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THEORY OF SOLVENT DISTURBANCE PEAKS AND EXPERIMENTAL DETERMINATION OF THERMODYNAMIC DEAD-VOLUME IN COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

Accurate definition and measurement of the thermodynamic dead-volume, V_m, in liquid chromatography is essential for the correct evaluation of capacity ratios. Many different recipies for determining V_m have been suggested. We propose that V_m be defined as the total volume of all eluent components within the column bed. It is shown that V_m, so defined, is given by

$$V_m = V_{A^*} x_A + V_{B^*} x_B + \ldots$$

where V_{A^*} etc. are the elution volumes of isotopically labelled eluent components A etc., and x_A etc. are the volume fractions of A etc. in the eluent fed to the column. For an $(N+1)$ component eluent there will be $(N+1)$ such peaks. If a mixture of same eluent components but with different composition is injected into the column, N solvent disturbance peaks will be obtained which, in general, will not coincide with the peaks for labelled eluent components.

The cases of binary and ternary mixtures are examined in detail and the transition from peaks due to trace components into solvent disturbance peaks is explored and clarified. The treatment is generalised to $(N+1)$ component mixtures and leads to important results relating to vacancy chromatography.

Experimental data are presented for binary and ternary eluents which provide practical validation of the above equation. For binary eluents, A + B, the same data allow calculation of partition isotherms for A and B between bulk eluent and space within the column bed, while the elution volumes of the solvent disturbance peaks in A + B give the gradient of the isotherm. This theoretical connection is accurately confirmed by our experimental data and by that of previous investigators [R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249].

On the basis of our theory and experimental data, a critique is given of the various methods currently claimed to give values for V_m.

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INTRODUCTION

The determination of the dead-volume of a column in liquid chromatography (LC) presents both theoretical and practical problems. First of all, there are two independent quantities which occur in the theoretical treatment: these are the dead-volume required for treatments of the kinetic and thermodynamic aspects of chromatography respectively. The kinetic dead-volume which we denote by V_0 is the volume of the mobile zone, that is the eluent in the interparticle space: the thermodynamic dead-volume includes an additional volume of eluent within the pores of the particles of column packing and is denoted by V_m.

This paper concerns only the definition and determination of V_m. A more serious theoretical difficulty arises from the definition of the mobile and stationary phases in LC, especially reversed-phase LC. Since the mean pore diameter in reversed-phase packings is around 10 nm and since the "thickness" of any liquid–liquid phase boundary is around 1 nm (*i.e.* going from composition virtually indistinguishable from bulk mobile phase to composition virtually indistinguishable from bulk stationary phase) it is not possible to define the position of this boundary sufficiently well to give an accurate measure of the relative volumes of the two phases. The situation is further complicated by the fact that any bonded stationary phase will preferentially adsorb certain components from eluents. Are these adsorbed eluent components to be considered as part of the mobile phase or of the stationary phase? If they are to be considered part of the stationary phase then how do we define and measure the amounts adsorbed?

An unambiguous definition of what constitutes mobile phase and what constitutes stationary phase in the context of LC using porous packing materials is impossible. Nevertheless, if we are to make any thermodynamic measurements, which include simple measurements of column capacity ratios, we must provide a clear-cut definition of dead-volumen V_m in order that the formula for capacity ratio, $k' = (V_R - V_m)/V_m$, can be used and data compared between laboratories. The value used for V_m and its definition should ideally be the same for all eluents and should be readily determined with adequate precision.

In a broad sense, V_m is very often stated to be elution volume of an unretained and unexcluded solute. Unfortunately, this statement begs many questions and has led to much confusion, especially in regard to how V_m should be measured. Horváth and Lin[1] give a clear introduction to this topic pointing out some of the pitfalls in determining V_m, but without explaining how exactly it should be measured in practice.

Berendsen *et al.*[2] reviewed the various experimental techniques which authors have claimed to give V_m, and compared them experimentally without coming to any final conclusion as to the best method. Wells and Clark[3] have also discussed the problem of determining V_m and measured the elution characteristics of a number of possible solutes which could act as markers. Unfortunately, neither Berendsen *et al.* nor Wells and Clark clearly define what they mean by V_m, apart from the rather general statement that it is the elution volume of an unretained and unexcluded solute. The problem, of course, is to decide which, if any, solute is both unretained and unexcluded. In fact, the measurements of elution volumes without any other criterion cannot provide an answer.

The following recipes have been advocated for the determination of V_m:

(1) V_m is the elution volume of a solvent disturbance or system peak obtained by injecting an eluent component[4–9].

(2) V_m is the elution volume of an unionised solute which gives the lowest retention volume and which is small enough not to be sterically excluded[8,10].

(3) V_m is the elution volume of an isotopically labelled component of eluent, for example ^2H_2O, in the case of a reversed-phase packing material[5,11].

(4) V_m is the elution volume of salt or ion, usually a UV-absorbing ion[12–17].

(5) V_m is the volume of liquid which the column contains (obtained, for example, by weighing full of liquid and then empty) less the volume of any adsorbed eluent components[18–20].

(6) V_m is the volume which, when subtracted from the elution volumes V_{Rn} of a series of homologues, provides a linear dependence of log $(V_{Rn} - V_m)$ against n, the number of carbon atoms in the homologues[2,21].

We are of the firm opinion that none of the above statements give an acceptable definition of V_m, and in this work we present experimental data to support a method of determining V_m, which we have previously proposed[22].

One of the first requirements for discussing the problem is to find a proper definition of V_m. It is essential that V_m has a clear physical meaning, and is not simply a theoretical concept: accordingly, any definition of V_m must, in principle, give a recipe for the measurement of V_m. We believe that the only satisfactory way to define V_m is as follows[6,7]:

"V_m, the column dead-volume, is the total volume of *all* the components of eluent present within the packed part of the column".

Slight uncertainties arise in the interpretation of this definition from (i) the extent to which partial molar volumes of components of eluent depend upon mixture composition and adsorption, and whether such changes should be allowed for, and (ii) the extent to which different components of the eluent are sterically excluded from the pore space of packings, particularly those containing very small pores. In most practical situations such uncertainties will be small, and although they could in theory be handled, it is simplest to assume (i) that there is no change in partial molar volume on mixing or adsorption of eluent components, and (ii) that exclusion from parts of the pore volume can be ignored. The most important feature of this definition is that it provides for direct experimental measurement of V_m.

V_m may, for example, be determined by weighing the column when full of eluent, removing the eluent from the column, reweighing the dry column and determining the density of the eluent extracted from the column (which will normally not be the same as that of the eluent fed to the column due to preferential adsorption of certain components of eluent). Alternatively, V_m may be determined by weighing the column when filled with two different pure liquids[18,20] of substantially different densities, d_A and d_B. V_m is then given from the two column weights, w_A and w_B, by

$$V_m = \frac{w_A - w_B}{d_A - d_B} \tag{1}$$

These direct procedures are not generally attractive to chromatographers since they involve disturbing the column and removing it from the equipment. In addition,

most chromatographers do not measure elution volumes directly but rather elution times. Column capacity ratios are nearly always obtained from time records, not from volume records. Thus, some kind of measurement of peak retention time is desirable in order to be able to calculate t_m and hence V_m through the measured volume flow-rate. Ultimately, we are interested in the ratio $t_R/t_m = V_R/V_m = (1 + k')$. The theory which is now developed derives values for the ratio V_R/V_m for various types of elution peak.

While we ultimately wish to determine V_m from measurements on chromatographic peaks, it is simplest first to consider the development of solvent concentration fronts which arise when the composition of eluent fed to the column is suddenly changed. When the concentration changes are small enough, these fronts follow the error integral and are sharp and symmetrical. If the composition of eluent is subsequently changed back to the original, a second series of fronts will move down the column with the same spacing as the original set. By making the interval between the two changes in composition short enough, the fronts come close together and eventually, in the limit of a pulse injection, produce Gaussian peaks (the Gaussian being the differential of the error integral curve). Thus, the retention times of appropriate fronts are the same as the retention times of the corresponding peaks arising from the injection of pulses of eluent with compositions slightly different from those of the eluent being fed continuously to the column[23]. Within this whole description we include the possibility that the second eluent or pulse may contain trace components which are absent from the first eluent.

