight of dried resin used for the resin bed was 1 g, and i_1 was approximately 2 ml. Hence v_1 was related to D by the simple equation—

$$v_1 = D + 2$$

D ml of solution must therefore be passed through the column to take an element, whose weight-distribution coefficient in the solution is D, from the top to the bottom of the column. If v ml of this solution is passed down the column ($v < D$), then $\frac{v}{D}$ is the fraction of the column, down which the maximum concentration of the element has travelled. If this fraction is calculated for a metal for each step of an elution sequence, the position of the maximum concentration of element on the column, *i.e.*, the peak of the distribution of element in the band, may be found by adding the fractions. The sum of the fractions may

be represented as $\Sigma\frac{v}{D}$. For an element, which is to be retained by the column, $\Sigma\frac{v}{D}$ must be considerably less than unity.

Results

Distribution coefficients for molybdenum, tungsten, niobium and tantalum between De-Acidite FF and hydrochluoric acid - ammonium chloride solutions—

Kraus, Nelson and Moore[7] used M hydrofluoric acid - 4 M ammonium chloride mixture for eluting niobium, and M ammonium fluoride - 4 M ammonium chloride mixture for eluting tantalum. However, they gave no indication of the behaviour of molybdenum and tungsten with such solutions. To see if solutions of these types would be of any value in separating these four elements, distribution coefficients were obtained for these metals in such solutions.

Fig. 2 shows the graphs of the distribution coefficients for these elements between De-Acidite FF and M hydrofluoric acid - 0 to 4 M ammonium chloride mixtures. A comparison of the results of the previous study[1] and Fig. 2 shows that the fluoride complexes of the four elements are displaced from the resin by increasing the chloride-ion concentration, but the formation of the chloro-complexes, which are held by the resin, is suppressed when the hydrogen-ion concentration is small. The tungsten and niobium curves are again similar, and although these ammonium chloride solutions are useful for removing these elements rapidly from the column, they are of no value for separating molybdenum, niobium and tungsten from each other.

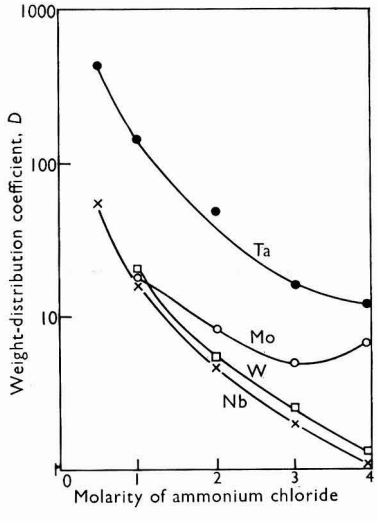

Fig. 2. Weight-distribution curves for tantalum, molybdenum, tungsten and niobium between De-Acidite FF resin and solutions. M in hydrofluoric acid, containing different concentrations of ammonium chloride

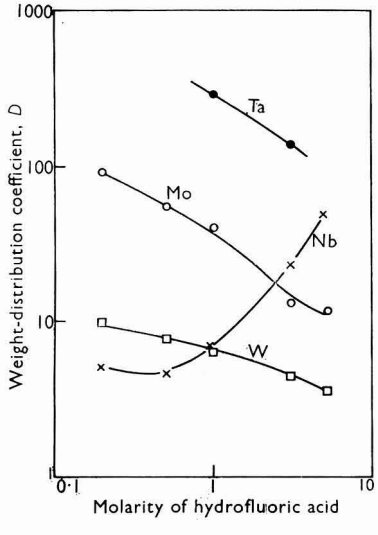

Fig. 3. Weight-distribution curves for tantalum, molybdenum, niobium and tungsten between De-Acidite FF resin and solutions, 6 M in hydrochloric acid, containing different concentrations of hydrofluoric acid

Even in M hydrofluoric acid - 4 M ammonium chloride mixture, tantalum is too strongly held by the resin for rapid elution. Distribution coefficients for tantalum were therefore determined in M hydrofluoric acid - 4 M ammonium chloride mixture, where the acidity was reduced still further by partly neutralising the hydrofluoric acid with ammonia solution. When the concentration of ammonium fluoride was 0·7 M, D for tantalum was 10, and this was reduced to 4 when the concentration of ammonium fluoride was increased to 0·9 M. These results confirm that M ammonium fluoride - 4 M ammonium chloride mixture, for which D is approximately 2, is the best eluting agent for tantalum. Weight-distribution coefficients for molybdenum, niobium and tungsten in this medium were all found to be less than 1.

DISTRIBUTION COEFFICIENTS FOR MOLYBDENUM, TUNGSTEN, NIOBIUM AND TANTALUM BETWEEN DE-ACIDITE FF AND SOLUTIONS OF 6 M HYDROCHLORIC ACID CONTAINING 0·2 TO 5·0 M HYDROFLUORIC ACID—

Kraus and Moore[8] have shown that, in 9 M hydrochloric acid, the adsorption of niobium on Dowex 1 resin varies widely over the range 0·01 to 7 M hydrofluoric acid. The adsorption was at a minimum at about 0·18 M. Tantalum was strongly adsorbed over the whole range, while zirconium was not adsorbed at all. However, they have published no corresponding values for molybdenum and tungsten. As De-Acidite FF is slowly attacked by high concentrations of hydrochloric acid, it was felt desirable to keep the acidity for determinations on the resin as low as possible. Fig. 3, therefore, shows graphs of weight-distribution coefficients for molybdenum, tungsten, niobium and tantalum between De-Acidite FF and solutions in 6 M hydrochloric acid (rather than in 9 M acid), containing 0·2 to 5 M hydrofluoric acid. At higher concentrations of hydrofluoric acid, the values of D for tungsten and niobium diverge widely. However, at the same time, the values of molybdenum become closer to those of tungsten. Neither titanium nor zirconium are adsorbed at all.

A study of the results in the previous paper[1] and Figs. 2 and 3 indicates a basic scheme for the separation of an alloy solution in M hydrofluoric acid. Initially iron, etc., are eluted with M hydrofluoric acid, then titanium *plus* zirconium with, say, 2 M hydrofluoric acid - 6 M hydrochloric acid mixture. Tungsten is eluted with a similar solution, and then niobium with 0·5 M hydrofluoric acid - 6 M hydrochloric acid mixture, and finally molybdenum and tantalum are eluted.

ELUTION STUDIES ON COLUMNS—

Preparation of a column for a separation—The column was filled with resin in the usual way. It was washed with 10 ml of M hydrofluoric acid and then with 10 ml of M ammonium fluoride - 4 M ammonium chloride mixture, to remove any anions that may have been adsorbed on to the resin. The column was then washed with 10 ml of 6 M hydrochloric acid, to ensure that the resin was entirely in the chloride form, and finally with 10 ml of M hydrofluoric acid to remove the hydrochloric acid. The solution was allowed to pass through the column until the level of the liquid was just above the resin.

All column work was done in an efficient fume cupboard.

Addition of metal solutions to the column—The solution of metal ions was transferred to the top of the column (see Fig. 1) in small portions and allowed to run down to the level of the resin. The top of the column was washed three times with about 1-ml portions of M hydrofluoric acid. When the last portion of wash solution was just below the level of the inlet tubes, the polythene stopper, lightly smeared with silicone grease, was slowly inserted into the top of the column. Care was taken to adjust the rate of this insertion to prevent air from being forced into any of the inlet tubes. As soon as the stopper was firmly in position, the clamp on the tube leading from the bottle containing M hydrofluoric acid was opened. The flow-rate could be adjusted, when necessary, by means of the clamp at the bottom of the column.

Determination of elution curves—About 0·5 to 1·0 g of the metal solution in dilute hydrofluoric acid was added to the column, which was then treated, where applicable, with previously determined quantities of earlier eluants and then with the solution for which the elution curve of the metal was to be determined. This effluent was collected in fractions of about 2 g and each was analysed for the metal. The concentration was determined in μg of metal per g of solution, and plotted against the weight of effluent collected, the mid-point of the particular fraction being taken for this purpose.

Elution curve for iron^{III} with M hydrofluoric acid—As iron is the major constituent of many alloys, it was felt that a large amount of iron should be used for the elution curve as some tailing could be expected with large amounts. An elution curve was obtained for 0·6 g of a solution containing 22 mg of iron, when M hydrofluoric acid was used as eluant. The column dimensions were 13 cm $\times$ 0·19 sq. cm, and it contained 1·0 g of De-Acidite FF (SRA 70). The elution curve is shown in Fig. 4 A.

The graph shows that 12 g of M hydrofluoric acid was necessary for the quantitative removal of iron from the column. If the weight of metal solution added to the column exceeds 1·0 g, then a corresponding increase in the weight of M hydrofluoric acid should be taken to ensure the quantitative recovery of iron. The first step in all subsequent elutions was to pass 12 g of M hydrofluoric acid through the column to remove iron, etc.

Elution curves for titanium and tungsten—Hague, Brown and Bright[9] have separated titanium from tungsten by using 2·5 M hydrofluoric acid - 6 M hydrochloric acid mixture, an anion-exchange resin of 200- to 400-mesh size and a flow-rate of approximately 0·08 ml per 0·19 sq. cm per minute. A similar separation was tried with our column, but it was considered unlikely that a complete separation would be achieved if the coarser resin and a faster flow-rate were used. The titanium was eluted with 12·8 g of M hydrofluoric acid, and then with 2 M hydrofluoric acid - 6 M hydrochloric acid mixture: tungsten is slightly more strongly adsorbed in this solution than in 2·5 M hydrofluoric acid - 6 M hydrochloric acid mixture. The elution curve is shown in Fig. 4 A, and the recovery of the titanium was quantitative in 10 g of this eluate. No titanium was found in the first 2·4 g of the 2 M hydrofluoric acid - 6 M hydrochloric acid effluent. Ten grams of 2 M hydrofluoric acid - 6 M hydrochloric acid were therefore considered necessary and sufficient for the quantitative recovery of titanium.

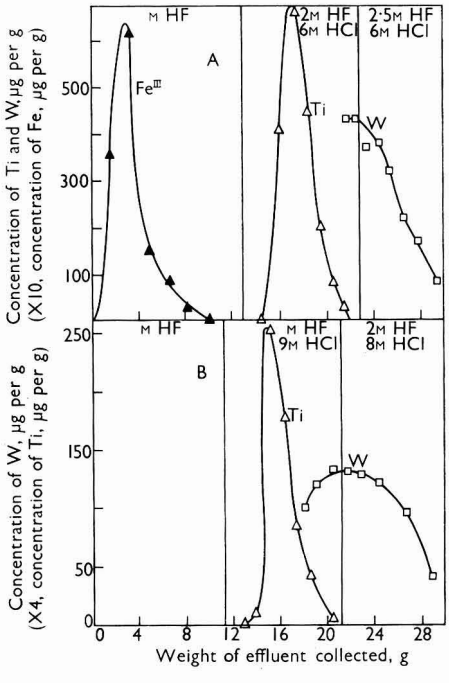

Fig. 4. A, elution curves for iron, titanium and tungsten; eluants as shown. (Average flow-rate, 0·45 g per minute): B, elution curves for titanium and tungsten; eluants as shown. (Average flow-rate, 0·40 g per minute)

The tungsten was eluted with 12 g of M hydrofluoric acid, 10 g of 2 M hydrofluoric acid - 6 M hydrochloric acid mixture, and then with 2·5 M hydrofluoric acid - 6 M hydrochloric acid mixture. Two fractions at the end of the 2 M hydrofluoric acid - 6 M hydrochloric acid effluent were analysed for tungsten, besides the fractions in the 2·5 M hydrofluoric acid - 6 M hydrochloric acid effluent. Part of the elution curve is shown in Fig. 4 A. As was expected, the overlap of the titanium and tungsten was considerable. In fact, 54 per cent. of the tungsten was eluted with the titanium eluant.

It was clear that the 2 M hydrofluoric acid - 6 M hydrochloric acid mixture was not a satisfactory eluant for titanium, since it also slowly removed tungsten from the column. A solution had to be found, in which tungsten was more strongly held by the resin, and from the previous study[1] and Fig. 3, M hydrofluoric acid - 9 M hydrochloric acid mixture

appeared to be the best, since tungsten, niobium, molybdenum and tantalum were all held quite strongly by the resin in the presence of this aqueous phase. Fig. 4B shows the elution curves for titanium and tungsten with M hydrofluoric acid - 9 M hydrochloric acid and 2 M hydrofluoric acid - 8 M hydrochloric acid mixture, respectively. The 2 M hydrofluoric acid - 8 M hydrochloric acid mixture was preferred to the 2·5 M hydrofluoric acid - 6 M hydrochloric acid mixture, since niobium and molybdenum would be more strongly adsorbed from the former solution. The two elution curves were obtained from separate experiments. Again considerable overlap of the curves occurred, only 55 per cent. of the tungsten being recovered in the 2 M hydrofluoric acid - 8 M hydrochloric acid effluent. The titanium recovery was quantitative in 10 g, as before.

Table I shows that the elution maxima for tungsten obtained in Figs. 4 A and B agree well with the values calculated by using the equation—

$$v_1 = D + 2.$$

TABLE I

COMPARISON OF PRACTICAL AND THEORETICAL ELUTION MAXIMA FOR TUNGSTEN

Eluant	$D + 2$	Elution maximum, ml
2 M hydrofluoric acid - 6 M hydrochloric acid mixture ..	7	7 to 8
M hydrofluoric acid - 9 M hydrochloric acid mixture ..	10	10

As 10 to 12 g of eluant were required to remove completely an element whose value of D was about unity, it was necessary that any element that was to remain on the column, should have a value of D greater than 20 and, probably, considerably more than 20. The results in the previous study[1] and those given in Fig. 3 indicated no conditions under which the value of D for tungsten is greater than 20, while, at the same time, those for titanium and zirconium are about unity. More work on distribution coefficients was therefore necessary.

DISTRIBUTION COEFFICIENTS FOR MOLYBDENUM, TUNGSTEN, NIOBIUM AND TANTALUM BETWEEN SOLUTIONS OF 9 M HYDROCHLORIC ACID CONTAINING 0·01 TO 3·0 M HYDROFLUORIC ACID AND DE-ACIDITE FF—

The weight-distribution coefficients for molybdenum, tungsten, niobium and tantalum are plotted *versus* the logarithm of the variable hydrofluoric acid concentration in Fig. 5. Neither titanium nor zirconium were adsorbed by the resin from any of the solutions indicated. Both molybdenum and tantalum are strongly adsorbed over the whole range. In 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture, molybdenum, tungsten, niobium and tantalum are all strongly adsorbed by the resin, and so this was the mixture chosen for eluting titanium and zirconium.

FURTHER ELUTION CURVES—

Fig. 6 A shows the elution curves for zirconium, tungsten and niobium when these ions were eluted successively with M hydrofluoric acid, 0·01 M hydrofluoric acid - 9 M hydrochloric acid, 2 M hydrofluoric acid - 8 M hydrochloric acid and 0·5 M hydrofluoric acid - 6 M hydrochloric acid mixtures. The recovery of zirconium was complete in 10 g of effluent, and tungsten was quantitatively recovered in 18 g of 2 M hydrofluoric acid - 8 M hydrochloric acid mixture. However, the recovery of niobium with 0·5 M hydrofluoric acid - 6 M hydrochloric acid mixture was only 81 per cent., the remainder having been eluted with the 2 M hydrofluoric acid - 8 M hydrochloric acid mixture. 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture was a satisfactory eluant for titanium and zirconium, and 12 g were collected in subsequent elutions.

Selection of suitable eluants for tungsten and niobium—A study of Fig. 5 shows that two possibilities exist for separating niobium and tungsten from each other, *viz.*, either (*a*) eluting tungsten with 2 or 3 M hydrofluoric acid - 9 M hydrochloric acid mixture, and then eluting niobium with 0·2 M hydrofluoric acid - 9 M hydrochloric acid or 0·5 M hydrofluoric acid 6 M hydrochloric acid mixtures, or (*b*) eluting niobium with 0·1 or 0·2 M hydrofluoric acid - 9 M hydrochloric acid mixture and then eluting tungsten with 2 or 3 M hydrofluoric acid - 9 M hydrochloric acid mixture.

Table II summarises the differences in the values of D for niobium and tungsten in these media.

