

CHROMSYMP. 756

## DISTRIBUTION OF A SOLUTE BETWEEN TWO PHASES

### THE BASIC THEORY AND ITS APPLICATION TO THE PREDICTION OF CHROMATOGRAPHIC RETENTION

E. D. KATZ, K. OGAN and R. P. W. SCOTT\*

*The Perkin-Elmer Corporation, 761 Main Ave, Norwalk, CT 06859-0089 (U.S.A.)*

---

#### SUMMARY

A simple theory that explains the distribution of a solute between two phases is put forward and is experimentally validated employing both previously published data and results from unambiguous liquid-liquid distribution experiments. The theory is then extended to liquid-liquid and liquid-solid distribution systems where association takes place between the components of the liquid phase. It is shown that under such circumstances a binary mixture becomes, in fact, a ternary system where the third component comprises the associated solvent. The properties of the ternary system are shown to be accounted for by the basic theory and, further, the theory can be employed to predict distribution behavior in liquid-liquid systems where association occurs. It is shown that the theory can also be used to predict solute retention in reversed-phase liquid chromatography.

---

#### INTRODUCTION

There have been very few papers published that deal with the theory of solute distribution between two phases and that provided an explicit equation relating distribution coefficient to phase composition. Such an equation could aid in the prediction of solute retention in liquid chromatography (LC). In 1976 Scott<sup>1</sup> put forward a simple theory that described the role played by phase composition in solute distribution and based on the concept that the concentration of a solvent component controls its probability of interaction with the solute. This paper aroused considerable controversy<sup>2</sup>. Since 1976, however, there have been a continuous number of publications reporting data that supports Scott's original hypothesis; notably, an extensive amount of data published by Purnell and Laub<sup>3</sup>, which has been summarized in two review papers by Laub<sup>4,5</sup>, and work in LC published by Scott and Kucera<sup>6</sup>, Robbins and McElroy<sup>7</sup>, Hurtubise *et al.*<sup>8</sup>, and Purnell *et al.*<sup>9</sup>.

One area where the theory appeared to fail was in reversed-phase chromatography, and this was due to incorrectly assuming that the solvent mixture comprising water and methanol was a binary system, whereas, due to water-methanol association, the system was, in fact, ternary. However, the apparent failure of the theory

to account for retention in reversed-phase systems resulted in further discredit. It follows that with new and significant experimental support for the theory, this is an appropriate time to extend the hypothesis and to develop procedures and equations to aid in the prediction of distribution coefficients and, consequently, solute retention in both normal- and reversed-phase chromatography.

Our paper consists of three parts. The first part extends the theory and demonstrates how published results provide convincing support for the theory. Some simple liquid-liquid distribution experiments are then described and the results used to demonstrate how the theory can be employed to explain the distribution behavior of more complex solvent systems.

In the second part the association of methanol-water, acetonitrile-water and tetrahydrofuran-water mixtures is examined theoretically and experimentally and by the determination of the change in volume on mixing, the exact composition of the solvent mixtures employed in reversed-phase chromatography is identified. Employing the correct solvent composition, the distribution theory is used to predict the distribution coefficients of a number of solutes in simple liquid-liquid reversed-phase systems and the results are compared with those obtained experimentally.

In the third part the true composition of reversed-phase solvent systems is used in conjunction with the distribution theory to predict retention in reversed-phase chromatographic columns and the results obtained are compared with those experimentally measured and the validity of the theory is again demonstrated.

#### BASIC DISTRIBUTION THEORY

The equation originally put forward by Scott<sup>1</sup> took the following form:

$$K = \frac{\sum_{r=1}^{r=n} \varphi_r v_r}{\sum_{p=1}^{p=m} \varphi'_p v_p} \quad (1)$$

where  $K$  is the overall distribution coefficient of the solute between the two immiscible solvents,  $\varphi_r$  and  $\varphi'_p$  are constants characteristic of the interaction of the solute with the respective solvent,  $v_r$  is the volume fraction of solvent  $r$  in phase 1 and  $v_p$  is the volume fraction of solvent  $p$  in phase 2.

The constants  $\varphi_r$  and  $\varphi'_p$  may be considered as being some measure of the interaction potential energy of the solute when completely surrounded by the solvent. They can probably be expressed in the form

$$\varphi_r = Ae^{-G_r/RT} \text{ and } \varphi'_p = A'e^{-G_p/RT}$$

where  $G_r$  or  $G_p$  are the free energies of the solute molecule when completely surrounded by the respective solvent molecules and  $T$  is the absolute temperature.

For two single immiscible solvents,  $n$  and  $m$ ,  $v_r$  and  $v_p$  become unity and the expression becomes simplified to

$$K = \varphi_r/\varphi'_p = Ae^{-G_r/T}/A'e^{-G_p/T} = A''e^{-\Delta G/T} \quad (2)$$

where  $A'' = A/A'$  and  $\Delta G$  is the excess free energy of the solute between the two phases.

Eqn. 2 is consistent with the normal expression for the distribution coefficient of a solute between two immiscible phases.

Eqn. 1 invokes the principle of the summation of distribution coefficients, which in general appears to be in conflict with accepted ideas of the nature of distribution coefficients. It is generally thought that it is the logarithm of the distribution coefficient that is additive and this assumption has arisen from the relationship between distribution coefficient and free energy, the logarithms of which are additive. Certainly, it is the logarithm of the distribution coefficient that is linearly related to the reciprocal of the temperature.

As  $\varphi_r/\varphi'_p = K_p^r$  is the distribution coefficient of a solute between phase component  $r$  and phase component  $p$ , eqn. 1 can be put into an alternative form

$$K = \sum_{r=1}^{r=n} \left( v_r \left/ \sum_{p=1}^{p=m} K_p^r v_p \right. \right)$$

Consider the form that eqn. 1 takes when the distribution of the solute is between a gas and a stationary phase consisting of a binary mixture. The value of  $r$  will be 2, the value of  $p$  will be unity, and eqn. 1 will take the form of

$$K = \frac{\varphi_1}{\varphi'} v_1 + \frac{\varphi_2}{\varphi'} v_2 = K_1 v_1 + K_2 v_2 \quad (3)$$

where  $\varphi_1/\varphi' = K_1$  is the distribution coefficient of the solute between the stationary phase component 1 and the gas and  $\varphi_2/\varphi' = K_2$  is the distribution coefficient of the solute between the stationary phase component 2 and the gas. The validity of eqn. 3 has been tested experimentally with many hundreds of solutes and stationary phase mixtures, and the results have been summarized by Laub<sup>4,5</sup>. The results are in complete agreement with those predicted by eqn. 3, with the exception of those stationary phases where the individual components became associated and, consequently, were in fact ternary stationary phase mixtures and not binary mixtures. It follows that retention behavior could not be described by eqn. 3, as this was derived for binary mixtures only. It will be seen later, however, that solute distribution can be predicted by eqn. 1 for solvent mixtures where association takes place, providing the appropriate form of eqn. 1 is utilized. Eqn. 1 can be also put into a form that is pertinent to liquid-solid chromatography:

$$K = \sum_{r=1}^{r=n} \varphi_r a_r \left/ \sum_{p=1}^{p=m} \varphi'_p v_p \right. \quad (4)$$

where  $a_r$  now is the area fraction of the surface  $r$  and the other symbols have the significance previously attributed to them.

Consider an LC system consisting of two solvents ( $m = 2$ ) and a single surface ( $n = 1$ ). Such a system would be typified by that of silica gel and a solvent mixture consisting of *n*-heptane and ethyl acetate, the level of ethyl acetate being in excess of

about 3% (v/v) to ensure a homogeneous surface coverage of the polar solvent<sup>6</sup>.

