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STUDY OF THE HYDROGEN BOND DONOR ACIDITY OF BINARY AQUEOUS MIXTURES AND THEIR ROLE IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

JUNG HAG PARK*, ANDREW J. DALLAS†, PHOEBE CHAU AND PETER W. CARR‡
Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, Minnesota 55455, USA

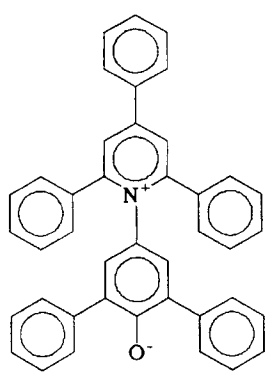
The solvatochromic hydrogen bond donor (HBD) acidity parameter (α_{mix}) of aqueous mixtures of methanol, acetonitrile, propan-2-ol and tetrahydrofuran were determined spectrophotometrically. The study was carried out at 25 °C as a function of composition. The indicators used were 2,6-diphenyl-4-(2,4,6-triphenyl-*N*-pyridino)phenolate, 2,6-dichloro-4-(2,4,6-triphenyl-*N*-pyridino)phenolate and $Fe(LL)_2(CN)_2$ (LL = *N*-(2-pyridylbenzylidene)-3,4-dimethylaniline). The HBD acidity of the aqueous organic mixtures was related to retention in reversed-phase liquid chromatography.

INTRODUCTION

Aqueous mixtures of organic solvents are very complex systems which undergo drastic changes in their chemical and physical properties as the composition of the mixture is varied. Examples of such changes include the effect of composition on dielectric constant,[1,2] surface tension,[3] dipolarity,[4] hydrogen bond donor (HBD) acidity[5,6] and hydrogen bond acceptor (HBA) basicity.[7,8] The strength of the interactions between the dissolved solutes and the solvent system changes significantly throughout the entire composition range.[4,5]

Aqueous–organic mixtures are used in many fields. For example, in reversed-phase liquid chromatography (RPLC) the solutes of interest are present at infinite dilution in aqueous–organic mobile phases. A solute's chromatographic retention can vary by several orders of magnitude as the mobile phase composition is altered. Changes in elution order with changes in mobile phase are common. Spectroscopic studies have shown that large changes in both dipolar and hydrogen bonding interactions between the mobile phase and the solute occur as the mobile phase composition is varied.[4–6,9–12]

In this work, we used the Kamlet–Taft solvatochromic approach to study the HBD acidity of the most common aqueous mixtures used in RPLC, namely, methanol, acetonitrile, propan-2-ol and tetrahydrofuran. Three solvatochromic indicators were used: 2,6-diphenyl-4-(2,4,6-triphenyl-*N*-pyridino)phenolate [denoted ET(30)] and 2,6-dichloro-4-(2,4,6-triphenyl-*N*-pyridino)phenolate [denoted ET(33)], which are chemically similar as shown in structures **I** and **II**, and $Fe(LL)_2(CN)_2$ (LL = *N*-(2-pyridylbenzylidene)-3,4-dimethylaniline) [denoted ET(Fe)], which is chemically different from ET(30) and ET(33) (see structure **III**).



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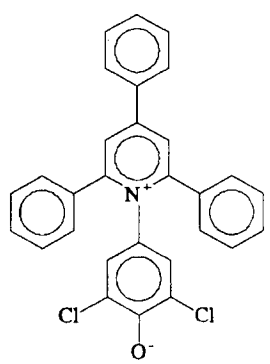

II

* On leave from Department of Chemistry, Yeungnam University, Kyongsan 712-749, Korea.
† Present address: Donaldson Co., Inc., 9250 W. Bloomington Fwy., Bloomington, MN 55431, USA.
‡ Author for correspondence.

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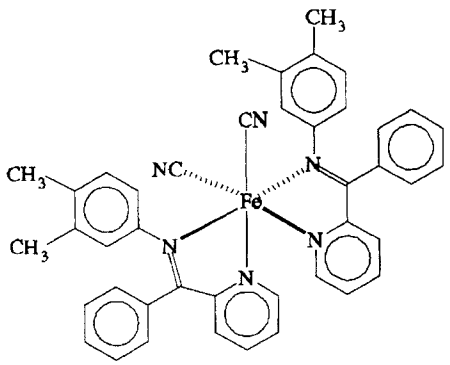

III

The energy of transition (E_T) at the wavelength of maximum absorbance is given by

$$E_T \text{ (kcal mol}^{-1}) = 28\,590/\lambda_{max} \text{ (nm)} \quad (1)$$

(1 kcal = 4·184 kJ). The E_T values for the three indicators [ET(30), ET(33) and ET(Fe)] in a wide variety of pure solvents can be related to the Kamlet–Taft α, β, π^* and δ solvatochromic solvent strength parameters by means of regression equations (2)–(5) discussed below. The above set of Kamlet–Taft parameters represent the ability of a solvent to stabilize by virtue of the solvent's dipolarity/polarizability (π^*), hydrogen bond donor acidity (α) and hydrogen bond acceptor basicity (β). The term δ represents a polarizability correction factor, usually minor, that takes values 0·0, 0·5 and 1·0 for aliphatic, polyhalogenated and aromatic solvents, respectively. In general, Kamlet and Taft use the following correlation equation for spectroscopic linear solvation energy relationships:

$$E_T = E_{T,0} + s\pi^* + d\delta + a\alpha + b\beta \quad (2)$$

For $E_T(30)$, equation (3) proved to be the best correlation equation for 100 pure organic solvents:

$$E_T(30) \text{ (kcal mol}^{-1}) = 30{\cdot}8 + 13{\cdot}68(\pm 0{\cdot}56)\pi^* - 3{\cdot}54(\pm 0{\cdot}34)\delta + 14{\cdot}51(\pm 0{\cdot}36)\alpha \quad (3)$$
$$n = 100, \text{ s.d.} = 1{\cdot}35, r = 0{\cdot}984$$

Note that $a/s = 1{\cdot}06$ [the coefficients s, d, a and b are defined implicitly in equation (2)]. For $E_T(33)$ we found equation (4) to be the best correlation equation:

$$E_T(33) \text{ (kcal mol}^{-1}) = 39{\cdot}09 + 14{\cdot}47(\pm 1{\cdot}19)\pi^* - 3{\cdot}18(\pm 0{\cdot}52)\delta + 14{\cdot}41(\pm 0{\cdot}57)\alpha \quad (4)$$
$$n = 49, \text{ s.d.} = 1{\cdot}45, r = 0{\cdot}977$$

The a/s ratio (0·996) for ET(33) is close to the value for ET(30). For E_T(Fe), equation (5) proved to be the best correlation equation:

$$F_T(\text{Fe}) \text{ (kcal mol}^{-1}) = 39{\cdot}71 + 3{\cdot}31(\pm 0{\cdot}33)\pi^* + 4{\cdot}50(\pm 0{\cdot}16)\alpha \quad (5)$$
$$n = 16, \text{ s.d.} = 0{\cdot}28, r = 0{\cdot}992$$

Note the a/s ratio is 1·36 and is still not very different from the other indicators.

