The thermodynamic properties of methanol–water association and its effect on solute retention in liquid chromatography

R. P. W. Scott

Chemistry Department, Georgetown University, Washington, DC, USA

Employing density data reported for a range of methanol–water mixtures measured at different temperatures, the methanol–water association constant was calculated at each temperature. The standard free enthalpy and entropy of association were then determined from the linear relationship that exists between the logarithm of the association constant and the reciprocal of the absolute temperature. It is shown that the standard free enthalpy is very high for simple association, *viz.*, 1.916 kcal mol^{-1}, and the standard free entropy of distribution was 2.05 cal K^{-1} mol^{-1}. These values indicate strong association and the relatively low standard free entropy shows a comparatively small change in molecular freedom after association. This indicates that the solvents are probably strongly associated with themselves when not associating with each other. Using the thermodynamic parameters and molar volumes derived from the calculation, the volume fractions of unassociated methanol, unassociated water and methanol–water associate were calculated for a range of initial methanol–water mixtures at different temperatures. The association decreased fairly rapidly with increased temperature as would be expected. From the calculated composition of the ternary mixtures, together with published retention data for the distribution coefficients of a series of aromatic hydrocarbons and aliphatic acids between methanol–water mixtures and hexadecane, it is shown that dispersive interactions are almost exclusively controlled by the volume fraction of free unassociated methanol. It is also shown that the other components (the methanol–water associate and the free water) exert significant control only over polar interactions.

Introduction

Liquid mixtures are occasionally employed as stationary phases in gas chromatography (GC) but are more frequently used as mobile phases in liquid chromatography. McCann *et al.*,[1] Laub and Purnell[2] and Laub[3] examined the effect of mixed phases on solute retention in GC and arrived at the startling conclusion that, for a wide range of binary mixtures, the corrected retention volume of a solute was linearly related to the volume fraction of either one of the two phases. Expressed mathematically:

$$V'_{r(AB)} = K_A \alpha V_S + K_B(1 - \alpha)V_S \qquad (1)$$

where $V'_{r(AB)}$ is the retention volume of the solute on a mixture of stationary phases A and B, K_A is the distribution coefficient of the solute with respect to the pure stationary phase A, K_B is the distribution coefficient of the solute with respect to the pure stationary phase B, V_S is the total volume of stationary phase in the column and α is the volume fraction of phase A in the stationary phase mixture, or

$$V'_{r(AB)} = \alpha(V'_A - V'_B) + V_B \qquad (2)$$

where V'_A is the retention volume of the solute on the same volume of pure phase A and V'_B is the retention volume of the solute on the same volume of pure phase B.

This relationship, however, was found not to be universal and appeared to break down where strong association occurred between the two components. Under such circumstances, the stationary phase would no longer be a simple binary mixture, but would also contain the associate of the two phases as a third component and the simple linear relationship obtained for a binary mixture would not be expected to hold. The linear relationship for a true binary mixture is not altogether surprising. The distribution coefficient of the solute with any pure component of the stationary phase is a constant. However, the volume fraction of each phase must determine the probability that a given solute molecule will interact with a molecule of that particular phase. This is much the same as the partial pressure of a vapor or gas determining the probability of

collision. It follows that doubling the concentration of one phase doubles the probability of interaction and consequently doubles its contribution to retention.

The same linear relationship between mobile phase composition and retention was noted by Katz *et al.*[4] for binary mixtures of non-associating solvents in LC. They examined liquid–liquid distribution systems by measuring absolute distribution coefficients of pentan-1-ol between water and mixtures of heptane and chloroheptane, heptane and toluene and heptane and heptyl acetate. The results they obtained are reproduced in Fig. 1. The same linear relationships between solvent composition and distribution coefficient were obtained for all three solvent mixtures, which closely matched the results that Purnell and Laub obtained in their GC experiments. The relationship
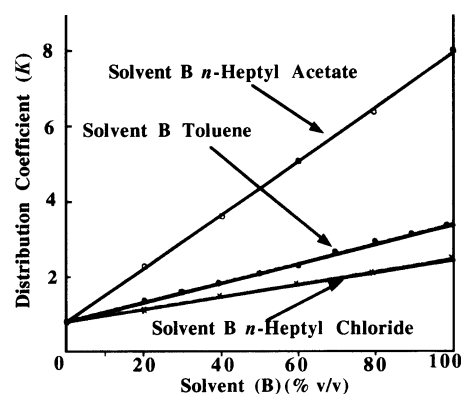
Solvent A	Solvent B
1 *n*-Heptane	*n*-Heptyl Acetate
2 *n*-Heptane	Toluene
3 *n*-Heptane	*n*-Heptyl Chloride

Fig. 1 Graphs showing the distribution coefficient of pentan-1-ol between water and three binary solvent mixtures plotted against solvent composition.

between distribution coefficient and solvent composition will be described by exactly the same equation.