Under the circumstances  $n = 1$ ,  $m = 2$ , and  $a_r = 1$  (a single surface), eqn. 4 can be put in the form

$$K = \frac{\varphi}{\varphi'_1 v_1 + \varphi'_2 v_2}$$

Now  $V' = KA_s$ , where  $V'$  is the corrected retention volume of a solute, and  $A_s$  is the total surface area of stationary phase in the column. Thus,  $1/V' = Av_1 + Bv_2$ , where  $A = \varphi'_1/\varphi A_s$  and  $B = \varphi'_2/\varphi A_s$ . Now, if there is no phase component association,  $v_2 = (1 - v_1)$ . Thus,

$$\frac{1}{V'} = B + A'v_1 \text{ where } A' = A - B \quad (5)$$

The relationship given in eqn. 5 has been experimentally supported by a number of workers in the field<sup>6-9</sup>. However, the data published employing LC systems with silica gel as the stationary phase suffered some criticism, as the linearity observed between the reciprocal of the corrected retention volume and solvent volume fraction was claimed to be due, at least in part, to surface effects on the silica gel. It follows that for conclusive proof of eqn. 1, it should be tested against liquid-liquid systems where the results can not be confused by any surface effects. It is convenient that the examination of the effect of solvent composition on solute distribution in such systems is experimentally very easy to carry out. However, solvents have to be employed that have a mutual insolubility with water to ensure that the composition of one phase is not affected by that of the other (see Table I).

### *Solute interactions with non-associated liquid mixtures*

#### *Experimental procedure*

The basic experimental procedure was as follows: 25 ml of water was placed in a 60-ml bottle, and then 25 ml of the solvent or solvent mixture was added. The latter contained about 0.1% of the solute to be examined, together with about 0.1% of a suitable gas chromatography standard, such as tetradecane. The bottle was

TABLE I  
SOLUBILITY DATA FOR SOLVENTS

<i>Solvent</i>	<i>Solubility of solvent in water (g/ml)</i>	<i>Solubility of water in solvent (g/ml)</i>
<i>n</i> -Heptane	$2.9 \cdot 10^{-6}$	$9.1 \cdot 10^{-5}$
Carbon tetrachloride	$7.7 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
<i>n</i> -Butyl chloride	$1.1 \cdot 10^{-3}$	$8.0 \cdot 10^{-4}$
Heptyl chloride	$< 1.1 \cdot 10^{-3}$	$< 8.0 \cdot 10^{-4}$
Toluene	$5.2 \cdot 10^{-4}$	$3.4 \cdot 10^{-4}$
Heptyl acetate	$< 1.7 \cdot 10^{-3}$	$< 1.1 \cdot 10^{-2}$
Trichloroethylene	$1.1 \cdot 10^{-3}$	$3.2 \cdot 10^{-3}$

sealed with a PTFE-lined cap and thermostated at 25°C. Prior to addition of the solvent mixture to the vessel, an aliquot of the mixture was taken and analyzed in triplicate by a gas chromatographic procedure. The average ratio of the area of the solute peak to that of the standard was noted. The bottle containing the water and solvent mixture was then shaken every 5 min for a period of 1/2 h while situated in the thermostated bath. Three replicate analyses were then carried out on samples taken from the solvent layer, and the ratio of peak area of the solute to that of the standard was again measured. It can be easily shown that the distribution coefficient,  $K$ , is given as

$$K = 1/(a/b - 1)$$

where  $a$  is the area ratio of the solvent peak to that of the standard before equilibrium with water and  $b$  is the area ratio of the solvent peak to that of the standard after equilibrium with water.

This procedure is valid, as the tetradecane standard is substantially insoluble in water. Consequently, the distribution coefficient of 1-pentanol was measured for different solvent mixtures of *n*-heptane and *n*-butyl chloride and of *n*-heptane and carbon tetrachloride. The results obtained are shown in Fig. 1. The same experiment was carried out again using binary mixtures of *n*-heptane and *n*-butyl chloride but, in this case, three other solutes were examined; ethyl acetate, tetrahydrofuran, and *n*-propanol. The results obtained, given as curves relating the distribution coefficient to solvent composition, are shown in Fig. 2. The effect of solvent composition on the distribution coefficient of 1-pentanol was then determined for mixtures of *n*-heptane and *n*-heptyl chloride and of toluene and *n*-heptyl acetate, respectively. The results obtained are shown in Fig. 3.

In Fig. 4, the relationship between the distribution coefficient and the solvent

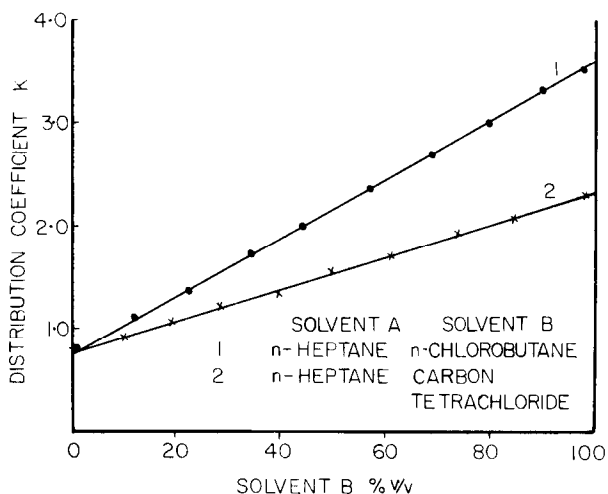


Fig. 1. Graphs showing the distribution coefficient of *n*-pentanol between water and a binary solvent mixture plotted against solvent composition.

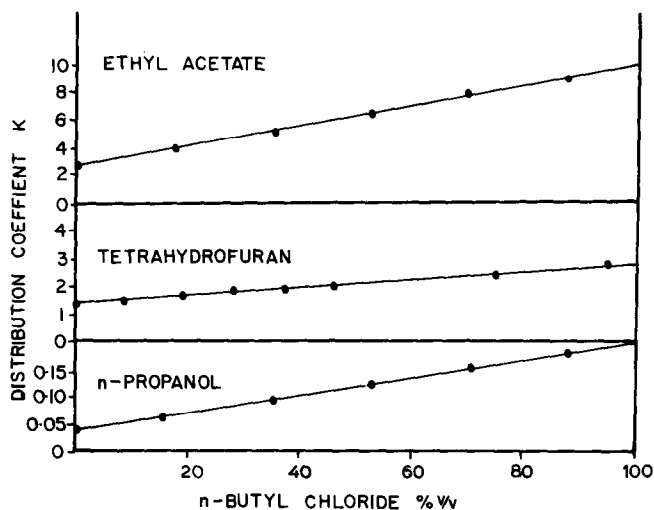


Fig. 2. Graphs showing the distribution coefficients for three solutes between water and a mixture of *n*-heptane and *n*-butyl chloride plotted against solvent composition.

composition, determined in the same manner, is shown for 1-pentanol, but in this case, between mixtures of carbon tetrachloride and toluene together with mixtures of *n*-heptane and *n*-heptyl chloride. It is seen that the distribution coefficient of *n*-pentanol between pure carbon tetrachloride and water is about 2.2; it is also seen that a mixture of approximately 82% *n*-heptyl chloride and 18% *n*-heptane gives an equivalent distribution coefficient to that of carbon tetrachloride. Consequently, the distribution coefficient of 1-pentanol was determined for solvent mixtures of 82%

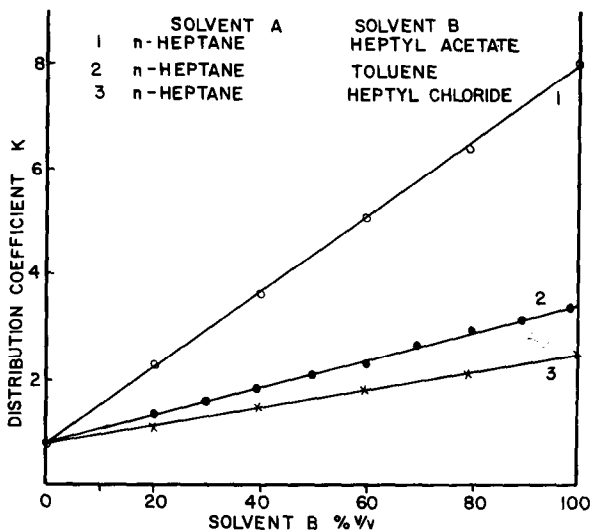


Fig. 3. Graphs showing the distribution coefficient of *n*-pentanol between water and a binary solvent mixture plotted against solvent composition.