These general procedures have been discussed in detail by Helferich and Klein[24] but, regrettably, in a way which the majority of chromatographers find very difficult to understand. Solns *et al.*[25] have carried out a computer study of the development of solvent fronts but without giving analytical solutions. McCormick and Karger[20] have also discussed this problem, and we shall comment on their paper later.

THEORETICAL DEVELOPMENT

We consider the passage of an eluent of two or more components, A, B, ... through a column containing a chromatographic packing material which, in general, will have different affinities for the different components of the eluent. Thus if an eluent containing volume fractions x_A, x_B, ... of the components A, B, ... is passed through the column until the column is fully equilibrated (*i.e.* eluent of the same composition is emerging from the column), the composition of the eluent within the packed bed taken as a whole will in general differ from x_A, x_B, ... and can be represented by volume fractions y_A, y_B, ... Since both x and y are volume fractions, we also have the relationships when x or y are summed over all components:

$$x_A + x_B + \ldots = 1$$
$$y_A + y_b + \ldots = 1 \qquad (2)$$

Two experiments may now be envisaged. The first enables V_m to be detemined, while the second shows how the elution volumes of solvent disturbance peaks are related to V_m.

Experiment A

A mixture of A, B and C is fed to the column and the column is equilibrated. This mixture is suddenly replaced by a mixture of identical composition but containing trace quantities of isotopically labelled components, denoted by A*, B* and C*. We assume that the distribution coefficients for molecules of the labelled components are identical to those of the unlabelled materials, that is, that there is no isotope effect. The breakthrough volumes, V_{A^*}, V_{B^*}, V_{C^*} etc., of the labelled eluent fronts can be detected by a scintillation counter, as detailed in the Experimental section below, if A*, B* and C* are radioactive. Deuterated compounds can be detected with a refractometer.

A theoretical expression for the breakthrough volume of each labelled solute is obtained by considering the volume of eluent fed to the column between the time when labelled eluent first meets the column and the appropriate front emerges from the end of the column. Since the amount of any labelled component, X*, must be conserved, we can state with complete certainty that: (the amount of X* fed to the column between the time when the labelled eluent first enters the column and when its front leaves the column) equals (the amount of X* which will be found within the column at equilibrium). Thus, we can write for each labelled component:

$$V_{A^*} x_A = V_m y_A; \; V_{B^*} x_B = V_m y_B; \; V_{C^*} x_C = V_m y_C \tag{3}$$

Using eqn. 2 we then obtain

$$V_m = V_{A^*} x_A + V_{B^*} x_B + V_{C^*} x_C \tag{4}$$

If, instead of making a sudden change of eluent composition to labelled eluent, we inject a pulse of labelled eluent, then labelled peaks will be eluted with the same elution volumes as the fronts just considered. V_m is thus obtained by injecting small samples of eluent with isotopically labelled solute components and determining the elution volume for each labelled component.

The experiments detailed below show that this method of determining V_m gives consistent results over a wide range of eluent composition when a reversed-phase column packing is used.

Eqn. 4 may obviously be generalised to any number of components. For $(N+1)$ components we have to determine $(N+1)$ elution volumes of labelled components in order to determine V_m. It is, of course, obvious that the method can be used with a pure eluent, and this is by far the simplest way of determining V_m. The use of an isotopically labelled eluent will thus provide a correct value of V_m only if the eluent is a pure substance or, at most, contains only very small proportions of other components. Thus, V_m for a column being eluted with a fairly weak aqueous buffer solution could be found by using ^2H_2O or ^3H_2O, provided that the peak due to the labelled water could be adequately detected and distinguished from the disturbance peaks arising say from the buffer components. This method has been thoroughly studied by McCormick and Karger[20].

The method of determining V_m just developed is clearly one which involves a substantial experimental effort, but for precise determination of k' values there is no real alternative to making such measurements. It may subsequently be possible to

use a nearly unretained marker as a subsidiary standard, provided that the dependence of its elution volume upon eluent composition is adequately established, say by absolute measurements of its retention volume under a variety of conditions.

Experiment B, solvent disturbance peaks

An original mixture of $(N+1)$ components A, B, C, ... with a volume composition x_A, x_B, x_C ... being fed to the column is replaced by a new mixture of composition x''_A, x''_B, x''_C ... The compositions of mixtures within the bed, corresponding to these two eluent compositions, are y_A, y_B, y_C ... and y''_A, y''_B, y''_C ... In general, a sequence of N fronts will be observed to emerge from the column following the step composition change in eluent. These N fronts clearly cannot correspond to the $(N+1)$ fronts observed by replacing unlabelled eluent A, B, C ... by eluent containing traces of radiolabelled A*, B*, C* ..., described in Experiment A.

Two-component systems

For two components the situation is as shown in Fig. 1. In this case

$$x_B = 1 - x_A; \qquad y_B = 1 - y_A \qquad \text{etc.} \tag{5}$$

If a volume, V_R, of new eluent has been added to the column, the changes in the amounts of A and B within the column are given in volume units by

$$\begin{aligned} \delta V_A &= V_R(x'' - x_A) = V_R\,\delta x_A \\ \delta V_B &= V_R(x''_B - x_B) = V_R\,\delta x_B = -V_R\,\delta x_A \end{aligned} \tag{6}$$

As new eluent is fed to the column, a front moves along the column and in traversing the whole column sweeps out a volume V_m of the original eluent. The changes in amounts of A and B within the column are then expressed as

$$\begin{aligned} \delta V_A &= V_m(y''_A - y_A) = V_m\,\delta y_A \\ \delta V_B &= V_m(y''_B - y_B) = V_m\,\delta y_B = -V_m\,\delta y_A \end{aligned} \tag{7}$$

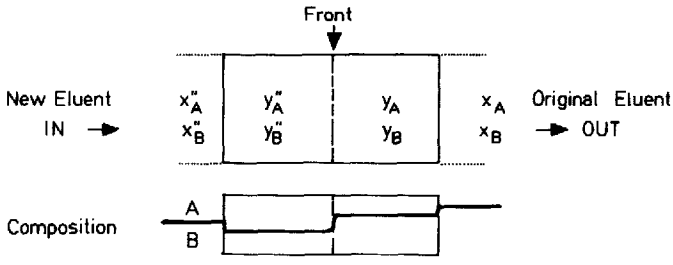

Fig. 1. Solvent disturbance front in a binary mixture of A + B arising from a sudden change in composition of eluent fed to the column from volume fractions (x_A, x_B) to volume fractions (x''_A, x''_B). The corresponding compositions within the column bed are (y_A, y_B) and (y''_A, y''_B). The lower part of the figure illustrates the compositions at different points outside and inside the column.

Combining eqns. 4 and 5 then gives

$$\frac{V_R}{V_m} = \frac{\delta y_A}{\delta x_A} = \frac{\delta y_B}{\delta x_B} \tag{8}$$

V_m/V_R may conveniently be denoted by R, the speed of the front or pulse relative to the speed of the eluent; it corresponds to the R_F value in planar chromatography. We note that for a finite change in composition, R is given by the gradient of the chord of the isotherm (which is the plot of y_A against x_A). For an infinitesimal change in composition R is given by the gradient of the isotherm itself; that is

$$R = \frac{V_m}{V_R} = \frac{dx_A}{dy_A} = \frac{dx_B}{dy_B} \tag{9}$$

If the first composition change is replaced almost immediately by the reverse composition change, in other words, if a pulse of new eluent is injected into the column, a peak will pass down the column with an elution volume, V_R, given by eqn. 9. In general, the (x,y) isotherm will be curved, and only at one particular composition will $V_R/V_m = 1$. The peak just described is the "solvent disturbance peak": it does not, in general, give a true value of V_m and its actual elution volume will depend upon the eluent composition.

