CHROM. 25 051

Review

Control of retention by molecular interactions in reversed-phase chromatography

R.P.W. Scott[*]

Chemistry Department, Georgetown University, Washington, DC 20057 (USA) and Chemistry Department, Birkbeck College, University of London, London (UK)

ABSTRACT

The procedures used in the synthesis of the different types of reversed phases are described, together with the physical and chemical properties of the products. The nature of the surface of the different reversed phases is discussed and the changes that take place when in contact with water explained on the basis of the dispersive character of the bonded phase and the high polarity of the water. The innate stability of the oligomeric phases is considered and the adsorption of solvent from aqueous solvent mixtures onto the surface of a reversed phase described, including the form of the adsorption isotherm. The different ways a solute molecule can interact with the reversed-phase surface is discussed and the interactions that take place in the mobile phase examined using simple liquid–liquid distribution systems. It is shown that the probability of solute interaction with a given solvent in a solvent mixture is proportional to the volume fraction of the solvent. Conditions are then considered where association between the components of the solvent mixture occurs using, as an example, aqueous solvent mixtures of methanol. The resulting ternary mixture is seen to add to the complexity of the retention mechanism. Finally, a general equation is given that describes the retention volume of a solute in terms of the distribution coefficient of the solute between each pair of stationary phase and mobile phase components and the fraction of each phase component available for solute interaction.

CONTENTS

---

[*] Address for correspondence: Sprucewood Lane, Avon, CT 06001, USA.

52

R.P.W. Scott / J. Chromatogr. A 656 (1993) 51–68

1. INTRODUCTION

Despite the fact that the introduction of silica gel made the renaissance of liquid chromatography (LC) possible, the vast majority of contemporary LC analyses are carried out using a reversed phase as the stationary phase. The first attempt to bond an organic moiety to the surface of silica gel was made by Halász and Sebestian in 1969 [1], who attached aliphatic hydrocarbon chains to the silica gel surface by means of the silicon-oxygen-carbon linkage. About the same time Simpson [2] filed a patent for a similar product which, however, was not granted until 1975. It is interesting to note, that the patent also contained the suggestion that the chlorosilanes could be used as alternative bonding reagents. Simpson's results were reported at an informal symposium organized by the Chromatography Discussion Group in May 1969. The original synthesis by Halász and Sebestian involved refluxing the silica gel with an aliphatic alcohol but, unfortunately, the silicon–oxygen–carbon bonds are very weak and the bonded hydrocarbon chain rapidly hydrolyzed from the surface, regenerating the original silica gel. Nevertheless, the material was sufficiently stable to allow Halász [1] to identify the highly desirable chromatographic properties of the bonded phase. In 1973, Gilpin and Burke [3] described the use of chlorsilanes as bonding reagents. When the hydroxyl group of the silica gel reacts with a chlorsilane, hydrogen chloride is released and the organic moiety is attached by means of the silicon–oxygen–silicon bond. The silicon-oxygen–silicon bond is far stronger than the silicon–oxygen–carbon bond and such bonded phases can be used satisfactorily in an LC column over long periods of time, provided extremes of pH are avoided. This type of bond became the basis for the synthesis of the vast majority of contemporary bonded phases.

There are basically three types of bonded phase: the "brush" phase, the "bulk" phase and the oligomeric phase. The brush phases are made by using the mono-functional silanes such as dimethyloctychlorsilane which reacts directly with the surface silanol groups with elimination of hydrogen chloride. This results in the surface being covered with dimethyloctyl chains like bristles on a brush, ipso facto the term "brush" phase evolved.

The "brush" phases, synthesized under carefully controlled conditions, are the most reproducible and consequently, are the most commonly used in LC analysis.

If bi-functional silanes are used in the synthesis, such as methyl-octyldichlorosilane, then it is possible to produce an oligomeric phase. The dichlorsilane reacts with a silanol group producing a methyloctylmonochlorsilyl group on the surface with the evolution of hydrogen chloride. If this monochlorosilane is then reacted with water more hydrogen chloride is generated and the bonded moiety now becomes a methyloctyl-monohydroxysilyl group. The bonded silica, containing the hydroxyl groups can then be again treated sequentially with the dichlorosilane and then water each time building another methyoctyl silyl group onto the surface. In this way a layer of hydrocarbon chains can be laid down on the silica surface as an oligomer, producing a very stable type of reversed phase. Employing a fluidized bed technique of synthesis [4,5] a series of oligomeric phases were prepared by Akapo et al. [6] and their properties reported.

A tri-functional silane, such as octyltrichlorosilane can produce the third type of bonded phase, the "bulk" phase. If the surface of the silica gel is saturated with water and treated with octyltrichlorosilane, reaction occurs between both the hydroxyl groups on the silica surface and the adsorbed water. The water causes an octylsilyl polymer to be formed which is cross-linked and, consequently, the stationary phase assumes a multi-layer character.

The synthesis can also be accomplished by using a procedure similar to that used in the preparation of the oligomeric phases; that is by employing a sequence of two-stage reactions involving firstly treatment with water and then by the trichlorosilane. In this way the polymeric layer can be increased to whatever thickness that is desired. After the last stage the product is treated with water and finally end capped. The multi-layer character of this type of bonded phase evoked the term "bulk", or polymeric bonded phase.

R.P.W. Scott / J. Chromatogr. A 656 (1993) 51–68

53

2. THE NATURE OF THE "BRUSH" AND "BULK" PHASES WHEN IN CONTACT WITH WATER

Scott and Simpson [7] carried out some retention measurements on samples of "bulk" and "brush" phases using methanol–water mixtures as the mobile phase. At very low methanol concentrations, they noted that the "bulk" phase performed in the expected manner and the retention of a solute decreased as the methanol concentration increased. However, solute retention on the "brush" phase initially *increased* with the methanol concentration until a maximum was reached. At higher concentrations the solute retention began to fall and subsequently solute retention progressively fell as the concentration of methanol continued to increase. Examples of the curves relating solute retention to solvent concentration for the two phases are shown in Fig. 1. It is seen that the retention volume of ethanol decreases continuously from 0 to 10% (w/w) of methanol on the "bulk" phase ODS-3. In contrast it is seen that on the "brush" phase, the retention volume of ethanol reaches a maximum at about 2.5% (w/w) of methanol in the mobile phase and subsequently falls, the graph of retention volume against methanol concentration finally becoming parallel to the curve of the "bulk" phase.

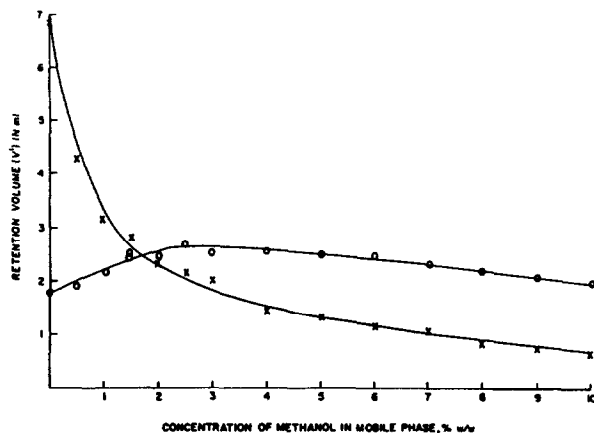Lochmüller and Wilder [7] and Gilpin and Squires [8] also noted the same phenomena and

Fig. 1. Graph of retention volume of ethanol (V') against concentration of methanol in the mobile phase. O = RP-18; × = ODS-3.

suggested that it was due to the dispersive forces between the hydrocarbon chains themselves being greater than the dispersive forces between the hydrocarbon chains and the mobile phase. As a consequence, the chains interacted preferentially with one another, collapsed onto the surface, forming clumps. This, in effect, reduced the available surface area of the bonded phase and, thus, reduced the magnitude of the solute retention. As the methanol concentration increased, the dispersive interactions of the hydrocarbon chains with the mobile phase eventually became sufficiently large to allow chains to disengage from one another and, consequently, increasing the effective surface area of the stationary phase.