The above pure solvent regressions are important in this work. They are used as the basis for calculating values of α for mixed solvents by measuring π^* of the mixtures based on the use of indicators which are only sensitive to solvent dipolarity/polarizability.

The HBD acidity of aqueous–organic mixtures, as measured by the Kamlet–Taft HBD acidity solvatochromic parameter, α, has been reported recently by several researchers.[5,6,13,14] Whereas α has been well established for a large number of neat solvents, this is not the case for binary solvent mixtures. It is far from proven that solvatochromic measures of solvent strength appropriate for pure solvents are valid for solvent mixtures. Preferential solvation effects and microheterogeneity (see below) in such systems greatly complicate the use of solvatochromic indicators to act as stand-ins for 'generalized' solutes relative to the case of a single (pure) solvent.[13] We attempt to address this question in this work. Additionally, we attempt to clarify the question of whether Kamlet–Taft α indicators probe bulk properties of the aqueous–organic mixtures.[13]

Recently, Marcus and Migron[13–16] reported on the extent of 'preferential solvation' and the degree of 'microheterogeneity' that exist in aqueous–organic mixtures. In their view, preferential solvation takes place when the local composition around a solute differs from the average bulk mixture. In essence, preferential solvation takes place only when a solute acts to establish its own microenvironment by preferentially extracting one component of the mixture from the bulk solvent. Preferential solvation is equivalent to solute-induced solvent sorting. In contrast, microheterogeneity takes place when a constituent of the mixed solvent prefers a molecule of the same type. The preference can be so strong that it extends over several concentric shells around a given type of molecule.

For aqueous–organic mobile phases used in RPLC, it is evident that 'microheterogeneous' environments exist in all mixtures except water–methanol.[13–18] Water–methanol mixtures are nearly ideal (random) mixtures. Using the Kamlet–Taft solvatochromic approach, in conjunction with methods for the determination of the local composition around each type of solvent molecule, Marcus and Migron[13,14] reported that the Kamlet–Taft π^*, β and α indicators are able to sense the actual bulk solvent environment in aqueous–organic mixtures without significantly perturbing this environment. That is, there is no preferential

solvation in these systems. Many earlier reports dispute this claim and have effectively shown that these indicators are preferentially solvated by one of the solvent components through dielectric enrichment (typically observed in non-aqueous mixtures),[17,19-21] hydrogen bonding[21,22] and/or hydrophobic interactions.[23,24] We investigate this issue in detail as it pertains to the Kamlet–Taft scale of HBD acidity strength.

The issue of preferential solvation in mixed solvents is complex, with very important consequences. A number of studies on the dipolarity/polarizability of solvent mixtures including aqueous–organic mixtures have concluded that preferential solvation is a real phenomenon.[4,25-28] However, it must be admitted that in previous work in this laboratory we did not consider the possible consequences of microheterogeneity. The phenomenon of preferential solvation is intrinsic to a number of important models of solution including the UNIQUAC and NRTL models.[29-31]

If preferential solvation does occur, it makes it impossible to define a probe-independent scale of solvent properties; it becomes meaningless to speak of the dipolarity or HBD acidity of a solvent mixture without specifying the probe.

The existence of preferential solvation has very important implications as to the validity of linear solvation energy relationships (LSERs) in mixed solvents. LSERs for liquid–liquid transfer processes such as RPLC and octanol–water partition coefficients are often given in the form

$$SP = SP_0 + mV_{x,2} + s\pi_2^* + a\alpha_2 + b\beta_2 \qquad (6)$$

The subscript 2 denotes a solute property; SP is a free energy-related solubility property of the solute species, $V_{x,2}$ is a characteristic volume of the solute and π_2^*, α_2 and β_2 are measures of solute dipolarity/polarizability, HB donor and acceptor strength, respectively.

Equations of the same form as equation (6) have been applied to RPLC.[32-35] The specific contributions to a solute's retention made by the mobile and stationary phases have been rationalized through the use of linear solvation energy relationships (LSERs) using the Kamlet–Taft multi-parameter scales:[32-35]

$$\log k' = \log k'_0 + M(\delta_s^2 - \delta_m^2)V_{x,2} + S(\pi_s^* - \pi_m^*)\pi_2^* + B(\alpha_s - \alpha_m)\beta_2 + A(\beta_s - \beta_m)\alpha_2 \qquad (7)$$

where the subscripts s and m denote the stationary and mobile phases, respectively, and k' is the capacity factor. The $\log k'_0$ term includes the volume phase ratio and dipolar/polarizability interactions between the solute and the chromatographic phases when π^* is zero. When a system with a fixed pair of mobile and stationary phases is considered, the above equation can be reduced to the form of equation (6):

$$\log k' = \log k'_0 + mV_{x,2} + s\pi_2^* + b\beta_2 + a\alpha_2 \qquad (8)$$

Table 1. LSER b coefficients in mobile phases of various organic composition[a]

Organic content (%, v/v)	Methanol	Acetonitrile	Tetrahydrofuran
50	−1·77(0·10)	−1·71(0·07)	−1·50(0·10)
40	−1·93(0·13)	−2·09(0·08)	−1·96(0·13)
30	−1·98(0·13)	−2·50(0·09)	−2·64(0·12)
20	−1·94(0·16)	−2·68(0·11)	−3·35(0·21)
10	−1·80(0·19)	NA[b]	NA[b]

[a] The b coefficients were obtained by regressing log k' values for 87 solutes of widely varying physico-chemical properties on an ODS column using mobile phases of various organic modifier compositions.[50] In parentheses are 95% confidence intervals for the coefficient.
[b] Data are not available.

The LSERs for log k' values in RPLC invariably show that the most significant solute–solvent interactions are solute size, which is determined by the cohesivity of the mobile phase, and the solute HBA basicity, which is determined by the mobile phases HBD acidity.[32-35] The solute HBD acidity and/or the mobile phase HBA basicity play a minor, almost insignificant, role.

Implicit in this equation is the concept that the phase (solvent)-dependent coefficients (m, s, b and a) are independent of the solute. If we consider that the solute establishes its own environment in a mixed solvent it follows that the coefficients will not be solute independent. It follows that if preferential solvation were to take place in RPLC, then very basic solutes would not be well fitted. On increasing the water content of the mobile phase, it is observed that the dependence on solute β_2 increases, i.e. the absolute magnitudes of the coefficient b in equation (8) increase as shown in Table 1.