TABLE II

COMPARISON OF DISTRIBUTION COEFFICIENTS FOR NIOBIUM AND TUNGSTEN IN VARIOUS MEDIA

Solution mixture	D for tungsten	D for niobium
0·1 M hydrofluoric acid - 9 M hydrochloric acid ..	45	7
0·2 M hydrofluoric acid - 9 M hydrochloric acid ..	28	3·5
2·0 M hydrofluoric acid - 9 M hydrochloric acid ..	5	28
3·0 M hydrofluoric acid - 9 M hydrochloric acid ..	4	50

At first sight, it would seem best to elute tungsten first with 3 M hydrofluoric acid - 9 M hydrochloric acid mixture, since the difference between the D values of niobium and tungsten is greatest in this medium. However, it is clear that when the eluant is changed from 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture to 3 M hydrofluoric acid - 9 M hydrochloric acid mixture, the concentration of a small portion of solution, where these two eluants mix, will have the approximate composition of a 0·2 M hydrofluoric acid - 9 M hydrochloric

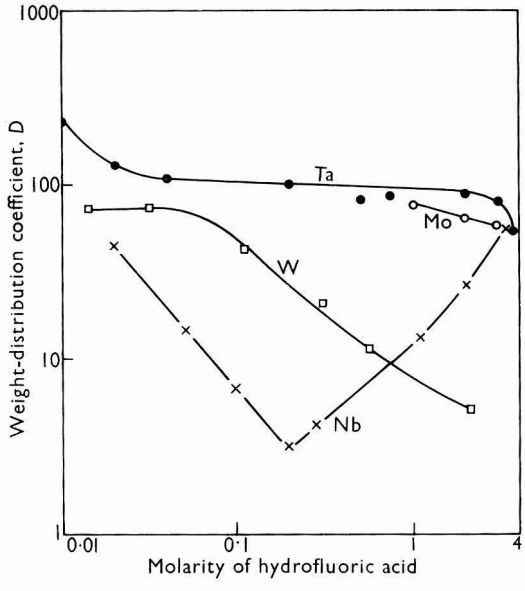

Fig. 5. Weight-distribution curves for tantalum, molybdenum, tungsten and niobium between De-Acidite FF resin and solutions, 9 M in hydrochloric acid, containing different concentrations of hydrofluoric acid

acid mixture, from which niobium is only slightly adsorbed. Further, when the eluant is changed from M hydrofluoric acid to 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture, a similar situation arises. Although it would be preferable to avoid passing through such adsorption minima, that caused by the second change of eluant is inevitable. The adsorption minimum caused by the first change of eluant can be avoided if niobium is eluted before tungsten, but in this instance the separation factor is worse. Both tungsten and molybdenum pass through a minimum when the eluant is changed from M hydrofluoric acid to 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture, but the effect is less pronounced for tungsten and considerably less pronounced for molybdenum.

To increase the separation factor for niobium, it was decided to reduce the flow-rate during the addition of the sample solution to the column and during the passage of one

column - volume (about 2 ml) after changing the eluant, when this change of eluant meant that a point of minimum adsorption for niobium, or any other element, was about to occur.

(*i*) *Elution of tungsten before niobium*—Fig. 6 B shows the elution curve for niobium when it was eluted successively with M hydrofluoric acid, 0·01 M hydrofluoric acid - 9 M hydro-

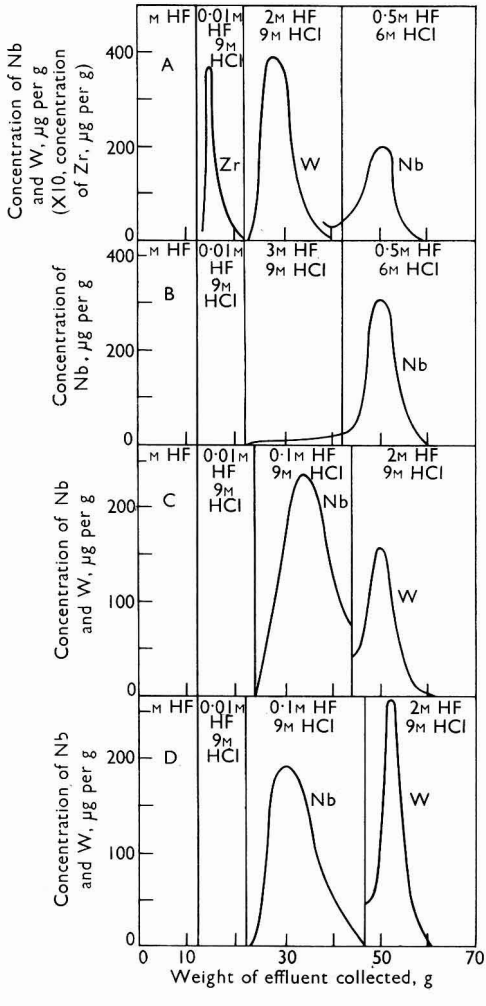

Fig. 6. A, elution curves for zirconium, tungsten and niobium; eluants as shown. (Average flow-rate, 0·40 g per minute): B, elution curves for niobium; eluants as shown. (Average flow-rate, 0·45 g per minute): C, elution curves for niobium and tungsten; eluants as shown. (Average flow-rate, 0·45 g per minute): D, elution curves for niobium and tungsten; eluants as shown. (Average flow-rate, 0·35 g per minute.)

Column dimensions: A, B and C, 13 cm × 0·19 sq. cm; D, 21 cm × 0·11 sq. cm

chloric acid, 3 M hydrofluoric acid - 9 M hydrochloric acid and 0·5 M hydrofluoric acid - 6 M hydrochloric acid mixtures. The flow-rate during the addition of the sample to the column and immediately after the first two changes of eluants was reduced to about 3 drops per minute (0·2 g per minute) from the normal flow-rate of 6 drops per minute.

The 0·5 M hydrofluoric acid - 6 M hydrochloric acid effluent contained 92 per cent. of the niobium, the remainder being found in the 3 M hydrofluoric acid - 9 M hydrochloric acid effluent. It would seem that some of the niobium had been carried near to the bottom of the column as its adsorption passed through these two minima, and was being progressively washed off with the 3 M hydrofluoric acid - 9 M hydrochloric acid eluant.

(*ii*) *Elution of niobium before tungsten*—Fig. 6 C shows the elution curves for niobium with 0·1 M hydrofluoric acid - 9 M hydrochloric acid mixture, and for tungsten with 2 M hydrofluoric acid - 9 M hydrochloric acid mixture, when a solution containing both niobium and tungsten was separated on a column of De-Acidite FF under conditions similar to those used to obtain the results given in Fig. 6 B. The niobium band was rather broader than expected, and 6 per cent. of the niobium had still not been eluted after 20 g of 0·1 M hydrofluoric acid - 9 M hydrochloric acid mixture had been passed through the column. Thirty per cent. of the tungsten was eluted before the change to 2 M hydrofluoric acid - 9 M hydrochloric acid eluant.

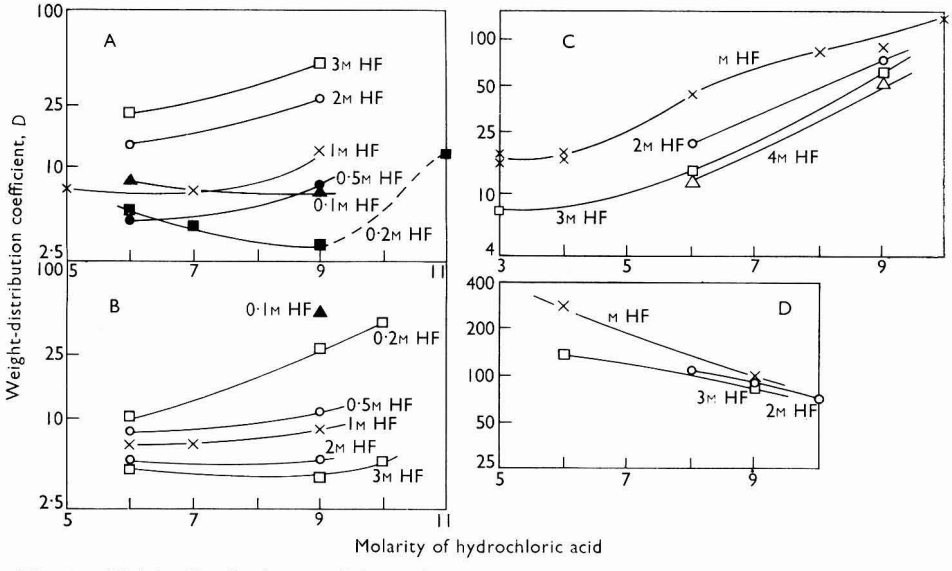

Fig. 7. Weight-distribution coefficients for: A, niobium; B, tungsten; C, molybdenum; and D, tantalum between De-Acidite FF resin and solutions containing fixed concentrations of hydrofluoric acid and different concentrations of hydrochloric acid

(*iii*) *Elution of niobium before tungsten on a longer column*—The columns used for all the above work were operated at a flow-rate of up to 6 drops per minute. Since the flow-rate could be increased to about 10 drops per minute when the tap at the foot of the column was fully open, it was obvious that a longer column giving a maximum flow-rate of 6 drops per minute could readily be constructed. Such a column, which should lead to an improved separation,[10] was constructed from polythene tube of internal diameter 3·5 mm and had dimensions of 21 cm × 0·11 sq. cm. In all other respects this column was identical to the columns used previously. The column also contained 1 g of dried resin and gave a flow-rate of 6 drops per minute under the usual head of about 20 cm of solution.

Fig. 6 D shows the elution curves for niobium and tungsten under conditions used to obtain Fig. 6 C, except that the longer column was used and 25 g of effluent were collected for the niobium fraction. The niobium recovery was almost quantitative (about 99 per cent.) and 80 per cent. recovery of tungsten was a considerable improvement on the results shown in Fig. 6 C.

At this stage, it was fairly certain that, if the separation of tungsten before niobium was carried out on the longer column, the recovery of niobium in the 0·5 M hydrofluoric acid - 6 M hydrochloric acid effluent (see Fig. 6 B) would be more quantitative. However, it was

decided to examine in detail all the information that had been obtained on distribution coefficients to see if better eluants for quantitatively separating niobium before tungsten could be found, since by using this sequence of elutions, the adsorption of niobium passes through only one minimum, but when tungsten is eluted, the niobium passes through two adsorption minima.

Discussion of distribution coefficients

Although Figs. 3 and 5 show how the hydrofluoric acid concentration affects the distribution coefficients of molybdenum, tungsten, niobium and tantalum, it is not easy to see from these graphs how the hydrochloric acid concentration affects the values of D. The values of D obtained from the previous study[1] and Figs. 3 and 5 are re-plotted together with some extra results, in Figs. 7 A, B, C and D, *versus* the hydrochloric acid concentration, each line having a constant hydrofluoric acid concentration. It was seldom easy to make up metal solutions more concentrated than 10 M in hydrochloric acid, and when the molarity of hydrofluoric acid exceeded 2, it was not easy to make the hydrochloric acid concentration exceed 9 M.

The retention of tungsten in the separation shown in Fig. 6 D would be improved by using 0·2 M hydrofluoric acid - 10 or 11 M hydrochloric acid mixture for eluting niobium. The values of D for niobium in Fig. 7 A tend to increase at the higher hydrochloric acid concentrations. Further, tantalum is less strongly held as the hydrochloric acid concentration increases, although molybdenum is more strongly adsorbed.

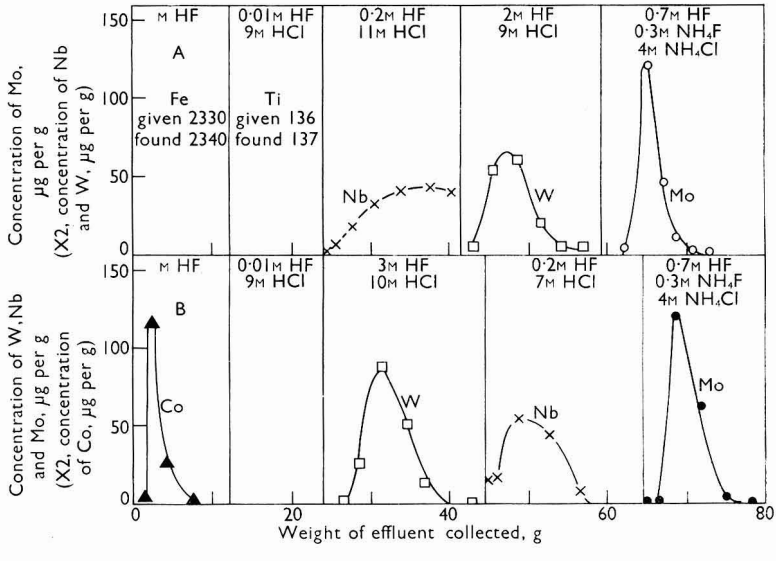

Fig. 8. A, recoveries for iron and titanium, and elution curves for niobium, tungsten and molybdenum; eluants as shown. (Average flow-rates, 0·48 g per minute for molybdenum and 0·25 g per minute for the rest): B, elution curves for cobalt, tungsten, niobium and molybdenum; eluants as shown. (Average flow-rates, 0·30 g per minute for cobalt and tungsten, 0·36 g per minute for niobium and 0·56 g per minute for molybdenum)

Fig. 8 A shows the recoveries of iron and titanium, and the elution curves for niobium, tungsten and molybdenum, when a solution containing these five elements was separated with M hydrofluoric acid, and 0·01 M hydrofluoric acid - 9 M hydrochloric acid, 18 g of 0·2 M hydrofluoric acid - 11 M hydrochloric acid, 18 g of 2 M hydrofluoric acid - 9 M hydrochloric acid and 0·7 M hydrofluoric acid - 0·3 M ammonium fluoride - 4 M ammonium chloride mixtures, respectively. The recoveries of iron, titanium, tungsten and molybdenum were all quantitative, but only 74 per cent. of the niobium was recovered in the 0·2 M hydrofluoric

acid - 11 M hydrochloric acid fraction. The recovery of molybdenum was completely satisfactory, since no trace of molybdenum could be detected in the first 1·0 g of effluent after the eluant had been changed to the 0·7 M hydrofluoric acid - 0·3 M ammonium fluoride - 4 M ammonium chloride mixture. The elution curve for niobium indicates that at least 27 and possibly 30 g of 0·2 M hydrofluoric acid - 11 M hydrochloric acid mixture are necessary for complete elution of the niobium. This would almost certainly involve considerable early elution of tungsten and excessive movement of tantalum on the column, and so 0·2 M hydrofluoric acid - 11 M hydrochloric acid mixture was considered unsuitable as an eluant for niobium.

Table III gives a comparison of the three eluants used so far for niobium, together with two possible alternatives.

TABLE III

COMPARISON OF VARIOUS ELUTING SOLUTIONS FOR NIOBIUM

Figure	6C	6D	8A	—	—
Molarity of hydrofluoric acid	0·1	0·1	0·2	0·2	0·2
Molarity of hydrochloric acid	9	9	11	11	10
Volume of effluent, v	17	21·5	15	22·5	17
D for niobium	7	7	11	11	5
D for tungsten, D_W	45	45	55	55	40
$\Sigma(v/D_W)$	0·38	0·48	0·27	0·41	0·43
Niobium eluant v/D_W	0·65	0·75	0·54	0·68	0·70
Niobium found, per cent.	94	99	74	—	—
Tungsten found, per cent.	70	80	100	—	—
Height of column, cm	13	21	21	21	21

The values of $\Sigma(v/D_W)$ for 27 g (22·5 ml) of 0·2 M hydrofluoric acid - 11 M hydrochloric acid mixture, and 20 g (17 ml) of 0·2 M hydrofluoric acid - 10 M hydrochloric acid mixture that would be necessary for quantitatively eluting niobium are only slightly lower than the value for 25 g (21·5 ml) of 0·1 M hydrofluoric acid - 9 M hydrochloric acid mixture, in which the recovery of tungsten was 80 per cent. It was considered that the slightly improved retention of the tungsten was not sufficient for a quantitative separation.

Attention was now returned to the alternative of eluting tungsten before niobium.

A study of the distribution coefficients of tungsten, niobium, molybdenum and tantalum for various possible eluants for tungsten, the results of which are given in Table IV, shows that the best solution for eluting tungsten before niobium is 3 M hydrofluoric acid - 10 M hydrochloric acid mixture. (Some of the values in this Table are approximate extrapolations from Figs. 7 A, B, C and D.)

TABLE IV

COMPARISON OF THE DISTRIBUTION COEFFICIENTS FOR TUNGSTEN, NIOBIUM, MOLYBDENUM AND TANTALUM

Eluant for tungsten	Distribution coefficients for—			
	tungsten	niobium	molybdenum	tantalum
2 M hydrofluoric acid - 9 M hydrochloric acid	5	28	70	90
3 M hydrofluoric acid - 9 M hydrochloric acid	4	50	57	83
4 M hydrofluoric acid - 9 M hydrochloric acid	4	80	51	60
3 M hydrofluoric acid - 10 M hydrochloric acid	5	60	90	70
4 M hydrofluoric acid - 10 M hydrochloric acid	5	80	80	50

A similar study of Table V shows that 0·2 M hydrofluoric acid - 7 M hydrochloric acid mixture is the most suitable eluant for niobium.

A solution containing cobalt, tungsten, niobium and molybdenum was separated on a 21-cm column of De-Acidite FF by using the solutions listed below for eluting the different fractions: 12 g of M hydrofluoric acid for cobalt, 12 g of 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture for titanium and zirconium, 20 g of 3 M hydrofluoric acid - 10 M hydrochloric acid mixture for tungsten, 20 g of 0·2 M hydrofluoric acid - 7 M hydrochloric acid mixture for niobium and 16 g of 0·7 M hydrofluoric acid - 0·3 M ammonium fluoride - 4 M ammonium chloride mixture for molybdenum. The elution curves for cobalt, tungsten, niobium and molybdenum are shown in Fig. 8 B. The recoveries of cobalt, tungsten and

molybdenum were quantitative, and the early elution of niobium was reduced to about 6 per cent. Since the 3 M hydrofluoric acid - 10 M hydrochloric acid mixture is the optimum eluant for tungsten and the loss of niobium was small, it was thought that if 100- to 200-mesh resin was used in place of 52- to 100-mesh resin, the separation would be made quantitative.