Reiterating the equation proposed by McCann *et al.*, for two solvents, A and B, in GC,

$$V'_{r(AB)} = \alpha(V'_A - V'_B) + V'_B \qquad (3)$$

The results of Katz *et al.* can be expressed algebraically in a similar form as in LC:

$$K_{AB} = \alpha K_A + (1 - \alpha)K_B \qquad (4)$$

$$K_{AB} = \alpha(K_A - K_B) + K_B \qquad (5)$$

Relationship between the equilibrium constant of a methanol–water mixture and its density

The relationship between water, methanol and methanol–water associate concentrations has been described elsewhere,[5] but as it is central to the following argument it will be briefly reiterated here. The equilibrium constant for the association of water with methanol can be described by the following equation;

$$\frac{[W][M]}{[MW]} = K \qquad (6)$$

where [W] is the molar concentration of water, [M] is the molar concentration of methanol and [MW] is the molar concentration of the methanol–water associate.

For mathematical convenience, the molar concentration will be considered to be expressed as mol ml^{-1}. Further, as the volume change on mixing is less than 3% v/v, the value of K calculated may also be in error by about 2%. However, as the magnitude of change in the value of K is about 220%, the error can be ignored and the respective molar concentrations will be considered independent of volume changes due to mixing. Assuming the original methanol–water mixture was made up with a volume fraction α, there will be a volume fraction $1 - \alpha$ of water.

Hence the original molar concentrations of methanol and water will be α/V_M and $(1 - \alpha)/V_W$ respectively, where V_M and V_W are the molar volumes of methanol and water, respectively. Consequently, after mixing and association has taken place to form the associate (MW),

$$[M] + [MW] = \frac{\alpha}{V_M} \qquad (7)$$

$$[W] + [MW] = \frac{(1 - \alpha)}{V_W} \qquad (8)$$

Thus, eqns. (6), (7) and (8) to solve for [W],

$$W = \frac{-b + (b^2 + 4c)^{0.5}}{2}$$

where

$$b = K + \frac{\alpha}{V_M} + \frac{\alpha}{V_W} - \frac{1}{V_W} \qquad (9)$$

and

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

Furthermore, having calculated [W], then it is clear that

$$[MW] = \frac{1 - \alpha}{V_W} - [W] \qquad (10)$$

and

$$[M] = \frac{\alpha}{V_M} - [MW] \qquad (11)$$

Hence the new volume after mixing will be

$$v_i = [W]V_W + [M]V_M + [MW]V_{MW} \qquad (12)$$

where V_{MW} is the molar volume of the methanol–water associate.

Similarly, the weight of 1 ml of the mixture will be

$$w_i = [W]M_W + [M]M_M + [MW]M_{MW} \qquad (13)$$

where M_W, M_M and M_{MW} are the molecular weights of water, methanol and the methanol–water associate, respectively. Hence the density of the mixture (d_i) will be given by

$$d_i = \frac{w_i}{v_i} \qquad (14)$$

It follows that if density data were available for a series of methanol–water mixtures, then both the equilibrium constant and the molar volume of the associate could be identified. This could be carried out iteratively by assuming values for the equilibrium constant and molar volume, calculating the density of the mixture employing eqns. (7)–(14) and comparing them with data obtained experimentally. The correct values of equilibrium constant and molar volume of the associate would be those that provide the minimum for the sum of variances of the error between the experimental data and the calculated data.

In addition, if density data were available for a range of methanol–water mixtures taken over a span of temperatures, then the standard free enthalpy and standard free entropy of association could be determined from the slope and intercept of the curve relating equilibrium constant to the reciprocal of the absolute temperature. Consequently, the relative volume fractions of methanol, water and methanol–water associate could be calculated for any practical operating temperature. Such data could be used to optimize chromatographic separations using methanol–water mixtures as the mobile phase to provide the minimum analysis time and the required resolution.