For a two-component mixture of A and B we can therefore identify three

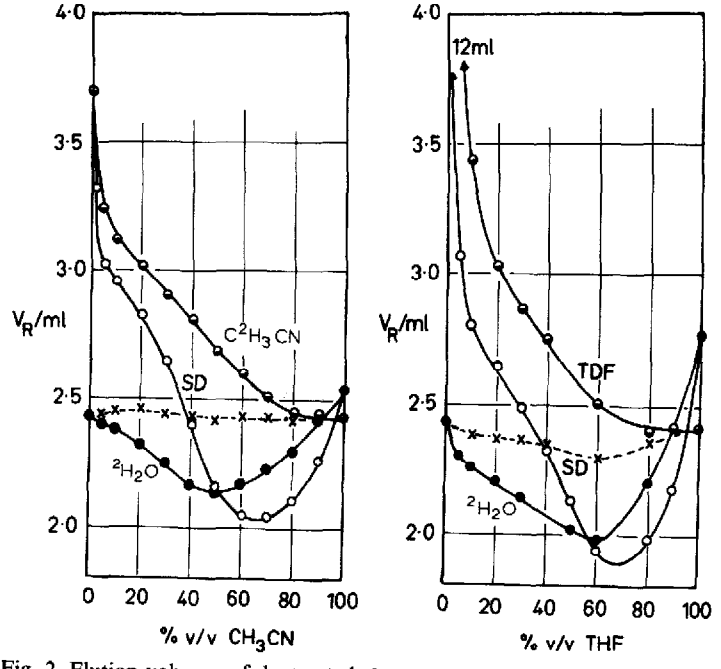

Fig. 2. Elution volumes of deuterated eluent components and of the solvent disturbance peak (SD) for acetonitrile–water (left) and tetrahydrofuran–water (right) mixtures. Data taken from McCormick and Karger[20]. Crosses and broken line show V_m as calculated from eqn. 4. TDF = Deuterated tetrahydrofuran.

measurable elution volumes (eqns. 3 and 9).

$$V_{A^*} = V_m \frac{y_A}{x_A}; \; V_R = V_m \frac{dy_A}{dx_A}; \; V_{B^*} = V_m \left\{\frac{1 - y_A}{1 - x_A}\right\} \tag{10}$$

None of these three will necessarily be equal to V_m!

This situation is well illustrated by Fig. 2, in which we have replotted the data of McCormick and Karger[20]. They used deuterium-labelled eluent components A* and B*, made up with the same volume composition as eluent, to determine the retention volume of the eluent components. To determine V_R for the solvent disturbance peak, they injected both pure A and pure B. Although the authors appear to have regarded the peaks so obtained as quite independent, their data show that the peaks obtained by injecting A and B have exactly the same retention volumes to within 0.5% over the entire composition range, confirming conclusively that only one solvent disturbance peak is obtained with a binary eluent. The retention volumes, V_{A^*}, V_{B^*} and V_R as seen from Fig. 2 are very different and clearly none of them, in general, gives V_m as we have defined it. McCormick and Karger obtained V_m, or what they called the "maximum possible V_m", by the weighing method, using methanol and carbon tetrachloride, and obtained a value of 2.42 ± 0.01 ml by this method. Application of eqn. 4 gives values very close to this, as shown by the x-points in Fig. 2, the mean value of V_m for the acetonitrile–water data being 2.435 ml and for tetrahydrofuran (THF)–water being 2.37 ml.

An interesting feature of the plots in Fig. 2 is that V_R can be smaller than either V_{A^*} or V_{B^*}. While McCormick and Karger recognise that V_{A^*} and V_{B^*} are determined by the chord of the isotherm while V_R is determined by the gradient of the isotherm, they do not explain this or attempt to correlate the two types of retention data. Accordingly, they provide an incomplete analysis of their highly accurate data. A full and self-consistent analysis of this and our own data is given in the Discussion section.

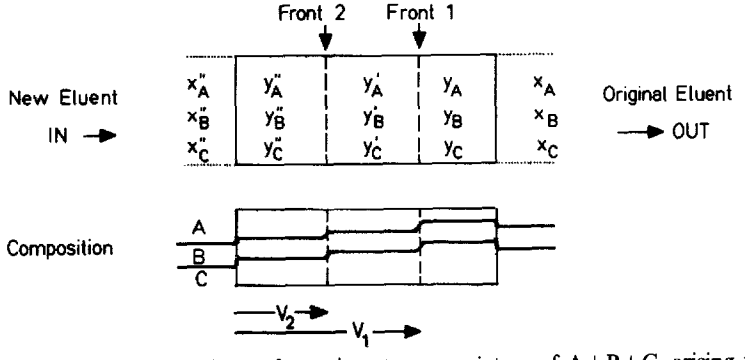

Fig. 3. Solvent disturbance fronts in a ternary mixture of A+B+C, arising from a sudden change in composition of eluent fed to the column from volume fractions (x_A, x_B, x_C) to (x''_A, x''_B, x''_C). The corresponding compositions within the column are (y_A, y_B, y_C) and (y''_A, y''_B, y''_C). Between the two fronts the composition within the column is (y'_A, y'_B, y'_C). The composition of eluent in equilibrium with this intermediate column composition is denoted by (x'_A, x'_B, x'_C).

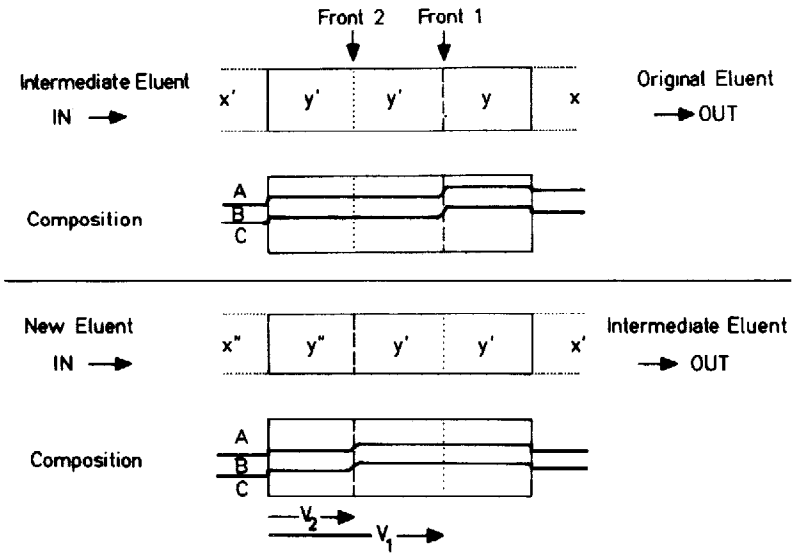

Fig. 4. Suppression of solvent disturbance fronts in a ternary mixture A + B + C by using eluent composition (x'_A, x'_B, x'_C) in equilibrium with the column composition (y'_A, y'_B, y'_C), either as new eluent or as original eluent [compositions such as (x'_A, x'_B, x'_C), are denoted by the summary notation x'].

Systems of three or more components

The situation for a three-component mixture, containing A, B and C, is more complex. Fig. 3 shows the general situation where two fronts develop. Between the two fronts, the composition within the column is denoted by y'_A, y'_B, y'_C. Such a composition must have an eluent with which it is in equilibrium, whose composition we denoted by x'_A, x'_B, x'_C. We now observe that either front 1 or front 2 can be suppressed by making either the initial eluent or the replacement eluent of this intermediate composition, as shown in Fig. 4.

Proceeding as before (see eqns. 6 and 7), we can write for the situation shown in Fig. 2:

$$\begin{aligned} \delta V_A &= V_R(x''_A - x_A) \\ \delta V_B &= V_R(x''_B - x_B) \\ \delta V_C &= V_R(x''_C - x_C) \end{aligned} \tag{11}$$

where V_R is the volume of new eluent added to the column, and for the contents of the column

$$\begin{aligned} \delta V_A &= V_2(y''_A - y'_A) + V_1(y'_A - y_A) \\ \delta V_B &= V_2(y''_B - y'_B) + V_1(y'_B - y_B) \\ \delta V_C &= V_2(y''_C - y'_C) + V_1(y'_C - y_C) \end{aligned} \tag{12}$$

where V_1 and V_2 are the volumes of column bed, swept out by fronts 1 and 2. Because of eqn. 2, we obtain

$$\delta V_A + \delta V_B + \delta V_C = 0 \tag{13}$$

Accordingly, only two of the three eqns. 11 and two of eqn. 12 are independent. We therefore quote only equations for A and B in the subsequent derivations.