This phenomena has also been described as due "hydrophobic forces", a most unfortunate term that implies some form of molecular repulsion (which of course, outside the Van der Waals radii, is impossible). The term "hydrophobic force" (literally meaning "fear of water" forces) was coined, for some reason, as an alternative to the well established term, *dispersive* forces. There are a number of different types of intermolecular forces that control solute retention but the two concerned here are dispersive and polar (including hydrogen bonding). Polar interactions arise from the charges residing on molecules resulting from permanent dipoles or induced dipoles. Examples of such molecules are those that have permanent dipoles such as alcohols, ketones esters, etc. and those that are polarizable such as the aromatic hydrocarbons. Dispersive interactions result from charge fluctuations in a molecule and not from permanent or induced dipoles, although polar substances will exhibit polar interactions as well as dispersive interactions. The forces between n-heptane molecules that keep them together as a liquid boiling at 100°C are typical dispersive interactions.

The reason that n-heptane and water are immiscible is *not* because water molecules *repel* heptane molecules. They are immiscible because the forces between two heptane molecules and the forces between two water molecules are much greater than the forces between a heptane molecule and a water molecule. Thus, water

molecules and heptane molecules associate *very much more strongly* with *themselves* than with *each other*. The fact that water has a small but finite solubility in n-heptane, and n-heptane has a small but finite solubility in water, eliminates the possibility of molecular repulsion being the reason for immiscibility. The saturated concentration of either solvent in the other, is that concentration where the probability of interaction of the minor components is equal to the probability of their disengagement. The molecules will disengage when the kinetic energy of the pair is equal or greater than the potential energy of their interaction.

The behavior of the brush phase in the above experiment is, in fact, a form of immiscibility. Until the interactive forces between the hydrocarbon chains themselves are *equal or less* than those between the hydrocarbon chain and the solvent (*i.e.* when there is adequate methanol present) their full availability for interaction with any solute will not be achieved. The "bulk" phase being a *rigid cross-linked* polymer hinders the collapse and self interaction of the hydrocarbon chains and thus, the availability of the chains to any solute is not reduced even in the presence of pure water. This might indicate that the "bulk" reversed phase would be advantageous when operating an LC system with aqueous mobile phases containing very low concentrations of solvent.

3. THE STABILITY OF THE OLIGOMERIC PHASES

The behavior of the oligomeric phases when in contact with water does not appear to have been examined, but as the polymer is linear, and not cross-linked, it is likely to have similar properties to the "brush" phase. However, the oligomeric phases do differ in one important respect to the "brush" and "bulk" phases and that is in their stability to mobile phases having very low pH values. Akapo *et al.* [6] synthesized four oligomeric phases containing 1, 3, 5 and 10 methyloctyl oligomers, respectively. They packed four columns with the material and measured the k' (capacity ratio) of aniline using a mobile phase consisting of 70% (v/v) of methanol and 30% (v/v) water and 0.1% (v/v) trifluoroacetic acid

(TFA). The mobile phase was passed continuously through the system and the measurement repeated after every 500 ml of mobile phase was eluted. The results they obtained, shown as a curve relating the k' of aniline against the volume of mobile phase passed through the column is shown in Fig. 2. It is seen that the mobile phase rapidly strips off the bonded phase from the column containing 1 oligomer. As the solute is basic, and the exposed silica is acidic, the value of k' rapidly rises as more and more mobile phase passes through the column. However, as the amount of oligomeric phase increases, and the layer becomes thicker, the loss of bonded phase becomes progressively less. It is seen that after 5 oligomers have been bonded to the surface the material has become quite stable. After 10 oligomers have been attached, it is very stable indeed, with very little bonded phase being removed even after the passage of over 2 l of mobile phase through the column. This inherent resistance of the oligomeric phases to degradation at low pH values could make them very useful in the separation of peptides and proteins where the use of TFA is often essential to achieve sample solubility.
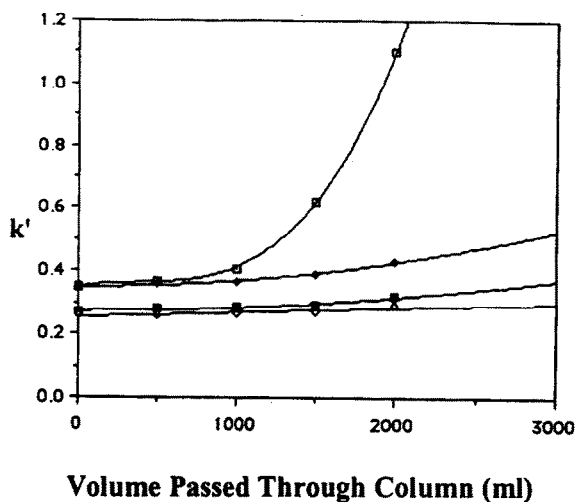

Fig. 2. Graph of k' of aniline against volume of mobile phase passed through the column. Oligomeric phases containing (□) 1, (♦) 3, (■) 5 and (◇) 10 methyloctyl oligomers.

4. SOLVENT–STATIONARY PHASE INTERACTIONS

The mechanism of interaction between solvent molecules and a reversed-phase surface are similar to the complementary interactions of solvent molecules with a silica gel surface. A layer of solvent is built up on the surface by absorption, the difference being that the interactive forces between the solvent and the reversed phase are dispersive in nature, as opposed to those with the silica gel which are mainly polar. The adsorption isotherm can also be shown to be described by the Langmuir equation. However, because the interactions with the reversed phase are almost exclusively dispersive and are not a mixture of dispersive and polar interactions as with the case of silica gel, a detailed study of the adsorption isotherms can provide a more exact understanding of the surface than is possible for silica gel.

4.1. The derivation of the Langmuir adsorption isotherm for reversed phases

$$c_m$$

$$c_s$$
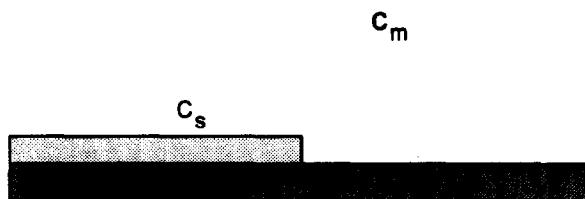
Consider 1 cm^2 of surface carrying an adsorbed layer of solvent at a concentration (C_s) g cm^{-2} in contact with a liquid containing (C_m) g of solvent per ml of the solvent mixture. Let the molecular mass of the solvent be M and the area covered by the solvent molecule when adsorbed on the surface be S.

Thus, assuming a mono-layer of solvent is formed on the surface, the area of exposed surface (ψ), is given by

$$\psi = 1 - \frac{C_s}{M} \cdot NS \tag{1}$$

where, N is Avogadro's number.

The number of molecules (N_1), leaving the surface will be proportional to the concentration of adsorbed molecules and a constant (β)

$$N_1 = \beta C_s$$

The constant β is that fraction of the adsorbed molecules that acquire sufficient kinetic energy to overcome the molecular forces holding the molecules to the surface at the temperature of adsorption.

The number of molecules striking and adhering to the exposed surface (N_2) will be proportional to the concentration of solvent in mobile phase, the unexposed area of surface and another constant (α).

Thus,

$$N_2 = \alpha \left(1 - \frac{CS_s}{M} \cdot NS \right) C_m$$

Now, under equilibrium conditions,

$$N_1 = N_2$$

Thus,

$$\alpha C_m - \alpha C_m \cdot \frac{NS}{M} = \beta C_s$$

and

$$C_s \left(\beta + \alpha \cdot \frac{NS}{M} \cdot C_m \right) = \alpha C_m$$

or

$$\frac{\alpha}{\beta + \alpha \cdot \dfrac{NS}{M} \cdot C_m} = \frac{C_s}{C_m} = K \tag{2}$$

where K is the net, effective distribution coefficient of the solvent between the stationary phase and a solution of the solvent in water at a concentration C_m. In practice, this means that if a neat sample of solvent is injected on to a column as a solute, when the mobile phase consists of an aqueous solution of the solvent at concentration (C_m), then the magnitude of its retention volume will be determined the distribution coefficient (K).