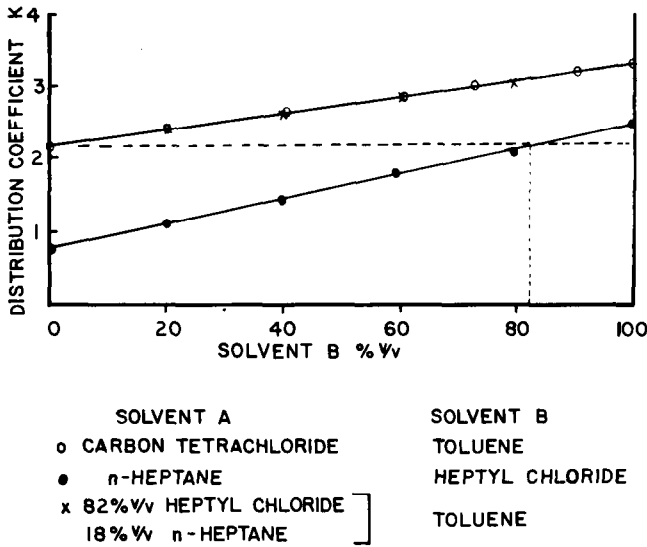


Fig. 4. Graphs showing the distribution coefficient of *n*-pentanol between water and a binary solvent mixture plotted against solvent composition.

*n*-heptyl chloride and 18% *n*-heptane and toluene as the equivalent to carbon tetrachloride, the proportion of *n*-heptyl chloride to *n*-heptane being kept constant at all times. The results obtained are also included in Fig. 4.

Finally, the distribution coefficient of 1-pentanol between water and a number of pure solvents was then determined, and these are shown in Fig. 5. The point for the distribution coefficient of 1-pentanol between toluene and water is joined by a

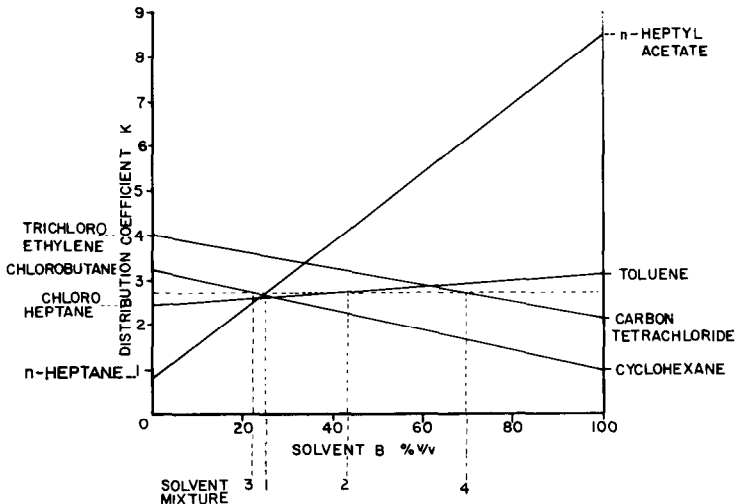


Fig. 5. Graphs showing the distribution coefficient of *n*-pentanol between water and binary solvent mixtures plotted against solvent composition.

TABLE II

COMPOSITION OF SOLVENT MIXTURE THAT PROVIDES A VALUE OF 2.75 FOR THE DISTRIBUTION COEFFICIENT OF 1-PENTANOL BETWEEN WATER AND THE SOLVENT MIXTURE

<i>Solvent mixture</i>	<i>Composition</i>	<i>Distribution coefficient (experimental)</i>
1	Heptyl acetate- <i>n</i> -heptane (25.4:74.6, v/v)	2.78
2	Heptyl chloride-toluene (57.1:42.9, v/v)	2.78
3	Cyclohexane- <i>n</i> -butyl chloride (22.6:77.4, v/v)	2.89
4	Carbon tetrachloride-trichloroethylene (69.5:30.5, v/v)	2.81
	Mean	2.82
	$\sigma$	0.051
	R.S.D.	1.85%

straight line to that for *n*-chloroheptane and water. Similarly, the point for trichloroethylene and water is joined to that of carbon tetrachloride and water, and that for *n*-heptyl acetate and water is joined to that for *n*-heptane and water. Finally, that for cyclohexane and water is joined to that for *n*-chlorobutane and water. Constructing a horizontal line at a distribution coefficient value of 2.8, it can be seen that this line intersects all the lines previously drawn and shows that a number of solvent mixtures containing quite different solvents can be chosen that would exhibit the same distribution coefficient for 1-pentanol between that solvent mixture and water. These solvent mixtures are shown in Table II. It was predicted that they should all exhibit a distribution coefficient for 1-pentanol of 2.8. The results obtained experimentally are also included in Table II.

#### Discussion of results

Consider once again eqn. 1. In the first liquid-liquid distribution coefficient measurements, binary solvent mixtures were employed for phase 1 and, thus,  $n = 2$ , whereas a single immiscible solvent, water, was used for phase 2 so that  $m = 1$  and, consequently,  $v_p = 1$ .

Thus, eqn. 1 can be rewritten in the form

$$K = (\varphi_1 v_1 + \varphi_2 v_2) / \varphi'$$

Now, bearing in mind that

$$\varphi_1 / \varphi' = K_1 \text{ and } \varphi_2 / \varphi' = K_2$$

where  $K_1$  is the distribution coefficient of the solute between the solvent 1 and water, and  $K_2$  is the distribution coefficient of the solute between the solvent 2 and water. Then

$$K = K_1 v_1 + K_2 v_2$$



Furthermore, for unassociated solvents where there is no change in volume on mixing

$$v_2 = (1 - v_1)$$

Consequently

$$K = K_1 v_1 + K_2(1 - v_1) \text{ or } K = K_2 + (K_1 - K_2)v_1 \quad (6)$$

Examination of Fig. 1 shows that the distribution coefficient of 1-pentanol increases linearly from that for pure *n*-heptane to that for pure *n*-butyl chloride, all the points for the intermediate solvent mixtures lying precisely on the same straight line. This relationship is exactly that which would be expected from eqn. 6.

The relationship between distribution coefficient and solvent composition for the three different solutes shown in Fig. 2 is again precisely that predicted by eqn. 6. It is also seen that for ethyl acetate, the distribution coefficient ranges from 2 to 10, indicating that the ethyl acetate is distributed preferentially in the solvent mixture over the complete concentration range. In contrast, the distribution coefficient of 1-propanol changes from 0.04 to about 0.2, indicating that the 1-propanol is preferentially distributed in the water. In Fig. 3, the same solute, 1-pentanol, is employed but with three different solvent mixtures, ranging in polarity from the relatively non-polar *n*-heptane-*n*-heptyl chloride mixtures to the relatively polar *n*-heptane-*n*-heptyl acetate mixtures. Again, as for the different solutes shown in Fig. 2, the same linear relationship between the distribution coefficient and solvent composition is shown for the different polarity solvents, as predicted by eqn. 6.

The results shown in Fig. 4 illustrate a somewhat different relationship between the distribution coefficient and solvent composition. The straight lines, relating the distribution coefficient of 1-pentanol between different solvent mixtures of *n*-heptane-*n*-heptyl chloride, and carbon tetrachloride-toluene and water, are completely expected from eqn. 6. The use of *n*-heptane-*n*-heptyl chloride (18:82, v/v), as an alternative to carbon tetrachloride results in a three component solvent mixture. It is seen, however, that all the points (marked as X) lie on the same straight line as those for the carbon tetrachloride-toluene mixtures. In fact, *n*-heptane-*n*-heptyl chloride (18:82, v/v) has exactly the same solvent properties with respect to 1-pentanol as carbon tetrachloride. The results shown in Fig. 4 also demonstrate the validity of eqn. 1 for three component mixtures.

