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RETENTION ANOMALY IN TERNARY SOLVENT SYSTEMS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

In ternary mobile phase reversed-phase liquid chromatography anomalous lags and even maxima in retention are observed as mobile phase composition is varied, *e.g.*, as acetonitrile replaces methanol in water–methanol–acetonitrile mixtures. A number of colligative properties have been determined for such mixtures in an attempt to give physical meaning to the anomaly. The association model of Scott is extended to ternary systems and it is found that there is a close correlation between 'free water' concentration and retention, density, surface and interfacial tension.

INTRODUCTION

In reversed-phase liquid chromatography (RPLC) a single stationary phase of low polarity is used for many chemically different solute types. By using mixtures of water and other miscible solvents, the solubility of sample components in the mobile phase can be varied continuously until retention reaches practical limits. For a given sample, there may be a binary mobile phase which produces adequate selectivity with reasonable total retention and different binary mobile phases of similar average 'polarity' can be prepared from different water-soluble modifiers to produce very specific selectivity effects[1]. However, there are only a limited number of organic modifiers that can be used to produce binary mixtures with water over wide composition ranges in practice and to circumvent this limitation one can use ternary or quaternary mixtures, since many more mobile phases of the same average polarity can be prepared. For example, methanol and acetonitrile solvents can be mixed in any arbitrary ratio with water to yield an infinite number of ternary mobile phases and thus greatly increase the flexibility of a search for optimal separation condition.

In recent years, many practical examples of the advantageous use of ternary mobile phases in RPLC have been published[2–5]. The first systematic investigation of ternary mobile phases was performed by Bakalyar *et al.*[2] and later by Glajch *et al.*[5]. In a more recent work, a systematic study of the behavior of two ternary mobile

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phase systems were reported by De Galan and co-workers[6–8]. In that work, the data were used to define ternary compositions of equal polarity and to analyze specific separation effects.

In this paper we report a systematic study of retention behavior in two ternary mobile phase systems: water–methanol–acetonitrile and water–methanol–tetrahydrofuran (THF) for a series of solutes in RPLC. In all the compositions that were studied, a very unusual trend was observed in the retention behavior of the solutes. As the amount of the second organic modifier (acetonitrile or THF) was increased in the ternary mixture of water and methanol the retention of some solutes would tend to lag or even increase over certain composition ranges. Past that region the retention would drop steadily with increase in stronger solvent as expected. In an effort to explain this observation we have carfully examined some of the common physical properties of ternary mobile phase which we suspected would reflect the origin of these unusual trends in retention behavior. Surface tension and densities of some solvent mixtures, and the interfacial tensions between an alkane phase (*n*-hexadecane) and the solvent mixtures were measured.

According to a mechanism proposed by Katz *et al.*[9], a binary mixture of water and an organic modifier (methanol, acetonitrile or THF) is no longer a binary but a ternary system, where the third component arises from the association of water and the organic modifier. Based on that theory, we have derived a mathematical model to predict the amount of "associated" and "free" (unassociated) components in a ternary solvent system. The physical properties of the ternary solvent system and ultimately the observed anomaly in the retention behavior is explained based upon the content of "free" water (unassociated water) in the ternary solvent mixture.

EXPERIMENTAL

All retention measurements were made using an IBM Model LC-9533 liquid chromatographic system and ultraviolet detection at the appropriate wavelength for individual solutes. All mobile phases were prepared independently of the gradient capability of the chromatographic system to insure integrity of the volume fractions used. Independent measurement of the volumetric flow-rate was achieved by timed collection of the effluent from the chromatographic column. The best estimate of retention time reproducibility is 1.0%.

The surface and interfacial tension measurements were done using a tensiometer, Cenco Model 70535, made by Central Scientific Co. For this study different mixtures of ternary mobile phase were interfaced with *n*-hexadecane (to simulate the stationary phase model) and allowed to equilibrate at 22°C for several hours. The container where the liquids were placed in was jacketed with circulating constant temperature water. It contained about 25 ml of the mixture and 25 ml of the alkane on top of the mixture (upper phase). The interfacial measurement was performed at the interface between the mixture and the paraffin using the ring method[10]. The surface tensions were measured using the same method in absence of the paraffinic phase.

The density of each phase (paraffin and mixture) was measured separately at 22°C utilizing the standard technique with a pycnometer.

The distribution coefficient of benzene between the two phases (paraffinic and mixture) was studied, using an ultraviolet spectrophotometer, Cary Model 2300 made by Varian. For each point, a solution of $1 \cdot 10^{-4}$ *M* benzene in that particular mixture was interfaced with equal volume of paraffin (50 ml) to equilibrate overnight. Then, each phase was studied spectroscopically, using absorbance measurements to determine the content of benzene in each phase. The molar absorptivity of each phase was determined in advance, by preparing and analyzing a series of known concentrations of the solute in each phase. The same measurements were done on a $1.5 \cdot 10^{-5}$ *M* solution of nitrobenzene and a $5 \cdot 10^{-5}$ *M* solution of benzonitrile.

All chemicals and reagents used for these experiments were high-performance liquid chromatography (HPLC) grade or best available commercially. Probe solutes were purchased in purified form from Chem Services (Westchester, PA, U.S.A.). *n*-Hexadecane was obtained from Aldrich Chemical Company, as of 99% pure.