Experimental data

Suitable density data, taken over a range of temperatures, were determined by Gutierrez[6] as part of a PhD Thesis at Georgetown University, Washington, DC. The results that were obtained and taken from his thesis are given in Table 1. Assuming values for the equilibrium constant and molar volume of the associate and

Table 1 Density of methanol–water mixtures taken at four temperatures

Volume fraction of methanol	Density/g ml^{-1}			
	25 °C	35 °C	45 °C	55 °C
0.00	0.9971	0.9941	0.9903	0.9857
0.10	0.9828	0.9800	0.9773	0.9745
0.20	0.9698	0.9659	0.9626	0.9591
0.40	0.9409	0.9354	0.9302	0.9249
0.45	0.9325	0.9264	0.9207	0.9149
0.50	0.9232	0.9167	0.9107	0.9046
0.55	0.9135	0.9066	0.9001	0.8934
0.60	0.9025	0.8957	0.8890	0.8821
0.65	0.8913	0.8841	0.8772	0.8702
0.70	0.8792	0.8718	0.8644	0.8571
0.75	0.8658	0.8586	0.8511	0.8435
0.80	0.8520	0.8444	0.8365	0.8287
0.85	0.8375	0.8292	0.8211	0.8130
0.90	0.8214	0.8130	0.8046	0.7962
0.95	0.8044	0.7954	0.7867	0.7778
1.00	0.7864	0.7770	0.7676	0.7582

using eqns. (7)–(14) the density of each mixture was calculated and compared with the data given in Table 1. The correct values of equilibrium constant and molar volume of the associate were taken as those that provided the minimum error between the experimental data and the calculated data. The results obtained from these iterative calculations for the equilibrium constant and the molar volume of the associate are given in Table 2.

The logarithm of the equilibrium constant was plotted against the reciprocal of the absolute temperature, which produced the curve shown in Fig. 2. The change in density between pure methanol and pure water is much greater than the change in density with temperature, the magnitude of which is close to the normal error of measurement. As a consequence, there is significant scatter between the points. However, the relationship is linear in nature (confirmed by an index of determination of 0.997) and the slope and intercept for the curve give realistic values that are proportional to the standard free enthalpy and standard free entropy of association, $i.e.$,

$$\log K = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} = \frac{-964.2}{T} + 1.03$$

Thus

$$\frac{-\Delta H}{R} = -964.2 \text{ or } \Delta H = 964.2R \qquad (15)$$

and

$$\frac{\Delta S}{R} = 1.03 \text{ or } \Delta S \text{ or } \Delta S = 1.03R$$

Taking a value of R of 1.987 kcal $K^{-1}mol^{-1}$, the standard free enthalpy and standard free entropy of distribution are 1916 cal mol^{-1} and 2.05 cal K^{-1} mol^{-1}, respectively. Bearing in mind that, in the pure state, water is strongly associated with water and methanol is strongly associated with methanol, it would appear that the energy involved in the association of water with methanol is much greater than either. This would mean, from the point of view of chromatography, that the interactive potential of both the methanol and the water with any dissolved solute is diluted by removing a significant proportion of both, in the formation of the strongly bound and consequently less interactive associate. The net effect is to reduce the overall eluting capacity of the solvent. It is also interesting to note that the standard free entropy of association is very small, indicating that the molecules are just as restrained when associated with themselves as when they are associated with each other, $i.e.$, their random character changes little.

Values for V_{MW}, the molar volume of the associate, were calculated simultaneously with the equilibrium constants (k) at each temperature and the values obtained, plotted against the temperature (t) in °C, are shown in Fig. 3. Again it is seen that the change in the molar volume with temperature over the restricted temperature range examined is relatively small, hence the scatter of the points around the linear curve is again fairly significant. Nevertheless, the data are sufficiently precise to give an expression for V_{MW} for the purpose of calculating the relative distribution of the components of the solvent mixture at any temperature, $viz.$,

$$V_{MW} = 0.0160t + 54.625 \qquad (16)$$

This expression, together with the equation for k, will allow V_{MW}, k and the volume fractions of methanol, water and methanol–water associate to be calculated for any known solvent mixture (expressed in volume fractions before mixing) and at any experimentally practical temperature.