Another goal in this work was to develop an understanding of the role of the mobile phase HBD strength in RPLC retention. Previous solvatochromic studies of retention in RPLC have stressed the importance of the solute HBA basicity (β_2) in establishing the solute capacity factor (k'). Large amounts of organic cosolvent and water are sorbed into a bonded stationary phase.[36,37] As the mobile phase becomes highly aqueous, less modifier and water are found in the bonded phase, which results in a lower HBD acidity of the bonded phase. The mobile phase HBD acidity increases as the mobile phase becomes more aqueous.[4,5] We therefore expect that the coefficient b will be directly related to α_{mix} for mobile phases of different organic composition.

EXPERIMENTAL

All solvents were HPLC grade and were used without further purification. The solvent mixtures were prepared

by mixing known volumes of each liquid. ET(33) was prepared and purified using a procedure given in the literature.[38] All spectroscopic measurements were made using a Varian *DMS* 200 spectrophotometer using a slit width of 0·2 nm, 20 nm min^{-1} scan rate, a smoothing constant of 5 s and 1 cm pathlength quartz cells. The wavelength of the spectrophotometer was calibrated daily using a holmium oxide filter and the stability of the instrument throughout this experiment is indicated by no poorer than a 0·10 nm variation in any of the six holmium oxide bands monitored. All samples were thermostated at 25 ± 0·2 °C for 15 min before scans were made. Each of the samples were gently rocked after sitting for 10 min in order to ensure temperature equilibrium throughout the sample. Peak maxima were determined using the '9/10' method in order to minimize the effect of changes in band shape with solvent.[39] Triplicate measurements of peak maxima agreed with one another to better than 0·5 nm. The indicator concentration was adjusted so as to give an absorbance in the range 0·5–0·8. At this concentration it was confirmed that the peak maxima are independent of solute concentration.

RESULTS AND DISCUSSION

Relationship between $E_T(30)$ and $E_T(33)$

Because the structures of ET(33) and ET(30) are so similar, we felt that ET(33) would sense the same intermolecular interactions as ET(30). Consequently, we expected that the absorption energy of ET(33) would be linearly related to that of ET(30) as the volume fraction of organic modifier was varied. Figure 1 shows the normalized E_T values for ET(33), $E_T^N(33)$, plotted against the corresponding $E_T^N(30)$ values for ET(30). The error bars indicate the magnitude of the random experimental error.

Surprisingly, for all four types of mixtures all the data lie below the 1 : 1 line. There are two potential simple explanations for the lack of linearity in these plots. First, as discussed above, E_T responds to changes in both a solvent's π^* and α. If both π^*_{mix} and α_{mix} were linear functions of composition or if they were colinear with one another, then $E_T(30)$ and $E_T(33)$ would have to be linearly related. This is required mathematically. However, we know from previous work[4,5] that neither π^*_{mix} nor α_{mix} is a linear function of compositions. In general they are not the same function of composition (see below). Even under this general condition, a plot of $E_T(30)$ versus $E_T(33)$ would still be linear if both indicators had the same a/s ratio, that is, the same blend of sensitivities to the solvent π^* and α.

As shown in equations (3) and (4), the a/s ratios for $E_T(30)$ and $E_T(33)$ are 1·06 ± 0·062 and 0·996 ± 0·12, respectively. These ratios are so similar that they cannot explain the non-linearity in the plots of $E_T^N(30)$ versus

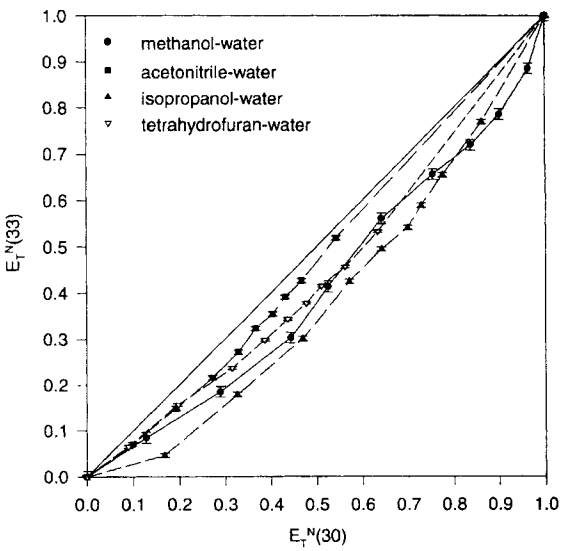

Figure 1. Plot of $E_T^N(33)$ versus $E_T^N(30)$ for aqueous mixtures of methanol, acetonitrile, propan-2-ol and tetrahydrofuran. E_T^N values were calculated with the equation $E_T^N = [E_T(\text{water}) - E_T(\phi_o)]/[E_T(\text{water}) - E_T(\text{pure organic})]$

$E_T^N(33)$ (see Figure 1) by any model that assumes a constant a/s ratio as the composition is varied. This is shown strikingly in Figure 2. To generate Figure 2 we used equations (3) and (4) to calculate theoretical values of $E_T^N(30)$ and $E_T^N(33)$ using previously measured values of π^*_{mix} and the average α_{mix} values estimated below. Thus we conclude that the non-linearity of the data in

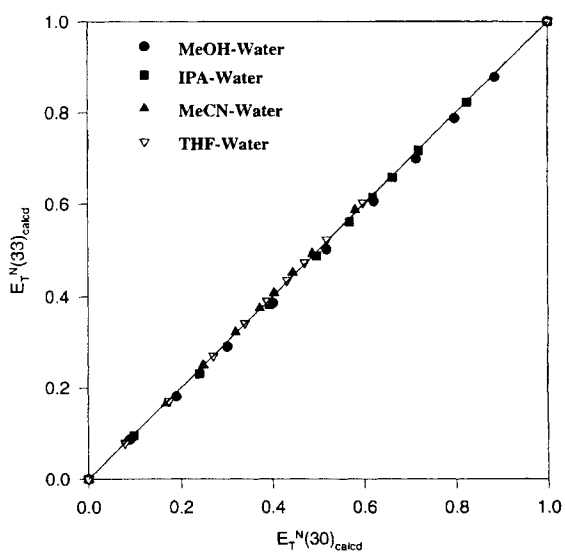

Figure 2. Plot of calculated $E_T^N(33)$ versus $E_T^N(30)$

Figure 1 can only be explained if either preferential solvation or microheterogeneity takes place so as to differentiate between these two structurally similar indicators. The non-linearity cannot be due to any intrinsic non-linearity in the dependences of $E_T(30)$ or $E_T(33)$ on composition or of a non-linear dependence of π^*_{mix} or α_{mix} on composition.

It is possible that the cybotactic region around ET(30) and ET(33) could differ. It seems reasonable, given that a chloro group is both a better electron acceptor and is less hydrophobic than a phenyl group, that the relative amount ofwater in the solvent adjacent to the dyes could differ.