Thus,

$$K = \frac{1}{g + \dfrac{NS}{M} \cdot C_m} \tag{3}$$

where $g = \beta/\alpha$ = the desorption–adsorption coefficient of the adsorbing solvent.

Now, if the reversed phase is considered to be packed into a column and operated with a mobile phase having a solvent concentration of C_m in water, the corrected retention volume (V') is given by the equation derived from the plate theory [2],

$$V' = K\varphi$$

where φ is the total chromatographically available surface area of the reversed phase in the column.

Substituting for K from eqn. 3

$$V' = \frac{\varphi}{g + \dfrac{NS}{M} \cdot C_m}$$

or

$$\frac{1}{V'} = \frac{g}{\varphi} + \frac{NS}{M\varphi} \cdot C_m \tag{4}$$

It is seen that eqn. 4 shows a linear relationship between the reciprocal of the corrected retention volume and the concentration of solvent in the mobile phase. Consequently, if retention data are measured over a range of solvent concentrations, employing the solvent itself as the solute, a linear relationship will be obtained by plotting $1/V'$ against C_m and from the intercept and slope of the graph, values for φ, g and K can be calculated.

For example, if the intercept and slope of a curve relating $1/V'$ to C_m are A and B, respectively, then

$$\frac{A}{B} = \frac{gM}{NS}$$

or

$$g = \frac{ANS}{BM} \tag{5}$$

and

$$\varphi = \frac{g}{A} \tag{6}$$

Scott and Simpson [10] made some accurate measurements of the retention volume (V_r) and C_m for a number of aliphatic alcohols and two aliphatic acids in an attempt to measure their adsorption-desorption coefficients and the surface area of the reverse phase available to each solvent. In practice, however, the approach described above suffers from one serious drawback. In order to measure V', the corrected retention volume, it is necessary to obtain an accurate value for the effective dead volume. The dead volume (V_0) of the LC column has been studied extensively from both a theoretical and experimental point of view [10–16]. There is much disagreement on the best method of dead volume measurement and in their work, Scott and Simpson, used sodium chloride as the dead volume marker. In subsequent review by other workers in the field, this solute was not considered appropriate and thus, many of their conclusions were considered in error. However, their data can be used very effectively without the need to know the dead volume of the column. The following approach, circumvents the uncertainties that arise from dead volume measurement and still provides adsorption–desorption coefficients and the surface area of the reversed phase that is available to each solvent.

Restating eqn. 4

$$\frac{1}{V'} = \frac{g}{\varphi} + \frac{NS}{M\varphi} C_m \tag{4}$$

Now

$$V' = V_r - V_0$$

Thus, substituting for V' in eqn. 4

$$\frac{1}{V_r - V_0} = \frac{g}{\varphi} + \frac{NS}{M\varphi} C_m \tag{7}$$

To aid in algebraic manipulation eqn. 7 can be put in the simple form

$$\frac{1}{V_r - V_0} = A + BC_m \tag{8}$$

where, as stated before

$$\frac{g}{\varphi} = A \quad \text{and} \quad B = \frac{NS}{M\varphi}$$

Now, when the solvent is chromatographed as the solute in pure water as the mobile phase, [*i.e.* $C_m = 0$ and $V_r = V_{r(0)}$], then

$$\frac{1}{V_{r(0)} - V_0} = A$$

Re-arranging

$$V_0 = \frac{AV_{r(0)} - 1}{A} \qquad (9)$$

Substituting for V_0 from eqn. 9 into eqn. 8

$$\frac{1}{V_{r(n)} - \dfrac{AV_{r(0)} - 1}{A}} = A + BC_{m(n)} \qquad (10)$$

where $V_{r(n)}$ is the retention volume of the solvent when chromatographed as a solute in a mobile phase containing the solvent at a concentration $C_{m(n)}$. Eqn. 10 relates the *retention volume* of the solvent (as opposed to the *corrected retention volume*) to the solvent concentration in the mobile phase and thus, *the need to determine the column dead volume is eliminated*.

4.2. Application of the theory to the adsorption of aliphatic alcohols

Eqn. 10 was used to develop the data of Scott and Simpson [10]. Using a simple iterative computer program the values of A and B were calculated that provided the minimum error between the two sides of eqn. 10 for values they obtained for all three alcohols. Having identified the values of A and B the corrected retention volume values were calculated from the retention volume data by the following equation.

$$V'_{(n)} = V_{r(n)} - \frac{AV_{r(0)} - 1}{A}$$

The results obtained for the series of aliphatic alcohols are shown as curves relating the reciprocal of the corrected retention volume to solvent concentration in Fig. 3 and it is seen that
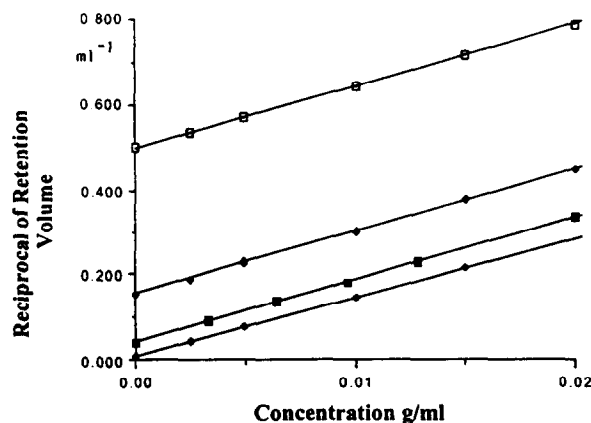

Fig. 3. Graph of $1/V'$ of a series of aliphatic alcohols against their respective concentrations in the mobile phase. $\square$ = Methanol, $y = 0.5014 + 14.2611x$ $(R = 1.00)$; $\blacklozenge$ = ethanol, $y = 0.1546 + 14.7714x$ $(R = 1.00)$; $\blacksquare$ = propanol, $y = 0.0431 + 14.6093x$ $(R = 1.00)$; $\lozenge$ = butanol, $y = 0.01 + 13.8x$ $(R = 1.00)$.

the expected linearity is precisely realized. Using the values for the slopes and intercepts obtained from the curve fitting procedure in eqns. 5 and 6, the effective surface area of the reversed phase to all four alcohols was calculated together with the distribution coefficient of each alcohol between water and the reversed phase. The results are shown in Table 1.

It is seen from Table 1 that all three alcohols give a mean value for the effective chromatographic surface area of 206.8 m^2 g^{-1} with a standard deviation of 4.8 m^2 g^{-1} which is only 2.35% of the mean. The reversed phase examined was ODS3 from Whatman which was reported by the manufacturers to be prepared from silica gel having a BET surface area of 350 m^2 g^{-1}. This would mean that the effective chromatographic surface area of the reversed phase was about 58% of the parent silica gel. It is interesting to note that the fraction of the silanol groups reacted with the silanizing reagent is usually between 50 and 60%.

The manner in which the solvent covers the surface is best revealed by the shape of the adsorption isotherm. An expression for the adsorption isotherm can be obtained by re-arranging eqn. 2

TABLE 1

CHROMATOGRAPHIC DATA FOR FOUR ALIPHATIC ALCOHOLS

Solvent	Molecular area ($Å^2$)	Intercept (ml^{-1})	Slope (g^{-1})	NS/M 10^{-7} cm^2	Surface area (m^2)	Distribution coefficient (K)
Methanol	15.5	0.5014	14.26	2.90	203.4	$0.981 \cdot 10^{-6}$
Ethanol	23.2	0.1546	14.77	3.04	205.8	$3.182 \cdot 10^{-6}$
Propanol	29.7	0.0431	14.61	2.98	204.0	$11.38 \cdot 10^{-6}$
Butanol	35.0	0.0100	13.80	2.95	213.8	$46.78 \cdot 10^{-6}$

$$C_s = \frac{\alpha C_m}{\beta + \frac{\alpha NS}{M} \cdot C_m}$$

Dividing throughout by α, the normal expression for the adsorption isotherm is obtained.

$$C_s = \frac{C_m}{g + \frac{NS}{M} \cdot C_m} \qquad (11)$$

Using the data given in Table 1 in conjunction with eqn. 5 the adsorption isotherm equations can be calculated for all four alcohols.