It may be noted that eqn. 11 can be written

$$\delta V_A = V_R (x''_A - x'_A) + V_R (x'_A - x_A) \tag{14}$$

and similarly for δV_B. Eqns. 11 and 12 then give

$$V_R(x''_A - x'_A) + V_R(x'_A - x_A) = V_2(y''_A - y'_A) + V_1(y'_A - y_A) \tag{15}$$

and similarly for component B. Reference to Fig. 4 shows that eqn. 15 can be split into two independent sets of equations:

$$\begin{aligned} V_R(x''_A - x'_A) &= V_2(y''_A - y'_A) \\ V_R(x'_A - x_A) &= V_1(y'_A - y_A) \end{aligned} \tag{16}$$

and similarly for component B. In general, eqn. 16 can be written in the shorthand form:

$$V_R\, \delta x_A = V_1\, \delta y_A \tag{17}$$

and similarly for component B. Further simplification is obtained by using the relative front speed $R_i = V_i/V_R$ giving, in general, for either front:

$$\begin{aligned} \delta x_A &= R\, \delta y_A \\ \delta x_B &= R\, \delta y_B \end{aligned} \tag{18}$$

Here, the δy values are the composition changes across any front within the column, and the δx values are the composition changes between the two eluents which would be in equilibrium with the mixtures in the column on either side of the front.

The compositions x and y are connected by the equations for the isotherm:

$$\begin{aligned} y_A &= f_A(x_A\, x_B) \\ y_B &= f_B\,(x_A\, x_B) \end{aligned} \tag{19}$$

Composition changes in the two phases can then be written as

$$dy_A = \left\{\frac{\partial y_A}{\partial x_A}\right\}_{x_B \text{const}} dx_A + \left\{\frac{\partial y_A}{\partial x_B}\right\}_{x_A \text{const}} dx_B \tag{20}$$

or, using a shorthand notation for the differentials,

$$\begin{aligned} dy_A &= f'_{AA}\, dx_A + f'_{AB}\, dx_B \\ dy_B &= f'_{BA}\, dx_A + f'_{BB}\, dx_B \end{aligned} \tag{21}$$

Proceeding to the limit of infinitesimal changes and substituting eqn. 21 into eqn. 18 gives

$$\begin{aligned} dx_A &= R(f'_{AA}\, dx_A + f'_{AB}\, dx_B) \\ dx_B &= R(f'_{BA}\, dx_A + f'_{BB}\, dx_B) \end{aligned} \tag{22}$$

or

$$\begin{aligned} \left(f'_{AA} - \frac{1}{R}\right) dx_A + f'_{AB}\, dx_B &= 0 \\ f'_{BA}\, dx_A + \left(f'_{BB} - \frac{1}{R}\right) dx_B &= 0 \end{aligned} \tag{23}$$

Since dx_A and dx_B can be arbitrarily chosen without changing the R values of the fronts, the determinant of the coefficients of eqn. 23 must be zero. That is

$$\begin{vmatrix} \left(f'_{AA} - \frac{1}{R}\right) & f'_{AB} \\ f'_{BA} & \left(f'_{BB} - \frac{1}{R}\right) \end{vmatrix} \tag{24}$$

$$= \left(f'_{AA} - \frac{1}{R}\right)\left(f'_{BB} - \frac{1}{R}\right) - f'_{AB}\, f'_{BA} = 0$$

or

$$(f'_{AA}\, f'_{BB} - f'_{AB}\, f'_{BA})\, R^2 - (f'_{AA} + f'_{BB})\, R + 1 = 0. \tag{25}$$

Eqn. 25 has two roots corresponding to the fronts 1 and 2 shown in Fig. 3.

An interesting special case arises if one component, say A, is not present in the original eluent, for example if $x_A = 0$ and x''_A is very small. This corresponds to the presence of a trace of A in the new eluent. In this case $f'_{AB} = (\partial y_A/\partial x_B)$ must be zero since, if no A is present, y_A must be zero and cannot change when the amount of B in the mixture is changed. (Note: $\partial \ln y_A/\partial x_B$ may well not be zero in the limit as $y_A \rightarrow 0$).

The second term in the first bracket of eqn. 25 is thus zero and the fronts occur at

$$R_1 = 1/f'_{AA} = \left\{\frac{\partial x_A}{\partial y_A}\right\} \text{ and } R_2 = 1/f'_{BB} = \left\{\frac{\partial x_B}{\partial y_B}\right\} \tag{26}$$

R_1 applies to the front or peak caused by the injection of the new trace component A, and R_2 applies to the solvent disturbance front or peak for the binary mixture B + C. Eqn. 26, as it relates to B, is thus seen to be equivalent to eqn. 9.

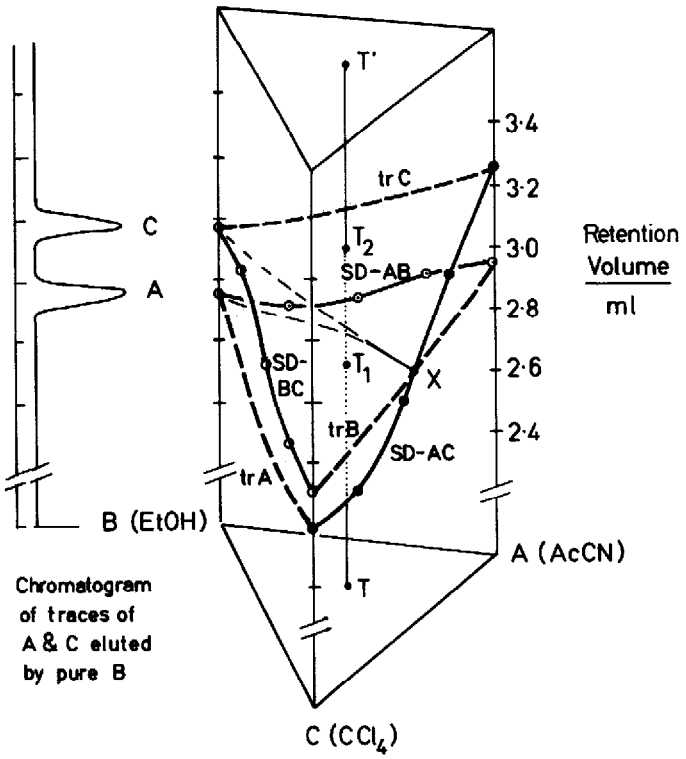

Fig. 5. Three-dimensional representation of the retention volumes (vertical axis) of the pairs of peaks obtained from ternary mixtures whose compositions are given by the triangular composition diagram forming the base of the cylinder. SD–AB refers to the solvent disturbance peak for the binary mixture A + B. trC refers to the peak given by injecting a trace of C into a mixture of A + B. Data points shown are detailed in Tables I and II. The diagram is to be seen as two winged surfaces, intersecting at a line through X and diverging towards the vertical through B. Symbols: AcCN = acetonitrile; EtOH = ethanol; CCl_4 = carbon tetrachloride.

It is interesting to explore in more detail the distinction between trace component peaks and solvent disturbance peaks for the three-component mixture just discussed, for clearly any of A, B and C can be present in trace amounts or can be injected in trace amounts into binary mixtures of the other two.