Finally, Table II shows that the predicted values for the distribution coefficient of 1-pentanol between the different solvent mixtures (graphically identified from Fig. 5) and water are, in fact, obtained experimentally. This result could only be achieved if the predictions arising from eqn. 6 were, indeed, valid. The results also show that the distribution coefficients can, in fact, be summed and that a solvent mixture having a particular solvent property can be obtained from an almost infinite number of different solvent mixtures, provided they contain the correct volume fraction of each component.

## ASSOCIATED SOLVENTS

In reversed-phase chromatography, aqueous mixtures of either methanol and water, acetonitrile and water, and sometimes a third solvent component, tetrahydrofuran, are commonly used as the mobile phase. Such solvent mixtures differ significantly from those previously considered. In a methanol-water mixture, for example, methanol and water are strongly hydrogen-bonded. Consequently, whereas the previous solvents were considered to interact only transiently with each other, the hydrogen bonded methanol and water will have a significant period of association. It follows that a methanol-water mixture is, in fact, a ternary mixture, consisting of free, unassociated methanol, free, unassociated water, and associated methanol and water. In this context, free, unassociated methanol is meant to signify that the methanol is not associated with water, but it may still be associated with itself. In a similar manner, free, unassociated water is water not associated with methanol, but almost certainly the water will be associated with itself. As a result of this more complex system, an alternative theoretical approach must be developed to take into account that any solvent mixture consisting of methanol and water or water and acetonitrile contains associated solvents as well as the individual components.

*Theory*

Consider a methanol-water mixture where there is an equilibrium between the water, methanol, and a 1:1 methanol-water complex. (This assumption will be confirmed or disapproved by subsequent experiment.) Then

$$\frac{[W][M]}{[MW]} = k \text{ or } [MW] = [W][M]/k \quad (7)$$

where  $[W]$  is the molar concentration of water,  $[M]$  is the molar concentration of methanol,  $[MW]$  is the molar concentration of associated methanol-water and  $k$  is the equilibrium constant. If the solution is originally made up with a volume fraction ( $\alpha$ ) of methanol and, consequently, a volume fraction  $(1 - \alpha)$  of water, then the original molar concentration of methanol and water will be  $\alpha/V_M$  and  $(1 - \alpha)/V_W$  respectively, where  $V_M$  is the molar volume of methanol and  $V_W$  is the molar volume of water. Then

$$[M] + [MW] = \alpha/V_M \quad (8)$$

and

$$[W] + [MW] = (1 - \alpha)/V_W \quad (9)$$

From eqns. 7 and 8

$$[M] + [W][M]/k = \alpha/V_M$$

or

$$[M](1 + [W]/k) = \alpha/V_M$$

or

$$[M] = \alpha / \{(1 + [W]/k)V_M\} \quad (10)$$

From eqns. 7 and 9

$$[W] + [M][W]/k = (1 - \alpha)/V_W \quad (11)$$

From eqns. 10 and 11

$$[W] + [\alpha / \{(1 + [W]/k)V_M\}][W]/k = (1 - \alpha)/V_W$$

or

$$[W] + \alpha[W] / \{(k + [W])V_M\} = (1 - \alpha)/V_W$$

Rearranging

$$[W]^2 + \left(k + \frac{\alpha}{V_M} + \frac{\alpha}{V_W} - \frac{1}{V_W}\right)[W] - k\left(\frac{1}{V_W} - \frac{\alpha}{V_W}\right) = 0$$

or

$$[W] = \left\{ -\left(k + \frac{\alpha}{V_M} + \frac{\alpha}{V_W} - \frac{1}{V_W}\right) \pm \left[ \left(k + \frac{\alpha}{V_M} + \frac{\alpha}{V_W} - \frac{1}{V_W}\right)^2 + 4k\left(\frac{1}{V_W} - \frac{\alpha}{V_W}\right) \right]^{1/2} \right\} / 2 \quad (12)$$

where if  $\alpha = 1$  then  $[W] = 0$  and if  $\alpha = 0$ ,  $[W] = 1/V_W$

Thus

$$[W] = [(-b + (b^2 + 4c)^{1/2})/2] \quad (13)$$

where

$$b = k + \frac{\alpha}{V_M} + \frac{\alpha}{V_W} - \frac{1}{V_W}$$

and

$$c = k\left(\frac{1}{V_W} - \frac{\alpha}{V_W}\right)$$

Consequently, the concentration of methanol and the associated methanol will, from eqns. 8 and 9, be given by

$$[M] = \alpha/V_M - [MW] \quad (14)$$

and

$$[MW] = (1 - \alpha)/V_W - [W] \quad (15)$$

It follows from eqns. 13–15 that if the equilibrium constant  $k$  is known, the molar concentrations of water ( $[W]$ ), methanol ( $[M]$ ) and associated methanol ( $[MW]$ ) can be calculated from the original volume fractions of methanol in the solvent mixture.

To determine the equilibrium constant ( $k$ ), it is necessary to fit the equations to some experimental parameters that would depend on changes in the individual concentrations of water, methanol, and associated methanol. It would be possible to identify  $k$  by developing equations that show the change in distribution coefficient of a given solute between methanol–water mixtures and  $n$ -hexadecane from initial values of  $\alpha$  and relate the equation to experimental results. Such a procedure, however, is vulnerable to criticism because it would be necessary to use results obtained from one set of distribution coefficient measurements to describe the properties of another distribution system and, consequently, could propagate a common error. It follows that a separate, unrelated set of experiments needs to be used to identify  $k$ .

From the concentration of water, methanol, and associated methanol the individual volume fractions of these components can be calculated from the initial volume fraction,  $\alpha$ , of the mixture, *viz.*

$$v_M = [M]V_M \quad (16)$$

$$v_W = [W]V_W \quad (17)$$

$$v_{MW} = [MW]V_{MW} \quad (18)$$

where  $v_M$  is the equilibrium volume fraction of methanol,  $v_W$  is the equilibrium volume fraction of water,  $v_{MW}$  is the equilibrium volume fraction of associated methanol and  $V_{MW}$  is the molar volume of the associated methanol. It follows that if the volume change in mixing is measured experimentally, the results can be used to identify  $k$  and  $V_{MW}$ , and this procedure will be a satisfactory method, independent of distribution phenomenon and, consequently, the values of  $k$  and  $V_{MW}$  can be subsequently used with confidence in a distribution equation. This procedure can be carried out by using eqns. 16–18 and an iterative procedure with the aid of a computer. The pertinent values for  $k$  and the molar volume ( $V_{MW}$ ) of the associated methanol can be identified as those which provide the minimum error between the calculated volume ( $v_M + v_W + v_{MW}$ ) of any given solvent mixture and that experimentally measured.

### Experimental

The experimental values for the volume change on mixing for methanol-water mixtures were determined over a range of initial volume fractions of methanol by mixing in a standard flask and thermostating it at 25°C. The change in volume of the mixture was measured by noting the amount of *n*-hexadecane necessary to bring the total volume of the mixture up to the graduation mark. The results obtained are included in Fig. 6. The numerical data are contained in Table III. The change in volume on mixing was also determined in the same way for mixtures of acetonitrile and water, together with mixtures of tetrahydrofuran and water. The numerical results are also included in Table III, and curves relating the volume change on mixing with initial solvent fractions are also given in Fig. 6.