We note that the two systems which show the greatest degree of microheterogeneity, tetrahydrofuran–water and acetonitrile–water, actually lie considerably closer to the 1 : 1 line in Figure 1 than does the nearly ideal mixture, methanol–water. This suggests that the lack of linearity is not due to microheterogeneity. However, plots of α_{mix} versus π^*_{mix} are more nearly colinear for the tetrahydrofuran–water ($r^2 = 0{\cdot}7657$) and acetonitrile–water mixtures ($r^2 = 0{\cdot}6164$) than for the methanol–water ($r^2 = 0{\cdot}0119$) and propan-2-ol–water mixtures ($r^2 = 0{\cdot}5114$), and this might explain why the data in Figure 1 for the tetrahydrofuran–water and acetonitrile–water mixtures lie closer to the 1 : 1 line.

Excess E_T

The differential behavior of probes which are sensitive to a solvent's HB donor strength has been extensively documented for the aqueous mixtures studied here.[23,24] Differences in the interaction of one indicator molecule with one component of the solvent mixture are better examined in terms of excess E_T (ΔE_T) values than by

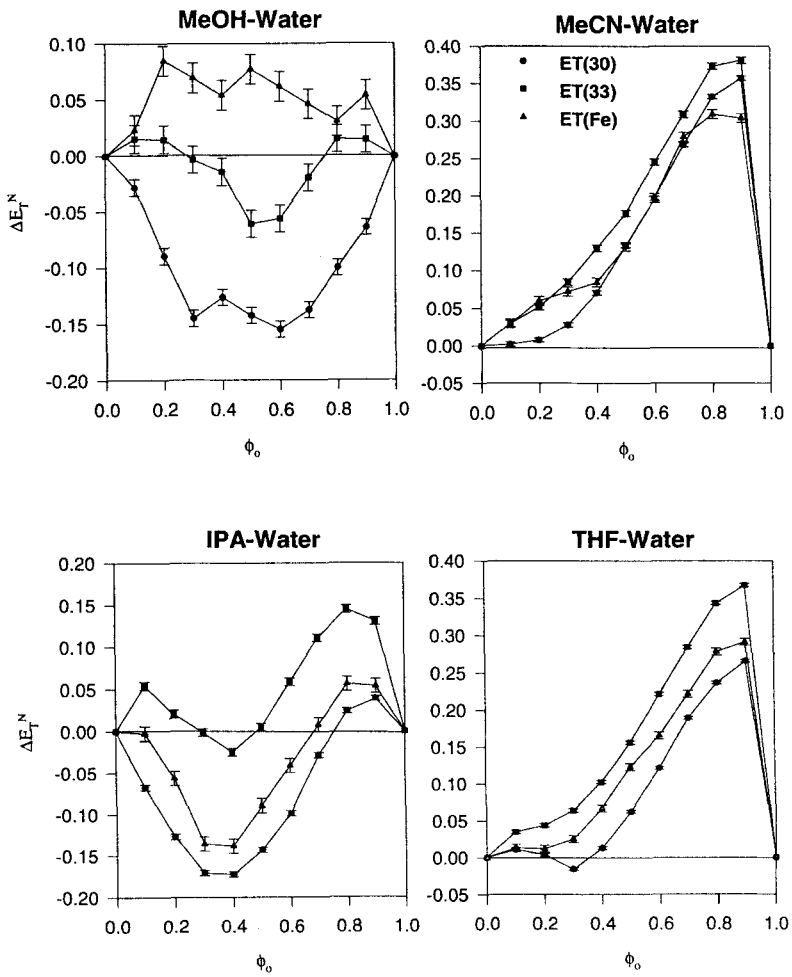

Figure 3. Plot of ΔE_T^N versus organic volume fraction (ϕ_o)

examination of plots of α_{mix}. The data are examined first from the perspective of ΔE_T because ΔE_T does not entail the complications of needing a pure solvent correlation. ΔE_T values are calculated by equation (9) using the volume fraction approach of Davis and Douheret[2] rather than the mole fraction approach taken by Kolling:[28]

$$\Delta E_T = E_T(\text{mix}) - E_{T,o}\phi_o - E_{T,w}(1 - \phi_o) \qquad (9)$$

The subscripts o and w indicate the organic component and water, respectively. Because the magnitude of ΔE_T values depends on the difference between $E_{T,o}$ and $E_{T,w}$, comparison of the plots of ΔE_T versus ϕ_o in different solvents can be misleading. To eliminate this dependence the ΔE_T values were normalized by dividing them by the difference between $E_{T,o}$ and $E_{T,w}$ to obtain ΔE_T^N.

A positive value of ΔE_T^N indicates that the probe indicator acts as if it were surrounded more by water than by the organic component of the mixture. When the solvents form a random mixture, i.e. they show no preference for one another, and when the probe solute interacts with both solvents equally, a plot of E_T versus ϕ_o will be a straight line and the ΔE_T^N will be zero at all compositions.

When the probe solute extracts one solvent preferentially from the bulk mixture then ΔE_T^N would be finite. Alternatively non-zero ΔE_T^N values can be explained based on microheterogeneity. The solute simply moves into the 'microphase' which it prefers. The finite excess E_T results from a non-linear variation in the amount of the microphases as the bulk composition is changed.

The ΔE_T^N values for the three indicators are plotted against volume fraction in Figure 3. The error bars indicate the magnitude of the random experimental error. Clearly, the ΔE_T values are non-zero. While the data shown in Figure 3 display several distinct patterns, the results can be summarized briefly as follows. First, relative to the two alcohol–water systems there is little differential behavior of the three indicators evident in the acetonitrile–water and tetrahydrofuran–water