Fig. 5 illustrates the various possibilities. The data points shown were obtained for binary mixtures of acetonitrile (A) ethanol (B) and carbon tetrachloride (C). A conventional triangular composition diagram forms the base of the figure, and the vertical dimension is used to represent retention volume: zero retention volume corresponding to the level of the base of the triangular cylinder. The corners of the triangle correspond to pure single eluent components and the edges to binary mixtures. Thus, the left-hand vertical shows the elution volumes of the two peaks obtained when traces of A and C are injected into a column eluted with pure B. A diagramatic representation of the chromatogram is shown to the left. The broken line connecting the verticals through corners B and C and so running along the left-hand face of the triangular cylinder gives the retention volumes for peaks obtained by injecting traces of A into mixtures of B + C, while the full line on the same

face gives the elution volumes for the solvent disturbance peaks in B + C. Similar lines are shown on the other faces of the cylinder. For compositions represented by points within the base of the cylinder, *e.g.* point T, corresponding to mixtures containing all three components, there will be two elution volumes, corresponding to the two solutions to eqn. 25. These are shown by the intersections T_1 and T_2 of the vertical through T and the two surfaces which are bounded by the broken and full lines drawn on the faces of the cylinder. It must be noted that, because of the nature of the lines bounding the surfaces, at least two of which must intersect, the two surfaces must themselves intersect at the intersection of these lines. This point is denoted by X. However, since the opposite sides of the surfaces above B are well separated, the surfaces must diverge at some distance from the point of intersection. At X the solvent disturbance peak for A + C and the peak for a trace of B in A + C coincide. As we move across the face of the cylinder near X, or away from the face a short distance towards B, it is necessary that the identities of the two peaks remain. This means that there cannot be an immediate divergence of the two surfaces: there must be a short distance over which they continue to touch and intersect. However, because of their eventual separation at the vertical through B, they must diverge at some point. At X the surfaces must intersect at a well-defined angle, yet when they diverge, they must do so from a tangential contact. Thus, a significant change in the geometry of the surfaces occurs quite close to the point X. A detailed experimental examination of the situation in a particular case would be of considerable interest.

We may also observe that peaks due to trace components gradually lose their identity as we move away from the faces of the cylinder. This distinction between trace component peaks and solvent disturbance peaks is quite clear when the composition lies on or close to a face of the cylinder. Thus, when a mixture of A + C, containing a small proportion of B, is used as eluent, a peak very close to the elution volume for B in pure A + C will be obtained, when an injection is made of an A + C mixture containing either a little more B (normal chromatography) or a little less B (vacancy chromatography). However, when this is done, a solvent disturbance peak will appear, and within this peak there will be disturbance of the proportion of B. Thus, the second peak also becomes associated with component B to a small extent. As the proportion of B in the eluent mixture increases, the distinction between the peak for B and the solvent disturbance peak gradually disappears, although there is a continuity in the elution volumes of the two peaks as the proportion of B increases.

The case of the three component mixture just considered may readily be generalised to $(N+1)$ component mixtures. If an $(N+1)$ component eluent is replaced by an eluent containing the same components in slightly different proportions, N fronts will develop whose R values will be given by solutions to the group of equations of the form of eqn. 27

$$(f'_{AA} - \frac{1}{R})\, dx_A + f'_{AB}\, dx_B + f'_{AC}\, dx_C + \ldots f'_{AN}\, dx_N = 0 \qquad (27)$$

Since the dx values can be arbitrarily chosen, the solutions are given by setting the determinant of the coefficients equal to zero:

$$D = \begin{vmatrix} \left(f'_{AA} - \frac{1}{R}\right) & f'_{AB} & f'_{AC} \ldots\ldots & f'_{AN} \\ f'_{BA} & \left(f'_{BB} - \frac{1}{R}\right) & f'_{BC} \ldots\ldots & f'_{BN} \\ \vdots & \vdots & \ddots & \\ f'_{NA} & f'_{NB} \ldots\ldots\ldots\ldots & & \left(f'_{NN} - \frac{1}{R}\right) \end{vmatrix} = 0 \quad (28)$$

where D is the determinant of the coefficients of the N equations of the form of eqn. 27 for the components A to N.

Where components A to J are trace components only present in the new eluent, D has zero elements of the form f'_{ij} above and to the right of the diagonal elements for all trace components, that is from $i = A$ to $i = J$. The determinant of the coefficients thus has the form

$$D = \begin{vmatrix} \left(f'_{AA} - \frac{1}{R}\right) & 0 \ldots & 0 & 0 \ldots\ldots & 0 \\ f'_{BA} & \left(f'_{BB} - \frac{1}{R}\right) \ldots & 0 & 0 \ldots\ldots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ f'_{JA} & f'_{JB} \ldots & \left(f'_{JJ} - \frac{1}{R}\right) & 0 \ldots\ldots & 0 \\ f'_{KA} & f'_{KB} \ldots & f'_{KJ} & \left(f'_{KK} - \frac{1}{R}\right) \ldots & f'_{KN} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ f'_{NA} & f'_{NB} \ldots & f'_{NJ} & f'_{NK} \ldots & \left(f'_{NN} - \frac{1}{R}\right) \end{vmatrix} = 0 \quad (29)$$

Since all the elements in the top right-hand sector of the determinant are zero, the determinant factorises into the product of the diagonal elements $\left(f'_{ii} - \frac{1}{R}\right)$ with i from A to J and the determinant shown within broken lines in eqn. 29.

The first J solutions to eqn. 29 are thus of the form

$$\left(f'_{ii} - \frac{1}{R}\right) = 0 \quad \text{or } R = 1/f'_{ii} = dx_i/dy_i;\ i = A \text{ to } i = J \tag{30}$$

These solutions correspond to the breakthrough fronts or peaks due to the trace components A to J and are those normally expected from the injection of a *J*-component mixture, dissolved in eluent. The remaining (*N–J*) solutions correspond to the fronts or peaks which arise from disturbance of the eluent composition. There is thus a clear distinction between the fronts or peaks arising from the trace components, one corresponding to each such component, and the fronts or peaks arising from the disturbance of eluent composition which cannot be identified with particular eluent components and whose elution volumes are given by complex functions of the differentials of the 2 (*N–J*) dimensional partition isotherms for the distribution of the eluent components between the bulk eluent and the column packing. As we showed above for the three-component system, this clear distinction is maintained so long as the proportions of the "trace" components remain small and disappears only gradually as their proportions increase.

It now becomes clear that vacancy chromatography will produce peaks identifiable with particular components only when these components are present as traces in a bulk solvent (which may itself be a mixture). The peaks obtained by injecting pure bulk solvent (or solvent mixture) will thus appear at exactly the same positions as they would have had if a mixture of the trace components had been injected into the pure bulk solvent (or solvent mixture). However, if there are no trace components in the mixture, vacancy chromatography, like normal chromatography, will give peaks which cannot any longer be identified with particular components of eluent.

In current LC practice, especially when UV spectrophotometric detectors are used, many of the composition disturbance peaks are likely to be either suppressed or to overlap one another. Thus, very careful experimentation with a non-specific detector would be required to isolate all such peaks when a multicomponent eluent is used. When a UV detector is used with any reasonably complex eluent, containing say water, one or two organic modifiers, buffering salts and ion pairing agents, the observed peaks, are likely to form a totally undecipherable pattern. Such patterns should therefore come as no surprise, and attempts to interpret them are likely to be a waste of time.

EXPERIMENTAL

Equipment and methods

Columns were of the Shandon pattern (Shandon Southern Products, Runcorn, U.K.) 250 × 5.00 mm I.D., made from internally polished stainless steel. Injection was by syringe into the bottom of a 5 mm deep layer of glass beads just above the inlet gauze, which retained the packing. The column was packed with 2.440 g of 5-μm ODS Hypersil (Shandon).

An Orlita microdosing pump, Model DMP1515 (Orlita, Giessen, G.F.R.) was used to supply eluent to the column. Solvent disturbance peaks were monitored by an Optilab Multiref 902 refractometer (Optilab, Vallingby, Sweden). Elution volumes

were measured in calibration runs by weighing eluate on an Oertling F22TD electronic balance (Oertling, Liverpool, U.K.) and allowing for the density of the eluent. The balance was coupled to a Servoscribe double-pen potentiometric recorder (Belmont Instruments, Glasgow, U.K.), which was also used to record chromatograms when the refractometer was used as detector.

Elution peaks resulting from the injection of radiolabelled components of eluent were counted by a Beckman LS-133 liquid scintillation counter (Beckman RIIC, High Wycombe, U.K.). The detailed procedure was as follows. The column outlet was connected to a short length of PTFE tubing, 1/16″ in. O.D. and 0.25 mm I.D. The eluate emerging from this tubing formed small droplets of extremely uniform volume. The drop volume for each eluent was found by weighing, and drop counting was then used to measure the elution volume. In a preliminary run with injection of any radiolabelled mixture, ten-drop samples of eluate were collected in scintillation vials, and the approximate position of the radiolabelled elution peak was thereby found. In a second experiment, individual drops were collected starting close to the point at which the peak elution was expected. Drop volumes were typically about 10 μl, while V_m was about 2.8 ml. Injections of about 10 μl were made, and the concentrations of radiolabelled eluent components were chosen to give about 5000 cpm for the drops of highest concentration collected. From the count rates determined for each drop a histogram was constructed, and the best fit of a Gaussian curve was determined using a computer program written by Dr. H. P. Scott[26].