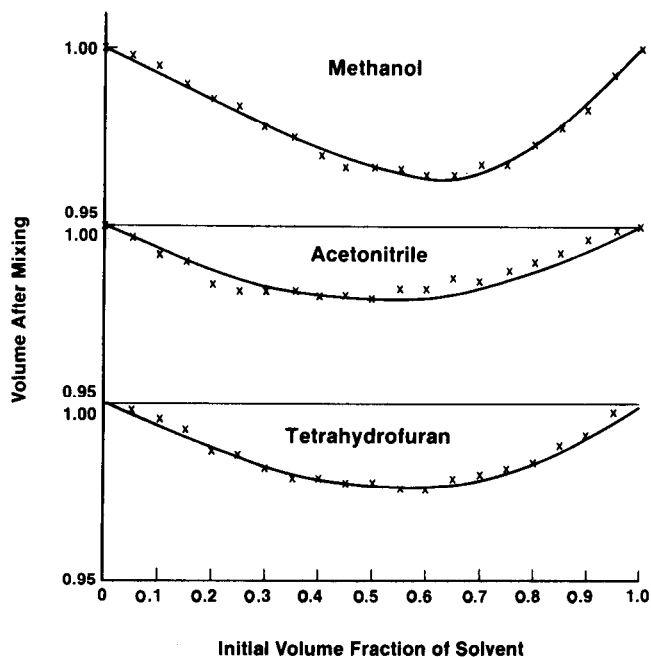


Fig. 6. Graphs of volume changes on mixing against initial volume fraction of solute.

The curves shown in Fig. 6 are calculated from the values of  $k$  and  $V_{MW}$  identified by the computer iteration process, and the plotted points are experimental. It is seen that there is a close agreement between the experimental and calculated data, although there is a significantly greater scatter in the experimental results obtained for the volume change in mixing for acetonitrile.

### Discussion of results

Employing eqns. 14-16 to calculate the molar concentrations of free water, free methanol, and associated methanol from assumed values for the equilibrium constant and molar volume of associated methanol, the volume of a mixture ( $v_M + v_{MW} + v_W$ ) was calculated by using eqns. 16-18. By the iterative procedure that tested

TABLE III

CHANGE IN VOLUME ON MIXING FOR METHANOL-WATER, ACETONITRILE-WATER AND TETRAHYDROFURAN-WATER MIXTURES

Initial volume fraction of solvent	Methanol-water		Acetonitrile-water		Tetrahydrofuran-water	
	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated
0	1.000	1.000	1.000	1.000	1.000	1.000
0.05	0.998	—	0.997	—	0.998	—
0.10	0.995	0.992	0.992	0.994	0.996	0.994
0.15	0.990	—	0.990	—	0.993	—
0.20	0.986	0.985	0.984	0.988	0.987	0.988
0.25	0.984	—	0.982	—	0.986	—
0.30	0.979	0.978	0.982	0.984	0.982	0.983
0.35	0.975	—	0.982	—	0.979	—
0.40	0.970	0.972	0.981	0.982	0.979	0.980
0.45	0.967	—	0.981	—	0.978	—
0.50	0.967	0.967	0.980	0.981	0.978	0.978
0.55	0.967	—	0.983	—	0.976	—
0.60	0.965	0.965	0.983	0.981	0.976	0.977
0.65	0.965	—	0.986	—	0.979	—
0.70	0.968	0.966	0.985	0.984	0.980	0.979
0.75	0.968	—	0.988	—	0.980	—
0.80	0.974	0.973	0.990	0.987	0.984	0.983
0.85	0.978	—	0.993	—	0.990	—
0.90	0.984	0.985	0.997	0.993	0.992	0.990
0.95	0.993	—	0.999	—	0.999	—
1.00	1.000	1.000	1.000	1.000	1.000	1.000

a wide range of values for  $k$  and  $V_{MW}$ , the correct and unique values for these constants were identified as those that provided the minimum error between calculated values and the experimental values for the volume change on mixing.

The values obtained for the equilibrium coefficients and the molar volumes of the associated solvent for methanol, acetonitrile, and tetrahydrofuran are shown in Table IV. The respective values of  $k$  and  $V_{MW}$  were then used to calculate the actual composition of the solvent mixtures, and the data are also included in Table IV. Curves relating volume fraction of free solvent and associated solvent against the original volume fraction of solvent are shown for methanol-water, acetonitrile-water and tetrahydrofuran-water mixtures in Figs. 7, 8 and 9, respectively. It is seen from the values of  $k$  for the three different solvents given in Table IV that the association of methanol and water is the strongest ( $k = 0.0045$ ), that of tetrahydrofuran and water somewhat less ( $k = 0.0468$ ), and the association of acetonitrile and water is the weakest ( $k = 0.206$ ).

It is clear from Fig. 7 that the apparently simple mixture of methanol and water is, in fact, very complex. This could account for the difficulties that arise when attempting to explain solute retention in reversed-phase chromatography on the assumption that the solvent behavior would be that of a simple binary mixture. The curves shown in Fig. 7 indicate that up to a nominal volume fraction of 0.4 methanol,

TABLE IV

COMPOSITION OF DIFFERENT AQUEOUS MIXTURES OF METHANOL, ACETONITRILE AND TETRAHYDROFURAN

Initial volume fraction	Methanol			Acetonitrile			Tetrahydrofuran		
	Volume fraction of components			Volume fraction of components			Volume fraction of components		
	Free water	Free solvent	Assoc. solvent	Free water	Free solvent	Assoc. solvent	Free water	Free solvent	Assoc. solvent
0.0	1.0000	0	0	1.0000	0	0	1.0000	0	0
0.10	0.8595	0.0088	0.1241	0.8934	0.0809	0.0193	0.8886	0.0049	0.0558
0.20	0.7203	0.0205	0.2444	0.7881	0.1653	0.0350	0.7786	0.1044	0.1048
0.30	0.5832	0.0369	0.3582	0.6841	0.2536	0.0468	0.6704	0.1676	0.1452
0.40	0.4498	0.0614	0.4609	0.5815	0.3461	0.0543	0.5643	0.2401	0.1753
0.50	0.3229	0.1008	0.5435	0.4805	0.4430	0.0575	0.4607	0.3238	0.1932
0.60	0.2085	0.1685	0.5875	0.3810	0.5444	0.0560	0.3601	0.4209	0.1964
0.70	0.1169	0.2873	0.5619	0.2831	0.6506	0.0498	0.2630	0.5340	0.1820
0.80	0.0557	0.4748	0.4427	0.1869	0.7617	0.0385	0.1701	0.6660	0.1469
0.90	0.0203	0.7203	0.2446	0.0925	0.8782	0.0220	0.0822	0.8203	0.0087
1.00	0	1.0000	0	0	1.0000	0	0	1.0000	0
	Equilibrium constant $K = 0.0045$ Molar volume of associated methanol-water = 55.46			Equilibrium constant $K = 0.206$ Molar volume of associated acetonitrile-water = 53.26			Equilibrium constant $K = 0.0468$ Molar volume of associated tetrahydrofuran-water = 88.89		

the solvent mixture actually consists of a binary mixture of free water and associated water. Similarly, between a nominal volume fraction of 0.8 methanol and 1.0 methanol, a different binary liquid mixture exists, consisting of associated methanol and

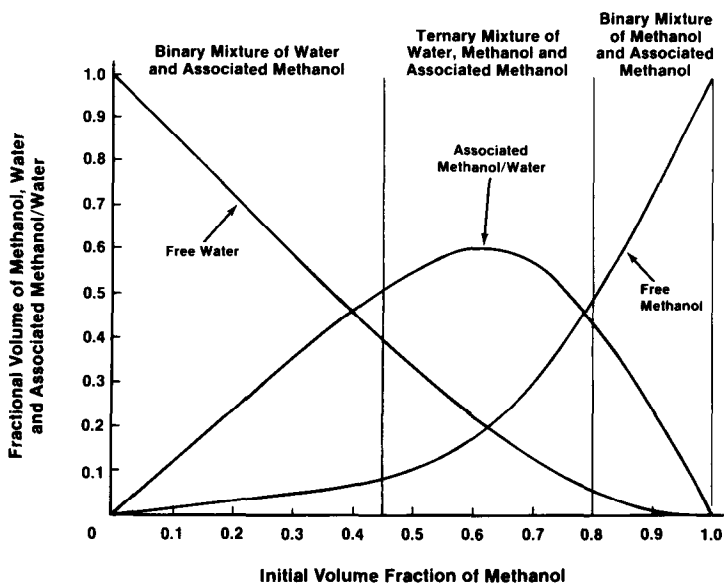


Fig. 7. Graphs showing relative volume fraction of water, methanol and associated methanol against original volume fractions of methanol in the mixture.