Calculations and discussion of results

Employing eqns. (15) and (16) to calculate the equilibrium constant and molar volume of the associate at different temperatures, the volume fraction of each associate was calculated for a range of initial volume fractions of methanol before mixing, using equations (9)–(12). In this way, curves relating the volume fraction of each component to the initial volume fraction of methanol before mixing could be obtained for temperatures of 0, 30 and 60 °C. The results obtained are shown in Fig. 4. The curves follow the form previously reported.[5] It is seen that at each temperature there are three ranges of solvent composition that have distinct and different interactive properties. Between 0 and 0.3 volume fraction of methanol, there is little or no free methanol to interact with the solute, the major solvent components being water and the

Table 2 Data generated by the iteration program to determine the equilibrium constant of methanol–water association and the molar volume of the associate

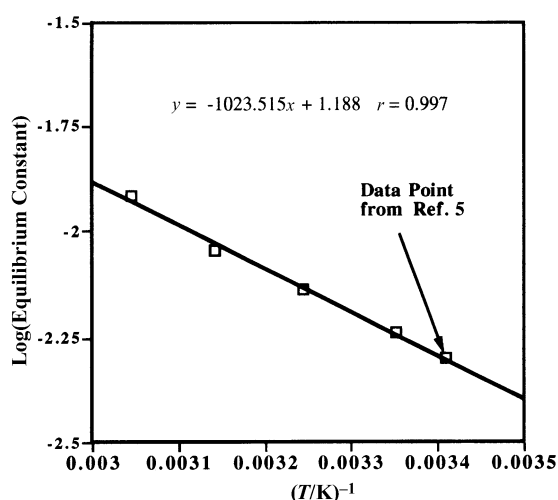| Temperature/ (°C)	Reciprocal of absolute temperature/ K^{-1}	Equilibrium constant	Log (equilibrium constant)	Molar volume of associate
20	0.003408	0.00504	−2.2976	54.90
25	0.003351	0.00580	−2.2366	54.836
35	0.003243	0.00733	−2.1349	55.456
45	0.003141	0.00902	−2.0448	55.528
55	0.003045	0.01218	−1.9144	55.285

Fig. 2 Graph of the logarithm of the water–methanol association equilibrium constant against the reciprocal of the absolute temperature.

$y = -1023.515x + 1.188$ $r = 0.997$
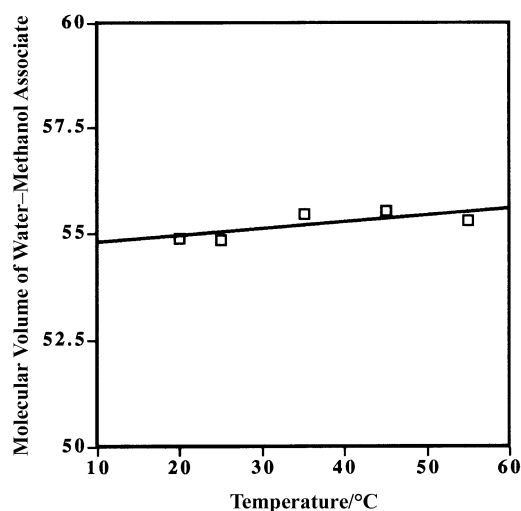
Data Point from Ref. 5

Fig. 3 Graph of molar volume of water–methanol associate against temperature.

methanol–water associate; the mixture is essentially binary. This explains why low concentrations of methanol can be used to elute proteins without denaturation, as there is little free methanol available to denature the solute because almost all the methanol is associated with water. Between 0.3 and 0.7 volume fraction of methanol, the solvent mixture is now ternary in nature, containing methanol, water and methanol–water associate, all in significant proportions with the methanol–water associate dominating. Between 0.7 and 1.0 volume fraction of methanol the mixture is again dominantly binary in nature containing largely methanol and the methanol–water associate.

Raising the temperature reduces the amount of association and thus, at any given methanol content, there is more methanol free to interact with the solute at the higher temperatures. As methanol is, in most cases, the stronger eluting solvent, raising the temperature increases the elution rate in two ways: it increases the interaction between the solutes and the methanol and it reduces the standard free enthalpy of distribution of the solute, which also reduces retention and elution time.

The effect of methanol–water association on solute retention, however, is complicated. If the solute is largely dispersive, the unassociated methanol will be the component that largely controls elution. If, however, the solute is strongly polar, then the water and possibly the methanol water associate will be the components of the mobile phase that largely control retention. If the solute has both strong dispersive and polar interactive centers then all three components may play a significant part in controlling retention.