Methanol: $$C_s = \frac{C_m}{1.02 \cdot 10^6 + 2.9 \cdot 10^7 C_m} \qquad (12)$$

Ethanol: $$C_s = \frac{C_m}{3.68 \cdot 10^5 + 3.07 \cdot 10^7 C_m} \qquad (13)$$

Propanol: $$C_s = \frac{C_m}{8.79 \cdot 10^4 + 2.98 \cdot 10^7 C_m} \qquad (14)$$

Butanol: $$C_s = \frac{C_m}{2.14 \cdot 10^4 + 2.95 \cdot 10^7 C_m} \qquad (15)$$

Eqns. 12–15 were used to construct the adsorption isotherms for all four alcohols and these are shown in Fig. 4. The effect of the dispersive hydrocarbon chain of the alcohol on the strength of the adsorption is clearly seen from the shape of the curves. The most strongly adsorbed alcohol, butanol, has only a four carbon chain and yet the surface is completely covered when the solvent concentration is only about 2% (w/v).
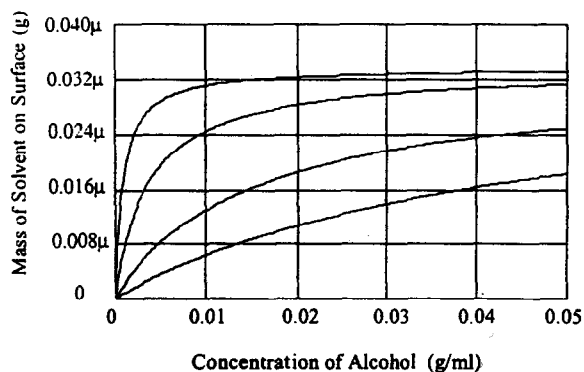

Fig. 4. The adsorption isotherms of a homologous series of aliphatic alcohols over the concentration range of 0 to 0.1 g ml^{-1}. It should be noted that the mass adsorbed is expressed as g cm^{-2}. On the y-axis, μ stands for μg.

This means that any water soluble material with a hydrocarbon chain length of four or more, will be rapidly adsorbed and extensively modify the reverse phase surface. This will effect the magnitude of solute retention significantly.

4.3. Application of the theory to the adsorption of aliphatic acids

The data obtained for acetic acid and propionic acid were also treated in the same way and the curves relating the reciprocal of the retention volume of each acid to the concentration of the acid in the mobile phase are shown in Fig. 5. It is observed that the same linear relationship is obtained between the reciprocal of the corrected retention volume and the concentration of solvent in the mobile phase. Again using the values for the slopes and intercepts obtained from the curve fitting procedure in eqns. 5 and 6, the
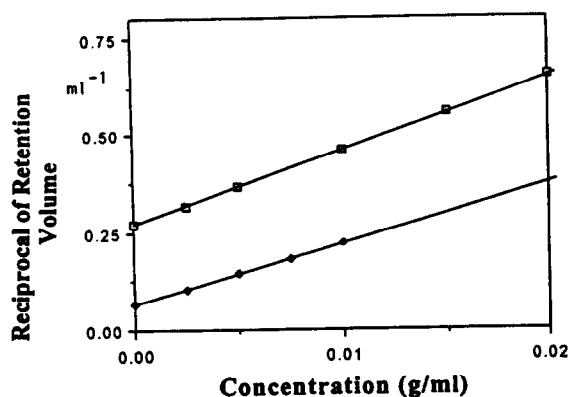
Fig. 5. Graph of $1/V'$ for acetic and propionic acids against their respective concentration in the mobile phase. $\square$ = Acetic acid, $y = 0.2689 + 19.3158x$ ($R = 1.00$); $\blacklozenge$ = propionic acid, $y = 0.0656 + 15.456x$ ($R = 1.00$).

effective surface area of the reversed phase available to both acids was calculated together with the distribution coefficient of each acid between water and the reversed phase.

The results are shown in Table 2. It is apparent that the surface area available to the acids is significantly less than that available to the alcohols. However, the aliphatic acids are partially ionized which would cause them to be ionically excluded from the pores of the stationary phase and, in fact, only the surface chains of the reversed phase would be accessible for interaction. It is also interesting to note that propionic acid, which is the weaker acid and less ionized is least excluded and has access to significantly more area of reversed phase than acetic acid.

Using the data given in Table 2 in conjunction with eqn. 5 as in the case of the alcohols, the adsorption isotherm equations were calculated for both acids.
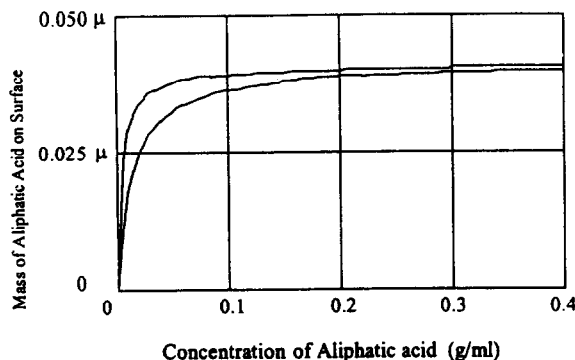

Fig. 6. The adsorption isotherms of acetic acid and propionic acid over the concentration range of 0 to 0.4 g ml^{-1}. It should be noted that the mass adsorbed is expressed as g cm^{-2}. On the y-axis, μ stands for μg.

Acetic acid:

$$C_s = \frac{C_m}{0.33 \cdot 10^6 + 2.4 \cdot 10^7 C_m} \quad (16)$$

Propionic acid:

$$C_s = \frac{C_m}{0.103 \cdot 10^6 + 2.43 \cdot 10^7 C_m} \quad (17)$$

In a manner, similar to that used with the alcohols, eqns. 16 and 17 were employed to construct the adsorption isotherms for the two aliphatic acids and these are shown in Fig. 6. It is seen that the acids exhibit the same type of isotherm as methanol and ethanol, the first two members of the alcohol series.

4.4. The relationship between K and the carbon number of a solvent/solute

The relationship between the log (K) and the carbon number of the aliphatic chain is shown for both the aliphatic alcohols and the two aliphatic acids in Fig. 7. It is seen that the curve

TABLE 2

CHROMATOGRAPHIC DATA FOR TWO ALIPHATIC ACIDS

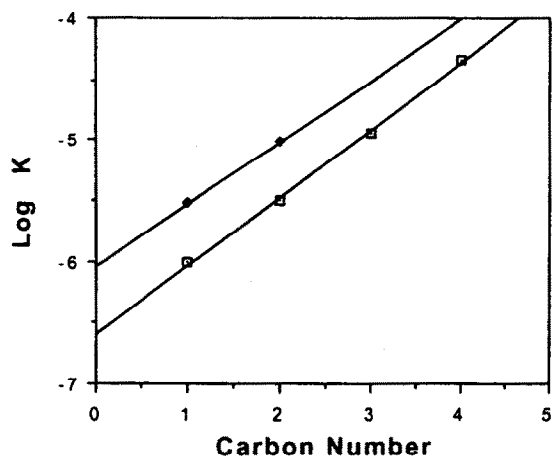| Solvent	Molecular area (Å^2)	Intercept (ml^{-1})	Slope (g^{-1})	NS/M 10^{-7} cm^2	Surface area (m^2)	Distribution coefficient (K)
Acetic acid	23.9	0.269	19.32	2.40	124.2	$3.00 \cdot 10^{-6}$
Propionic acid	29.8	0.0656	15.45	2.43	157.3	$9.69 \cdot 10^{-6}$

Fig. 7. Graphs of log K against carbon number of solute. $\square$ = Alcohols, $y = -6.5921 + 0.5589x$ ($R = 1.00$); $\blacklozenge$ = acids, $y = -6.0321 + 0.5092x$ ($R = 1.00$).

for the homologous series of alcohols is an excellent straight line and this linear relationship between log K and carbon number was observed by Martin [18], Colin and Guiochon [19] and Berendsen [20]. It is also seen that the slope of the line for the alcohols is very similar to that for the two acids, albeit the line for the aliphatic acids is drawn through only two points. The similarity in slopes would indicate that the contribution to log K by each methylene group tends to be independent of the terminal functional group.