TABLE V

COMPARISON OF THE DISTRIBUTION COEFFICIENTS FOR NIOBIUM, MOLYBDENUM AND TANTALUM

Eluant for niobium	Distribution coefficients for—		
	niobium	molybdenum	tantalum
0·2 M hydrofluoric acid - 6 M hydrochloric acid ..	5	90	400
0·5 M hydrofluoric acid - 6 M hydrochloric acid ..	5	55	300
0·2 M hydrofluoric acid - 7 M hydrochloric acid ..	4	95	250
0·5 M hydrofluoric acid - 7 M hydrochloric acid ..	5	70	200
0·2 M hydrofluoric acid - 9 M hydrochloric acid ..	3	100	100

None of the ammonium chloride solutions used for obtaining the results shown in Fig. 2 gave a sufficient difference in D values between tantalum and molybdenum to permit their separation to be made by using reasonable flow-rates. However, a study of the results of the previous paper[1] and Figs. 7 C and D indicates that 3 M hydrofluoric acid - 3 M hydrochloric acid mixture is the best eluant for molybdenum; D for molybdenum is about 6, whereas that for tantalum is about 200.

SEPARATIONS IN WHICH 100- TO 200-MESH RESIN WAS USED IN THE SHORT COLUMNS—

An average flow-rate in our resin columns of less than 6 drops per minute was undesirable, since it would mean that a separation and subsequent determination of six elements would not be achieved in a working day. Under a 20-cm head of solution, a flow-rate of 6 drops per minute was not possible with the 21-cm column filled with 100- to 200-mesh resin. However, if the 13-cm column packed with 100- to 200-mesh resin was used, an average flow-rate of 6 drops per minute was achieved under a 20-cm head, with the screw clamp on the tube at the bottom of the column fully open.

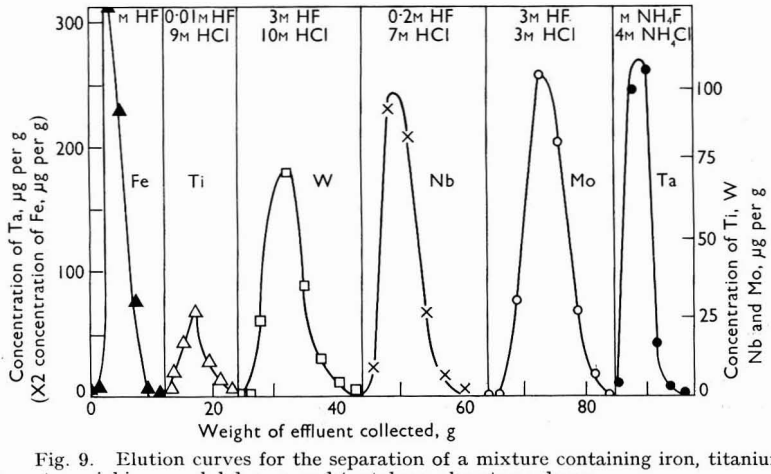

Fig. 9. Elution curves for the separation of a mixture containing iron, titanium, tungsten, niobium, molybdenum and tantalum; eluants as shown

A solution containing iron^{III}, titanium, tungsten, niobium, molybdenum and tantalum in dilute hydrofluoric acid was separated on the short column containing 100- to 200-mesh De-Acidite FF into six separate effluents, by successively using 12 g of M hydrofluoric acid, and 12 g of 0·01 M hydrofluoric acid - 9 M hydrochloric acid, 20 g of 3 m hydrofluoric acid - 10 M hydrochloric acid, 20 g of 0·2 M hydrofluoric acid - 7 M hydrochloric acid, 20 g of 3 M hydrofluoric acid - 3 M hydrochloric acid and 17 g of ammonium fluoride - 4 M ammonium

chloride mixtures. Each effluent was collected in 6 to 8 fractions, and the elution curves obtained for each of the six metals are shown in Fig. 9. The recoveries of all six elements were quantitative, and no early elution of any element could be detected. The molybdenum curve was rather broader than those for tungsten and niobium, but 20 g of 3 M hydrofluoric acid - 3 M hydrochloric acid mixture was sufficient for the full recovery of molybdenum. Thirteen grams of M ammonium fluoride - 4 M ammonium chloride mixture was sufficient to remove all the tantalum.

The recoveries of the six metals, together with the average flow-rates and times taken for the various elutions, are shown in Table VI.

TABLE VI

RECOVERIES OF IRON, TITANIUM, TUNGSTEN, NIOBIUM, MOLYBDENUM AND TANTALUM AFTER ANION-EXCHANGE SEPARATION

	Iron	Titanium	Tungsten	Niobium	Molybdenum	Tantalum
Average flow-rate, g per minute ..	0·21	0·29	0·33	0·38	0·50	0·65
Time for fraction, minutes ..	55	41	60	53	40	26
Metal added, μg	2161	124	442	630	819	1132
Metal found, μg	2225	126	462	632	788	1100

The satisfactory recoveries of molybdenum and tantalum, and the symmetrical shape of their elution curves justified the use of the higher flow-rates.

The total time taken for the separation was 4½ hours and this was considered to be satisfactory.

CONCLUSIONS ON ELUTION STUDIES

It can now be concluded from all of the elution studies described above that, for the column conditions used by us, the *D* values for elements to be retained on the resin column, must be as high as possible and certainly greater than 50. The *D* value for an element that is to be removed from the resin colmun should be as low as possible, and be preferably less than 5.

As the *D* value for an element to be removed increases, the elution curve becomes broader. For examples of this, see Fig. 6 D, where *D* for niobium is 7, Fig. 8 A where *D* for molybdenum is 2 and *D* for tungsten is 5, but *D* for niobium is 12, and Fig. 9 where the *D* values of iron and titanium are less than or equal to 1, but *D* for molybdenum is 6.

METHOD FOR SEPARATING TITANIUM, ZIRCONIUM, NIOBIUM, TANTALUM, MOLYBDENUM AND TUNGSTEN FROM THE REMAINING CONSTITUENTS OF AN ALLOY AND FROM EACH OTHER—

Construct a polythene column of dimensions 13 cm × 0·19 sq. cm, as described on page 186, fill it with 1·00 ± 0·05 g of dry, 100- to 200-mesh De-Acidite FF (SRA 71) and wash the resin column as described on page 187. Add dropwise to the column a portion (by weight) of 0·5 to 1·0 g of a solution containing about 15 mg of alloy per g of approximately M hydrofluoric acid. Open the outlet tap so that the flow-rate is about, but does not exceed, 3 drops per minute and collect the effluent in a dry, weighed 30-ml polythene bottle. Allow the level of the solution to fall just to the top of the resin bed. Carefully fill the top of the column with M hydrofluoric acid from a side-tube, and allow the level of the solution to fall to the top of the resin bed again. Wash traces of solution on to the resin column twice more with M hydrofluoric acid. When the level of the liquid has fallen just below the side-tubes, insert the stopper (lightly coated with silicone grease) slowly but firmly, and then immediately open the tap from the M hydrofluoric acid stock-bottle.

Now open the tap at the bottom of the column fully so that the flow-rate is about 6 drops per minute. Collect a total of 12 g (12 ml) of this effluent containing the iron, chromium, nickel, cobalt, copper, manganese, aluminium and vanadium^{IV}. Then close the tap from the M hydrofluoric acid stock-bottle, and immediately open the tap from the 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture stock-bottle. Partially close the tap at the bottom of the column so that the flow-rate is reduced to between 2 and 3 drops per minute, and change the receiver to another 30-ml polythene bottle. After 10 minutes open the tap at the bottom of the column fully, so that the flow-rate is again about 6 drops per minute. When a total of 12 g (11 ml) of this effluent, containing titanium, zirconium and vanadium^{V},

has been collected, change the eluant to 3 M hydrofluoric acid - 10 M hydrochloric acid mixture, again reduce the flow-rate to between 2 and 3 drops per minute and change the receiver to another 30-ml polythene bottle. After 10 minutes increase the flow-rate to about 6 drops per minute. When 20 g (18 ml) of this effluent, containing tungsten, has been collected, change the eluant to 0·2 M hydrofluoric acid - 7 M hydrochloric acid mixture and change the receiver to another 30-ml polythene bottle. Collect 20 g (18 ml) of the eluate, containing niobium, at the rate of 6 drops per minute. Change the eluant to 3 M hydrofluoric acid - 3 M hydrochloric acid mixture and collect 20 g (20 ml) of the eluate, containing molybdenum, in a polythene bottle at the rate of 6 drops per minute. Change the eluant to M ammonium fluoride - 4 M ammonium chloride mixture, and immediately change the receiving vessel. Collect 13 g (12 ml) of effluent, containing tantalum, in a polythene bottle.

TABLE VII

RESULTS OBTAINED FROM THE ANALYSIS OF SYNTHETIC SOLUTIONS

All figures are expressed in μg

	Iron	Nickel	Titanium	Tungsten	Niobium	Molybdenum	Tantalum
Taken	14,210	—	—	445	—	783	—
Found	14,420	—	—	445	—	771	—
Error	(+1·5%)	—	—	0	—	−12	—
Taken	20,023	—	601	403	562	710	299
Found	20,460	—	600	396	547	698	312
Error	(+2%)	—	−1	−7	−15	−12	+13
Taken	—	1711	—	643	515	—	—
Found	—	1726	—	632	528	—	—
Error	—	+15	—	−11	+13	—	—
Taken	189	986	16·0	370	311	—	8·0
Found	—	—	14·2	381	330	—	10·0
Error	—	—	−1·8	+11	+19	—	+2·0
Taken	3710	—	—	4·5	292	7·9	156
Found	—	—	—	4·4	314	9·0	153
Error	—	—	—	−0·1	+22	+1·1	−3

The collecting vessels were all calibrated at the appropriate volumes so that the amount of effluent collected was within ± 1 ml of the optimum volume.

NOTES—

1. If the flow-rate is not reduced at the start of the collections of the titanium and tungsten eluates, losses of niobium may occur.
2. The capacity of the resin is 3·5 milliequivalents per dry gram; 1 g of resin, for a 1 per cent. loading (single charge), will therefore accommodate the weights of strongly adsorbed metals (if adsorbed singly) listed below: titanium, 1·7 mg; zirconium, 3·2 mg; tungsten, 6·4 mg; niobium, 3·3 mg; molybdenum, 3·4 mg; and tantalum, 6·3 mg.
3. The total column loading of strongly adsorbed metals must not exceed 5 per cent., and preferably should not exceed 3 per cent.
4. As the resin is slowly attacked by the strong acids, it is advisable to change the resin after about 2 months' use.
5. It is possible to leave a trace of metal solution at the top of the column during a separation; this would be washed on to the resin during the next separation. Although the loss will be less than 0·1 per cent. of the solution added, and so could not be detected even in a volumetric analysis, it can become important when a trace of a metal is analysed in the next separation. Therefore it is advisable, when the composition of the alloy is greatly different from that of the previous alloy, to wash the top of the column several times with M hydrofluorice acid, allowing the washings to pass through the resin bed, which is then treated with 10 ml of M hydrofluoric acid and 12 ml of M ammonium fluoride - 4 M ammonium chloride mixture. This operation will remove traces of any metal left at the top of the column from the previous separation. The stopper should also be cleaned thoroughly between separations.
6. If a metal is known to be absent, the particular step in the elution sequence may be omitted.
7. If a determination of titanium and zirconium is not required, the 0·01 M hydrofluoric acid - 9 M hydrochloric acid elution may be omitted, and the titanium and zirconium eluted together with tungsten, with whose spectrophotometric determination they will not interfere.
8. If the determination of tungsten and niobium is not required, or if one is absent, the element (or elements) can be eluted with 25 g of 0·5 M hydrofluoric acid - 7 M hydrochloric acid mixture, without affecting the molybdenum or tantalum. However, no solution could be found that would elute niobium and molybdenum together without affecting the retention of the tantalum.
9. If a determination of the metals towards the end of the sequence is not required, they may be eluted together with M ammonium fluoride - 4 M ammonium chloride mixture.

Analysis of synthetic solutions

The metals in a number of synthetic solutions, containing widely different amounts of titanium, tungsten, niobium, molybdenum and tantalum, were separated by using the proposed scheme, and the separated elements were determined spectrophotometrically by the methods described earlier. For the spectrophotometric determinations of iron, titanium and niobium, part or all of the effluents were evaporated to a small volume in silica beakers and heated with 1 ml of concentrated sulphuric acid until fumes of sulphur trioxide appeared. The nickel, molybdenum and tungsten effluents were treated in a similar way, but Pyrex glass beakers instead of silica beakers were used. Fractions of the tantalum effluents were analysed directly. Table VII shows the recoveries for five synthetic solutions.

These recoveries were considered to be reasonable. In most instances, the errors were within the limits of error attributable to the spectrophotometric determinations. The first two synthetic mixtures gave good recoveries for all the elements strongly adsorbed on the resin column from M hydrofluoric acid, when the amount of iron added to the column was large. Twenty milligrams of iron would give a column loading of about 10 per cent. for a singly charged complex if the iron were strongly adsorbed, but since the distribution coefficient of $iron^{III}$ in M hydrofluoric acid is less than 1, the column loading is probably under 5 per cent.

Analysis of alloys

Method for dissolving an alloy—

Weigh accurately 0·5 to 1·0 g of the alloy into a 100-ml polytetrafluoroethyelene beaker, add about 10 ml of 40 per cent. w/w hydrofluoric acid (in a fume cupboard) and carefully add 2 to 3 ml of nitric acid, sp.gr. 1·42, a few drops at a time, covering the beaker with a nylon cover. Most alloys dissolve in about 2 minutes. Heat the beaker and contents on a hot-plate to break down any carbides, and evaporate almost to dryness to remove as much as possible of the excess of nitric acid. Warm the residue with about 15 ml of M hydrofluoric acid and transfer the solution to a dry, weighed 30-ml polythene bottle. Most, if not all, of the salts should have redissolved. If necessary, dissolve the remainder by warming with a further 5 ml of M hydrofluoric acid, and transfer the solution to the polythene bottle. Wash the beaker twice with M hydrofluoric acid, and add the washings to the polyethylene bottle to make a total volume of about 30 ml. Re-weigh the bottle, and hence calculate the concentration of the alloy in mg of alloy per g of solution.

Note—

If the iron concentration of the alloy is more than 60 per cent., only 0·5 g of alloy should be dissolved in 30 ml of solution, since $iron^{III}$ fluoride is not too soluble in M hydrofluoric acid. Steels and ferro-alloys normally gave a clear solution with up to 1·0 g of alloy per 30 ml of solution.

Table VIII

Results obtained for the analysis of mild steels

Alloy	Metal	Certificate composition, per cent.	Found, per cent.			
British Chemical Standard No. 273 ..	Tungsten	0·28	0·277,	0·272,	0·283	
	Niobium	$0·000_3$	<0·001,	<0·001,	<0·001	
	Molybdenum	$0·04_5$	0·041,	0·041,	0·043	
British Chemical Standard No. 275 ..	Tungsten	0·05	0·043,	0·041,	0·042	
	Niobium	$0·03_5$	0·035,	0·043,	0·043,	0·039
	Molybdenum	$0·09_5$	0·094,	0·091,	0·088,	0·094
British Chemical Standard No. 276 ..	Tungsten	0·20	0·196,	0·191,	0·189	
	Niobium	$0·05_5$	0·054,	0·048,	0·050	
	Molybdenum	<0·01	0·004,	0·003,	0·004	
British Chemical Standard No. 277 ..	Titanium	$0·03_5$	0·035,	0·038,	0·039	
	Tungsten	0·12	0·126,	0·110,	0·121	
	Niobium	$0·02_1$	0·020,	0·025,	0·027	
	Molybdenum	$0·01_5$	0·011,	0·012,	0·013	

Analysis of steels—

Four mild steels, containing about 98 per cent. of iron and traces of other metals, were analysed by the proposed method. Spectrophotometric finishes were used for determining

the separated elements. Because the elements that are strongly adsorbed on De-Acidite FF resin were present in only trace amounts, about 4 g of the alloy solution (instead of 0·5 to 1·0 g) were added to the column in several portions. Under these circumstances, 16 g of M hydrofluoric acid were passed down the column to ensure complete removal of iron and other non-absorbed metals. The results are given in Table VIII.

These results, obtained for the determination of titanium, niobium, molybdenum and tungsten in these mild steels, were considered to be satisfactory. No results are reported for tantalum in these steels, because their tantalum contents were too low (of the order of 0·01 per cent.) to be determined with even moderate precision by using the spectrophotometric method described earlier (p. 188).

There is no doubt that tantalum in the tantalum effluents could be determined with an accuracy and precision of $\pm 0{\cdot}001$ per cent. for the tantalum content of the steel, if a more reliable spectrophotometric method for determining traces of tantalum was available.