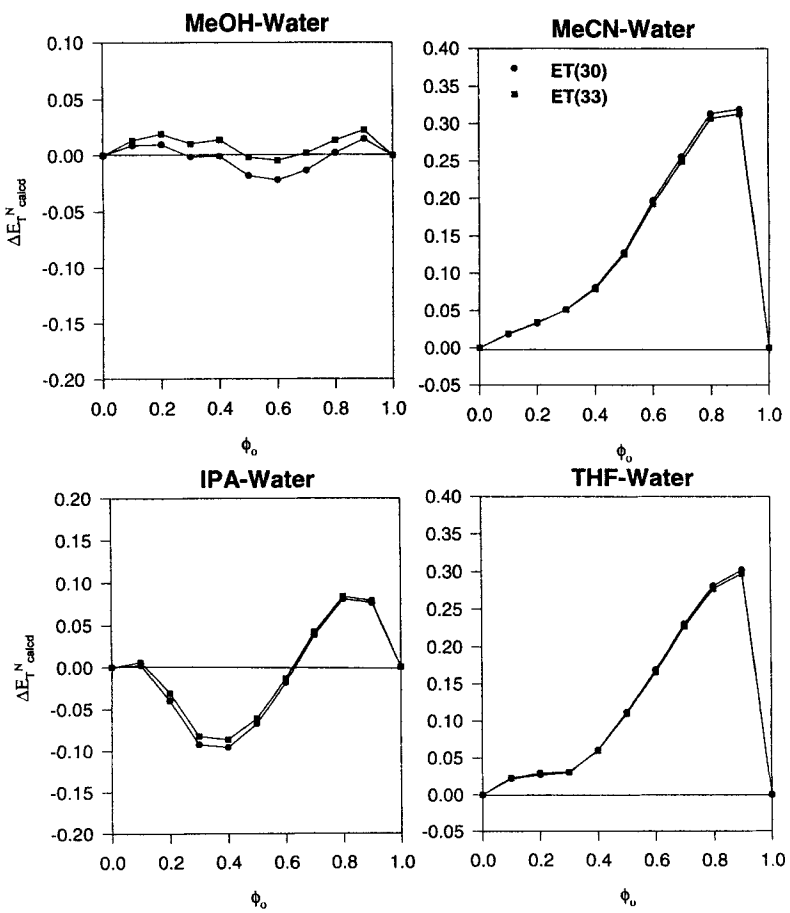

Figure 4. Plot of calculated ΔE_T^N versus organic volume fraction (ϕ_o)

mixtures. All three indicators behave very similarly in the two non-alcohol solvent systems. The positive ΔE_T^N values in both of these systems show that the three indicators are better solvated by water than by the organic component. This could be due either to indicator-independent preferential solvation effects or to microheterogeneity. However, microheterogeneity is more plausibly indicator independent than is indicator-independent preferential solvation. Second, the three indicators behave differentially in the two alcohol–water systems but the pattern of solvation is complex. Only ET(30) consistently interacts more strongly with the organic component whereas ET(33) and ET(Fe) show different preferences in methanol–water and propan-2-ol–water.

As shown in Figure 4, the average π^*_{mix} and average α_{mix} values when used in conjunction with equations (3) and (4) reproduce the ΔE_T^N values for acetonitrile–water and tetrahydrofuran–water systems very well. However, for the alcohol–water mixtures, especially for the methanol–water mixtures, the ΔE_T^N values are not well reproduced. The above observations lead us to believe that while differential indicator behavior is minor in the tetrahydrofuran–water and acetonitrile–water mixtures, there is considerable microheterogeneity as demonstrated by the deviation from random mixing, that is, the ΔE_T values are large. In contrast, in the alcohol–water mixtures there is considerable differential behavior among the three indicators, which we attribute to the preferential solvation and not microheterogeneity. This is especially true for the methanol–water system since this mixture is nearly ideal.

Hydrogen bond donor acidity

The solvatochromic characteristics of the five pure solvents used in this work are summarized in Table 2. It is evident that these liquids span a wide range in properties. We therefore expect that mixtures with water, particularly of tetrahydrofuran and acetonitrile, will show large changes in α as the composition is varied.

Determination of the α values for aqueous organic mixtures was carried out by the use of a method reported in previous papers on the properties of aqueous–organic mixtures.[4,5] The correlation between the observed transition energy of maximum absorption of an indicator (E_T) and the solvatochromic properties in a wide variety of pure solvents was used as a calibration line [see equations (3)–(5)]. Once a solvent's π^* parameter is established, one can back-calculate the solvent's α value. In order to minimize potential errors due to self-association of strong HB donor solvents and errors in the π^* values, Kamlet *et al.*[40] suggested that properties which meet the following criteria be chosen for formulating an α scale: (a) the properties should involve sufficiently strong HB acceptors that competitive solvent self-association should not materially influence the enhanced solvatochromic effects due to hydrogen bonding; and (b) the a/s ratio should not be too low (preferably >1·0) so that uncertainties in the π^* values, which are necessarily less reliable for HB donor than for non-HB donor solvents, should not introduce unacceptable uncertainties in the α values. The three indicators chosen in this work meet the second requirement but it is not clear that they satisfy the first, given the strength of water as an HB donor and acceptor.

Table 2. Solvatochromic properties of pure solvents

Solvent	α	β	π^*
Water	1·17	0·47	1·09
Methanol	0·98	0·66	0·60
Propan-2-ol	0·76	0·84	0·48
Acetonitrile	0·19	0·40	0·75
Tetrahydrofuran	0·00	0·55	0·58

Marcus[41] also noted that a four-parameter regression equation including the solvents' β parameter gave a slightly improved fit over that given in equation (3). However, the improvement was only marginal and the structure of ET(30) indicates there are no HBD sites in the molecule. We therefore used equation (3) as the calibration line. Assuming that the correlations for pure solvents also hold for solvent mixtures, the α values for mixtures (α_{mix}) are calculated as follows:

$$\alpha_{mix} = [E_T(30) - 30{\cdot}8 - 13{\cdot}68\pi^*_{mix}]/14{\cdot}51 \quad (10)$$

The $d\delta$ term drops out since δ is zero for all the aqueous–organic mixtures studied here. Similarly, α_{mix} values were calculated with the indicators ET(33) and ET(Fe) as follows:

$$\alpha_{mix} = [E_T(33) - 39{\cdot}09 - 14{\cdot}47\pi^*_{mix}]/14{\cdot}41 \quad (11)$$

$$\alpha_{mix} = [E_T(Fe) - 39{\cdot}71 - 3{\cdot}31\pi^*_{mix}]/4{\cdot}50 \quad (12)$$

The α_{mix} were calculated using the π^*_{mix} values given by Cheong and Carr[4] (see Table 3).

The use of equations (10)–(12) was validated to some extent by principal component analysis. We performed principal component analysis on a matrix of E_T values for the three indicators used here and also the ^{13}C NMR chemical shifts of dialkylbenzamides used by Schneider *et al.*[6] in methanol–, acetonitrile– and tetrahydrofuran–water mixtures at eleven volume fraction compositions. The data were mean centered and the range normalized prior to analysis. We found that only two factors are needed to explain slightly more than 99% of the total variance. We presume that these factors are related to π^*_{mix} and α_{mix}.