In summary the use of binary solvent mixtures as mobile phases in reversed-phase chromatography changes the reversed phase surface in a predictable manner according to the Langmuir adsorption isotherm. The adsorption isotherm of any solvent or moderator, on a reversed phase, can be determined by a relatively simple chromatographic procedure without involving measurements where the methodology is uncertain or a subject of controversy. It follows, that if the adsorption isotherm is known for the moderator or solvent that is being used, then the nature of the interacting surface can also be identified and can be changed in a predictable manner to achieve specific changes in solute retention. The same will be basically true for a ternary mixture of solvents, but the surface will be more complex and the adsorption isotherms equations, al-

though essentially the same basic form as the Langmuir function, will be far more cumbersome. Nevertheless, if the Langmuir isotherm equation is determined for a given mobile phase system then the area occupied by each component of the mobile phase can be calculated and the net effect on solute retention predicted.

5. MOLECULAR INTERACTION KINETICS AND SOLUTE RETENTION

Solutes are distributed between two phases as a result of the relative strengths of the intermolecular forces between the solute molecules and those of the two phases together with their probability of interaction. The nature of the different intermolecular forces that can be involved have already been discussed and need no further consideration but the *probability* of molecular interaction is a relatively new concept and the factors that control it must now be considered.

5.1. Molecular interactions and retention in gas chromatography

The first major contribution to the molecular interaction theory of solute retention was discovered in gas chromatography (GC) where retention is solely controlled by interactions in the stationary phase and was made by McCann et al. [21] supported by the work of Laub and Purnell [22] and Laub [23]. These workers examined the effect of mixed phases on solute retention and arrived at the startling conclusion that the corrected retention volume of a solute was linearly related to the volume fraction of either one of the two phases.

That is

$$V'_{AB} = \alpha V'_A + (1 - \alpha)V'_B \tag{18}$$

where V'_{AB} is the corrected retention volume of a solute on the mixture of phases, V'_A is the corrected retention volume of the solute on phase A, V'_B is the corrected retention volume of the solute on phase B, and α is the volume fraction of phase A. Re-arranging eqn. 18

$$V'_{AB} = \alpha(V'_A - V'_B) + V'_B \qquad (19)$$

This remarkably simple relationship is depicted in Fig. 8. This relationship was confirmed for many substances by Laub and Purnell [22] and also by Laub [23]. There were, however, certain mixtures of stationary phases that did not exhibit the above relationship but these could be explained on the basis that there was either association between the phases such as in methanol water mixtures (which will be discussed later) or the self association of one component. In these cases the mixtures were no longer binary but ternary in nature and the simple relationship for the binary mixture would no longer apply. The distribution coefficient of the solute with any pure component of the stationary phase is not a variable. Consequently, the volume fraction of each phase must control the probability of interaction in much the same way that the partial pressure of a gas determines the probability of collision. Laub and Purnell [22] confirmed the above relationship in a number of interesting ways. He showed that the following alternative chromatography systems all provided the same value for the corrected retention volume of a substance. A given fraction α of phase A could be mixed with a fraction $(1 - \alpha)$ of phase B and coated on a support and packed in a column. The two fractions could, individually be coated on some support and the coated supports mixed and packed in a column. Finally each fraction could be coated on a support and packed into separate columns and the columns joined in series. Purnell experimentally demonstrated that all three columns gave exactly the
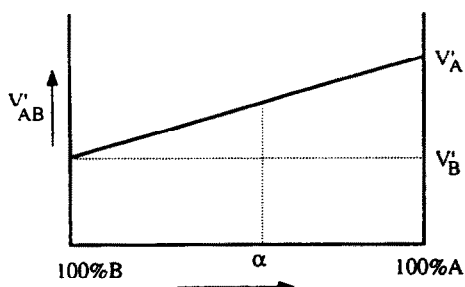

Fig. 8. Graph of corrected retention volume against volume fraction of stationary phase.

same corrected retention volume for a given solute. The effect of volume fraction of stationary phase was confirmed. More importantly, it shows that for the distribution systems examined, in effect, distribution coefficients can be summed but *not* their logarithms. It is now of interest to see if the same relationship can be obtained in liquid chromatography.

5.2. Molecular interactions and retention in liquid chromatography

The same experiments cannot be directly carried out in liquid chromatography using an LC column, because, as has already been shown, any change in volume fraction of one component of the mobile phase will simultaneously change both the nature of the stationary phase surface as well as the interactions in the mobile phase. Katz et al. [24] avoided the problem by employing a liquid–liquid distribution system using water and a series of immiscible solvent mixtures and by measuring absolute distribution coefficients as opposed to retention volumes. They measured the distribution coefficient of n-pentanol between water and mixtures of n-heptane and chloroheptane, n-heptane and toluene and n-heptane and heptyl acetate. The two-phase system was thermostatted at 25°C and, after equilibrium had been established, the concentration of solute in the two phases was determined by GC analysis. The results they obtained are shown in Fig. 9.

It is seen that the same linear relationships between solvent composition and distribution coefficient was obtained for all three solvent mixtures simulating the results that Purnell and Laub obtained in their GC experiments. The results obtained will be described by exactly the same equation and thus, knowing the distribution coefficient of pentanol between water and any of the pure solvents then the distribution coefficient can be calculated for any binary mixture of those solvents and water. Again the results demonstrate, that in the distribution systems examined, the actual distribution coefficients can be summed but not their logarithms. It is seen that the concentration of any solvent in the mixture controls the probability of interaction between the solute and that solvent. For
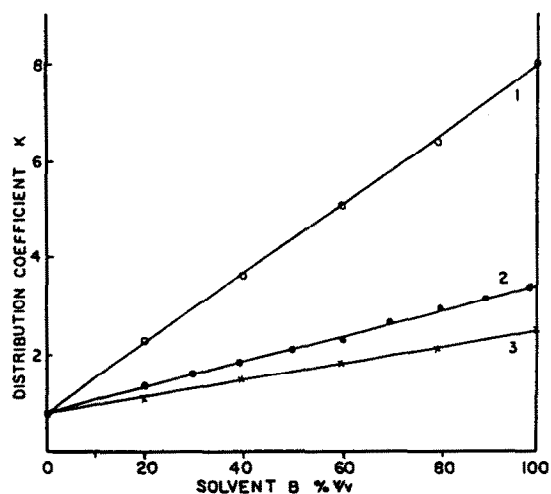
Fig. 9. Graphs showing the distribution coefficient of *n*-pentanol between water and a binary solvent mixture plotted against solvent composition. Solvent A: *n*-heptane; solvent B: 1 = heptyl acetate, 2 = toluene, 3 = heptyl.
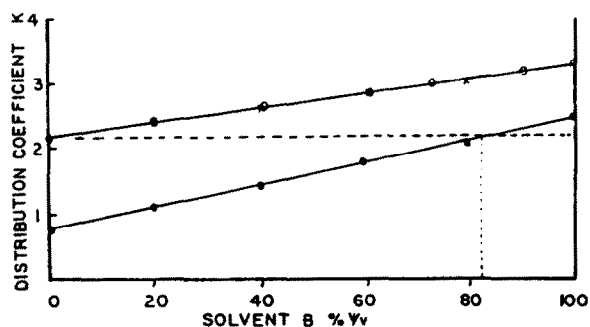

Fig. 10. Graphs showing the distribution coefficient of *n*-pentanol between water and a binary solvent mixture plotted against solvent composition. (O) Solvent A: carbon tetrachloride, solvent B: toluene; (●) solvent A: *n*-heptane, solvent B: heptyl chloride; (×) solvent A: heptyl chloride–*n*-heptane (82:18, v/v), solvent B: toluene.

example in the pure solvent the probability of interaction is unity. In a 50% (v/v) mixture of two solvents the probability of interaction between the solute and either solvent is 0.5.