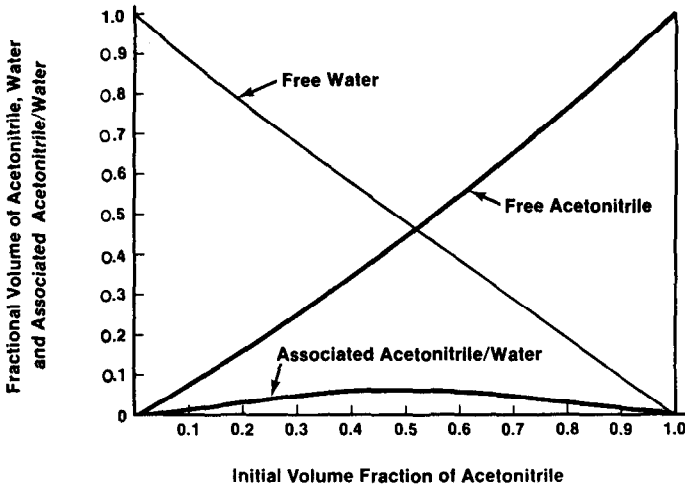


Fig. 8. Graphs showing relative volume fraction of water, acetonitrile and associated acetonitrile against original volume fraction of acetonitrile.

free methanol. Between nominal volume fractions of 0.4 and 0.8 of methanol, an even more complex situation arises, since there is a ternary mixture consisting of free water, free methanol, and associated methanol. The real nature of the solvent system, as depicted in Fig. 7, explains a number of anomalous results that have been noted in the past.

Scott and Simpson<sup>10</sup> measured the adsorption isotherms for methanol on a reversed-phase matrix and demonstrated that a Langmuir-type isotherm was involved. However, Lochmuller<sup>11</sup> claimed that at the low nominal concentrations of

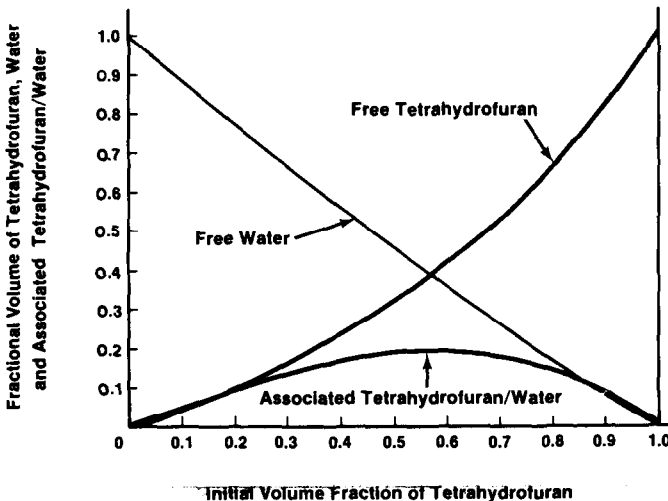


Fig. 9. Graphs showing relative volume fraction of water, tetrahydrofuran and associated tetrahydrofuran against original volume fraction of tetrahydrofuran.



methanol over which the measurements were taken, the reversed-phase material contains a considerable amount of water on the surface as well as the methanol. From the curves shown in Fig. 7, it is apparent that the adsorption isotherms measured by Scott and Simpson were probably for associated methanol-water, not methanol, and, furthermore, that the water identified by Lochmuller was not free water but the water contained in the associated methanol-water complex. Scott and Simpson also calculated the surface area of some reversed-phase matrices from the maximum amount of methanol adsorbed and the area of the methanol molecule. The values they obtained were rather low, and it is now obvious that this error arose, at least in part, as a result of employing the area of the methanol molecule in their calculations and not that of associated methanol.

A linear gradient of nominal volume fraction of methanol with water, in fact, can now be seen to be a very complex gradient system, if the nature and concentration of the individual interacting solvent species are taken into account. Initially, there is an approximately linear change from pure water to associated water until the nominal volume fraction of water is about 0.4. Subsequently, from a nominal volume fraction of methanol of 0.4 to 0.8, the volume fraction of free water falls to almost zero, the volume fraction of associated methanol goes through a maximum, and the free volume of methanol begins to rise very rapidly. Finally, the volume fraction of free methanol increases almost linearly, while the volume fraction of associated methanol decreases linearly. As, in general, the strength of the interactions of methanol with a solute are likely to be much greater than that between the solute and free water and associated methanol (*i.e.*, the eluting strength of free methanol is much greater than that of free water or associated methanol), then solutes would be eluted very rapidly towards the end of the linear program. The rapid manner in which the free methanol volume fraction changes towards the end of a linear program, as deduced from the curves in Fig. 7, explains the need for using convex program gradient profiles with methanol-water mixtures.

In Fig. 8, the association of acetonitrile with water relative to the initial volume fraction of acetonitrile is shown. It is clearly seen that the association is very much weaker than that in the methanol-water system and that only 5% of associated acetonitrile exists at an initial volume fraction of acetonitrile of about 0.5. Consequently, it is likely that the free acetonitrile is the major factor that controls the overall distribution coefficient of the solute with respect to the reversed-phase material. Consequently, the relationship between distribution coefficient (if defined with respect to the solvent mixture) and initial volume fraction of acetonitrile is likely to have a more closely (but not completely) linear relationship.

In Fig. 9, the same set of curves for the tetrahydrofuran-water mixtures is given. The degree of association of tetrahydrofuran with water is intermediate between that of the acetonitrile-water mixtures and the methanol-water mixtures. Under these circumstances, it is seen that at an initial volume fraction of tetrahydrofuran of 0.6, about 20% (v/v) of the resulting mixture contains associated tetrahydrofuran. The relationship between free tetrahydrofuran and the initial volume fraction of tetrahydrofuran is certainly no longer linear and, thus, the behavior of the system is likely to be more similar to that of the methanol-water mixture than that of acetonitrile-water.

### *Solute interaction with associated liquid mixtures (methanol-water)*

It is now necessary to test the validity of eqn. 1 when applied to solvent mixtures where association occurs between individual components and, consequently, the associated solvent becomes another interacting moiety in the system. The associated solvent will exhibit its own unique interactive characteristics, which in most cases will be quite different from those of the free unassociated solvents. In order to eliminate any uncertainties resulting from the use of chromatographic measurements involving surfaces, the equation was tested against distribution data obtained from simple liquid-liquid systems.

### *Experimental*

The experimental procedure used was very similar to that employed for the unassociated solvents. In this case, however, *n*-hexadecane was used as one phase and mixtures of methanol-water as the other phase. *n*-Hexadecane was chosen to simulate, as closely as possible, the bonded stationary phases normally employed in reversed-phase chromatography. Due to the different nature of the phase system, internal standards could not easily be used in the GC analysis. Consequently, average peak areas of a number of replicate samples of the methanol-water phase, containing the solute before and after equilibration, were used to determine the distribution coefficient. The distribution coefficient of the solute between the two phases was calculated as follows:

$$K = a_2 / (a_1 - a_2)$$

where  $K$  is the distribution of the solute between the methanol-water mixture and *n*-hexadecane,  $a_1$  is the average peak area of the solute before equilibration and  $a_2$  is the average peak area of the solute after equilibration.

Two solutes were examined, vinyl acetate and 1-pentanol. The numerical results obtained for each solute are included in Table V and the  $K$  value for each solute at each methanol concentration plotted in Fig. 10.