To prepare samples for scintillation counting each one- or ten-drop sample was collected in a 20-ml scintillation vial containing 10 ml of BDH scintillator cocktail in dioxane. In a typical run with water–acetonitrile (25:75, v/v), 5 μl of a mixture of eluent, containing 0.125 μCi of ^3H and 0.025 μCi ^{14}C, was injected into the column, and gave the histograms shown in Fig. 6. The drop volume in this case was 11.52 μl at a flow-rate of 1.06 ml/min, and the elution volumes of ^3H_2O and $^{14}CH_3CN$ were 233.8 drops (2.69 ml) and 261.5 drops (3.01 ml), respectively. The solvent disturbance peak, as measured by the refractometer, was eluted at 2.74 ml.

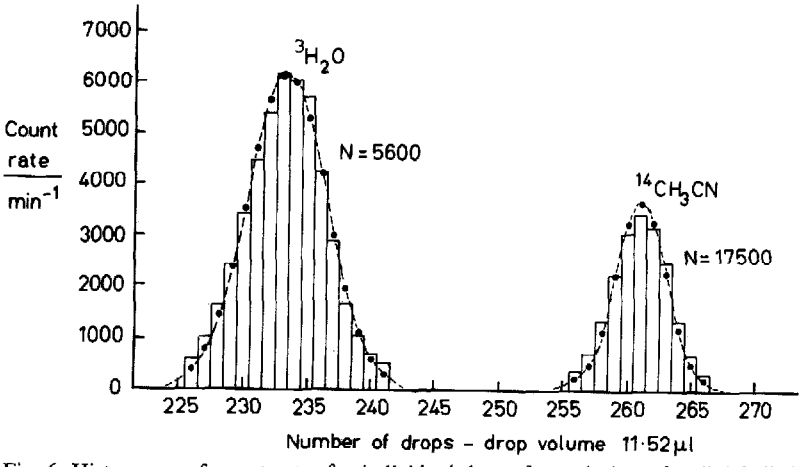

Fig. 6. Histograms of count rates for individual drops from elution of radiolabelled water (^3H_2O) and acetonitrile ($^{14}CH_3CN$) from a 250 × 5 mm I.D. column, packed with ODS Hypersil. Eluent, acetonitrile–water (75:25, v/v). Flow-rate, 1.06 ml/min. N value is the number of plates to which column is equivalent.

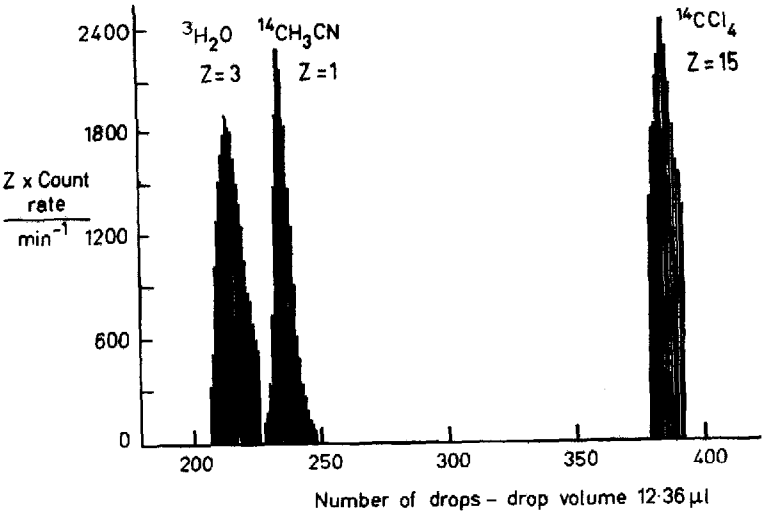

Fig. 7. Composite histogram of count rates for individual drops from elution of radiolabelled water (^3H_2O), acetonitrile ($^{14}CH_3CN$) and carbon tetrachloride ($^{14}CCl_4$) injected sequentially. Column as for Fig. 6. Eluent: acetonitrile–water–carbon tetrachloride (71:27:2, v/v/v). Flow-rate, 0.69 ml/min.

From eqn. 4, the column void volume is $V_m = 2.69 \cdot 0.25 + 3.01 \cdot 0.75 = 2.93$ ml. Fig. 7 shows results for a three-component eluent of water–acetonitrile–carbon tetrachloride (27:71:2, v/v/v). The elution volumes of the labelled peaks were respectively 2.66, 2.92 and 4.46 ml, and those of the solvent disturbance peaks were 2.46 and 4.98 ml. V_m, calculated by eqn. 4, was 2.89 ml, in good agreement with the value of 2.93 ml derived from the results of Fig. 6. The solvent disturbance peaks were eluted far from V_m in both cases.

Materials

Methanol and acetonitrile were HPLC-grade solvents (Rathburn Chemicals, Walkerburn, U.K.). Ethanol and carbon tetrachloride were BDH AnalaR-grade (BDH, Poole, U.K.). Water was doubly distilled in quartz. When using carbon tetrachloride, it was necessary to dry and de-gas the liquid under nitrogen, and to exclude oxygen by sparging continuously with nitrogen. Otherwise photo-oxidation occurred, producing phosgene and other products, which were highly corrosive to stainless steel to such an extent indeed that the eluate emerging from the system was yellow in colour.

Radiolabelled chemicals, ^3H_2O, [1-^{14}C]ethanol, $^{14}CCl_4$, [1-^{14}C]acetonitrile, and [*u*-^{14}C]benzene were obtained from the Radiochemical Centre (Amersham, U.K.).

RESULTS

The experimental results are presented in Table I for binary mixtures, and in Table II for ternary mixtures. The values of V_m calculated by eqn. 4 are highly consistent and provide an overall mean from 36 observations of $V_m = 2.86 \pm 0.01$ ml (the outlying value of 2.53 ml for one ternary mixture has been omitted). Slightly

TABLE I

ELUTION VOLUMES OF PEAKS DUE TO ELUENT COMPONENTS OF BINARY MIXTURES

Components		*Volume composition (%)*		*Elution volumes (ml)*				*Mean*
A	*B*	*A*	*B*	V_{A^*}	V_{B^*}	V_{SD}	V_m	
Ethanol	Water	100	–	2.92	–	–	2.92	
		75	25	2.93	2.86	2.78	2.91	
		50	50	3.03	2.82	2.70	2.92	
		25	75	3.20	2.81	2.90	2.91	
		17	83	–	–	2.92	–	
		5	95	3.96	2.83	3.36	2.89	
		–	100	–	2.91	–	2.91	2.91 ± 0.01
Acetonitrile	Water	100	–	2.84	–	–	2.84	
		75	25	3.00	2.67	2.74	2.92	
		50	50	3.21	2.46	2.67	2.83	
		41	59	–	–	2.74	–	
		25	75	3.48	2.59	3.03	2.81	
		5	95	3.91	2.77	3.52	2.83	
		–	100	–	2.91	–	2.91	2.86 ± 0.04
Acetonitrile	Carbon tetrachloride	100	–	2.84	–	–	2.84	
		75	25	2.70	3.25	3.04	2.84	
		60	40	–	–	2.89	–	
		50	50	2.57	2.99	2.76	2.78	
		25	75	2.55	2.85	2.59	2.78	
		–	100	–	2.79	–	2.79	2.81 ± 0.03
Carbon tetrachloride	Ethanol	–	100	–	2.92	–	2.92	
		25	75	3.04	2.83	–	2.88	
		50	50	3.04	2.77	–	2.90	
		75	25	2.82	2.63	–	2.77	
		100	–	2.79	–	–	2.79	2.85 ± 0.03
Acetonitrile	Ethanol	–	100	–	2.92	–	2.92	
		25	75	2.73	2.81	–	2.79	
		50	50	2.78	2.78	–	2.84	
		75	25	2.76	2.89	–	2.79	
		100	–	2.84	–	–	2.84	2.82 ± 0.02
Benzene	Ethanol	–	100	–	2.92	–	2.92	
		25	75	3.01	2.79	–	2.84	
		50	50	2.94	2.71	–	2.82	
		75	25	2.87	2.70	–	2.83	
		100	–	3.05	–	–	3.05	2.88 ± 0.03

different mean values are obtained from different binary pairs, ranging from 2.91 ± 0.01 for ethanol–water mixtures to 2.81 ± 0.03 for acetonitrile–carbon tetrachloride mixtures, with the ternary mixtures (acetonitrile–carbon tetrachloride–water) giving a mean of 2.86 ± 0.03.