Katz *et al.* tested the theory further and measured the distribution coefficient of *n*-pentanol between mixtures of carbon tetrachloride and toluene and pure water and mixtures of *n*-heptane and *n*-chloroheptane and pure water. The results they obtained are shown in Fig. 10. The linear relationship between the distribution coefficient and the volume fraction of the respective solvent is again clearly demonstrated. It is seen that the distribution coefficient of *n*-pentanol between water and carbon tetrachloride is about 2.2 and that an equivalent value for the distribution coefficient of *n*-pentanol was obtained between water and a mixture of chloroheptane–*n*-heptane (82:18, v/v). Katz *et al.* repeated the experiment with toluene but this time using a chloroheptane–*n*-heptane (82:18) mixture in place of carbon tetrachloride. In fact, a ternary mixture was used comprised of toluene, chloroheptane–heptane and *n*-heptane but the chloroheptane–heptane and *n*-heptane was always in the ratio of 82:18 by volume to simulate carbon tetrachloride. It is seen that the chloro-

heptane–heptane/*n*-heptane mixture behaves in an identical manner to carbon tetrachloride and all the points are on the same straight line as that produced using a mixture of carbon tetrachloride and toluene. These experiments are similar to normal-phase chromatography using pure water instead of silica gel except that the water phase is not modified by the solvents in the way a silica gel surface would be.

6. AQUEOUS SOLVENT MIXTURES

When the relationship between the distribution coefficient of a solute and solvent composition, or the corrected retention volume and the solvent composition, was tested with aqueous solvent mixtures it was found that the relationship identified by Purnell and Laub and Katz *et al.* failed. It was suspected that the failure was due to the solvent strongly associating with the water and, in fact, an aqueous solution of methanol, for example, contained methanol, water and methanol associated with water. The solvent mixture was thus a ternary system and the prediction of net distribution coefficient or net retention volume required the use of three distribution coefficients. One representing the distribution of the solute between the stationary phase and water, one representing that between the stationary phase and methanol and one be-

R.P.W. Scott / J. Chromatogr. A 656 (1993) 51–68

63

tween the stationary phase and the methanol–water associate.

The association of methanol and water was examined by Katz et al. [25] using volume change on mixture and refractive index data and established that the methanol–water solvent system was indeed a complex ternary system. They calculated both the association equilibrium constant and the distribution of the different components of a methanol water mixture from zero to 100% methanol. The curves they obtained are shown in Fig. 11. It is seen from Fig. 11 that there are three distinct ranges of methanol concentration where the solvent will behave very differently. From zero to 40% (v/v) of methanol in the original mixture the solvent will behave as though it were a binary mixture of water and methanol associated with water. From 40 to 80% (v/v) of methanol in the original mixture the solvent will behave as though it were a ternary mixture of water, methanol and water associated with methanol. From 80 to 100% (v/v) of methanol in the original mixture the solvent will behave as though it were again a binary mixture but this time a mixture of methanol and water associated with methanol. The curves shown in Fig. 11 shed some light on the unique characteristics of mobile phases consisting of methanol–water mixtures when used in reversed-phase LC. From Fig. 11 it is seen that when the original methanol mixture contains 50% (v/v) of
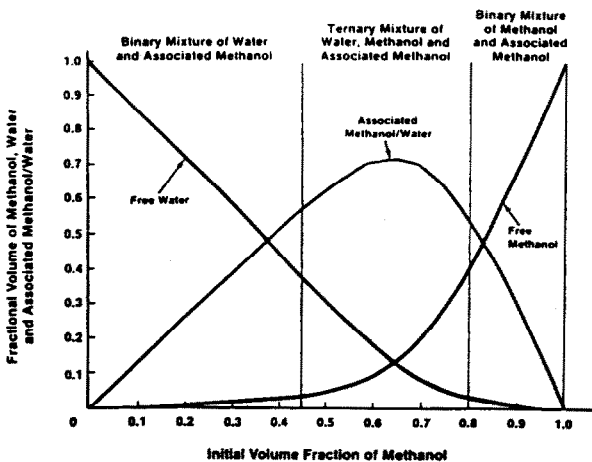

Fig. 11. Graphs showing relative volume fraction of water, methanol and associated methanol against original volume fractions of methanol in the mixture.

methanol there is little free methanol available in the mobile phase to elute the solutes as it is mostly associated with water. Subsequently, however, the amount of methanol unassociated with water increases rapidly in the solvent mixture and this rapid increase must be accommodated by the use of a convex gradient profile when employing gradient elution. The convex gradient will compensate for the strongly concave form of the unassociated methanol concentration profile shown in Fig. 11 which will be the strongest eluting component of the mobile phase. The strong association of methanol with water could also account for the fact that proteins can tolerate a significant amount of methanol in the mobile phase without them becoming denatured. This is because there is virtually no unassociated methanol present in the mixture which could cause protein denaturation since all the methanol is in a deactivated state by association with water.

Katz et al. [25] also examined acetonitrile–water, and tetrahydrofuran (THF)–water mixtures in the same way and showed that there was significant association between the water and both solvents but not to the same extent as methanol–water. At the point of maximum association for methanol, the solvent mixture contained nearly 60% of the methanol–water associate. In contrast the maximum amount of THF associate that was formed amounted to only about 17% and for acetonitrile the maximum amount of associate that was formed was as little as 8%. It follows that acetonitrile–water mixtures would be expected to behave more nearly as binary mixtures than methanol–water or THF–water mixtures.

7. A BASIC DISTRIBUTION THEORY

Employing the principles experimentally demonstrated by Laub and co-workers [22,23] and Katz et al. [24], Scott [26] put forward the following equation to describe the distribution coefficient of a solute between two phases:

$$K_2^1 = \sum_{r=1}^{r=n} \varphi_r \alpha_r \; \frac{1}{\sum\limits_{p=1}^{p=m} \varphi_p' \nu_p} \tag{20}$$

where K_2^1 is the distribution coefficient of a solute between two solvent mixtures containing n and m components, respectively; φ_r and φ_p' are constants characteristic of the interaction forces between the solute and solvents r and p, respectively; α_r and ν_p are the volume fractions of solvents r and p in phases 1 and 2, respectively; and n and m refer to the stationary phase and mobile phase components, respectively.

The constants φ_r and φ_p' may be considered as some measure of the interaction potential energy of the solute when completely surrounded by the solvent. They can be expressed in the form

$$\varphi_r = A\,e^{-G_r/RT} \quad \text{and} \quad \varphi_p = A'\,e^{-G_p/RT}$$

where G_r and G_p are the free energies of the solute molecules when completely surrounded by solvent molecules.

For two single immiscible solvents n, m, α_r and ν_p become unity and eqn. 20 simplifies to:

$$K = \frac{\varphi_r}{\varphi_p'} = \frac{A\,e^{-G_r/RT}}{A'\,e^{-G_p/RT}} = A''\,e^{-\Delta G/RT} \tag{21}$$

where $A'' = A/A'$ and ΔG is the excess free energy of the solute when exchanging between the two solvents.

Eqn. 21 is consistent with the normal expression for the distribution coefficient of a solute between two immiscible phases.

As $\varphi_r/\varphi_p' = K_p^r$, the distribution coefficient of a solute between phase component r and phase component p, eqn. 20 can, if so desired for convenience, be put in the form

$$K_2^1 = \sum_{r=1}^{r=n} \left(\frac{\alpha_r}{\sum_{p=1}^{p=m} K_r^p \nu_p} \right) \tag{22}$$

Consider eqn. 18 and the experiments carried out by Purnell and Laub by GC.

In their experiments, $n = 2$ and $m = 1$, and thus, $\nu = 1$ and eqn. 20 reduces to:

$$K = \frac{\varphi_1}{\varphi'} \cdot \alpha_1 + \frac{\varphi_2}{\varphi'} \cdot \alpha_2 = K_1\alpha_1 + K_2\alpha_2$$

Now

$$\alpha_2 = (1 - \alpha_1)$$

Thus,

$$K = K_1\alpha_1 + K_2(1 - \alpha_1)$$

or

$$V_r' = K_1\alpha_1 V_1 + K_2(1 - \alpha_1)V_1 \tag{23}$$

where V_1 is the total volume of stationary phase in the column.