VOLUMETRIC FINISH FOR THE DETERMINATION OF THE MAJOR CONSTITUENTS OF AN ALLOY—

Pure solutions of iron^{III}, titanium^{IV}, niobium^{V}, molybdenum^{VI} and tungsten^{VI} in hydrofluoric acid - hydrochloric acid mixtures are suitable for determining the metal by a volumetric reduction method. Headridge and Taylor[11] have shown that niobium^{V} in 0·5 M hydrofluoric acid - 6 M hydrochloric acid solution is quantitatively reduced in a Jones reductor to niobium^{III}. The reduced species is collected in ammonium ferric sulphate solution, and the equivalent amount of ferrous iron thus produced is titrated with standard dichromate solution. Similar methods have been devised by the same workers[12,13] for iron^{III}, tungsten^{VI} and molybdenum^{VI} in fluoride solutions, by using a Jones reductor or a silver reductor.

Such volumetric methods are suitable for determining major constituents (10 to 100 per cent.) of an alloy after anion-exchange separation. Three such alloys were analysed, and the results of the volumetric determinations are shown in Table IX.

TABLE IX

RESULTS OBTAINED FROM THE ANALYSIS OF ALLOYS BY USING A VOLUMETRIC FINISH AFTER ANION-EXCHANGE SEPARATIONS

Alloy	Element	Certificate value, %	Found, %
B.C.S. 241/1 (Cr–V–W–Co–Mo steel)	Tungsten	19·61	19·57, 19·64
B.C.S. 231/2 (Ferro-molybdenum)	Molybdenum	70·1	70·2, 70·2
	Iron	28·9*	28·8, 28·9
Ferro-niobium-tantalum†	Niobium	59·1	59·2, 59·3

* Value obtained by Headridge and Taylor.[13]
† Supplied by London and Scandinavian Metallurgical Co. Ltd.

The volumetric determinations were considered to be completely satisfactory and to furnish conclusive proof of the clean separation of all of the metals by the scheme described in this paper. As the separations are quantitative, the accuracy and precision of an analysis is dependent only on the accuracy and precision of the final determination of a metal in the pure solution.

EFFECT OF OTHER METALS ON THE SEPARATION SCHEME—

Several elements, such as boron, lead, magnesium, phosphorus, silicon, sulphur and tin, that can be encountered in steels, high-temperature and ferro-alloys, have not been studied in this work. Of these only boron and tin might cause interference.

Boron—Boron forms an anionic complex with hydrofluoric acid and would be adsorbed by the anion-exchange resin from M hydrofluoric acid. However, as it is not complexed by chloride, it would be displaced from the resin along with titanium and zirconium by the 0·01 M hydrofluoric acid - 9 M hydrochloric acid mixture.

Tin—Kraus and Nelson[14] have reported distribution coefficients for tin^{IV} in hydrofluoric acid - hydrochloric acid solutions. A study of their results shows that tin^{IV} would be eluted with molybdenum by 3 M hydrofluoric acid - 3 M hydrochloric acid mixture. The recovery of tin would probably be quantitative in the molybdenum fraction.

Conclusions

The method described should be useful for determining titanium, zirconium, niobium, tantalum, molybdenum and tungsten in steels, high-temperature and ferro-alloys, and could be readily adapted for determining these elements in ores, mineral dressings and other materials.

The analysis of a complex alloy can be completed by two chemists in a working day.

While it would certainly be quicker to determine these elements in alloys by using instrumental techniques such as emission spectrography, X-ray fluorescence spectrography and atomic-absorption spectroscopy, the method should be useful for the analysis of standard alloys, and for non-routine analysis of special alloys, where the use of an instrumental technique is not justified.

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The Spectrographic Analysis of Zinc and Cadmium Chloride Solutions and Solid Zinc and Cadmium Sulphides by a Solution - Powder Method

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The development of a spectrographic d.c. arc, solution - powder method for determining impurities (less than 500 p.p.m.) in cadmium and zinc chloride solutions and solid cadmium and zinc sulphides is described.

Sufficient solution or solid containing 20 mg of cadmium or zinc is taken as a sample, converted to the sulphate and evaporated to dryness. The arc is struck between graphite electrodes with cadmium sulphate present, and twenty-two impurity elements are determined, some with barium as an internal standard. The zinc sulphate is mixed with graphite, and the arc is struck between graphite electrodes in a Stallwood Jet with the sample present in an argon - oxygen atmosphere, thus eliminating the effect of selective volatilisation. Nine impurity elements are determined.

A method was needed in this laboratory for the simultaneous determination of many trace impurity elements in solid cadmium sulphide and zinc sulphide phosphors, as well as in products from the intermediate stages of their production, *i.e.*, cadmium and zinc chloride solutions. This was because of the rôle that small traces of impurity elements can play in varying the physical characteristics of a phosphor.

An estimate of the possible impurity elements likely to be found in the zinc materials was nine (see Table I), and in the cadmium materials, twenty-two (see Table II). The conversion of the samples to sulphate appeared to be the most promising approach for determining the impurities in both sulphides and chlorides. As this necessitates dissolving the cadmium or zinc salt, it was thought that the addition of the impurity elements in the standards at this stage would eliminate the tedious preparation and standardisation that is necessary with the "doping" of pure cadmium and zinc sulphide powders, especially with the large number of impurity elements required for the cadmium sulphide. This procedure is similar to the solution - powder method mentioned by Young[1] in his review of spectrographic solution methods.

Development of the Method

Dropping-plate exposures of "doped" solid cadmium sulphate were prepared by using a 6-A d.c. arc and graphite electrodes, and showed that the impurity elements could be divided into two categories—

Volatile: boron, thallium, sodium, copper, lead, zinc, silver, arsenic, tin, bismuth, gold and indium.

Non-volatile: aluminium, iron, cobalt, manganese, gallium, nickel, antimony, chromium, magnesium and titanium.

Cadmium was satisfactory as an internal standard for the volatile elements, whereas barium was found to be applicable for the less volatile elements.

Dropping-plate exposures of "doped" zinc sulphate were prepared by using the same conditions as for the cadmium sulphate, and showed non-uniform rates of volatilisation for most elements. The volatilisation curves for zinc and the impurity elements were too dissimilar for zinc to be of any use as an internal standard. Thus, the simple method used for cadmium sulphate could not be applied to zinc sulphate.

At this stage it was considered necessary to try various conditions in an attempt to improve the volatilisation rates. A series of dropping-plate exposures was conducted, with a range of arc currents ranging from 6 to 12 A, with a change in the atmosphere in which the arc was struck from air to an argon - oxygen mixture by means of a Stallwood Jet,[2] and finally with graphite mixed in the sample.

The effect of an increase in arc current reduced the selective volatilisation, in that all the impurity elements were boiled off in a shorter time, but with this short exposure time (10 to 20 seconds) the precision of the method suffered badly. The use of a Stallwood Jet seemed to offer a solution to this problem. A number of exposures were made to determine the optimum operating conditions for the jet as applied to this compound. Several mixtures

TABLE I

SENSITIVITY OF THE ZINC SULPHIDE - ZINC CHLORIDE METHOD

Line densities are measured at 100 per cent. transmission

Element	Analytical line, Å	Internal zinc standard line, Å	Concentration range, p.p.m. in ZnS
Cadmium	2288·0	2463·5	3 to 500
Gold	*2428·0	2684·2	20 to 500
Manganese	2794·8	2684·2	3 to 500
Lead	*2833·1	2684·2	3 to 500
Nickel	*3002·5	2684·2	3 to 500
Iron	*3020·6	2684·2	3 to 500
Aluminium	*3092·7	2684·2	3 to 500
Copper	*3274·0	2684·2	0·4 to 500
Silver	*3382·9	2684·2	3 to 500

* Line intensities are corrected for background.

TABLE II

SENSITIVITY OF THE CADMIUM SULPHIDE - CADMIUM CHLORIDE METHOD

Element	Analytical line, Å	Internal standard line, Å	Concentration range, p.p.m. in CdS
Arsenic	2349·8 (*a*)	Cd 2775·0	20 to 500
Gold	2428·0 (*a*)	Cd 2775·0	3 to 120
Boron	2497·7 (*a*)	Cd 2775·0	20 to 500
Antimony	*2598·1 (*a*)	Ba 3071·6	3 to 500
Thallium	2767·9 (*a*)	Cd 2775·0	20 to 500
Manganese	2794·8 (*a*)	Ba 3071·6	0·5 to 500
Magnesium	2795·5 (*b*)	Ba 3071·6	3 to 500
Lead	2833·1 (*a*)	Cd 2775·0	3 to 500
Chromium	*2849·8 (*a*)	Ba 3071·6	3 to 500
Sodium	2852·8 (*a*)	Cd 2775·0	20 to 500
Gallium	2944·2 (*a*)	Ba 3071·6	3 to 500
Nickel	3002·5 (*a*)	Ba 3071·6	3 to 500
Iron	3020·6 (*a*,*b*)	Ba 3071·6	3 to 500
Tin	3034·1 (*a*)	Cd 2775·0	3 to 500
Indium	3039·4 (*a*)	Cd 2775·0	3 to 500
Bismuth	*3067·7 (*a*)	Cd 2775·0	0·5 to 500
Aluminium	3092·7 (*a*,*b*)	Ba 3071·6	3 to 500
Copper	3274·0 (*a*,*b*)	Cd 2775·0	0·5 to 120
	2824·4 (*a*)	Cd 2775·0	20 to 500
Silver	3280·7 (*b*)	Cd 2775·0	0·5 to 120
Zinc	*3345·0 (*a*)	Cd 2775·0	20 to 500
Titanium	3349·0 (*a*)	Ba 3071·6	20 to 500
Cobalt	3412·6 (*a*)	Ba 3071·6	3 to 500

* Line intensities corrected for background.
(*a*) = 100 per cent. transmission. (*b*) = 12½ per cent. transmission.

of oxygen and argon were tried at several different flow-rates, and with the arc current at settings from 6 to 15 A. Several electrode configurations were also tried with the sample as the anode or as the cathode. The electrode configuration finally decided upon was that shown in Fig. 1 (*a*). A gas mixture of 20 per cent. of oxygen in argon at a flow-rate of 5 litres per minute was used. An arc current of 12 A was used with the sample as the anode. These conditions are similar to those used in rare-earth element analysis by Hammaker.[3] By implementing these conditions, the volatilisation time of the elements was increased, but selective volatilisation was not eradicated until the sample was mixed with graphite in the ratio of 2 parts of sample to 1 part of graphite. It is now possible to use zinc as an internal standard, with the best sensitivity and optimum reproducibility.

METHOD

Zinc sulphide and zinc chloride analysis

Reagents—

Zinc sulphate solution—Prepare a solution from Specpure material such that—

1 ml of solution ≡ 10 mg of zinc.

Sulphuric acid, 5 N—Prepare an approximately 5 N solution by diluting micro analytical-reagent grade sulphuric acid, sp.gr. 1·84.

Nitric acid saturated with bromine—Saturate "electronic-grade" nitric acid with bromine.

Standard solutions—Prepare separate solutions of manganese, iron, copper, aluminium, cadmium, lead, silver, nickel and gold from Specpure materials. The concentrations of three solutions of each metal should be such that—

1 ml of solution ≡ 10 μg of metal, 1 ml of solution ≡ 1 μg of metal and 1 ml of solution ≡ 0·1 μg of metal.

Preparation of standards—

Prepare standards by adding the impurity elements in the required proportions to 2 ml of zinc sulphate solution and 0·2 ml of 5 N sulphuric acid. Evaporate the mixture to dryness, taking care that all the excess of sulphuric acid is removed by fuming. Mix the residue, consisting of 50 mg of zinc sulphate plus impurities, with 25 mg of graphite powder for five minutes in a small agate mortar.

Preparation of sample—

Dissolve 30 mg of solid zinc sulphide in 0·1 ml of bromine-saturated nitric acid, or take a portion of zinc chloride solution containing 20 mg of zinc. Then add 0·2 ml of 5 N sulphuric acid and evaporate the solution to dryness. Treat the residue as for the preparation of standards above.

Cadmium sulphide and cadmium chloride analysis

Reagents—

Cadmium sulphate solution—Prepare a solution from Specpure material such that—

1 ml of solution ≡ 20 mg of cadmium.

Barium sulphate solution—Prepare a solution from Specpure material such that—

1 ml of solution ≡ 1 mg of barium.

Sulphuric acid, 5 N—Prepare an approximately 5 N solution by diluting micro analytical-reagent grade sulphuric acid, sp.gr. 1·84.

Nitric acid saturated with bromine—Saturate "electronic-grade" nitric acid with bromine.

Standard solutions—Prepare separate solutions of silicon, arsenic, gold, boron, antimony, thallium, manganese, magnesium, lead, chromium, sodium, gallium, nickel, iron, tin, indium, bismuth, aluminium, copper, silver, zinc, titanium and cobalt. The concentrations of three solutions of each metal should be such that—

1 ml of solution ≡ 10 μg of metal, 1 ml of solution ≡ 1 μg of metal and 1 ml of solution ≡ 0·1 μg of metal.

Preparation of standards—

Prepare standards by adding the impurity elements in the required proportions to 1 ml of cadmium sulphate solution and add 0·1 ml of 5 N sulphuric acid. Evaporate the mixture to dryness, taking care that all the excess of sulphuric acid is removed by fuming.

Preparation of sample—

Dissolve 26 mg of solid cadmium sulphide in 0·1 ml of bromine-saturated nitric acid, or take a portion of cadmium chloride solution containing 20 mg of cadmium, and add 0·1 ml of 5 N sulphuric acid. Evaporate the solution to dryness, and treat the residue as for the preparation of standards above.

Spectrographic procedure

For both zinc and cadmium samples, prepare the solutions and evaporate them in 5-ml Pyrex beakers. Thoroughly mix the residue and transfer it quantitatively to pre-burned

graphite electrodes. The shape and dimensions of these electrodes are given in Fig. 1 (*a*) for zinc and 1 (*b*) for cadmium.

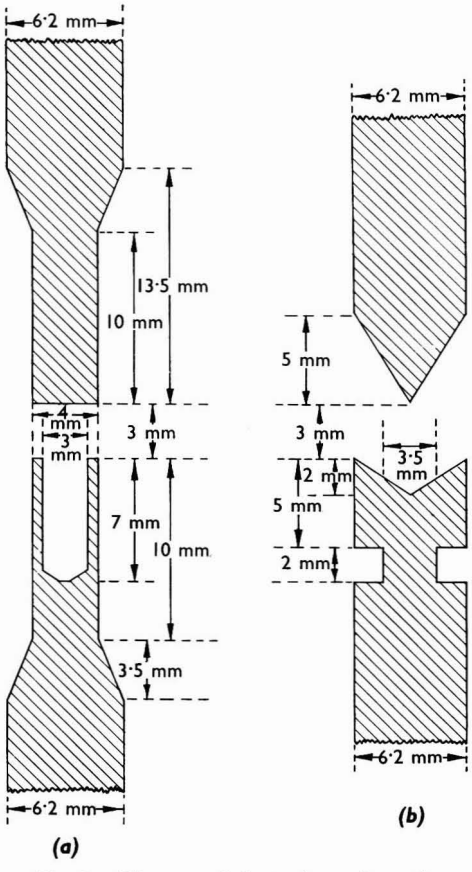

Fig. 1. Diagram of electrode configurations

SPECTROGRAPHIC CONDITIONS—

	Cadmium sulphide and chloride	Zinc sulphide and chloride
Spectrograph ..	Hilger, medium quartz, E 498	Hilger, medium quartz, E 498
Slit width	20 μ	20 μ
Slit length	2 mm	2 mm
Analytical gap ..	3 mm (electrodes were adjusted during exposure to keep the gap constant)	3 mm (electrodes were adjusted during exposure to keep the gap constant)
Rotating sector ..	12½ per cent. and 100 per cent. in front of spectrograph slit	12½ per cent. and 100 per cent. in front of spectrograph slit
External optics ..	Lens F1026, focusing the discharge on the collimator lens	Lens F1026, focusing the discharge on the collimator lens
Plate	Ilford R50	Ilford R50
Development ..	ID2 (1 + 2), 4½ minutes at 20° C	ID2 (1 + 2), 4½ minutes at 20° C
Excitation	6-A d.c. arc (15-kV initial spark)	12-A d.c. arc (no spark initiation) in controlled atmosphere of 20 per cent. oxygen in argon at a flow-rate of 5 litres per minute
Exposure	40 seconds	50 seconds
Graphite	Ringsdorff RWIII, 6·15-mm diameter rod	Ringsdorff RWO, 6·15-mm diameter rod
Electrodes	See Fig. 1 (*b*)	See Fig. 1 (*a*)

Photometry—Measure the densities of spectral lines by using a Joyce - Loebl recording microdensitometer and convert to logarithmic relative intensities by means of a plate calibration curve. Analytical curves may be constructed by plotting—

$$\log\left(\frac{\text{intensity of the analytical line}}{\text{intensity of the internal-standard line}}\right) \textit{versus} \log (\text{concentration}).$$

Analytical line pairs are presented in Tables I and II, together with the concentration ranges of the analytical curves prepared by these methods. Tables III and IV show the coefficient of variation obtained for each impurity element.