The α_{mix} values given in Table 3 are plotted versus the volume fraction of organic solvent (ϕ_o) in Figure 5. The standard deviations of the measurements at each

Table 3. α_{mix} values for aqeuous–organic mixtures[a]

	Methanol				Propan-2-ol				Acetonitrile				Tetrahydrofuran			
ϕ_o[b]	ET(30)	ET(33)	ET(Fe)	Av.	ET(30)	ET(33)	ET(Fe)	Av.	ET(30)	ET(33)	ET(Fe)	Av.	ET(30)	ET(33)	ET(Fe)	Av.
0·0	1·12	0·97	1·24	1·11	1·12	0·97	1·24	1·11	1·12	0·97	1·24	1·11	1·12	0·97	1·24	1·11
0·1	1·07	0·95	1·20	1·07	0·95	0·93	1·16	1·01	1·03	0·92	1·16	1·03	0·98	0·86	1·11	0·99
0·2	0·99	0·92	1·17	1·03	0·79	0·80	1·02	0·87	0·94	0·87	1·08	0·96	0·83	0·72	0·98	0·84
0·3	0·95	0·91	1·15	1·00	0·72	0·77	0·90	0·80	0·90	0·86	1·01	0·92	0·71	0·67	0·90	0·76
0·4	0·94	0·90	1·10	0·98	0·69	0·74	0·88	0·77	0·90	0·87	0·95	0·91	0·69	0·66	0·86	0·74
0·5	0·92	0·89	1·07	0·96	0·72	0·77	0·89	0·79	0·90	0·86	0·90	0·88	0·66	0·64	0·83	0·71
0·6	0·91	0·90	1·04	0·95	0·72	0·79	0·89	0·80	0·89	0·87	0·90	0·89	0·64	0·63	0·78	0·68
0·7	0·94	0·95	1·02	0·97	0·73	0·79	0·88	0·79	0·91	0·89	0·88	0·89	0·63	0·60	0·74	0·66
0·8	0·99	1·01	1·02	1·01	0·73	0·78	0·86	0·79	0·88	0·86	0·82	0·85	0·58	0·57	0·68	0·61
0·9	1·03	1·05	1·02	1·03	0·72	0·75	0·81	0·76	0·83	0·81	0·71	0·78	0·52	0·49	0·58	0·53
1·0	1·14	1·15	1·02	1·10	0·73	0·69	0·78	0·73	0·34	0·36	0·32	0·34	0·01	−0·23	0·04	−0·06

[a] The α_{mix} values were calculated based on equations (10), (11) and (12).
[b] Volume fraction of organic component in the mixture

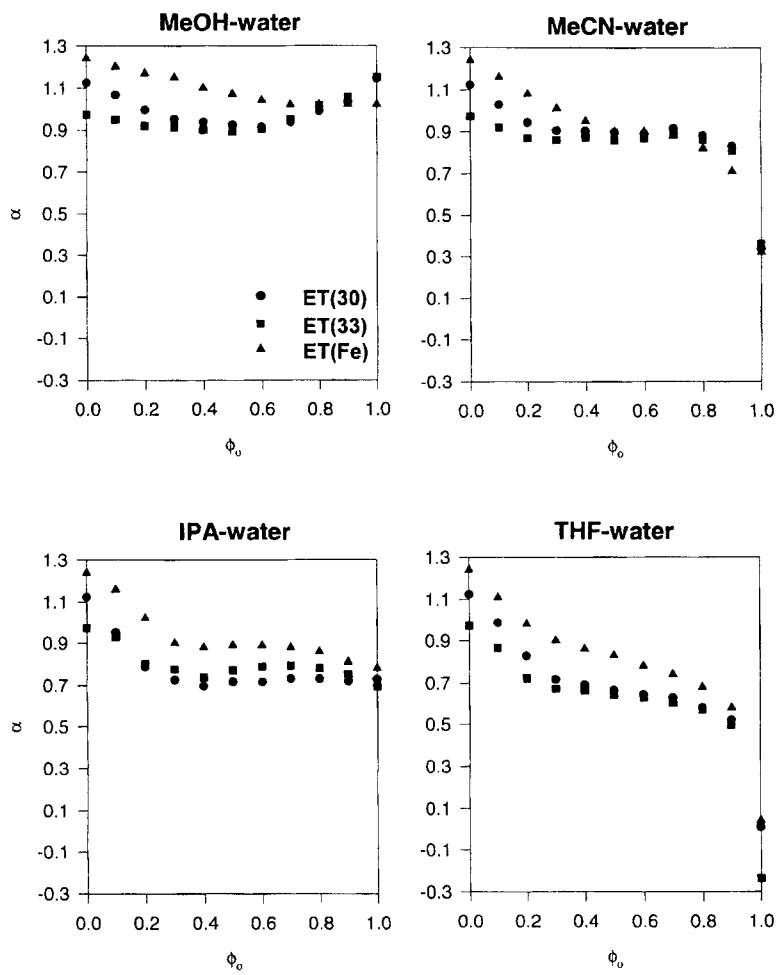

Figure 5. Variation of α_{mix} with organic volume fraction (ϕ_o)

composition are smaller than the size of the symbols and therefore the error bars are not given for these data.

Inspection of Figure 5 shows that the HB donor acidities of all the aqueous mixtures increase in a non-linear fashion as water is added to the organic solvent. For aqueous mixtures of acetonitrile and tetrahydrofuran, α_{mix} increases very dramatically on addition of even a small amount of water to the neat solvent; it then reaches a plateau and finally rises slowly to the HB acidity of pure water. In contrast, the α_{mix} for the mixtures of methanol and propan-2-ol, which are acidic, increase only slightly as the first small amount of water is added and then increase more rapidly as the composition approaches pure water. In methanol–water mixtures there is a strong sign of a local minimum in α_{mix}.

Differential behavior of the indicators

Inspection of Figure 5 strongly suggests that the different indicators behave differently. Before examining the differences in detail, we first ask whether they are real or simply a consequence of how we measure them.

We must point out that the differences in α_{mix} values shown in Figure 5 do not provide incontrovertible evidence for differential behavior of the different indicators in mixed solvents due to either preferential solvation or microheterogeneity. The experimental error of measurement, that is, the precision, is far smaller than the spread between the three indicators. This seems to validate the idea that the three indicators behave differentially. However, we point out that the spread between the three indicators is greatest in pure water,