As an example, distribution coefficients have been published by Schantz[7] for a series of aromatic hydrocarbons and some homogenous series of aliphatic acids between methanol–water mixtures and hexadecane and the results obtained are given in Tables 3 and 4. The measurements were made at 20 °C and hence employing eqn. (15) the association constant and the volume fractions of methanol, water and methanol–water associate were calculated for a range of different initial methanol–water mixtures, and the results are given in Table 5. The values of Schantz were determined with respect to hexadecane as the distribution phase, hence the reciprocal of each value was taken so that the distribution coefficient was referred to the methanol–water mixtures. Considering first the aromatic hydrocarbons, the only significant interactions that could retain the solutes in the methanol–water mixture would be dispersive (hydrophobic) in nature. This results from the absence of any polar groups on the solutes and their relatively weak polarizability. Hence, their distribution in methanol–

water mixtures would be expected to depend heavily on the free unassociated methanol in those mixtures. This dependence is confirmed by the curves shown in Fig. 5 relating the distribution coefficient to the volume fraction of unassociated methanol in the methanol–water mixtures. It should be pointed out that in measuring the distribution coefficients of the solutes in pure methanol, the reported values varied by nearly an order of magnitude, apparently owing to experimental difficulties. It was impossible to assess an accurate value for this particular data set and values for pure methanol are therefore not included.

It is clear that the distribution coefficient of each aromatic hydrocarbon is linearly related to the volume fraction of free methanol in the mixtures (at least at initial concentrations of methanol below a volume fraction of 0.7). The linear curve with seven points for benzene has an index of determination of 1.000, for the three points for toluene 1.000 and for the three points for propylbenzene 0.999. There are a limited number of points for the last two hydrocarbons but as all three sets give closely linear curves with high indices of determination the evidence for linearity is very strong. This linear relationship is supported by

Table 3 Distribution coefficients of various hydrocarbons between hexadecane and methanol–water mixtures

Volume fraction of methanol before mixing	Distribution coefficient		
	Benzene	Toluene	Propylbenzene
100	0.0085	0.0019	0.00015
80	0.0185	0.0067	0.00078
50	0.0935	0.0373	0.00775
40	0.1588		
30	0.2322		
20	0.4404	0.2253	0.07577
10	0.6637		

Table 4 Distribution of coefficients of various alcohols between hexadecane and methanol–water mixtures

	Distribution coefficient				
Compound	0% v/v MeOH (before mixing)	20% v/v MeOH (before mixing)	50% v/v MeOH (before mixing)	80% v/v MeOH (before mixing)	100% v/v MeOH (before mixing)
Butan-1-ol	9.11	12.81	24.05	56.83	91.84
Pentan-1-ol	2.3	3.19	8.94	27.66	65.37
Hexan-1-ol	0.29	1.07	3.35	14.01	46.99
Heptan-1-ol	0.08	0.23	1.23	9.39	28.22
Octan-1-ol	0.012	0.068	0.533	4.014	18.357
Nonan-1-ol	0.002	0.013	0.210	2.829	13.330

Table 5 Volume fractions of methanol, water and methanol–water associate for different initial methanol–water mixtures at 20 °C

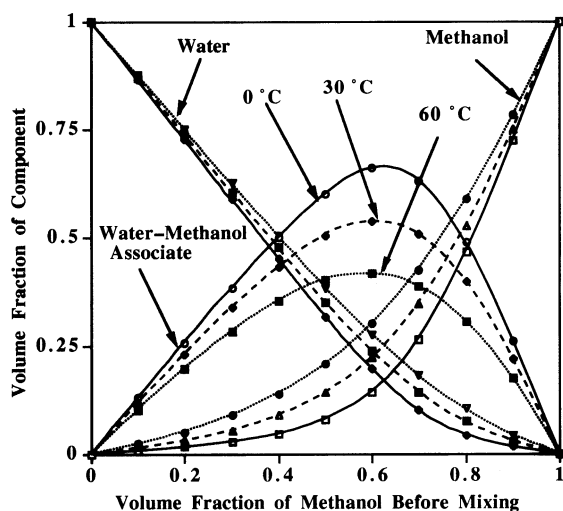| Volume fraction of methanol before mixing	Volume fraction of methanol	Volume fraction of water	Volume fraction of methanol–water associate
0	0.0000	1.0000	0.0000
0.1	0.0063	0.8655	0.1282
0.2	0.0151	0.7295	0.2554
0.3	0.0278	0.5928	0.3794
0.4	0.0475	0.4567	0.4958
0.5	0.0809	0.3241	0.5951
0.6	0.1432	0.2022	0.6547
0.7	0.2642	0.1053	0.6305
0.8	0.4654	0.0459	0.4886
0.9	0.7223	0.0156	0.2621
1.0	1.0000	0.0000	0.0000