TABLE III

PRECISON OF THE ZINC SULPHIDE - ZINC CHLORIDE METHOD

Element	Coefficient of variation	Average value,* p.p.m.
Cadmium	33	115
Gold	17	150
Manganese	11	65
Lead	9	51
Nickel	13	48
Iron	17	55
Aluminium	22	43
Copper	14	64
Silver	8	58

* 10 determinations.

TABLE IV

PRECISION OF THE CADMIUM SULPHIDE - CADMIUM CHLORIDE METHOD

Element	Coefficient of variation	Average value, p.p.m.	Number of determinations
Arsenic	8	101	8
Gold	24	42	8
Boron	36	73	8
Antimony	26	76	10
Thallium	17	34	8
Manganese	26	39	8
Magnesium	43	300	10
Lead	6	350	14
Chromium	31	52	8
Sodium	44	43	8
Gallium	25	61	8
Nickel	27	40	10
Iron	20	387	14
Tin	26	49	10
Indium	12	25	10
Bismuth	18	50	8
Aluminium	14	360	14
Copper	16	347	14
Silver	22	41	8
Zinc	6	330	14
Titanium	24	85	8
Cobalt	22	50	8

DISCUSSION

The zinc sulphide - zinc chloride method is satisfactory for all the elements so far used, with the exception of cadmium and to a lesser extent aluminium. The cadmium line, 2288·0 Å, is in that region of the photographic plate not normally considered useful, owing to the low gamma and sensitivity of the emulsion at this wavelength. Unfortunately, all other cadmium lines suffer from low sensitivity, high background interference and interference from other element lines. The lack of a suitable zinc line of similar wavelength to the cadmium contributes towards the high coefficient of variation.

The Stallwood Jet with the argon - oxygen atmosphere caused a marked diminution of the cyanogen bands. The background interference above 3200 Å is still somewhat excessive, but is a continuum rather than a band structure, and is probably present owing to glowing particles of sample and graphite being carried into the arc stream.

The reproducibility of the zinc sulphide - chloride method could probably be improved if a vibratory-electrode packer was used instead of a glass rod for tamping the charge. This also relieves the tedium of this part of the method. This has not, however, been attempted by the authors.

The cadmium sulphide - cadmium chloride method gives poor reproducibility for sodium, boron and silicon, and gives non-ideal standard curves that were non-linear and of low gradient. This is probably largely due to the glass-ware used in the preparation of the samples. Although the standard curve for magnesium was satisfactory, the reproducibility was poor. This was almost certainly due to varying amounts of magnesium present as an impurity in the electrodes.

These two methods should be suitable for the analysis of any zinc or cadmium compound capable of conversion to the sulphate, and needs little or no modification.

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Subtracting the Blank Value

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A widely used method for preparing some calibration curves, in instances where these curves extend to zero concentration, is to "subtract the blank value." Some of the consequences of this action are examined in this paper, and it is suggested that the statistical approach leads to more efficient and objective results.

THE method often used for rapidly determining the concentration of some compound in a solution, by colorimetric means, is to prepare a set of standard concentrations, add reagents to each standard to produce a colour and to measure the optical density of the colour obtained with an absorptiometer. The results will be such as are shown below—

Concentration	x_1	x_2	$x_3 \ldots x_n$
Drum reading	y_1	y_2	$y_3 \ldots y_n$

where n different concentrations are taken, of which x_1 represents zero concentration so that y_1 is the blank value. This blank value could be zero, but if the reagents absorbed some light (owing to active compounds in the reagents themselves or by virtue of colours present from some other cause) then the blank value would be greater than zero. In this latter instance the blank value is often subtracted from each of the other readings as indicated below—

Concentration	x_2	x_3	x_n
Corrected drum reading ..	$(y_2 - y_1)$	$(y_3 - y_1)$	$(y_n - y_1)$

The results are then displayed graphically by joining the points with a smooth curve passing through the origin. In the experiment the reagents are added to a solution of unknown concentration, and a drum reading is obtained. Simultaneously, a new blank reading is made; the difference between the sample value and the concurrent blank value is then calculated, and the concentration of the unknown solution is determined from the calibration curve.

THE MATHEMATICAL MODEL

If Beer's law holds, if the drum has a logarithmic scale and if there are no experimental errors, then the initial uncorrected readings are connected by the relationship given in the equation below—

$$y_r = A + Bx_r \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where $r = 1,2,3, \ldots n$,
and the calibration curve is a straight line of which A is the blank value and B is the increase in drum reading per unit increase in concentration.

Unfortunately, in practice, even if the first two conditions are valid, it is found that the points do not lie exactly on a straight line and that the relationship between the uncorrected readings are more accurately described by the equation given below—

$$y_r = A + Bx_r + e_r \ldots \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where $r = 1,2,3, \ldots n$,
and e_r (the deviations of the y values from the "true" straight line) are the experimental errors arising from inaccuracies in standards, variation in drum response, etc.

The introduction of such errors complicates the determination of A and B; the properties of equation (2) must be stated more explicitly before further mathematical treatment can be applied.

It is assumed that—

(*a*) The values of x_r are not in error (or are the values of a controlled variable).[1]

(*b*) The constants A and B are initially unknown and are to be estimated from the results.

(*c*) The errors $e_1, e_2, e_3, \ldots e_n$ have the following properties—

(*i*) Their averages are zero; this means that in a long series of identical repeated calibration experiments, the errors in, *e.g.*, each of the blank values, e_1, will sometimes be positive and sometimes negative but will average out to zero. The same holds for the other errors.

(*ii*) They are uncorrelated; this means that the products of the errors in, *e.g.*, the first and second readings, e_1 and e_2, in the same calibration experiment, will average out to zero in a long series of repeated calibrations. The same assumption holds for the other products.

(*iii*) They have a common variance; this means that the average of the squares of the errors in, *e.g.*, the blank readings over a large number of repeated calibrations, is σ^2, where σ is the standard deviation. The same assumption holds for the other errors.

The analysis

In terms of the foregoing mathematical model, the process of subtracting the blank value and deducing the corrected readings is equivalent to the set of equations derived from equation (2) and given below—

$$(y_r - y_1) = (A + Bx_r + e_r) - (A + Bx_1 + e_1)$$

which as $x_1 = 0$ reduces to—

$$(y_r - y_1) = Bx_r + (e_r - e_1) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where $r = 2,3,4, \ldots n$.

An estimate, B_1, of B is calculated by using the "Method of Least Squares"; B_1 is chosen so that the sum of the squares of the deviations is a minimum, *i.e.*, so that $\sum_{r=2}^{n} (y_r - y_1 - Bx_r)^2$ is a minimum. When this is differentiated with respect to B and equated to zero, it is found that—

$$B_1 = \frac{\sum_{r=2}^{n} (y_r - y_1)\, x_r}{\sum_{r=2}^{n} x_r^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

As B_1 is only an estimate of B, its value will alter from calibration to calibration and its variance is required, so that the accuracy of the determination may be estimated. By using the properties listed in (*c*) above it can be shown that[2]—

$$B_1 = B + \frac{\sum_{r=2}^{n} (e_r - e_1)\, x_r}{\sum_{r=2}^{n} x_r^2} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

$$\text{and Var } (B_1) = \frac{\sigma^2 \left[\sum_{r=2}^{n} x_r^2 + \left(\sum_{r=2}^{n} x_r \right)^2 \right]}{\left(\sum_{r=2}^{n} x_r^2 \right)^2} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Another estimate of B can be obtained, however, without first subtracting the blank value, if both A and B are estimated from equation (2) by using the "Method of Least Squares," *i.e.*, if estimates A_2 and B_2 are obtained by differentiating $\sum_{r=1}^{n} (y_r - A - Bx_r)^2$ with respect to A and B and equating the result to zero.[3] This gives—

$$B_2 = \frac{\sum_{r=1}^{n} (y_r - \bar{y})\, (x_r - \bar{x})}{\sum_{r=1}^{n} (x_r - \bar{x})^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

$$A_2 = \bar{y} - B_2\bar{x} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where $\bar{x}$ and $\bar{y}$ are the arithmetic means of the x and y values, respectively. (It may be noted that A_2 is the statistical estimate of the "true" blank value.)

By using methods similar to those used for deriving equations (5) and (6) it can be shown that[2]—

$$B_2 = B + \frac{\sum_{r=1}^{n} (e_r - \bar{e})(x_r - \bar{x})}{\sum_{r=1}^{n} (x_r - \bar{x})^2} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

$$\text{and Var } (B_2) = \frac{\sigma^2}{\sum_{r=1}^{n} (x_r - \bar{x})^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10)$$

It is now possible to compare the merits of the two estimates of the slope, B_1 and B_2.

Firstly, they are both unbiased in the sense that the average of all such estimates derived from repeated calibration experiments (having the same x values) is the true slope B. If property $c(i)$ is assumed to hold for equations (5) and (9), this can be proved with ease. From this point of view there is nothing to choose between the two estimates. In any particular experiment they may give slightly different results, but on the average these differences would be zero.

The second criterion for choice between the two slope estimates is the relative size of their variances; the estimate having the smaller variance being the more acceptable, since a small variance implies that the observed values lie, on the average, close to the true value. The ratio R of equation (6) to equation (10) is—

$$R = \frac{[\Sigma x_r^2 + (\Sigma x_r)^2] \Sigma (x_r - \bar{x})^2}{(\Sigma x_r^2)^2} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

and since it can be shown that R is always greater than unity (whatever the values of x), the estimate given by equation (7) is preferable to that given by equation (4). This may be seen clearly in the common practical case of equidistant values of x.

Let—

$$x_r = (r - 1)\mathrm{d}$$

where d is a constant and $r = 1,2,3, \ldots n$.

Then—

$$\Sigma x_r = 0 + \mathrm{d} + 2\mathrm{d} + \ldots + (n-1)\mathrm{d} = \tfrac{1}{2}(n-1)n\mathrm{d}$$
$$\Sigma x_r^2 = 0 + \mathrm{d}^2 + 4\mathrm{d}^2 + \ldots + (n-1)^2\mathrm{d}^2 = \tfrac{1}{6}(n-1)\,n\,(2n-1)\mathrm{d}^2$$
$$\bar{x} = \tfrac{1}{2}(n-1)\mathrm{d}.$$

If these equations are substituted into equation (11), it is found that—

$$R = \frac{(3n-2)(n+1)^2}{4(2n-1)^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (12)$$

The values of R for selected values of n are given below—

n	1	3	5	8	12
R	1	1·12	1·44	1·98	2·72

The inefficiency of the first method increases with the number of calibration points (in fact with the number of times the blank value is subtracted) so that with eight points, for example, the variance of B_1 is about twice that of B_2.

Another reason for choosing equation (7) rather than equation (4) is that all the standard statistical tests can be applied to the former (B_2), whereas no such tests can be used on the latter (B_1).[4] This is chiefly owing to the fact that estimates of slope and error variance in the first method are not statistically independent.

Predicting a result

When it has been decided to use the slope estimate B_2, the accuracy with which the determination of a concentration of an unknown solution can be made has to be calculated.

Let Y = drum reading of unknown sample *minus* drum reading of unknown blank.

Then Var $(Y) = 2\sigma^2$ where σ is the standard deviation, since Y is the difference of two independent readings having errors whose variances are each σ^2.[5]

With no experimental error, x would be estimated from the equation $Y = Bx$ or $x = \frac{Y}{B}$ but as Y is in error and as we only have B_2 as an estimate, x has to be estimated from—

$$x = \frac{Y}{B_2}$$

Now it can be shown approximately[6] that—

$$\text{Var } (x) = \frac{\text{Var } (Y)}{B^2} + \frac{Y^2}{B^4} \text{ Var } B \quad .. \quad .. \quad .. \quad .. \quad (13)$$

so that by using equation (10) and the expression for Var (Y) above in equation (13), we have—

$$\text{Var } (x) = \frac{\sigma^2}{B^2}\left[2 + \frac{x^2}{\Sigma (x - \bar{x})^2}\right] \quad .. \quad .. \quad .. \quad .. \quad (14)$$

Hence the error in any value of x is the sum of two terms, the first, $\frac{2\sigma^2}{B^2}$, arising from the error in the readings of the sample and its associated blank value and the second, $\frac{\sigma^2 x^2}{B^2 \Sigma (x - \bar{x})^2}$, arising from the uncertainty of the slope of the calibration curve. If the percentage error is taken as a criterion of accuracy, the coefficient of variation of $x\left(= \frac{100\sqrt{\text{Var}(x)}}{x}\right)$ is more informative, when equation (14) is used.

The coefficient of variation, V, is given by the equation—

$$V = \frac{100\sigma}{B}\sqrt{\frac{2}{x^2} + \frac{1}{\Sigma (x - \bar{x})^2}} \quad .. \quad .. \quad .. \quad .. \quad (15)$$

From this it can be seen that the percentage error increases indefinitely at small values of x, and that it can never be less than $\frac{100\sigma}{B\sqrt{\Sigma (x - \bar{x})^2}}$. This term occurs as a result of the uncertainty in estimating the slope.

Practical example

In order to assess the size of errors encountered in practice, a calibration curve of the concentration of iron in sample solutions was made using thioglycollic acid as a complexing agent.

Sufficient analytical-grade reagent, ammonium ferrous sulphate, was weighed out and diluted with water to provide 250 ml of a solution containing 100 μg of iron per ml. A 25-ml portion of this solution was diluted to 250 ml and to 0-, 1-, 2-, 3-, 4- and 5-ml portions of this latter solution 5 ml of 10 per cent. thioglycollic acid was added. A slight excess of ammonia solution, sp.gr. 0·88, was added to develop the colour, and then the portions were diluted to 100 ml with water. The optical density of each solution was measured in 4-cm cells against a water blank solution by using a Hilger-Spekker absorptiometer, fitted with Ilford 605 filters. The results obtained are shown below—

Concentration, μg per ml, x ..	0	10	20	30	40	50
Drum reading, y	0·009	0·035	0·061	0·083	0·109	0·133

$$\bar{x} = 25 \qquad \bar{y} = 0{\cdot}072 \qquad \Sigma (x - \bar{x})(y - \bar{y}) = 4{\cdot}32$$

$$\Sigma (x - \bar{x})^2 = 1750 \qquad \Sigma (y - \bar{y})^2 = 0{\cdot}010669.$$

By using equation (7) we have—

$$B_2 = \frac{4{\cdot}32}{1750} = 0{\cdot}0024686$$

To calculate the coefficient of variation, equation (15) requires that the value of σ^2, the error variance should be known; since this is initially unknown, it has to be estimated as[7]—

$$\sigma^2 = \tfrac{1}{4}\left[\Sigma\,(y-\bar{y})^2 - B_2\,\Sigma\,(x-\bar{x})\,(y-\bar{y})\right] = 1{\cdot}28 \times 10^{-6}$$

By using the above result and B_2 for B, equation (15) becomes—

$$V = \frac{100\,\sqrt{1{\cdot}28 \times 10^{-6}}}{0{\cdot}0024686} \times \sqrt{\frac{2}{x^2} + \frac{1}{1750}}$$

Values of V for selected values of x are given below—

x	10	20	50	∞
V, per cent.	6·6	3·4	1·7	1·1

Although V is only an estimate, it is reasonable to suppose that it shows the correct order of magnitude to be expected for the percentage error, and that the true value lies inside a range $\pm 2V$. If this range is too large, it can be reduced only by reducing the error variance. This may be achieved by repeating the calibration experiment and averaging the results obtained for each concentration.

I thank my colleague N. Halstead who provided the practical example and the assessors who were extremely patient and helpful in clarifying a number of obscurities.

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The Determination of Certain Sulphonamides

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A new titrimetric method is described for determining certain sulphonamides. The proposed method is based on the fact that *N*-bromosuccinimide readily and quantitatively brominates an aqueous acidic solution of the sulphonamide and is itself irreversibly reduced to succinimide. The mechanism of the reaction is illustrated. The experimental error is within ±1 per cent.

Certain sulphonamides, such as sulphanilamide, sulphaguanidine and sulphacetamide, cannot be determined by the silver nitrate method.[1] Further, sulphanilamide and sulphaguanidine cannot be estimated by the recent complexometric method,[2] whose error in case of sulphacetamide sodium amounts to more than 20 per cent.

This paper describes a new method for determining these sulphonamides. The proposed method is based on the fact that *N*-bromosuccinimide readily and quantitatively brominates an aqueous acidic solution of sulphanilamide, sulphaguanidine or sulphacetamide to the corresponding 3,5-dibromo derivative and is itself irreversibly reduced to succinimide, as represented by the equation—

where

R = —H for sulphanilamide, R = —C(=NH)—NH_2 for sulphaguanidine and

R = —CO—CH_3 for sulphacetamide.

In the presence of glacial acetic acid, the reaction is quantitative for the molecular ratio of two molecules of *N*-bromosuccinimide to one molecule of sulphonamide at room temperature; methyl red was used as an indicator. Succinimide was isolated from the reaction mixture and identified by comparing the melting-point of the compound produced by the reaction with the melting-point obtained from a mixture of the product and a pure sample. The 3,5-dibromo derivative of sulphanilamide or sulphaguanidine was isolated from the reaction mixture and identified by comparing the melting-points of the products with the melting-points of admixtures with pure samples of 4-amino-3,5-dibromobenzene sulphonamide[3] and 4-amino-3,5-dibromophenyl sulphonylguanidine,[4] respectively.