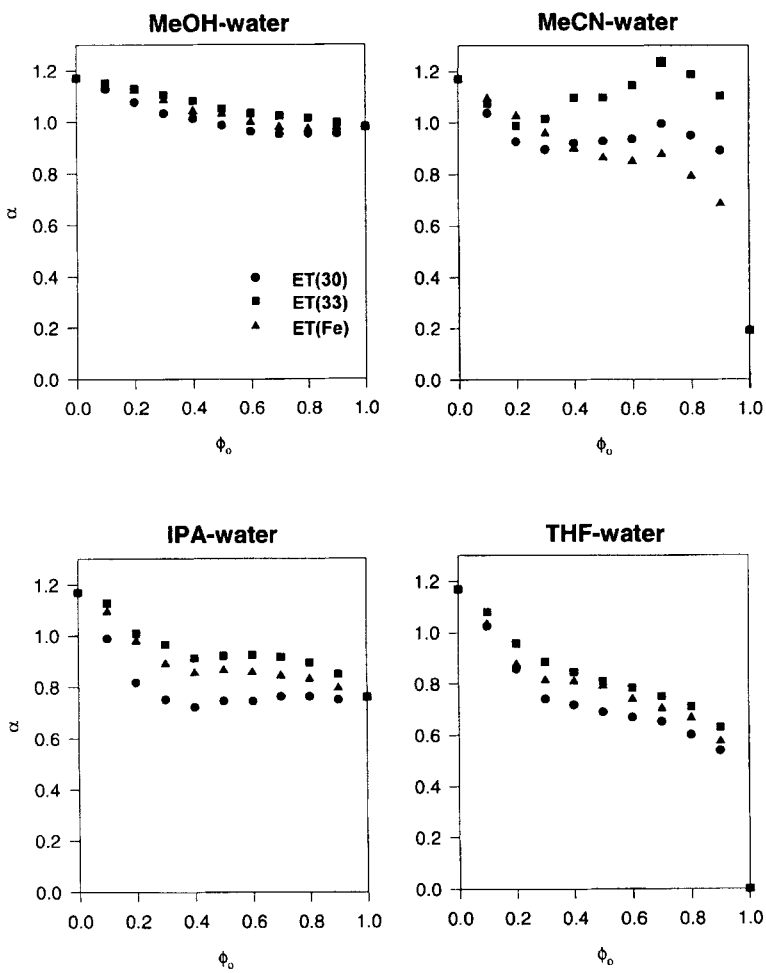

Figure 6. Variation of α_{mix} with organic volume fraction (ϕ_o). The α_{mix} values were calculated by forcing exact agreement in the α values in both water and pure organic solvent

and this cannot be due to either preferential solvation or microheterogeneity. We conclude that simple comparisons such as shown in Figure 5 here or figures presented by Schneider *et al.*[6] cannot be used as evidence for differential behavior of different indicators.

The differences among the three indicators in pure water result from the lack of fit in the pure solvent correlations [see equations (3)–(5)] in fitting the behavior of the three indicators in water. We can force agreement in water by using the E_T values in pure water as the basis for computing the a coefficient for the three indicators. When we did this the curves became discrepant at the pure organic end of the plot. We decided to develop a method that forced exact agreement in both water and the pure organic solvent. This can be done by using the pure solvent correlations [equations (3)–(5)] to derive an intercept and then for each indicator solve two simultaneous equations inputing the π^* and α values for water and the organic solvent to establish both the a and s coefficients. The results of calculating α_{mix} in this way are shown in Figure 6. All curves shown in both Figures 5 and 6 are plotted on the same scale to facilitate comparison. The symbols used are about 0·035 units in size and we are confident that α values can be reproduced to better than 0·02 from day to day. Hence the differences are real. However, it is clear from comparing Figures 5 and 6 that the method of converting a measured transition energy into an α value has a tremendous effect on the value of α obtained and on the trend observed as a function of composition. This is strikingly evident in the results obtained for acetonitrile–water mixtures where in Figure 5 we see a monotonic dependence on ϕ_o whereas in Figure 6 a distinct local maxima is seen with all three indicators.

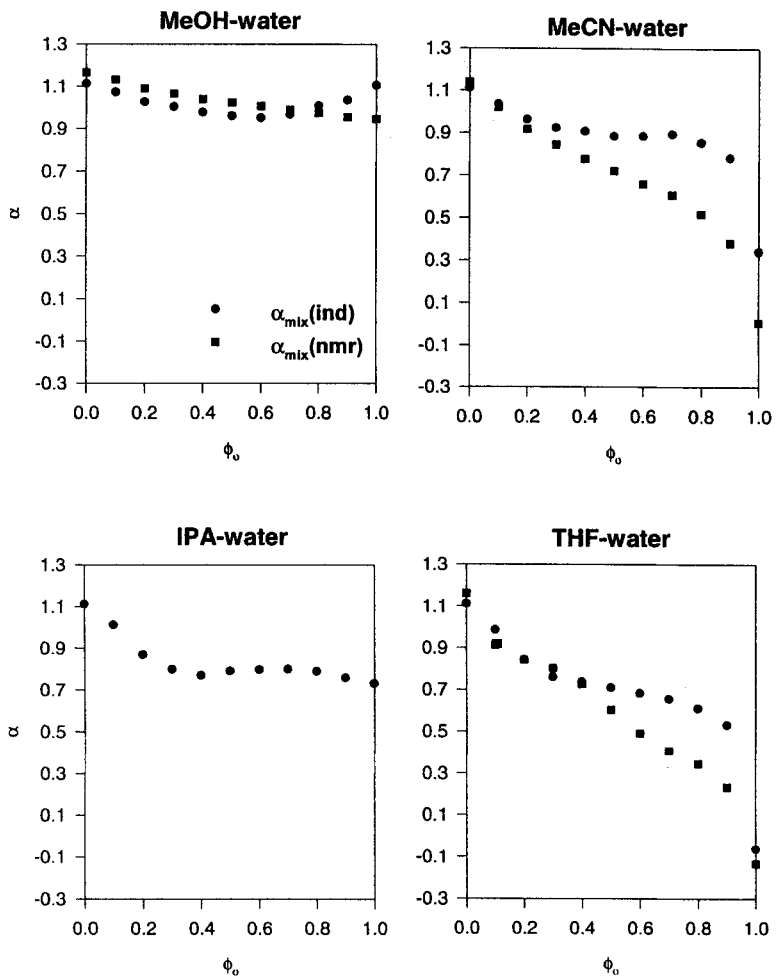

Figure 7. Plot of average α_{mix}(ind) from the three indicators and α_{mix}(nmr) from the band of the highest a/s ratio versus organic volume fraction (ϕ_o)

Since the method of converting the raw transition energies into α values is arbitrary, we see no point in discussing in any greater detail the differential behavior of the three indicators. It is best to consider only the average α_{mix} of the solvents as shown in Figure 7, where we have also plotted the α_{mix} values obtained by NMR spectroscopy.[6] One of the NMR bands studied by Schneider *et al.*[6] was found to be very weakly dependent on solvent π^*. Its a/s ratio is 4·68. We therefore used this band to estimate α_{mix} as shown in Figure 7.