Fig. 4 Curves relating volume fractions of methanol, water and methanol–water associate to volume fraction of methanol before mixing for three temperatures.
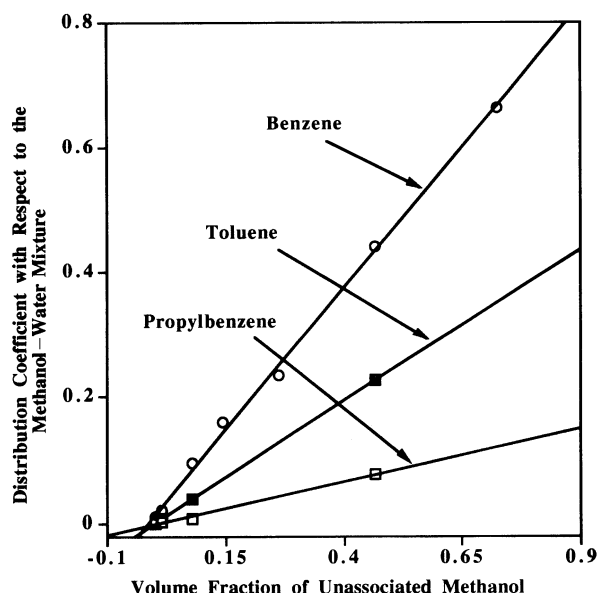
Fig. 5 Graph of distribution coefficient against volume fraction of unassociated methanol for three aromatic hydrocarbons.

Fig. 6 Graph of distribution coefficient against volume fraction of unassociated methanol for four aliphatic alcohols.

the results of Katz *et al.*[5] and confirms that the dispersive interactions that occur between the solute and a methanol–water mixture are due solely to interactions between the solute and the free unassociated methanol. If the solute can exert polar interactions with the solvent mixture (*e.g*, the molecule contains strongly polar groups), then all three components probably contribute to elution.

Schantz[7] also published data for a series of aliphatic alcohols and from his data curves relating the distribution coefficient of each solute to the volume fraction of unassociated methanol were constructed and are shown in Fig. 6. It is seen that the linear relationship no longer holds and components other than the unassociated methanol must now contribute to solute retention. The curves were fitted to a second order polynomial and a reasonably good fit was obtained. However, at present, there is no obvious theoretical reason why this fit should be anything other than fortuitous. The two sets of curves do show, however, that elution control can depend exclusively on the
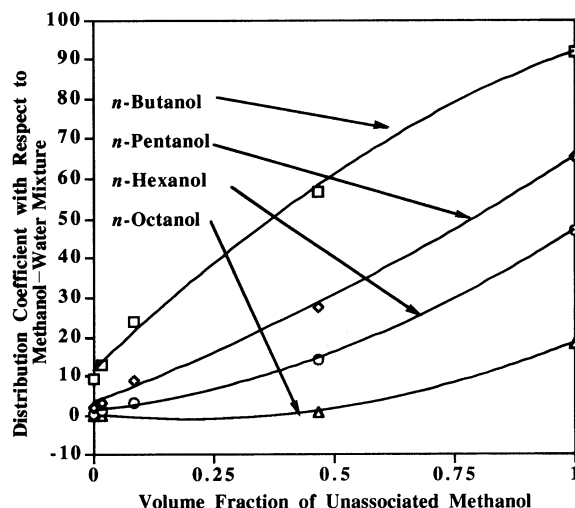
volume fraction of one component of the mixture only, *i.e.*, unassociated methanol. Furthermore, if a series of solutes could be identified on which polar interactions strongly dominated the interactive character of the molecule (dispersive interactions were minimal), the distribution coefficient might well be found to be determined solely by the volume fraction of unassociated water.

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