### *Results and discussion*

Due to the presence of the associated methanol, there are three components in the methanol-water phase and, thus,  $n = 3$  in eqn. 1. Furthermore, as there is a single solvent component present in the hydrocarbon phase,  $m = 1$ , eqn. 1 becomes

$$K = \frac{\varphi_1 v_1 + \varphi_2 v_2 + \varphi_3 v_3}{\varphi'}$$

Thus,  $\varphi_1/\varphi' = K_M$ , the distribution coefficient of solute between free methanol and *n*-hexadecane,  $\varphi_2/\varphi' = K_{MW}$ , the distribution coefficient of solute between associated methanol and *n*-hexadecane and  $\varphi_3/\varphi' = K_W$ , the distribution coefficient of solute between water and *n*-hexadecane. Or

$$K = K_M v_1 + K_{MW} v_2 + K_W v_3 \quad (19)$$

where  $v_1$  = volume fraction of free methanol,  $v_2$  = volume fraction of associated

TABLE V

## EXPERIMENTAL AND CALCULATED DISTRIBUTION COEFFICIENTS FOR TWO DIFFERENT SOLUTES

Phase 1: different volume fractions of methanol in water. Phase 2: *n*-hexadecane.  $K_M$  is the distribution coefficient of the solute between the pure methanol and *n*-hexadecane;  $K_{MW}$  is the distribution coefficient of the solute between the associated methanol and *n*-hexadecane;  $K_W$  is the distribution coefficient of the solute between the water and *n*-hexadecane.

Initial volume fraction of methanol	Vinyl acetate		<i>n</i> -Pentanol	
	$K$ actual	$K$ calculated	$K$ actual	$K$ calculated
0	0.331	0.200	2.80	2.80
0.10	0.347	0.277	3.45	3.11
0.20	0.328	0.364	4.12	3.65
0.30	0.544	0.464	4.63	4.49
0.40	0.415	0.591	7.46	5.93
0.50	0.738	0.771	8.58	8.58
0.60	1.045	1.044	13.8	13.3
0.70	1.468	1.480	21.9	21.9
0.80	2.150	2.150	34.4	35.7
0.90	2.969	3.007	—	—
1.00	—	—	—	—
	$K_M = 3.98, K_{MW} = 0.56, K_W = 0.20$		$K_M = 74.59, K_{MW} = 0.26, K_W = 2.8$	

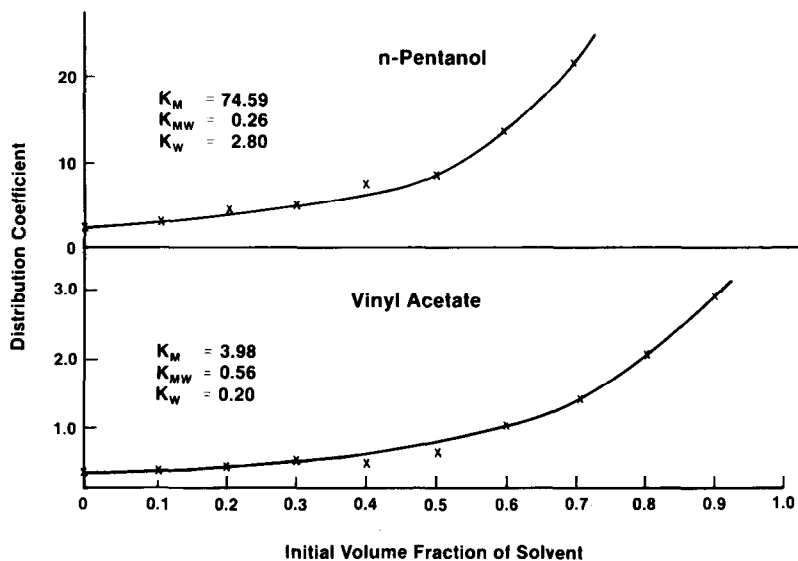


Fig. 10. Graphs relating solute distribution coefficient between methanol-water mixtures and *n*-hexadecane against initial volume fraction of methanol (the lines are theoretical, the points experimental).

methanol and  $v_3$  = volume fraction of free water. Now, the results for each solute will be considered separately.

*Vinyl acetate.* The distribution coefficient of vinyl acetate between methanol-water mixtures and *n*-hexadecane is found to depend on the interaction of the solute with free methanol and associated methanol and also with free water. Consequently, eqn. 19 must be used and values have to be allotted to  $K_M$ ,  $K_{MW}$ , and  $K_W$ . The value for  $K_W$  was obtained from the intercept of a polynomial curve fit to the data on the distribution coefficient axis at zero concentration of methanol. Employing this procedure, the value of  $K_W$  was found to be 0.200. Having arrived at a value of  $K_W$ , simultaneous equations were set up at initial volume fractions of methanol of 0.6 and 0.8. The values obtained for  $K_M$  and  $K_{MW}$  were found to be 3.98 and 0.56, respectively, and are included in Table V. Using the values of  $K_M$ ,  $K_{MW}$ ,  $K_W$  in eqn. 19, the theoretical values of  $K$  were calculated, and the values obtained are represented by the line for vinyl acetate shown in Fig. 10. It is seen that the theoretical curve closely matches the experimental values. It is also seen that the distribution of vinyl acetate between methanol-water and *n*-hexadecane, as well as being controlled by the concentration of free methanol and associated methanol, is also influenced by the amount of free water present.

*1-Pentanol.* The same procedure was adopted for 1-pentanol. The value for  $K_W$  was taken as 2.8 from the experimental data and the values for  $K_M$  and  $K_{MW}$  were calculated from simultaneous equations, employing the data arrived at for initial volume fractions of methanol of 0.5 and 0.7. Using the values of  $K_M$ ,  $K_{MW}$ , and  $K_W$  in eqn. 19, values for  $K$  were calculated over the pertinent range of initial volume fractions of methanol and the results are given as the curve for 1-pentanol in Fig. 10. It is seen that agreement between the theoretical curve and experimental values is again very good.

#### CORRELATION OF DISTRIBUTION DATA WITH RETENTION ON REVERSED-PHASE COLUMNS

Discussions in the literature and in this work have so far largely involved GC systems where the stationary phase is a liquid, liquid-liquid systems, and liquid-solid systems, for which the properties of the interacting surface remain sensibly constant over a wide range of solvent composition. Reversed-phase chromatography is far more complex, and there is a much more restricted range of solvent composition over which the properties of the surface remain constant and where the validity of eqn. 4 can be tested. For example, when mixtures of methanol and water are employed as the mobile phase, Scott and Simpson<sup>10</sup> have shown that the solvent, now understood to be in its associated form, is adsorbed on the surface of the reversed-phase material. Furthermore, the adsorption process is not complete until the fraction of methanol in the original solvent reaches about 0.35. Examination of Fig. 7 indicates that the situation could be even more involved, since beyond a volume fraction of about 0.75 of methanol, a significant amount of free methanol exists, and this could result in the nature of the surface of the reversed-phase matrix changing yet again. The surface originally covered with associated methanol-water is likely to be replaced by a surface of pure methanol. It follows that, if solute retention is to be related to solute distribution between the respective methanol-water mixture and *n*-hexadecane,

the range of solvent composition must be confined between 0.35 and 0.80 of methanol. In order to predict the retention of a solute over the complete range of methanol concentrations, the adsorption isotherms of associated methanol and free methanol must also be known over the entire range. In this paper, it will be considered sufficient to establish the validity of the distribution theory over that range of concentration where the properties of the surface of the reversed-phase matrix are known to remain sensibly constant.

### Experimental

The column used was 25 cm × 4.6 mm I.D. and was packed with 10- $\mu$ m reversed-phase column material (Whatman ODS-2). The detector employed was a Perkin-Elmer LC-85B, operated at wavelengths appropriate to the solute. The injection valve was a Rheodyne 7125, having an external loop of 10  $\mu$ l, and a Perkin-Elmer Series 3B pump. The column was placed in a thermostated bath and controlled at 25°C. The measured flow-rate used was 2 ml/min, and retention volumes were taken as the product of the retention time and the flow-rate. The dead-volume of the column was taken as the retention volume of sodium nitrate (700  $\mu$ g injected), and the corrected retention volume was taken as the difference between the retention volume of the solute and the retention volume of sodium nitrate. Measurements were made in triplicate and the average taken as the true retention volume. The solutes examined were again vinyl acetate and 1-propanol. The elution of 1-pentanol was detected by means of a Perkin-Elmer LC-25 refractometer detector. The results obtained are given in Table VI.