The values of V_{A^*} and V_{B^*} allow an isotherm to be constructed for each binary pair, using eqn. 3 and the value of V_m for any pair. Such isotherms are shown in Fig.

TABLE II

ELUTION VOLUMES OF PEAKS DUE TO ELUENT COMPONENTS OF TERNARY MIXTURES

Volume compositon (%)			*Elution volumes (ml)*					
Aceto-nitrile (A)	*Carbon tetrachloride (B)*	*Water (C)*	V_{A^*}	V_{B^*}	V_{C^*}	$V_{SD,1}$	$V_{SD,2}$	V_m
71	2	27	2.92	4.46	2.66	2.46	4.98	2.88
74	3	23	–	–	–	2.41	4.57	–
78	7	15	–	–	–	2.61	3.94	–
82	8	10	2.78	3.44	2.80	2.79	3.63	2.83
83	10	7	–	–	–	2.90	3.61	–
87	8	5	2.83	3.42	3.16	2.84	3.40	2.89
82	13	5	2.78	3.33	3.10	2.84	3.39	2.87
85	13	2	–	–	–	2.90	3.26	–
68	30	2	–	–	–	3.14	3.14	–
58	40	2	2.40	2.77	1.61	2.88	2.88	(2.53)

Mean (excluding the value in parentheses) 2.86 ± 0.03

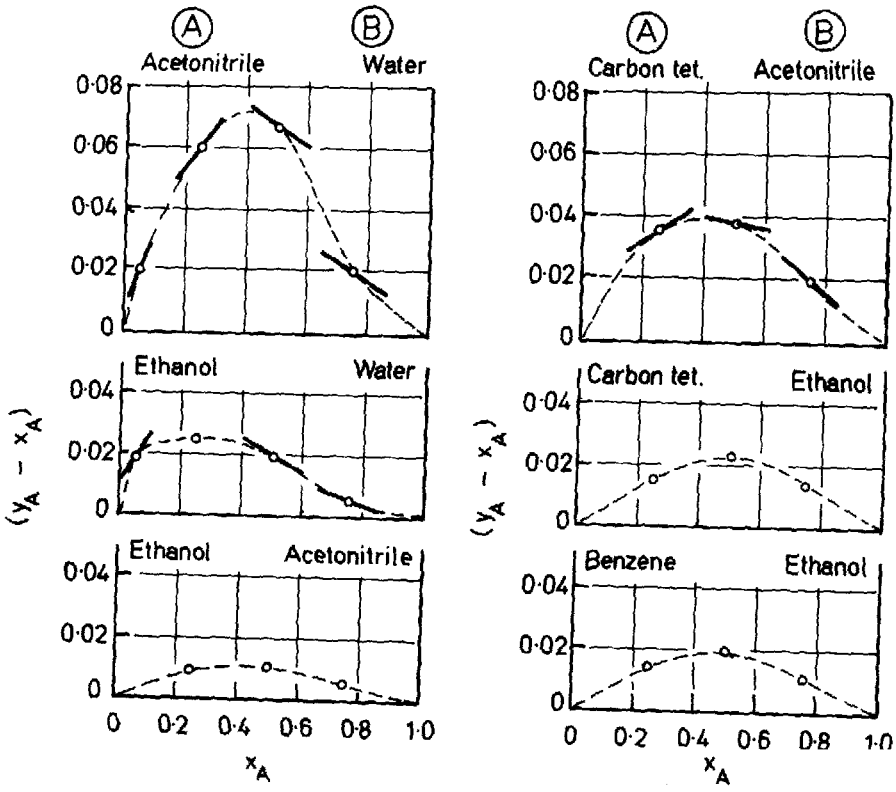

Fig. 8. Partition isotherms for binary mixtures on ODS Hypersil. Points and broken lines derived from retention volumes of radiolabelled components (see Tables I and II and eqn. 3). Heavy, short lines indicate gradients, calculated from retention volumes of solvent disturbance peaks (see Tables I and II, and eqn. 9).

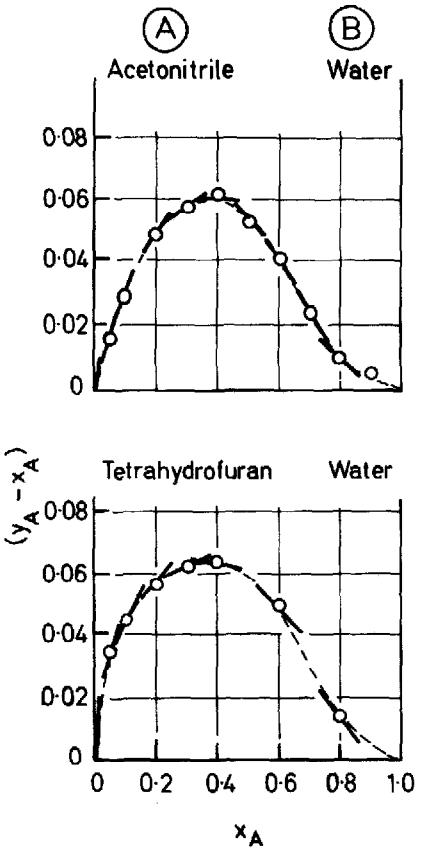

Fig. 9. Partition isotherms for binary mixtures on octyl-bonded Hypersil. Points and gradients as for Fig. 8. Data taken from McCormick and Karger[20].

8 as plots of $(y-x)$ against x. According to eqn. 9, the gradient of the isotherm should provide the ratio V_R/V_m for the solvent disturbance peak. These gradients are also shown in Fig. 8 which shows that excellent agreement is found between the gradients of the smooth curves through the data calculated from measurement of V_{A^*} and V_{B^*} and those obtained V_R. Fig. 9 shows isotherms calculated from the data of McCormick and Karger[20] for acetonitrile–water and labelled THF–water. The column packing was Hypersil which had been C_8-bonded and capped in their laboratories. This material is probably very similar to the commercial MOS Hypersil. Once again, the gradients of isotherms obtained from the solvent disturbance peaks are in excellent agreement with the gradients of the smooth lines through the curves from the labelled data. It may further be noted that McCormick and Karger's data[20] for the acetonitrile–water system are almost identical to ours for ODS Hypersil, despite some difference in bonding chemistry. The composition of maximum adsorption of acetonitrile are the same, only the maximum percent adsorbed is slightly higher for the ODS material.

Comparison of the data of Fig. 8 for different binary mixtures shows the maximum excess for the different systems given in Table III.

TABLE III
MAXIMUM EXCESS FOR DIFFERENT BINARY MIXTURES

System		*Maximum excess* $y_A - x_A$ *(vol. %)*	*Composition at maximum excess* x_A	*Reference*
A	*B*			
Acetonitrile	Water	6.0	0.40	20
		6.8	0.45	This paper
THF	Water	6.5	0.35	20
Ethanol	Water	2.6	0.20	This paper
Carbon tetrachloride	Acetonitrile	7.0	0.38	This paper
Carbon tetrachloride	Ethanol	2.3	0.50	This paper
Ethanol	Acetonitrile	1.2	0.40	This paper
Benzene	Ethanol	2.0	0.45	This paper

The pattern of preferential adsorption is in general agreement with a polarity index, such as the ε_0 values of Snyder[27] or the Hildebrand solubility parameters[28], but the detailed effects are not self-consistent. Thus, acetonitrile is strongly adsorbed from water and carbon tetrachloride strongly adsorbed from acetonitrile. Ethanol is more weakly adsorbed from water than acetonitrile (as expected), yet both carbon tetrachloride and benzene are only weakly adsorbed from ethanol. Contrary to expectation, ethanol is preferentially adsorbed from acetonitrile. Adsorption by residual silanol groups is the most probable explanation of these inconsistencies, but more experimental work is clearly required.