This reaction does not appear to have been described in the literature. *N*-Bromosuccinimide is an effective brominating agent,[5] and can decolorise methyl red[6] in aqueous acidic medium, but it brominates sulphonamides preferentially. Until all the sulphonamide in the reaction mixture has been brominated, the colour of methyl red does not disappear. A slight excess of *N*-bromosuccinimide added after all the sulphonamide has been brominated causes the methyl red to be decolorised, and consequently the end-point can be easily detected.

Experimental

Reaction between *N*-bromosuccinimide and sulphanilamide—

A 0·86 g portion of sulphanilamide (0·005 mole) was dissolved in 20 ml of 10 per cent. hydrochloric acid, and 1·78 g of *N*-bromosuccinimide (0·01 mole) were dissolved in 100 ml of hot distilled water. When the *N*-bromosuccinimide solution was cool, it was gradually added, with shaking, to the cold sulphanilamide solution. Colourless crystals separated out and these were filtered off. When the solid was re-crystallised from ethanol, colourless needles, m.p. 237° C, were obtained. The melting-point of the solid in admixture with 4-amino-3,5-dibromobenzene sulphonamide, prepared by halogenation of sulphanilamide with bromine water,[3] was also 237° C. The yield was 1·45 g. The filtrate was distilled under reduced pressure. The solid residue, re-crystallised from benzene, was succinimide, m.p. 125° C, identified by melting-point and mixed melting-point determinations with an authentic sample. The yield was 0·4 g.

REACTION BETWEEN *N*-BROMOSUCCINIMIDE AND SULPHAGUANIDINE—

Similarly, 4-amino-3,5-dibromophenyl sulphonylguanidine,[4] m.p. 283° C, and succinimide were prepared and identified by the melting-point procedure described above.

VALIDITY OF THE REACTION FOR QUANTITATIVE DETERMINATIONS—

The quantitative nature of the reaction between *N*-bromosuccinimide and sulphanilamide was checked. An accurately measured volume, *e.g.*, 2 ml, of a solution containing 0·172 g (1 millimole) of sulphanilamide per 100 ml of 10 per cent. hydrochloric acid, was placed in a 50-ml conical flask, an equal volume of glacial acetic acid, to dissolve the produced 3,5-dibromo derivative, and 2 drops of methyl red indicator solution were added. The mixture was titrated with 0·178 per cent. w/v *N*-bromosuccinimide solution, added gradually from a microburette, with continuous shaking after each addition until the colour of methyl red just disappeared, and the titre was noted. A similar series of experiments was carried out with *N*-bromosuccinimide solution containing twice the number of molecules of solute present in the reagent. It was found that the reaction was stoicheiometric in acid medium at room temperature; the results are shown in Table I.

TABLE I

RESULTS OF TITRATION OF SULPHANILAMIDE WITH *N*-BROMOSUCCINIMIDE

Vol. of sulphanilamide solution (1 mmole per 100 ml) used, ml	Titre of *N*-bromosuccinimide solution (1 mmole per 100 ml), ml	Titre of *N*-bromosuccinimide solution (2 mmole per 100 ml), ml
10	19·95	10·0
5	9·95	4·95
4	8·0	4·05
3	5·95	2·95
2	4·07	2·05
1	2·05	1·05

Sulphaguanidine and sulphacetamide sodium also gave similar results.

METHOD

REAGENTS—

Methyl red solution, 0·04 per cent. w/v, alcoholic.
Hydrochloric acid, 10 per cent. w/v, aqueous.
Acetic acid, glacial.
N-Bromosuccinimide solution—Freshly prepare a 0·1 per cent. w/v aqueous solution.

PROCEDURE—

Dissolve 100 mg of the sample containing sulphonamide, *viz.*, sulphanilamide, sulphaguanidine, or sulphacetamide sodium, in 100 ml of diluted hydrochloric acid. Transfer by means of a pipette a suitable volume, *e.g.*, 5 ml, of this solution to a 50-ml conical flask, add 5 ml of acetic acid, *i.e.*, an equal volume, and 2 drops of methyl red indicator solution. Shake the mixture continuously and titrate it with *N*-bromosuccinimide solution from a microburette, graduated at 0·01-ml intervals. The end-point is reached when the last drop of titrant added decolorises the methyl red indicator. Perform a blank experiment under parallel conditions, without the sulphonamide, and subtract the blank value from the titre before calculating the sulphonamide content of the sample.

Calculate the sulphonamide content of the sample from the expression—

$$\text{Sulphonamide present, mg} = \frac{Mvc}{356}$$

where M is the molecular weight of the sulphonamide in the sample,
v is the titre of the *N*-bromosuccinimide solution in ml and
c is the concentration of the *N*-bromosuccinimide solution in grams per litre.

APPLICATIONS OF THE METHOD

PURE SULPHONAMIDES—

A 0·1 per cent. solution of the pure sulphonamide was prepared by dissolving 100 mg of the sulphonamide in sufficient 10 per cent. hydrochloric acid to produce, in a calibrated flask, 100 ml of solution. The sulphonamide was then determined by the proposed method; the results are shown in Table II.

TABLE II

DETERMINATION OF PURE SULPHONAMIDES BY THE PROPOSED METHOD

Sulphonamide	Volume of 0·1 per cent. sulphonamide solution used, ml	Sulphonamide content, mg	Titre of of 0·1 per cent. w/v *N*-bromo-succinimide solution, ml	Sulphonamide found, mg	Error, %
Sulphanilamide	1	1	2·05	0·99	1·00
	2	2	4·15	2·00	Nil
	3	3	6·25	3·02	0·67
	4	4	8·25	3·99	0·25
	5	5	10·40	5·02	0·40
	6	6	12·40	5·99	0·17
	7	7	14·55	7·03	0·43
	8	8	16·55	8·00	Nil
	9	9	18·70	9·03	0·33
	10	10	20·80	10·05	0·50
Sulphaguanidine ..	1	1	1·53	1·00	Nil
	2	2	3·05	1·99	0·50
	3	3	4·55	2·97	1·00
	4	4	6·15	4·01	0·25
	5	5	7·65	4·99	0·20
	6	6	9·10	5·94	1·00
	7	7	10·80	7·05	0·71
	8	8	12·35	8·06	0·75
	9	9	13·90	9·07	0·78
	10	10	15·35	10·02	0·20
Sulphacetamide sodium	1	1	1·40	1·00	Nil
	2	2	2·80	2·00	Nil
	3	3	4·25	3·03	1·00
	4	4	5·65	4·03	0·75
	5	5	7·05	5·03	0·60
	6	6	8·40	6·00	Nil
	7	7	9·85	7·03	0·43
	8	8	11·30	8·07	0·88
	9	9	12·70	9·07	0·78
	10	10	14·15	10·10	1·00

1 ml of 0·1 per cent. *N*-bromosuccinimide ≡ 0·4831 mg of sulphanilamide,
0·6525 mg of sulphaguanidine or
0·7140 mg of sulphacetamide sodium.

A series of comparative analyses was carried out on 0·5 g of each pure sulphonamide by the proposed method and by the recent nitrite method,[7] in which *p*-dimethylaminobenzaldehyde is used as an internal indicator; the results are shown in Table III.

COMPOUNDED SULPHONAMIDES—

The proposed method was used for determining sulphaguanidine in sulphaguanidine tablets B.P. and in a specially prepared mixture, containing—

Streptomycin sulphate	1·00 g
Sulphaguanidine B.P.	8·00 g
Light Kaolin B.P.	8·00 g
Sucrose up to	37·00 g

One tablet or 1 g of the special mixture was assayed by the proposed method; 0·5 g of charcoal, subsequently filtered off, was used for decolorising the yellow solution. The results are given in Table IV.

TABLE III

COMPARISON OF RESULTS FOR PURE SULPHONAMIDES BY THE PROPOSED METHOD AND THE NITRITE METHOD

Sample contained 500 mg of sulphonamide

Sulphonamide	Nitrite method		*N*-Bromosuccinimide method	
	Found, mg	Error, %	Found, mg	Error, %
Sulphanilamide	485	3	502·42	0·48
Sulphaguanidine	490·62	1·88	496·55	0·69
Sulphacetamide	488·06	2·39	504·80	0·96

TABLE IV

ASSAY OF COMPOUNDED SULPHONAMIDES

Sulphaguanidine	Content, mg	Found, mg	Error, %
Tablets B.P.	500	505·4	1·08
Preparation	216·2	213·9	1·05

DISCUSSION OF RESULTS

The end-point of the nitrite method[7] is not easily detected, since the colour change of the indicator (*p*-dimethylaminobenzaldehyde) from yellow to colourless is not sharp.

The proposed method can be applied to determine amounts of as little as 1 mg of the sulphonamide. The experimental error, as deduced from the results in Tables II and III, does not exceed ± 1 per cent. The results suggest that the proposed method is superior to the nitrite method of Fischbach[7] in accuracy and sensitivity.

The method has been successfully applied to pharmaceutical preparations that have had excipients such as starch, kaolin and sucrose, and gave reproducible results.

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SHORT PAPERS

The Paper Chromatography of Some Organo-tin Compounds Part II*. Reversed-phase Systems

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Since our first paper,[1] Franc, Wurst and Moudry[2] have separated a number of organo-tin compounds by using reversed-phase paper chromatography with white spirit or a high boiling-range petroleum fraction as the stationary phase and developing with aqueous ethanol - acetic acid mixtures, and diaryl-tin compounds have been separated on an olive oil stationary phase.[3] Thin-layer chromatography with silica gel has been used for the separation of dialkyl-tin compounds with acidic solvents,[4] and for the separation of trialkyl- and aryl-tin compounds with basic solvents.[5]

We have used reversed-phase paper chromatography to separate a number of organo-tin compounds from one another.

Experimental

Organo-tin compounds—

These were supplied by Prof. G. J. M. Van der Kerk, Organic Chemistry Institute, T.N.O., Utrecht, The Netherlands, or by Pure Chemical Ltd., Liverpool, and suitably purified, if necessary.

Systems used—

The systems used in the investigations were as given below—

(*i*) *Stationary phase*—Dinonylphthalate.
Developing solvent—Methanol - N hydrochloric acid in the ratios (3 + 1), (1 + 1) and (1 + 3), v/v.

(*ii*) *Stationary phase*—Tritolylphosphate.
Developing solvent—Methanol - N hydrochloric acid, (1 + 1).

(*iii*) *Stationary phase*—2-Phenoxyethanol.
Developing solvent—2,2,4-Trimethylpentane - acetic acid, (92·5 + 7·5).

Preparation of paper—

Strips of Whatman No. 4 chromatographic paper, 19 cm wide, were cut to allow up to 45 cm for movement of the solvent. These were immersed in a 10 per cent. solution of the stationary phase in ethanol and hung up by a short edge until the ethanol had evaporated (about 45 minutes). It was found that the direction of drainage of this solution in relation to the direction of flow of the developing solvent had no effect on the R_F values obtained.

As previously mentioned,[1] in the case of tetra- or tri-compounds, it is necessary to irradiate the paper for 5 to 10 minutes with an ultraviolet lamp before spraying.

Spray reagent—

Catechol violet solution—A 0·1 per cent. v/v solution in 95 per cent. ethanol.

Procedure

Solutions of organo-tin compounds (1 per cent. in ethanol) were applied at 3-cm intervals across the strips by means of a microlitre syringe. Optimum amounts were about 25 μg; spots containing 150 μg and above showed tailing owing to overloading. Descending-solvent chromatograms were developed in the dark at 20° ± 1° C. By using systems (*i*) and (*ii*), times of development were about 15 hours, during which time the solvent moved 35 to 40 cm. In these systems the developing solvent formed two fronts, the first consisting of pure methanol and the second, 5 to 6 cm behind, was composed of the bulk solvent. Measurements were made to this second (acid) front, whose position was readily detected when the spray reagent was applied. With system (*iii*), the movement of the developing solvent was much more rapid (development time 3½ hours), and only a single front was formed.

* For details of Part I of this series, see reference list, p. 222.

After development, the chromatograms were allowed to dry and were irradiated with ultra-violet light. They were then sprayed with the catechol violet solution and any acid remaining on the paper neutralised with ammonia vapour. R_F values were measured to the centres of the blue spots obtained.

Results

R_F values obtained by using system (*i*) are shown in Table I.

Table I

R_F values obtained for various organo-tin compounds by using system (*i*)

Compound	R_F value Developing solvent, MeOH - N HCl 3 + 1	1 + 1	1 + 3
Me_2SnCl_2	0·96	0·96	0·96
$EtSnCl_3$	0·96	0·96	0·91
Et_2SnCl_2	0·96	0·93	0·88
Et_3SnOH	0·83	0·58	0·29
Pr_2SnCl_2	0·81	0·58	0·32
$(Pr_3Sn)_2O$	0·44	0·06	0·00
$BuSnCl_3$	0·96	0·96	0·88
Bu_2SnCl_2	0·65	0·24	0·06
Bu_3SnCl	0·21	0·00	0·00
Bu_4Sn	0·00	0·00	0·00
Hex_2Sn (laurate)$_2$	0·22	0·00	0·00
Oct_2SnCl_2	0·04	0·00	0·00
Oct_3SnCl	0·00	0·00	0·00
Oct_4Sn	0·00	0·00	0·00
Et_2 lauryl SnOAc	0·05	0·00	0·00
$PhSnCl_3$	0·96	0·93	0·84
Ph_2SnCl_2	0·82	0·32	0·14
Ph_3SnCl	0·28	0·01	0·00
Ph_4Sn	0·00	0·00	0·00

It can be seen from Table I that the R_F values of dialkyl- and trialkyl-tin compounds depend on the total number of carbon atoms in the alkyl chains; thus triethyltin hydroxide has a similar R_F value to dipropyltin dichloride and the R_F values of dihexyltin dilaurate and tributyltin chloride are coincident. If the hydrochloric acid in the developing solvent is replaced by acetic acid, the R_F values obtained are similar, but the above observation regarding the number of carbon atoms no longer applies, so that further separations may be effected. However, the use of acetic acid causes less ideally shaped spots; mono-alkyl-tin and diphenyltin compounds tend to streak.

The mobilities of organo-tin compounds are increased by increasing the alcohol concentration so that the lower-alkyl-tin compounds are best examined with a developing solvent containing 25 per cent. of methanol, whereas the higher alkyls, whose mobility is lower, are best separated with a solvent containing 75 per cent. of methanol. For the separation of di- and trioctyltin compounds, the methanol can, with advantage, be replaced by ethanol, giving R_F values of 0·16 and 0·02 respectively, instead of 0·04 and 0·00.

With any of these solvents, di- and trimethyl- and diethyltin compounds remain close together. However, if dinonylphthalate is replaced by tritolylphosphate, system (*ii*), the R_F values of organo-tin compounds are considerably reduced, so that separation of methyl- and ethyltin compounds is more easily effected. Some R_F values obtained with this stationary phase are shown in Table II.

Table II

R_F values obtained for various organo-tin compounds by using system (*ii*)

Compound	R_F	Compound	R_F
Me_2SnCl_2	0·96	Pr_2SnCl_2	0·20
Me_3SnCl	0·80*	$(Pr_3Sn)_2O$	0·03
Et_2SnCl_2	0·64	Bu_3SnCl_2	0·06
Et_3SnOH	0·29	$(Bu_3Sn)_2O$	0·00

* Rather diffuse spot.

The mobilities of these compounds may be reversed if 2-phenoxyethanol is used as the stationary phase and 2,2,4-trimethylpentane containing 7·5 per cent. of acetic acid as the eluting solvent, system (*iii*). R_F values obtained with this sytem are shown in Table III.

TABLE III

R_F VALUES OBTAINED FOR VARIOUS ORGANO-TIN COMPOUNDS BY USING SYSTEM (*iii*)

Compound	R_F	Compound	R_F
Me_2SnCl_2	0·02	Bu_4Sn	0·96
$EtSnCl_3$	0·00	Hex_2Sn (laurate)$_2$	0·69
Et_2SnCl_2	0·13*	Oct_2SnCl_2	0·85
Et_3SnOH	0·44	Oct_3SnCl	0·95
Pr_2SnCl_2	0·38†	Oct_4Sn	0·96
$(Pr_3Sn)_2O$	0·76	$PhSnCl_3$	0·00
$BuSnCl_3$	0·00	Ph_2SnCl_2	0·10
Bu_2SnCl_2	0·49	Ph_3SnCl	0·27
Bu_3SnCl	0·84	Ph_4Sn	Streaks extending for the length of the paper

* Elongated spot. † Tailing.

By using pieces of Whatman 3 MM chromatographic paper (15 cm × 15 cm), simple mixtures can be adequately separated in 20 to 30 minutes by the ascending-solvent technique by using system (*iii*). It is possible to detect 1 per cent. of tributyltin chloride in dibutyltin dichloride by using this method, and organo-tin stabilisers in poly(vinyl chloride) can be examined. About 0·5 to 1 g of the poly(vinyl chloride) is chopped into small pieces, which are refluxed for 1 hour with 10-ml of petroleum spirit. The solvent is decanted, and 10 or 20 μl are applied to a square of Whatman 3 MM chromatographic paper and the paper developed. In this way it is easy to ascertain whether the stabiliser is a dibutyl- or a dioctyltin compound, and semi-quantitative estimations may be obtained by comparing the colour and size of the spots with those produced by known amounts of stabiliser.