TABLE VI

VALUES OF  $1/V'$  FOR TWO SOLUTES ELUTED FROM A REVERSED-PHASE (OCTADECYL) COLUMN BY DIFFERENT METHANOL-WATER MIXTURES, TOGETHER WITH THEIR COMPLEMENTARY DISTRIBUTION COEFFICIENTS BETWEEN *n*-HEXADECANE AND THE RESPECTIVE METHANOL-WATER MIXTURES

Initial volume fraction of methanol	Vinyl acetate		<i>n</i> -Pentanol	
	$1/V'$	<i>K</i>	$1/V'$	<i>K</i>
0.40	0.126	0.591	0.077	5.93
0.45	0.170	0.675	—	7.10
0.50	0.205	0.771	0.127	8.58
0.55	0.258	0.891	—	10.6
0.60	0.328	1.05	0.224	13.3
0.65	0.420	1.24	0.309	17.0
0.70	0.543	1.48	0.400	21.9
0.75	0.685	1.79	—	28.1
0.80	—	2.15	0.676	35.7
0.85	1.220	2.56	—	—
0.90	—	—	—	—
0.95	—	—	—	—

### Discussion of results

Considering eqn. 4 and remembering that the range of concentrations employed maintained a surface of constant properties, then  $r = 1$  and  $a = 1$ . Furthermore, as an associated solvent system is also used,  $p = 3$ .

Thus, eqn. 4 can be put in the form

$$K' = \frac{1}{K_1 v_1 + K_2 v_2 + K_3 v_3}$$

Now, as  $V' = KA_s$ ,  $1/V' = (K_1 v_1 + K_2 v_2 + K_3 v_3)/A_s$ . But from eqn. 19  $1/K' = K = K_M v_M + K_{MW} v_{MW} + K_W v_W$  (because  $K$  in eqn. 19 is defined with respect to the methanol/water phase). Consequently,

$$\frac{1}{V'} = \frac{K}{A_s} \quad (22)$$

It follows from eqn. 22 that a graph relating  $1/V'$  to  $K$  should give a straight line.

In Fig. 11, values of  $1/V'$ , taken from Table VI, are plotted against  $K$  at each respective concentration of methanol between 0.35 and 0.85 and for each solute. It is seen that the graphs are linear for both solutes.

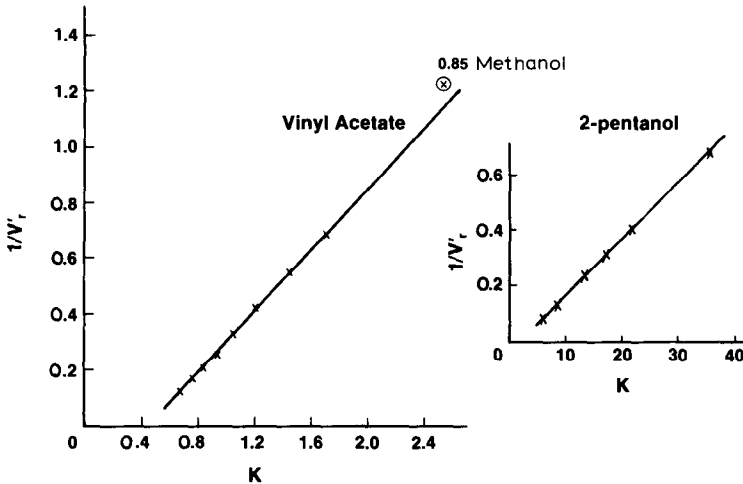


Fig. 11. Graphs of  $1/V'$  for two solutes against their distribution coefficients.

Examination of these curves reveals certain interesting facts about the interaction of the solute with the surface. Firstly, the linearity of the curves again validates the basic principles of distribution, as defined by eqn. 1. Secondly, the point at high concentrations of methanol, *i.e.* 0.85 for vinyl acetate, falls outside the respective straight line. This supports the previous premise that the high methanol concentration changes the nature of the surface. More interestingly, the slopes (0.54 and 0.02

for vinyl acetate and 1-pentanol, respectively) and intercepts ( $-0.22$  and  $-0.04$  for vinyl acetate and 1-pentanol, respectively) of the curves are different. From eqn. 22 it might be assumed that the solutes would exhibit the same slope. The fact that they, indeed, have different slopes means either that each solute interacts with a surface of different area or that the distribution of the solute, although proportional to its respective distribution coefficient with *n*-hexadecane is modified by the surface layer of associated methanol–water or methanol. The possibility of different solutes interacting with significantly different surface areas seems remote. It is more likely that the contribution of the layer of adsorbed solvent at the interface is different for each solute. Consequently, the solutes displace the adsorbed associated methanol–water in much the same way as anisole displaces butyl chloride from silica gel, as described by Scott<sup>12</sup>.

It would appear that, despite the claims that methanol is the ideal solvent to mix with water, the converse is the case. Fig. 7 shows solvent changes in three distinct stages across the entire concentration range. Furthermore, and as a result, there are at least two distinct changes in the nature of the stationary phase surface. In contrast, from Fig. 8, it is seen that the degree of association of acetonitrile with water is much less than that of methanol and, consequently, acetonitrile exhibits far more ideal behavior than methanol. Although not experimentally established, it is likely that, as the concentration of free acetonitrile increases almost linearly throughout the entire concentration range, only two types of stationary phase surface can exist. Furthermore, the nature of the surface is likely to remain constant throughout the whole concentration range, and hence, beyond an initial acetonitrile concentration of 0.1, the prediction of solute retention should be simpler.

The fact that there is little free methanol in the solvent mixture until the nominal volume fraction of methanol is in excess of about 0.5 also accounts for the fact that proteins and other biological materials that readily are denatured in the presence of free methanol can be separated by reversed-phase chromatography, provided the nominal methanol volume fraction is less than *ca.* 0.6. The surprising fact that proteins are not denatured when methanol–water mixtures are used as the mobile phase is explained by the fact that virtually all of the methanol is associated with water and thus is sufficiently less active than free methanol in causing denaturation.

The work described in this paper strongly supports the distribution theory, as mathematically defined by eqn. 1. However, the concept of the additivity of distribution coefficients has to be made rationally compatible with the fact that distribution coefficients vary exponentially with the excess free energy of the partition system. Nevertheless, the theory put forward in 1976, supported by a considerable amount of data generated over the intervening years and further confirmed by the experimental work described here, will, without doubt, eventually help to arrive at the goal of an explicit equation or equations that will allow the accurate prediction of chromatographic retention. Such equations could also be invaluable in the optimization of solvent extraction processes.

#### ACKNOWLEDGEMENT

The authors wish to thank Ms. Carol D. Halfmann for her skillful assistance in the experimental work of this paper.

## REFERENCES

- 1 R. P. W. Scott, *J. Chromatogr.*, 122 (1976) 35.
- 2 L. R. Snyder and H. Poppe, *J. Chromatogr.*, 184 (1980) 363.
- 3 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
- 4 R. J. Laub, in J. C. MacDonald (Editor), *Inorganic Chromatographic Analysis*, Wiley Interscience, New York, 1985, Ch. 2.
- 5 R. J. Laub, in P. Kuwana (Editor), *Physical Methods in Modern Chemical Analysis*, Academic Press, New York, 1983, Ch. 4.
- 6 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 149 (1978) 93.
- 7 W. K. Robbins and S. C. McElroy, *Liquid Fuel Technol.*, 2 (1984) 113.
- 8 R. J. Hurtubise, A. Hussain and H. F. Silver, *Anal. Chem.*, 53 (1981) 1993.
- 9 M. McCann, S. Madden, J. H. Purnell and C. A. Wellington, *J. Chromatogr.*, 294 (1984) 349.
- 10 R. P. W. Scott and C. F. Simpson, *Faraday Discussions of the Royal Society of Chemistry*, No. 15 (1980) 69.
- 11 C. Lochmuller and M. Wilder, *Anal. Chim. Acta*, 130 (1981) 31.
- 12 R. P. W. Scott, *Faraday Discussions of the Royal Society of Chemistry*, No. 15 (1980) 49.