In Fig. 5 the binary elution data for pairs of the three components acetonitrile, ethanol and water are shown. No experiments were carried out with ternary mixtures. It would undoubtedly be of interest to extend our results by obtaining detailed information on retention of traces of a third component in each binary pair and on the positions of the solvent disturbance peaks for a ternary mixture generally.

The most important conclusion to be drawn from our results, shown in Tables I and II and Figs. 7 and 8, is undoubtedly that the basic theory developed in the first part of this paper is fully confirmed by independent experiments, carried out by ourselves (this paper) and by McCormick and Karger[20]. Both groups have shown that with a binary eluent A + B, a *single* solvent disturbance peak is produced when trace amounts of A or B of a mixture of A and B, are injected into the column: the V_R value is the same, whichever sample is injected, if the disturbance of the eluent concentration is kept small. When isotopically labelled samples of A* and B* are injected, two peaks are obtained, one for A* and one for B*. The three retention times are connected through the distribution isotherm for A and B between the eluent and the column bed.

Additionally, our results provide a clear, unequivocal method for determining the column dead-volume, defined as "the total volume of all eluent components within the column bed".

We will now comment specifically on the various other methods proposed and often used for determination of V_m and which were referred to in the Introduction.

(1) Our work shows clearly that the elution volume of the solvent disturbance peak V_{SD} does not provide a correct value of V_m, except by chance. However, if the eluent components are more or less equally sorbed by the packing, V_{SD} may not be

seriously in error. Unfortunately, V_{SD} may be outside the range of V_R for labelled eluent components, if the gradient of the isotherm is steep.

(2) The work of Knox *et al.*[22] showed clearly that enthalpic exclusion of small molecules could occur, and the present work confirms that V_R for some labelled components of eluent (and hence of solutes similar to eluent components) are eluted before V_m. The identification of V_m with V_R of the unionised solute giving the lowest retention can be subject to substantial error.

(3) The present work shows that with mixed eluents it is necessary to determine V_R for labelled samples of *all* eluent components, not just a single eluent component. However, if the eluent consists of a high volume fraction of one component, V_R for a labelled sample of that component will be very close to V_m (see Fig. 2).

(4) Knox *et al.*[22], amongst others, have shown that the use of salts is to be avoided. With ODS-bonded silicas, which bear a fixed negative charge, anions are excluded, whereas cations can be strongly retained. The degree of exclusion or retention depends upon the ionic strength of the solution. In practice it is, of course, the elution volume of a particular ion which is measured, not that of the salt as a whole.

(5) We have advocated that V_m be taken as the volume of all eluent components within the column without any allowance for preferential adsorption of one or more of the components. The calculation of the volume of adsorbed components is very arbitrary, since one is in essence assuming that the "adsorbed phase" can be clearly distinguished from eluent, even in the pores of a material such as silica gel. That is, it is a phase with a uniform composition, different from the phase it contacts, which is assumed to be pure eluent. In fact, there can never be a clear distinction between phases on the distance scale of a typical pore diameter (100–500 Å). As one moves from the silica surface through the bonded layer (maybe 10–20 Å thick) towards the centre of any pore, the average composition of eluent components will gradually and

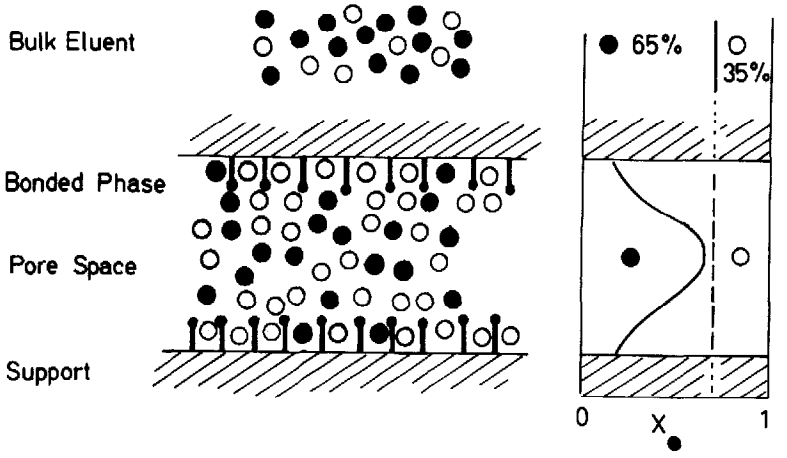

Fig. 10. Illustration of the partitioning of molecules of a binary eluent (○ and ●) between the extra particle space (bulk eluent) and the pores of a bonded packing material, where ○-molecules are preferentially adsorbed by the bonded layer. It is to be noted that there is a continuous variation in composition with no clear phase boundary as one moves from the surface of the support towards the centre of a pore, whose diameter is of the order of 200 Å.

continuously change, as suggested by Fig. 10, and even in the centre of a pore the composition may well differ from that of bulk eluent outside the particles of packing. The assumption that the bulk eluent composition is maintained right up to the bonded layer and then changes suddenly to that of an adsorbed layer cannot, in our view, be sustained. The only situation where we can reasonably distinguish between two phases is where we are dealing with liquid–liquid partition chromatography and where the pores of the particles are fully filled with a mixture of eluent components whose composition differs substantially from that outside the particles. The particle surface then coincides with a phase boundary between two liquid phases: V_m becomes the volume outside the particles, and V_s becomes the pore volume. We believe that all intermediate situations should be dealt with by taking V_m equal to the total volume of eluent components within the column.

(6) The use of linearisation formulae for the free energy of retention of homologues to determine V_m is undesirable for many reasons. First of all, it assumes that $\ln(V_{Rn} - V_m)$ is, in fact, a linear function of n (the number of carbon atoms in successive homologues). This assumption is equivalent to assuming that the increment in the free energy of partition per CH_2 group is constant, irrespective of n. It implies that there is no effect of the rest of the molecule in protecting this group from solvation as n increases, in other words, that the alkyl chains of the homologues are fully extended in both the eluent and sorbed states. Such an assumption cannot be universally true, especially when dealing with adsorbent surfaces and thin, bonded layers. Colin *et al.*[29] have, for example, shown that plots of $\ln(V_{Rn} - V_m)$ show gradient changes at specific values of n. Linearisation in such a case is impossible.

A second criticism of this method is that, as admitted by Kaiser and Oelrich[21], extremely precise measurements of V_{Rn} are required to obtain reasonable precision in V_m. Such measurements are very time-consuming.

A third objection to the method is that it does not necessarily give V_m in any case. At least it is not clear that it gives the volume of eluent components in the column or the retention volume of an unretained, unexcluded solute. It provides a certain theoretical value for a volume which is likely to be somewhat similar to V_m. The value provided by the technique does not appear to have any fundamental or universal validity.

CONCLUSIONS

(1) We re-emphasise the distinction between the kinetic dead-volume, V_0 (which is the volume of the interparticle void), and the thermodynamic dead-volume, V_m (which includes the additional volume of eluent components within the porous particles of packing).

(2) We recommend that the following definition of the thermodynamic dead-volume be adopted: "The thermodynamic dead-volume, V_m, is the total volume of all eluent components within the column bed".

(3) We advocate that the primary method for determining V_m should be to measure the retention volumes, V_R, of isotopically labelled samples of *all* eluent components and calculate V_m from eqn. 4. We have established that, over a wide range of composition and with a range of eluent components, eqn. 4 gives essentially identical values of V_m.

(4) In practice, it will be simplest to determine V_m by flushing the column with a one-component eluent and determining the single V_R of an isotopically labelled sample.

(5) Since the determination of V_m by the isotope labelling method can be time-consuming and can require the use of a different-from-normal detector, a subsidiary standard is desirable. The subsidiary standard could be a solvent disturbance peak, but we then advocate that the absolute retention volume of this peak be measured directly as a volume for each eluent composition and related to the true V_m value in a special experiment.

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