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NOTE—Reference 1 is to Part I of this series.

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An Examination of the Occurrence of Honeydew in Honey, Part III*

BY A. C. COWAN AND T. J. MITCHELL

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HONEYDEW honey is formed in periods of prolonged drought, when the normally sufficient supply of nectar is greatly diminished. In order to augment their supply of honey, bees turn to other sources of sugar and frequently collect honeydew, a sweet and sticky fluid excreted on foliage by leaf-sucking insects. The identification of honeydew honey is important because of its detrimental effect on the bee colony, and its dark colour and rank flavour, which makes it unsuitable for public use.

From the analysis of forty-two honeys, Kirkwood, Mitchell and Smith[1,2] derived the equation—

$$X = -8{\cdot}3x_1 - 12{\cdot}3x_2 + 1{\cdot}4x_3$$

where X is a linear discriminant function, x_1 is the pH, x_2 is the percentage of ash and x_3 is the percentage of reducing sugars. The numerical value of X of any sample identifies the presence of honeydew honey; X is less than 73·1 for honeydew honeys and greater than 73·1 for floral honeys.

It is apparent, from the equation, that several analytical determinations are required to classify a honey sample. Thus it would assist the beekeeper if a simple test was available by which the harmful honey could be detected.

* For details of Parts I and II of this series, see reference list, p. 226.

This paper summarises work confirming the efficiency of the linear discriminant function X, and investigating the use of simple tests as a means of honey classification. Two such tests were examined—

(*i*) determination of uric acid and

(*ii*) determination of hydroxymethyl furfural.

Uric acid, which forms the principal end-product of the nitrogenous metabolism of insects, is probably the only excretory substance present in detectable amounts in honeydew honeys. Hydroxymethyl furfural, $C_6H_6O_3$, may be formed in honeys by the acid hydrolysis of the reducing sugars, but other factors, including age and climatic conditions, may also be of importance.

Methods

Ash, pH and moisture—

The samples were cleaned and the pH, ash and moisture contents were determined as described previously.[3]

Reducing sugars—

Lane and Eynon's volumetric method,[4] involving reduction of Fehling's solution with methylene blue as internal indicator, was used.

Uric acid—

Uric acid is normally determined by colorimetric methods, but because of the high sugar content and colour of the honey, these methods were rejected, and a chromatographic procedure was adopted. The interfering neutral constituents of the honey were removed by passing the honey solution through an Amberlite IRA-400(OH) resin. The uric acid was eluted with dilute hydrochloric acid and partitioned by using the n-butanol - acetic acid - water system. The chromatograms were prepared by the standard method by using an ascending-solvent front. Eight spots, including a uric acid standard, were put on each paper, and the chromatograms were run overnight and tested for uric acid with several location reagents—

(*a*) *Ultraviolet light*—The chromatograms were studied under an ultraviolet lamp and the dark spots produced were examined.

(*b*) *Silver nitrate - bromophenol blue reagent*—This reagent was prepared by Smith's[5] method. The chromatograms were dipped in the reagent, hung up for 10 minutes, washed and dried, and the spots were then studied.

(*c*) *Folin's test*—Brown's[6] preparation of the reagent was used. The chromatograms were sprayed with a 12 per cent. solution of sodium cyanide and then with Folin's reagent.

(*d*) *Mercuric acetate - diphenyl carbazole test*—The procedure of Dikstein[7] was used.

Hydroxymethyl furfural—

Since an aqueous solution of hydroxymethyl furfural exhibits the property of absorbing light, an ultraviolet spectrophotometric method was used for determining the hydroxymethyl furfural content of honey. A 10 per cent. honey solution was analysed by using the method described by Winkler.[8]

Results

The results of the various tests are shown in Table I. The values of the discriminant function, X, gave a satisfactory differentiation between honeydew and floral honeys.

The results of the chromatographic tests for uric acid with the four different reagents varied extensively; they showed little correlation either with each other or with the values of the discriminant function. They were thus so inconclusive as to be of little value, except possibly as a sorting procedure, in the sense that when all four tests are negative the sample may be accepted as floral (in our series of samples there is no exception to this), but if one of the tests is positive the sample may be either floral or honeydew and an estimation of the discriminant function is called for.

The analyses for hydroxymethyl furfural produced contents ranging from 0 to 26 mg of hydroxymethyl furfural per 100 gm of honey. Little correlation was obtained between the hydroxymethyl furfural content and the discriminant function, X, but it was observed that two-thirds of the samples over ten years old had a relatively high hydroxymethyl furfural content. The method gave reproducible results and was straightforward in operation.

TABLE I

VALUES FOUND FOR VARIOUS HONEYS

Sample No.	Location	Type of honey	pH	Ash on dry material, per cent.	Invert sugars on dry material, per cent.	Hydroxymethyl furfural content of dry material, mg per 100 g
1	Dinnet	F	4·13	0·43	88·5	
2	Dinnet	F	4·06	0·35	84·5	
3	Ballindarroch	F	3·91	0·35	81·8	
4	Muir of Ord	F	4·09	0·12	92·3	
5	Muir of Ord	H	4·16	0·33	67·7	
6	Ballindarroch	H	4·40	0·63	77·5	
7	Muir of Ord	F	4·05	0·20	87·8	
8	Alness	F	3·81	0·12	83·9	
9	Ballindarroch	H	4·69	0·71	82·2	
10	Alness	F	3·98	0·20	85·6	
11	Ballindarroch	H	4·06	0·81	77·4	
12	Ballindarroch	F	3·82	0·30	87·9	
13	Ballindarroch	F	3·78	0·41	83·5	
14	Ballindarroch	F	3·44	0·24	83·3	
15	Ballindarroch	F	3·58	0·58	83·0	
16	Ballindarroch	F	3·64	0·54	83·7	
17	Ballindarroch	F	3·52	0·31	88·9	
18	Dinnet	F	3·30	0·10	88·7	
19	Pyrenees	F	4·18	0·41	89·1	
20	Spain	F	3·98	0·43	81·0	
21	California	F	3·38	0·09	82·2	
22	Warsaw	F	4·24	0·28	87·8	
23	Canada	F	3·83	0·68	86·1	
24	Greece	H	4·53	0·58	69·3	
25	Hungary	F	3·93	0·07	84·5	
26	Scotland	F	3·92	0·07	93·9	
27	Tasmania	F	4·36	0·36	90·7	0·41
28	Greece	F	4·16	0·35	89·5	5·18
29	Greece	H	4·25	0·52	70·2	2·15
30	Argentina	F	3·86	0·01	86·5	2·60
31	Oxford	F	4·37	0·56	88·8	Nil
32	Pembrokeshire	F	3·82	0·12	88·1	2·69
33	California	H	4·69	0·79	80·5	3·42
34	New York	H	4·80	0·67	87·7	26·75
35	New Zealand	F	4·00	0·69	87·4	2·80
36	Austria	H	4·70	0·99	81·7	0·85
37	Switzerland	H	4·58	0·91	74·8	5·10
38	Germany	H	4·92	0·95	77·9	0·60
39	Germany	H	4·83	0·88	67·7	Nil
40	Germany	H	4·96	1·01	76·7	0·78
41	Germany	H	4·48	1·18	75·2	2·38
42	Austria	H	4·78	1·14	75·5	2·61
43	Pitmaduthy, Ross-shire	F	4·31	0·62	84·6	12·32
44	Muir of Ord	F	4·74	0·64	91·9	Nil
45	Alness	F	4·30	0·70	85·2	6·63
46	Ballindarroch	F	4·17	0·65	84·0	6·56
47	Drummondhill	F	4·24	0·16	94·3	3·16
48	Perthshire	F	4·00	0·76	92·6	2·28
49	Kyle of Lochalsh	F	4·99	0·31	95·9	3·18
50	Kyle of Lochalsh	F	5·42	0·56	96·3	Nil
51	Loch Lomondside	F	4·48	0·57	86·2	10·10
52	Kincardineshire	F	4·42	0·43	93·8	6·68
53	Aberdeen	F	4·70	0·49	96·4	Nil
54	Aberdeen	F	4·76	0·85	88·3	Nil
55	Ballindarroch	F	4·82	0·50	91·3	Nil
56	Bristol	F	4·28	0·69	85·5	19·75
57	Greenock	F	4·38	0·51	87·1	3·38
58	New York	F	4·68	0·50	89·3	25·62
59	Perthshire	F	4·60	0·40	85·0	4·32

TABLE I—*continued*

Sample No.	Moisture, per cent.	Uric acid detection with— ultraviolet light	$AgNO_3$ – BPB	Folin's reagent	diphenyl carbazole	Discriminant factor
1	15·4	+	–	–	+	84·9
2	17·8	–	–	–	–	80·9
3	16·8	–	–	–	–	77·5
4	16·7	–	–	–	–	93·2
5	18·0	+	–	–	–	56·2
6	18·7	+	–	–	+	64·1
7	16·4	–	–	–	–	86·9
8	17·8	–	–	–	–	84·5
9	19·8	–	+	+	–	67·4
10	16·0	–	–	–	–	84·7
11	15·9	–	–	–	+	64·8
12	18·0	–	+	–	–	87·4
13	18·5	+	–	–	–	80·0
14	16·0	–	–	–	–	90·2
15	17·5	–	–	–	+	79·4
16	18·6	+	–	–	–	79·0
17	17·7	–	–	–	–	91·5
18	15·9	–	+	–	+	96·2
19	21·1	–	–	–	–	93·1
20	17·2	–	–	–	–	75·5
21	18·2	–	–	–	–	91·1
22	17·2	–	–	–	–	84·0
23	16·9	–	–	–	–	80·2
24	17·1	+	+	–	–	52·6
25	17·1	–	–	–	–	84·8
26	18·2	–	–	–	–	98·9
27	17·6	+	–	–	–	86·8
28	18·0	+	–	–	–	86·7
29	17·8	+	–	+	–	56·6
30	18·3	–	–	–	–	89·1
31	23·0					81·0
32	16·8					88·1
33	14·5					62·2
34	17·7					72·6
35	15·3					78·6
36	15·0					63·2
37	15·0					55·6
38	13·1					55·5
39	16·3					43·8
40	14·1					53·7
41	13·4					53·5
42	16·4					52·1
43	16·6					74·9
44	18·7					81·3
45	16·2					74·9
46	17·8					75·1
47	16·3					94·8
48	19·0					87·1
49	17·9					88·9
50	19·9					82·8
51	17·7					76·5
52	16·2					89·1
53	18·7					89·8
54	18·3					73·5
55	19·5					81·8
56	17·1					73·7
57	16·6					77·3
58	17·6					77·9
59	16·2					73·9

We are indebted to D. Goldrich, N. McInnes and W. I. Raybould for collaboration in the practical work, and also thank beekeepers in various parts of the world who supplied samples of honey.

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NOTE—References 1 and 2 are to Parts I and II of this series, respectively.

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The Micro-determination of Hydrazine Salts and Certain Derivatives

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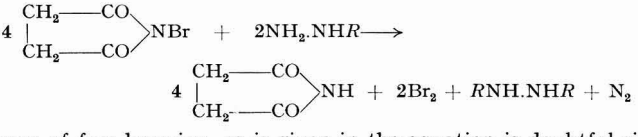
THE titrimetric method for determining hydrazine and certain derivatives with *N*-bromosuccinimide in the presence of dilute sulphuric acid described by Barakat and Shaker[1] has been used successfully for determining hydrazine. However, we are unable to agree with the equation for the reaction between *N*-bromosuccinimide and mono-substituted aromatic hydrazines as set out in their published article.[1] This equation is given below—



The presence of free bromine, as is given in the equation is doubtful since it is as effective, both as an oxidising agent for hydrazines, and in decolourising methyl red, as is *N*-bromosuccinimide itself. The oxidation of an aromatic hydrazine normally gives the corresponding azo compound[2] and not hydrazobenzene as shown in the equation.

Studies in this laboratory on the reaction between *N*-bromosuccinimide and phenylhydrazine hydrochloride under the conditions described by Barakat and Shaker[1] have shown—

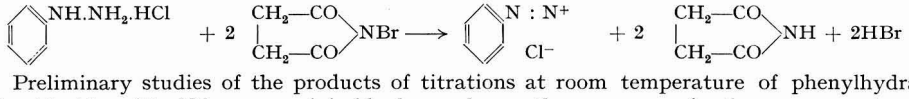
(*i*) An accumulation of free bromine is not detectable during the titration because the indicator does not change until the equivalent of 2 molecules of *N*-bromosuccinimide per molecule of aromatic hydrazine have been added.

(*ii*) Bromide ions are detected at the end-point by subsequent titration with silver nitrate solution.

(*iii*) When the reaction liquors are subsequently cooled to 0° C, and are added to an excess of β-naphthol in sodium hydroxide at 0° C, a precipitate of phenylazo-β-naphthol, identified by its melting-point and infrared spectrum, is recovered.

(*iv*) Hydrazobenzene reacts with *N*-bromosuccinimide to give coloured products.

From the above facts it is thought that the reaction proceeds initially according to the equation—



Preliminary studies of the products of titrations at room temperature of phenylhydrazine hydrochloride with *N*-bromosuccinimide have shown the presence of other compounds, some derived from the decomposition of the benzenediazonium salt, but including others brominated on the aromatic nucleus.

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Received *June* 4*th*, 1963

A Simple All-glass Dispenser

By G. M. LEET and BARBARA M. LEE
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The need has frequently arisen in this laboratory for a rapid and reliable dispenser capable of dealing with corrosive liquids of various viscosities. The apparatus described in this paper can be constructed from standard items of laboratory glassware.

The apparatus

The unit (see Fig. 1) is clamped to the laboratory bench at a suitable height. The outlet tube of the Dreschel-bottle head is connected to a water-operated suction pump via a T-joint. The inlet tube of the Dreschel-bottle head is connected to a polythene tube that leads to the vessel containing the liquid to be dispensed. This vessel is positioned lower than the dispenser.

When the side-arm of the T-joint is closed, a partial vacuum is created, and liquid is drawn from the storage vessel into the dispenser. When the side-arm of the T-joint is re-opened, any excess of liquid in the dispenser is automatically syphoned back into the storage vessel. The amount of liquid dispensed by the unit is thus determined by the length of the tube, A. The appropriate length of this tube can readily be found by placing the required volume of liquid into the dispenser, noting the level of liquid on the tube, and cutting the tube off at this level.

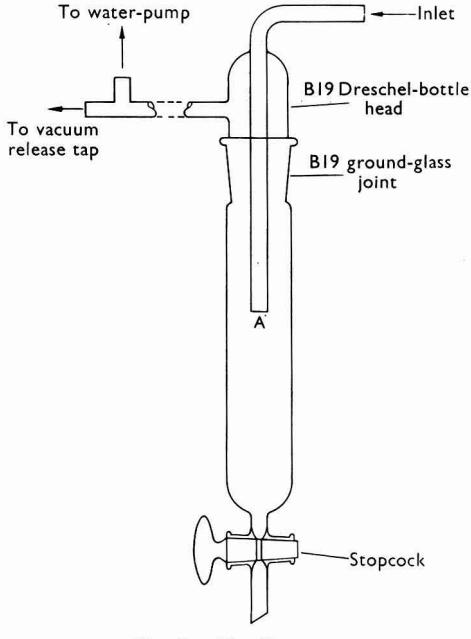

Fig. 1. The dispenser

Advantages of the dispenser—

(*i*) It is accurate and simple in operation.

(*ii*) The dispenser may be connected directly to the storage vessel, thus eliminating any need for transferring concentrated acids manually; there is, therefore, no hazard to the operator.

(*iii*) As all parts of the apparatus are standard items of laboratory equipment, the dispenser can be constructed by laboratory staff who have a minimum knowledge of glass-working.

Received *September 24th*, 1963

Book Reviews

CHARACTERISTIC FREQUENCIES OF CHEMICAL GROUPS IN THE INFRA-RED. By M. ST. C. FLETT. Pp. x + 98. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 25s.

The title of this slim monograph, in which *infra-red* is used as a noun, might cause some surprise. I believe that the acceptance of the title by the publishers is merely a tribute to the wide acceptance of infrared-absorption spectroscopy by modern industrial and academic chemists, The volume, which has been compiled by Mr. Flett, a specialist in spectroscopic methods at the Dyestuffs Division of Imperial Chemical Industries Ltd., consists essentially of a set of correlation tables, listing in order of decreasing wave-number, the absorption ranges for various organic chemical groups and of a series of short tables listing the ranges of the principal absorption bands associated with seventy-four classes of organic compounds; most of the lists are accompanied by short bibliographies containing selected references for further reading. When available, apparent and absolute extinction-coefficient values are also included.

The band lists, so far as they go, are fairly reliable, but specialists will notice several gaps, such as the absence of bands for assigning the structures of sugars and of steroidal sapogenins; likewise the bands used to distinguish between axial and equatorial substituents in cyclohexane and steroid derivatives are not mentioned. Unfortunately, in a book of this size it is not possible to discuss, in detail, factors such as steric effects, hydrogen bonding and conjugation, that can cause absorption bands to move widely inside, and sometimes outside, the ranges quoted in the tables. For detailed information of this kind, which can often help to identify the precise position of a particular group in a complex molecule, the reader must consult the references given in the bibliographies.