Eqn. 23 is the relationship experimentally confirmed by Purnell and Laub.

Now consider a situation in LC where a binary mixture of solvents is employed as the mobile phase and the stationary phase is covered with solvent and thus, represents a single surface. Under such circumstances in eqn. 20, $n = 1$ thus, $\alpha = 1$ and $m = 2$,

Then eqn. 20 becomes,

$$K = \frac{\varphi_1}{\varphi_1'\nu_1 + \varphi_2'\nu_2}$$

Now, $V' = KA_s$, where V' is the corrected retention volume of a solute and A_s is the surface area of the stationary phase in the LC column.

$$\frac{1}{V_r'} = A\nu_1 + B\nu_2$$

where

$$A = \frac{\varphi_1'}{\varphi A_s} \quad \text{and} \quad B = \frac{\varphi_2'}{\varphi A_s}$$

Now, if there is no phase component association,

$$\nu_2 = (1 - \nu_1)$$

Thus,

$$\frac{1}{V_r'} = B + A'\nu_1 \tag{24}$$

where

$$A' = A - B$$

The validity of eqn. 24 has been established by a number of workers. McCann *et al.* [21] measured the corrected retention volumes of a number of different solutes using silica gel as the stationary phase and a binary mixture of diethyl ether and carbon tetrachloride as the mobile phase. The column was 25 cm long and 4.6 mm in diameter packed with Hypersil silica gel having a mean diameter of 5 μm. They measured the corrected retention volume of the solutes phenol, nitromethane and nitroethane and 3-phenyl-propane-1-ol and confirmed the linear relationship between the reciprocal of the corrected retention volume and the volume fraction of one component (diethyl ether) of the binary mixture. An example of the results they obtained for phenol is given in Fig. 12.

It is seen that the curve is clearly linear with a high correlation coefficient demonstrating unambiguously the validity of eqn. 24. They also noted a deviation at the very low levels of diethyl ether (0–10%, v/v, of diethyl ether) in the data for nitromethane and nitroethane indicating that the character of the stationary phase was changing. This was probably due to a layer of ether being adsorbed on to the silica surface and, according to the Langmuir equation, changing the nature of the interacting stationary phase surface. The layer was probably complete when the mobile phase contained 10% v/v of diethyl ether and any subsequent changes in retention
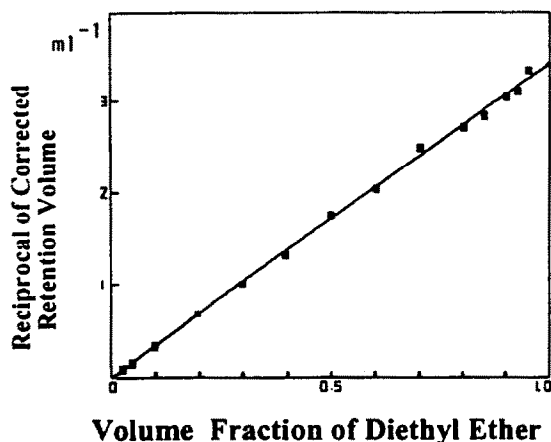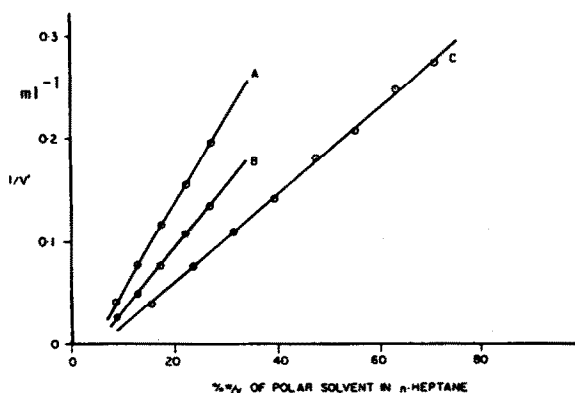

Fig. 13. Graph of the reciprocal of the corrected retention volume against the volume fraction of the polar solvent in *n*-heptane. (A) Solute: benzyl alcohol, polar solvent: tetrahydrofuran; (B) solute: 3-phenyl-1-propanol, polar solvent: tetrahydrofuran; (C) solute: desoxycorticosterone, polar solvent: isopropanol.

were due to interactions in the mobile phase defined by eqn. 24. Further support for eqn. 24 was afforded by the work of Scott and Kucera [26] who used a column of 25 cm × 4.6 mm I.D. packed with Partisil 10 silica gel. The solvent systems used was a mixture of tetrahydrofuran and *n*-heptane and isopropanol and *n*-heptane with concentrations of polar solvent ranging from about 8 to 70% (v/v). The solutes used were benzyl alcohol, 3-phenyl-1-propanol and desoxycorticosterone; the results are shown in Fig. 13 as curves relating the reciprocal of the corrected retention volume to the volume fraction of the polar component of the mobile phase. It is seen that the same linear curves are obtained as those of Laub and Purnell [22], again supporting the predictions of eqn. 24.

8. SOLUTE INTERACTIONS WITH ASSOCIATED SOLVENTS

Testing the applicability of eqn. 20 to liquids where the solvent components associate with themselves and each other is experimentally difficult. Katz *et al.* [25] attempted to do this by measuring the distribution coefficients of some solutes between an hydrocarbon and methanol water mixtures; the solvents they used were *n*-pentanol, cyclohexyl acetate, vinyl acetate and benzene. The distribution system chosen was

Volume Fraction of Diethyl Ether

Fig. 12. Graph of the reciprocal of the corrected retention volume of phenol against the volume fraction of diethyl ether in the mobile phase.

hexadecane (to closely emulate a reversed phase) and methanol–water mixtures having compositions ranging from 0 to 80% (v/v) of methanol in the original made up mixture. Obviously, due to the strong association of water and methanol the actual quantity of methanol unassociated with water in the solvent mixture will be very much less than the actual methanol added.

Considering the hexadecane/water–methanol system with respect to eqn. 20, since, due to association, there are three components present in the water–methanol mixture, $n = 3$. Furthermore as the other phase hexadecane is a single component solvent then, $m = 1$ and $v = 1$.

Thus, eqn. 20 for the system used by Katz et al. [25] reduces to

$$K = \frac{\varphi_1 \alpha_1 + \varphi_2 \alpha_2 + \varphi_3 \alpha_3}{\varphi'}$$

Now, $K_M = \varphi_1/\varphi'$ where K_M is the distribution coefficient of the solute between methanol unassociated with water and hexadecane; $K_{MW} = \varphi_2/\varphi'$ where K_{MW} is the distribution coefficient of the solute between methanol associated with water and hexadecane; $K_W = \varphi_3/\varphi'$ where K_W is the distribution coefficient of the solute between water unassociated with methanol and hexadecane.

Thus,

$$K = K_M \alpha_1 + K_{MW} \alpha_2 + K_W \alpha_3$$

where α_1, α_2 and α_3 are the volume fractions of methanol unassociated with water, methanol associated with water and water unassociated with methanol, respectively.