Relationship between hydrogen bond donor acidity and RPLC retention

As shown above, there are large changes in α as the composition is varied. Consequently, the retention of strong HB acceptor solutes in RPLC should experience these large changes in the mobile phase HB donor acidity. Equation (7) shows that the LSER b coefficient is determined by the difference between the HB donor acidity of the stationary and mobile phases ($\alpha_s - \alpha_m$). This difference manifests itself as a negative contribution to retention. This can only result when the mobile phase is a significantly better HB donor acid than is the stationary phase. From the work of Yonker *et al.*[36,37] and many other groups,[42–46] it is evident that a large amount of organic cosolvent and water are sorbed into the bonded stationary phase. As the mobile phase becomes more aqueous, less modifier and water are found in the bonded phase. This ought to result in a decrease in the HBD acidity of the bonded phase. Since mobile phase HBD acidity increases as the mobile phase becomes more water-like, it follows that the mobile phase HBD acidity ought to be inversely related to that of the bonded phase. This is in agreement with reports by Carr and Harris[47] and Jones and Rutan.[48] We therefore expect

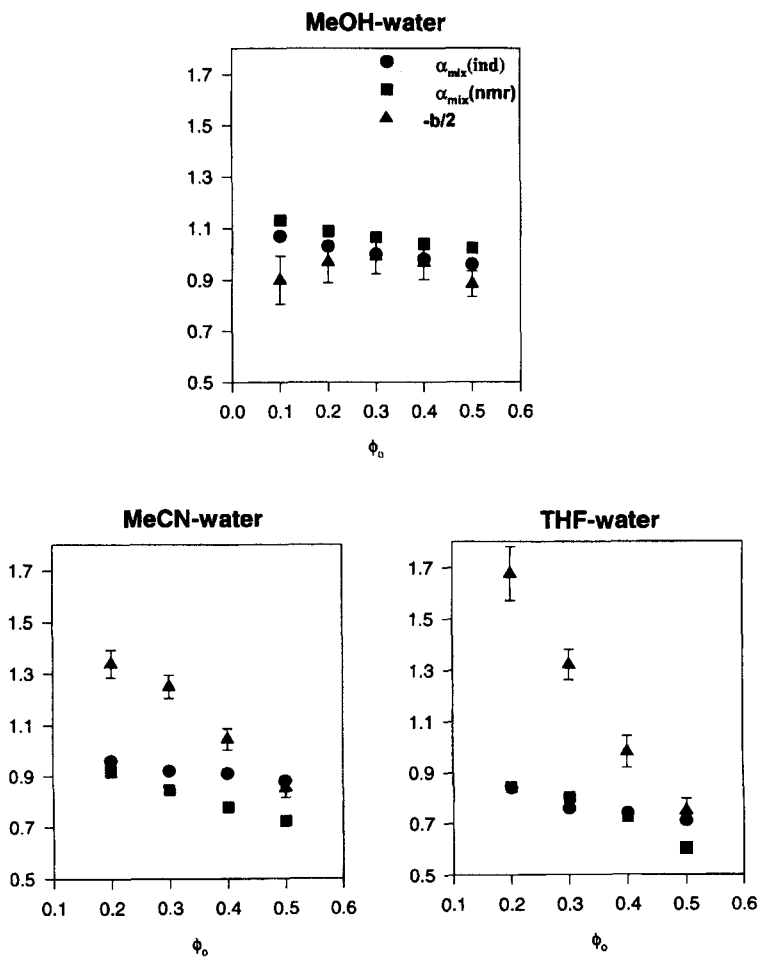

Figure 8. Variation of $-b/2$, α_{mix}(ind) and α_{mix}(nmr) with organic volume fraction (ϕ_o)

that the magnitude of the b coefficient will be monotonically related to α_{mix} of the mobile phase.

Figure 8 shows the variation in $-b$, average α_{mix}(ind) based on the three indicators and α_{mix}(nmr) from the band of the highest a/s ratio versus ϕ_o for methanol–water, acetonitrile–water and tetrahydrofuran–water mixtures. As can be seen, in the case of acetonitrile–water and tetrahydrofuran–water mobile phases the $-b$ coefficient, α_{mix}(ind) and α_{mix}(nmr) decreases monotonically with ϕ_o. However, in methanol–water mobile phases a maximum is observed in the plot of $-b$ versus ϕ_o. Inspection of Table 1 indicates that within the range of organic composition where the b coefficients were obtained, the α_{mix} values measured by all three indicators increase monotonically as the mixtures become more water-like. However, the magnitude of b first increases as more water is added to the 50% methanol mobile phase, and then seems to decrease at a methanol volume fraction of 20%. Considering the magnitude of errors associated with the b coefficients, the curvature in the plot may not be significant. We also note that the magnitude of b in the case of methanol–water mobile phases is in general small compared with those for the acetonitrile–water and tetrahydrofuran–water phases. This may be due to the fact that methanol is a much stronger HBD acid than acetonitrile and tetrahydrofuran, and hence bonded phases modified by sorption of methanol have greater HBD acidities than do those modified by the weak HBD acid acetonitrile or non-HBD acid tetrahydrofuran. This in turn will yield a smaller differential HBD acidity between the mobile and stationary phases and hence a decreased dependence of solute retention on solute HBA basicity and a smaller magnitude of b result.

Recently, Helburn *et al.*[49] reported measurements of the HBD acidities of solvated ODS bonded stationary phases. They observed that the HBD acidities of the stationary phases modified by sorption in acetonitrile–water and methanol–water mobile phases are actually greater than those of the bulk aqueous–organic mixtures in equilibrium with them. We believe that their measurements are in error. If the HBD acidity of the modified stationary phase is greater than that of the bulk mobile phase, the differential term $(\alpha_s - \alpha_m)$ will be positive. This in turn will result in a positive b coefficient. However, as shown in Table 1, the signs of the b coefficients for RPLC retention measured in the mobile phases of the same volume fractions as studied by Helburn *et al.* are all negative. Helburn *et al.* noted the possibility that their values for the solvated stationary phases were overestimated owing to the very different hydrophobicities of the indicator dyes used. This causes the dyes to sorb into very different active sites on the stationary phase and thus sense differently the environment in the stationary phase. It is also possible that silanol group interactions strongly influence the behavior of the indicators.

CONCLUSION

For acetonitrile–water and tetrahydrofuran–water mixtures, which are known to be microheterogeneous, the three indicators of sufficiently different structures behave very similarly in terms of ΔE_T. In both of these systems the three indicators are better solvated by water than by the organic component. This happens when polar and HB accepting indicators move into the water-rich 'microphase.' The indicators sense the bulk property of these mixtures which are intrinsically microheterogeneous and thus can be used as stand-ins for the 'general solute.' However, for methanol–water and propan-2-ol–water mixtures, which are not microheterogeneous, the three indicators behave differently. In the methanol–water system, which is known to be a random mixture, ET(30) is preferentially solvated by methanol molecules whereas ET(Fe) is preferentially solvated by water molecules. The indicators are no longer able to probe bulk properties of this random mixtures due to preferential solvation. If preferential solvation occurs, it makes it impossible to define a probe-independent scale of solvent properties; it becomes meaningless to speak of the dipolarity or HBD acidity of a solvent mixture without specifying the probe. These indicators cannot be used as stand-ins for the 'general solute' in these alcohol–water systems.

The LSER b coefficients are always negative in sign and vary monotonically with α_{mix} of the aqueous–organic mixtures over the composition range studied. This indicates that the RPLC stationary phase is less HB acidic than the mobile phase. This is opposite to the observation made by Helburn *et al.*[49] However, we believe that their results are in error for reasons discussed in the previous section.

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