Throughout the book, the positions of bands are reported in both wave-numbers and wavelengths, the latter being given in several places, for no apprent reason, to three decimal places. Space would have been saved and the text made easier to read by using wave-numbers and providing a table of reciprocals for the benefit of chemists who prefer to use wavelength units.

The monograph is, in general, well-produced and easy to use; there are, however, a number of printing errors, most of which, fortunately, are fairly obvious and, beyond causing the reader some irritation, are unlikely to impair the practical value of the book. The volume is reasonably up-to-date and can, within the limitations mentioned, be recommended as an aid to organic chemists for the qualitative interpretation of infrared spectra. J. E. PAGE

MODERN METHODS OF PLANT ANALYSIS. Volume VI. Founded by K. PAECH and M. V. TRACEY. Continued by H. F. LINSKENS and M. V. TRACEY. Pp. xxiv + 512. Berlin, Göttingen and Heidelberg: Springer-Verlag. 1963. Price DM 98.

As with the preceding volumes, this new, sixth volume is the work of several experts, some contributing in English and some in German.

The book divides into two main parts. The first 295 pages follow the broad pattern of Volumes II to IV, and are concerned with the assay of groups of naturally occurring substances. The remainder of the volume, about 200 pages, is concerned with general enzyme chemistry.

In the first part, some chapters are about the assay of substances classified according to chemical constitution: these include sulphydryl, silicon and acetylene compounds, phosphatides, glycolipids, chromones and giberellins. Other chapters consider substances that occur in hops, bacterial cell-walls and lichens. Biological activity is the basis of classification in chapters on substances promoting cell division, substances toxic to plants and on phytaglutinines.

The first part of the book, as usual, provides not only information about assay procedures, but also of many aspects of the occurrence, preparation, purification and general chemistry of the substances treated.

The section on enzyme chemistry is necessarily different. Information essential for intelligent enzyme assay is set out in chapters on the assay of enzyme activity, general characteristics of enzymes and interpretation of results. There is a chapter on the Thunberg technique for the assay of dehydrogenases and reference to earlier volumes for manometric and spectrophotometric methods. Unfortunately, there are no references to recent developments of electrochemical assay procedures, especially those for dissolved oxygen. It seems likely that such omissions may be made good in the next volume. The chapter on the preparation of enzymes gives an excellent

précis of the various procedures available for the preparation of both cell-free extracts and subcellular particles. Two chapters systematically set out methods available for enzyme purification; they include the most recent developments of ion-exchange chromatography, electrophoresis and gel-filtration. Basic theories of enzyme inhibition are clearly and concisely set out in a chapter that also gives useful lists of enzyme inhibitors and activators. Chapters on the assay of enzymes in soil and on the application of enzymes to the assay of some amino- and keto-acids and the assays of a variety of metabolites including adenylic acids, organic acids and sugars and their phosphates complete this section.

This second part of the book on enzyme chemistry provides much more than an introduction to the subject. There are obvious gaps, but in an extensive and rapidly growing subject this is to be expected. Its great value is the practical approach to the subject without neglecting the basic theories.

In the volume as a whole, treatment is understandably not uniform, because of the diversity of subject matter. Analysis, be it chemical, biochemical or biological is the unifying theme, but not the preoccupation of the authors. The information gathered in this volume has a wider application than analysis, and it should be valuable to anyone interested in the subjects treated.

K. A. LORD

TECHNIQUE OF ORGANIC CHEMISTRY. Volume VIII. Part II. INVESTIGATION OF RATES AND MECHANISMS OF REACTIONS. Edited by S. L. FRIESS, E. S. LEWIS and A. WEISSBERGER. Second Edition. Pp. xii + 703–1582 + Index (21 pages). New York and London: Interscience Publishers, a division of John Wiley & Sons. 1963. Price £11.

The appearance of a further volume of Weissberger will always excite the interest of any chemist concerned with the precise measurement of the physical properties of highly purified chemical compounds. Volume VIII, which deals with rates and mechanisms of reactions, has been issued in two parts, of which Part I deals exclusively with kinetic methods, whereas the volume under review, Part II, discusses both kinetic and non-kinetic methods.

While it is a truism to state that such treatises will inevitably become out-dated in a relatively short time, it is extremely refreshing to find such a combination of acknowledged authorities as Roughton, Melville, Eigen, Burnett, Porter, etc., writing so concisely on their own individual fields of work. The style is eminently readable and sufficient detail is included so that it can be consulted profitably by anyone confronted with a problem in an unfamiliar field of investigation. Owing to the wide range of topics falling under the "kinetic umbrella," few chemists will find the whole compilation of equal interest; but it should be emphasised that there is scarcely anyone whose interest and resourcefulness would not be whetted by reading some of the contributions.

Chapter 14 (Roughton and Chance), which should be read by all seeking information on kinetic methods, is a judicious blend of theory and practice and bears the stamp of experienced investigators giving the uninitiated the benefit of their unrivalled experience. Chapters 15 to 20 deal extensively with the kinetics of extremely rapid reactions in solution (half-time < 1 millisecond) and obviously owe much to Eigen, who has contributed a valuable section on relaxation methods. The latter may be rather heavy going for the non-mathematical reader, but some effort should be made to appreciate its relevance to the technique of subjecting systems in equilibrium to artificial shock treatments. In this way the succeeding excellent chapters on flash photolysis and active intermediates in solution by Porter and Burnett, respectively, will be more thoroughly comprehended. Chapter 15, which describes electrochemical methods, provides much valuable information for workers in the polarographic and potentiometric fields, who may not profess any particular interest in kinetics.

Burnett has written a good, up-to-date section on polymerisation, a subject that can scarcely be disregarded by any organic chemist, whatever his particular predilections. Just as advances in the structural chemistry of, say, nucleic acids have tended to lag behind those of classical organic chemistry, the kinetic study of enzyme reactions is much less advanced than that of other reactions in solution, judging from Chapter 22.

The second portion dealing with non-kinetic methods, *i.e.*, mechanisms of reactions, should be regarded as complementary to the first portion. In some respects it appears less valuable than the former, as a considerable amount of the subject matter requires a more detailed treatment, such as will be found in Ingold's "Structure and Mechanism in Organic Chemistry." This comment does not apply to Chapter 26, which gives a valuable survey in fair depth of the applications and limitations of isotopes and tracers in the elucidation of the mechanism of organic reactions.

Finally, this volume is likely to remain a valuable work of reference for a long time, in spite of the increasing pace of new developments in this field. It is well printed, the few misprints that occur are of a non-technical character and the numerous references to the literature at the end of each chapter extend in several instances to 1962. There are several references to some of the earlier volumes; but as the price of this volume is necessarily high it will be found mainly in the research libraries, where the companion volumes can be consulted for purposes of cross-reference. At the end of Part II there is a cumulative subject index to both parts of Volume VIII, which appears rather brief coverage for 1582 pages. F. G. ANGELL

ANALYTICAL METHODS FOR PESTICIDES, PLANT GROWTH REGULATORS, AND FOOD ADDITIVES. Edited by GUNTHER ZWEIG. Volume 1, Principles, Methods, and General Applications. Pp. xiv + 637. New York and London: Academic Press. 1963. Price 171s. 6d.

This is the first of a four-volume work on analytical methods and, as such, is an introductory volume. Those in preparation are to deal with insecticides; fungicides and miscellaneous chemicals; and herbicides. The present volume is divided into twenty-three chapters, written between them by twenty-seven authors, including the series editor, all, with one exception, from the United States. It is divided into three sections according to the title, the first six chapters constituting the first section and dealing with the basic processes of formulation and (this being the main theme) residue analysis and toxicological testing. The second section (thirteen chapters) deals with individual analytical methods, from spectrophotometry to neutron-activation analysis and includes a separate, but rather brief, chapter on the organisation of the pesticide residue laboratory. The final section (four chapters) includes applications of the methods to the analyses of specific types of food.

It is perhaps not clear from the series title that the work is concerned almost exclusively with pesticides (in the broadest sense); preservatives, anti-oxidants, colours and other food additives are considered only relatively briefly and certainly not systematically. Even so, the subject is a large one and any sub-division is bound to face difficulties of duplication. In describing firstly the types of analytical methods available and then the applications of those that are appropriate to individual classes of food, some care has been taken to give the latter chapters a general review character to avoid the repetition of detail from previous chapters. There are cross-references where necessary. As might be expected, the text is written with North American legislation in mind: the chapter on analysis in government laboratories is thus somewhat parochial. There is, perhaps, an emphasis on residues in relation to field trials, as in the chapters on the principles of residue analysis. In several of the practical chapters the descriptions are a mixture of the definitive and the tentative. This is a consequence of the fact that residue analysis has advanced rapidly in recent years; and is still advancing. The applications of gas chromatography, for example, were only beginning to reach the periodical literature when the present volume was compiled and many of the references are to conference abstracts or to commercial literature. Despite the difficulties, there is need for speed in the textbook presentation of such material: the obvious risk of obsolescence has attracted an alternative, review-type periodical. But the present volume, notwithstanding its somewhat misleading title, promises the prospect of a most useful series on the analysis of pesticide residues. H. EGAN

ION EXCHANGE SEPARATIONS IN ANALYTICAL CHEMISTRY. By OLAF SAMUELSON. Pp. 474. Stockholm, Goteborg and Uppsala: Almqvist & Wiksell; New York and London: John Wiley & Sons. 1963. Price 72s.

This book is based on the author's *Ion Exchangers in Analytical Chemistry*, first published in 1953. In general, this is not so much a new book as an extended version of the old. This does not necessarily detract from the value of the book since the grafting operation has been done with skill. As an example, a short section on the application of the plate theory to ion-exchange columns is included where previously the subject was only briefly mentioned in three separate places. This is characteristic of much of the book. It gives the general impression that considerable work has been done in collecting information for more useful presentation to the reader.

The number of applications of the ion-exchange method cited has been considerably extended and the reader should find the field adequately covered. Only in one respect is there a serious omission; organic applications of the technique, which formerly occupied some thirty pages, are

now deliberately discarded. Although this allows a fuller treatment of the inorganic applications, the book is of less value to the general reader on account of the change.

Chapter XV comprises some hundred-odd pages of text dealing with metal separations. This is new matter as far as Samuelson is concerned.

Tables of distribution coefficients of both cations and anions are given, and numerous inorganic separation systems are discussed. These tables are concerned with alkali metals, alkaline-earth elements, rare-earth elements and actinides by using both cation and anion exchangers. The separation of the elements of groups IB, IIIB, IVA and IVB and of the periodic table are given relatively full treatment. A short chapter is devoted to the separation of anionic species.

The extended inorganic section is not in itself sufficiently comprehensive to render the book valuable for that alone; the omission of much of the organic field previously covered leaves the reader with an unbalanced view of the subject.

The system of textual references in which the initial letter of the first name cited precedes the reference number, causes the reader intense irritation since it appears to serve no purpose. The references are, in any case, listed with the first author's name in alphabetical order. This is perhaps more a matter for editor and publisher than for the author.

The production of the book is excellent and the indexing adequate. Fuller treatments of the inorganic and organic fields exist but, in spite of this, the book is both readable and useful in its content.

E. Q. LAWS

ADVANCES IN X-RAY ANALYSIS. Volume 6. Edited by WILLIAM M. MUELLER and MARIE FAY. Pp. xii + 480. New York: Plenum Press Inc. 1963. Price $17.50.

The techniques of X-ray analysis are now well established among the many valuable physicochemical tools available to the modern analyst. X-ray diffraction methods have long been used in the identification, and sometimes in the determination, of phases and in the measurement of crystallite size and orientation; X-ray fluorescence spectrometry is now widely used for elemental analysis, at trace to major-component levels, to obtain results in a much shorter time than is possible by classical methods with no loss in precision, and electron-probe spectrometric methods are coming into use for elemental analysis of selected very small surface volumes of poly-component materials. Progress in this field during the past 10 to 15 years has been so rapid that it has been difficult for the practising analyst to keep abreast of developments, which, because of the borderline nature of the subject, have appeared in papers published in a wide variety of journals. To help in this direction, the University of Denver in 1952 sponsored the first of a series of annual conferences on the industrial application of X-ray analysis. From a small beginning these conferences have increased in scope and support, and since the VIth, the Proceedings have been published in book form. These volumes have proved to be most useful in bringing together papers on advances in the theory, instrumentation and practical applications of the several X-ray analytical techniques.

The sixth volume at present under review includes all the 44 papers delivered at the XIth conference held in August, 1962, and again there is much valuable information for the analyst using X-rays, whatever his speciality. With the wide range of topics covered, however, any selection of papers for comment must be arbitrary and reflect the interests of the reader.

In the field of X-ray diffraction analysis, papers on applications of diffractometry also give information on high- and low-temperature and helium-path techniques. There is a critical assessment of diffractometric methods used in the determination of crystallite size. A useful survey is given of the factors governing the precision obtainable in powder diffractometry and methods are given for indexing powder-diffraction patterns.

In the field of X-ray fluorescence spectrometry, much useful information is given on the application of the method to the analysis of a variety of materials ranging from petroleum products and polymers, to minerals, metals and alloys. The use of filters in fluorescence spectrometry is discussed, and details are given of methods used to improve the sensitivity and precision of determination of sodium and magnesium, which are, at the present, the lower atomic-number limit of the method. One paper discusses design considerations for on-stream X-ray fluorescence analysis.

Recent advances in the field of electron-probe analysis are described in three papers, and in particular, information is given on work that is being carried out to extend the atomic-number limit of the method to elements below sodium in the periodic table.

The main value of this book lies in the information given on developments in the application of X-ray analytical techniques, and for this reason doubt may perhaps be cast on the virtue of

including papers on the theoretical discussion of results, in particular in the field of structure analysis, unless there is special interest in the method by which the results are obtained.

Unlike the earliest volumes in this series, the present volume is very well produced, and, in spite of the obvious difficulties, a real attempt has been made to include a useful index. Although the price of the book is high, it must join the earlier volumes of the series on the bookshelf of every analyst involved in work with X-rays. D. E. PALIN

CHROMATOGRAPHIE SYMPOSIUM II: 1962. Pp. 310. Brussels: Société Belge des Sciences Pharmaceutiques. 1963. Price Belg. Fr. 150.

This paper-back volume presents the lectures and communications delivered to the second symposium on chromatography organised by the Société Belge des Sciences Pharmaceutiques and held in Brussels on September 14th and 15th, 1962. It contains four conference lectures and thirty-one contributed papers, two of the lectures and five of the papers being in summary only. The language is mainly French (two lectures and sixteen papers), but one lecture and seven papers are in English, and one lecture and six papers in German.

The standard of both lectures and papers is generally high, but somewhat mixed. All important chromatographic techniques are represented, including column, paper and thin-layer chromatography. A number of papers describe methods in which gas chromatography is used, and a few are concerned with ion-exchange resins. Radiochemical methods are incorporated in some procedures and are used as a means of identifying, or following, chromatographic fractions. There is a distinct biochemical and medical bias in the communications, but some papers deal with purely pharmaceutical problems. The reason for the inclusion of one or two papers in a conference of this type is obscure.

The titles of the lectures and communications of analytical importance are listed in *Analytical Abstracts*, **1964**, **11**, 2.

This is a collection of papers that only the specialist in a narrow field will wish to own, but users of chromatography, especially in the field of biochemistry, will find it useful for reference.

G. F. REYNOLDS

ZONE MELTING OF ORGANIC COMPOUNDS. By E. F. G. HERINGTON, D.Sc., A.R.C.S. Pp. viii + 162. Oxford: Blackwell Scientific Publications. 1963. Price 35s.

This book presents an adequate account of the subject. The underlying theory is developed and full practical details of a number of zone-melting techniques is given. Chapter 5 should be of interest to readers of this journal in providing a clear account of some of the ingenious methods of thermal analysis.

In Chapters 2 and 7 the author summarises the complex mathematics in a manner that should not prove too difficult for the chemist with a limited mathematical background to understand. This is achieved in part by the omission of a few basic derivations for which there is some justification when one considers that for organic systems the results must be regarded as a guide rather than given any exact quantitative significance. For those desiring mathematical rigour, a comprehensive list of references is given. I feel, however, that the author has carried the simplification a little too far in places, with the result that the reasoning occasionally becomes a little obscure.

Perhaps a more appropriate title could have been chosen for Chapter 7, greater attention being given to predicting the outcome of zone melting from the study of phase relationships than to the construction of phase diagrams from the analysis of results of zone-melting experiments.

These are, however, minor criticisms and the author is to be congratulated in having so successfully condensed a great deal of information, much of which was quite obviously obtained by first hand experience, into a concise and readable form. K. J. WHITE

Errata

JANUARY (1964) ISSUE, p. 1, 15th line. *For* "to test both" *read* "both to test".

IBID., p. 4, 9th line from foot of page. *For* "calcium DL-pantothenate" *read* "calcium D-pantothenate".

IBID., p. 5, 3rd line from foot of page. *For* "pH 6 to 8" *read* "pH 6·8".