The results obtained by Katz et al. [25] are shown as experimental points on the curves relating the distribution coefficient of the solute against volume fraction of methanol added to the original mixture in Fig. 14. Due to the difficulty of measuring the distribution coefficient of each solute between pure water and hexadecane because of their extremely high retention, the values were obtained from a polynomial curve fit to the data giving a value for K at $\alpha = 0$. From the data obtained for each of the solutes and a knowledge of each respective value of α_1, α_2 and
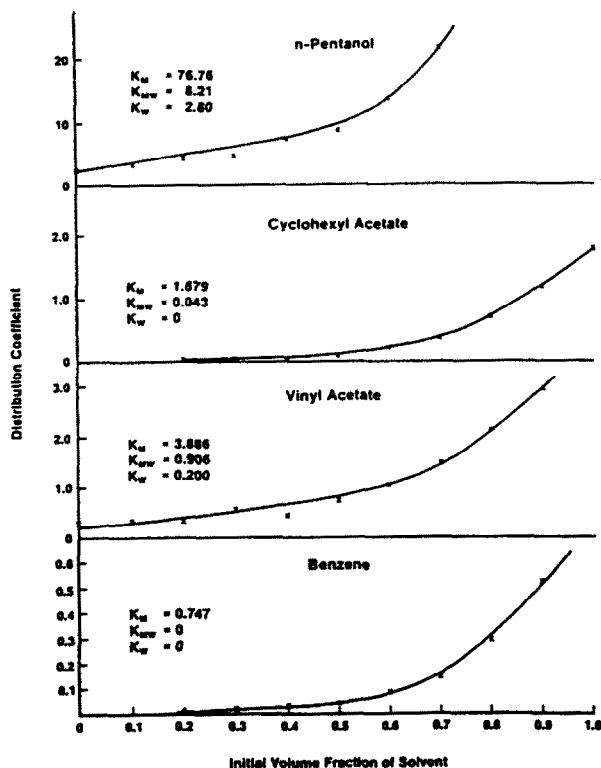

Fig. 14. Graph of distribution coefficient of solute between methanol–water mixtures and hexadecane against volume fraction of methanol in solvent mixture.

α_3 from the work of Katz et al. [25], the values of K_M and K_{MW} were calculated with the aid of a simple iterative computer program and the results obtained are included in each graph. Using the values of K_M and K_{MW} for each solute the theoretical relationship between volume fraction of methanol and solute distribution coefficient was calculated and the values obtained are shown as the curves in Fig. 14. The agreement between the calculated curves and the experimental points is encouraging.

The values of the individual distribution coefficients shown in Fig. 14 give insight into the nature of the solutes. Obviously, pentanol the most polar has significant interactions with both the methanol associated with water and water itself. In contrast benzene apparently has little or no interaction with methanol associated with water or water as the values of K_{MW} and K_W are zero. Interaction between benzene and the aque-

ous solvent mixture appears to occur solely with the methanol unassociated with water. Cyclohexyl acetate associates weakly with methanol associated with water but not with water itself whereas vinyl acetate, like pentanol, interacts with all three components of the aqueous mixture albeit only very weakly with water.

The results for benzene suggests an interesting possibility. If interaction only occurs with the methanol unassociated with water then the system resembles that of Laub and Purnell [22] and Katz et al. [24] in that the distribution coefficient of benzene between methanol–water mixtures and hexadecane should be linearly related to the volume fraction of methanol that is *unassociated* with water.

This is because, from the results in Fig. 14, benzene does not appear to interact with methanol associated with water or water itself. The data for the volume fraction of unassociated methanol is available from the work of Katz et al. [25] and in Fig. 15 the distribution coefficient of benzene is plotted against volume fraction of methanol unassociated with water.

It is seen that the linear curve is obtained with zero intercept indicating that the distribution of benzene in the aqueous phase relative to hexadecane is solely due to that portion of the methanol in the aqueous mixture that is unassociated with water. In fact, the methanol associated with water plays no significant part in competing for the benzene against the dispersive interactions of

the hexadecane. This is hardly surprising as, although benzene is polarizable, it has no permanent dipole, and the dispersive interactions between the methyl group of the methanol and benzene will be very much grater than any polar interactions that might occur with water or water associated with methanol.

9. GENERAL COMMENTS

Despite the theories discussed in this paper and the help they give in accounting for solvent composition and solvent association, the full explanation of solute retention on silica, bonded phases, or for that matter between liquid/liquid distribution systems, is still elusive. The long sought after *kinetic theory of liquids* that will allow the distribution coefficient of any substance between any two immiscible phases to be calculated from basic physical chemical data remains in obscurity. It would appear that the "philosophers stone" of the chromatographer is yet to be found.

Considerably more work appears to be necessary and, in the meantime, we must content ourselves with empirical equations, based on arbitrary relationships, in the hope that one of them will provide an approximate estimate of the optimum phase system we require for a particular separation.
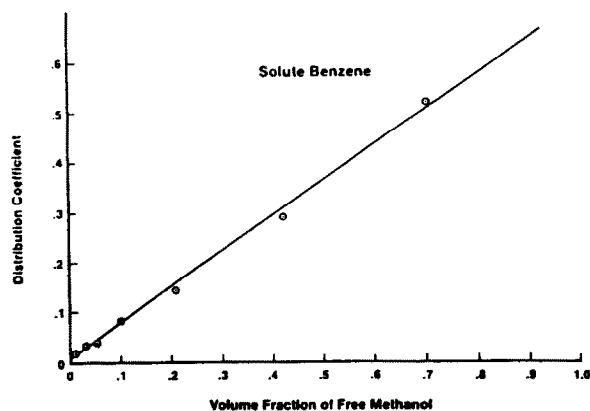

Fig. 15. Graph of distribution coefficient of benzene between methanol–water mixture and *n*-hexadecane against volume fractions of free methanol.

REFERENCES

1 I. Halász and I. Sebestian, *Angew. Chem., Int. Ed. Engl.*, 8 (1969) 453.
2 C.F. Simpson, *UK Pat.*, 1 310 872 (1975).
3 R.K. Gilpin and M.F. Burke, *Anal. Chem.*, 45 (1973) 1383.
4 S.O. Akapo, *Ph. D. Thesis*, University of London, London, UK, 1989.
5 T.M. Kong and C.F. Simpson, *Chromatographia*, 24 (1987) 385.
6 S.O. Akopo, R.P.W. Scott and C.F. Simpson, *J. Liq. Chromatogr.*, 14 (1991) 217.
7 R.P.W. Scott and C.F. Simpson, *J. Chromatogr.*, 197 (1980) 11.
8 C.H. Lochmüller and D.R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
9 R.K. Gilpin and J.A. Squires, *J. Chromatogr. Sci.*, in press.

10 R.P.W. Scott and C.F. Simpson, *Faraday Symp. Chem. Soc.*, 15 (1980) 69.

11 D.N. Northrop, R.P.W. Scott and Daniel E. Martire, *Anal. Chem.*, 63 (1991) 1354.

12 P.L. Zhu, *Chromatographia*, 20 (1985) 425.

13 J.H. Knox and R.J. Kaliszan, *J. Chromatogr.*, 349 (1985) 211.

14 R.J. Smith and C.S. Nieass, *J. Liq. Chromatogr.*, 9 (1986) 1387.

15 H. Colin, A. Krstulovic and G. Guiochon, *Anal. Chem.*, 54 (1982) 2438.

16 R.A. Djerki and R.J. Laub, *J. Liq. Chromatogr.*, 10 (1987) 1749.

17 E. Grushka, *J. Liq. Chromatogr.*, 5 (1982) 1392.

18 A.J.P. Martin, *Biochem. Soc. Symp.*, 3 (1949) 4.

19 H. Colin and G. Guiochon, *J. Chromatogr. Sci.*, 18 (1980) 54.

20 G.E. Berendsen, *Doctorate Thesis*, Technische Hogeschool Delft, Delft, 1979.

21 M. McCann, J.H. Purnell and C.A. Wellington, *Faraday Symp. Chem. Soc.*, (1980) 83.

22 R.J. Laub and J.H. Purnell, *J. Chromatogr.*, 112 (1975) 71.

23 R.J. Laub, in P. Kuwana (Editor), *Physical Methods in Modern Chromatographic Analysis*, Academic Press, New York, 1983, Ch. 4.

24 E.D. Katz, K. Ogan and R.P.W. Scott, *J. Chromatogr.*, 352 (1986) 67.

25 E.D. Katz, C.H. Lochmüller and R.P.W. Scott, *Anal. Chem.*, 61 (1981) 344.

26 R.P.W. Scott, *J. Chromatogr.*, 122 (1976) 35.

27 R.P.W. Scott and P. Kucera, *J. Chromatogr.*, 112 (1975) 425.