THEORY

In a recent paper, Katz *et al.*[9] suggest that once water is mixed with an organic solvent, such as methanol, the mixture is no longer a binary system but a ternary mixture of "free" unassociated methanol, "free" unassociated water and a third component arising from the association of methanol and water. Such a complex presumably arises from the strong hydrogen bond between water and methanol. Katz *et al.*[9] have shown that this kind of association does take place in aqueous solutions of acetonitrile or tetrahydrofuran (THF) as well, but to a lesser extent.

The general form of such equilibria is

$$W + O \rightleftharpoons W \cdot O \qquad K = \frac{[W]\,[O]}{[W \cdot O]}$$

where "W" represents water and "O" is any organic solvent which could associate with water. "W · O" is the complex and the equilibrium constant is *K*. The equilibrium constant, *K*, can be determined by fitting the experimental data to a model which is based on the above equilibrium process. The experimental data are obtained by measuring the volume change in a series of binary mixtures of water and an organic modifier from 0 to 100%, by volume as shown by Katz *et al.*[9]

(A) Two-step equilibrium processes in binary mixtures of water and methanol

There is literature precedent for aqueous solution complexes of the W · O and $W \cdot O_2$ type [11,12]. Pursuing the reexamination of the data of Katz *et al.*[9] and using the same approach as theirs, we have been able to show that the experimental data could fit far better into a model with two consecutive equilibrium constants, the first one arising from the association of one methanol and one water and the second one from the association of one methanol and the newly formed complex:

$$M + W \overset{K_1}{\rightleftharpoons} M \cdot W \tag{1}$$

$$M + M \cdot W \overset{K_2}{\rightleftharpoons} M_2 \cdot W \tag{2}$$

The equilibrium constants for the above processes can be written as

$$K_1 = \frac{[\mathrm{M}][\mathrm{W}]}{[\mathrm{MW}]} \text{ or } [\mathrm{MW}] = \frac{[\mathrm{M}][\mathrm{W}]}{K_1} \tag{3}$$

$$K_2 = \frac{[\mathrm{M}][\mathrm{MW}]}{[\mathrm{M_2W}]} \text{ or } [\mathrm{M_2W}] = \frac{[\mathrm{M}]^2[\mathrm{W}]}{K_1K_2} \tag{4}$$

where [M] is the molar concentration of methanol, [W] is the molar concentration of water, [MW], [M_2W] are the molar concentrations of the first and second complexes, respectively. For water and methanol we can show

$$[\mathrm{M}] = \frac{\alpha_\mathrm{M}}{V_\mathrm{M}} - [\mathrm{MW}] - [\mathrm{M_2W}] \tag{5}$$

$$[\mathrm{W}] = \frac{(1 - \alpha_\mathrm{M})}{V_\mathrm{w}} - [\mathrm{MW}] \tag{6}$$

where α_m is the volume fraction of methanol and V_M and V_W are the molar volumes of methanol and water, respectively. Substituting for [MW] and [M_2W] from eqns. 3 and 4 in the above equations, we get:

$$[\mathrm{M}] = \frac{\alpha_\mathrm{M}}{V_\mathrm{M}} - \frac{[\mathrm{M}][\mathrm{W}]}{K_1} - \frac{[\mathrm{M}]^2[\mathrm{W}]}{K_1K_2} \tag{7}$$

$$[\mathrm{W}] = \frac{(1 - \alpha_\mathrm{M})}{V_\mathrm{W}} - \frac{[\mathrm{M}][\mathrm{W}]}{K_1} \tag{8}$$

Rearranging eqn. 8, one gets:

$$[\mathrm{W}] = \frac{(1 - \alpha_\mathrm{M})K_1}{(K_1 + [\mathrm{M}])V_\mathrm{W}} \tag{9}$$

Substituting [W] from eqn. 9 into eqn. 7 and rearranging it, one obtains a second order equation in terms of [M] as:

$$[\mathrm{M}]^2 + \frac{K_2\left(V_\mathrm{W}K_1 + 1 - \alpha_\mathrm{M} - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot \alpha_\mathrm{M}\right)}{(1 - \alpha_\mathrm{M} + V_\mathrm{W}K_2)} \cdot [\mathrm{M}] - \frac{V_\mathrm{W}K_2K_1\alpha_\mathrm{M}}{(1 - \alpha_\mathrm{M} + V_\mathrm{W}K_2)V_\mathrm{M}} = 0 \tag{10}$$

Eqn. 10 can be solved as:

$$[\mathrm{M}] = \frac{-b + \sqrt{b^2 + 4c}}{2} \tag{11}$$

where

$$b = \frac{K_2\left(V_W K_1 + 1 - \alpha_M - \frac{V_W}{V_M} \cdot \alpha_M\right)}{(1 - \alpha_M + V_W K_2)}$$

$$c = \frac{V_W K_1 K_2 \alpha_M}{(1 - \alpha_M + V_W K_2) V_M}$$

From eqns. 11 and 9 [W] can be determined as

$$[W] = \frac{(1 - \alpha_M) K_1}{(K_1 + [M]) V_W} \tag{12}$$

and finally from eqns. 5 and 6 the concentration of the two associated components can be given as

$$[MW] = \frac{1 - \alpha_M}{V_W} - [W] \tag{13}$$

and

$$[M_2W] = \frac{\alpha_M}{V_M} - [M] - [MW] \tag{14}$$

From eqns. 11–14 it follows that if the equilibrium constants, K_1 and K_2, are known, the molar concentration of water, [W], methanol, [M], and the complexes, [MW] and $[M_2W]$, can be calculated from the original volume fractions of methanol in the mixture.

From the equilibrium concentration of water, methanol and the complexes the individual volume fractions of these components can be determined from the initial volume fraction, α_M, of the mixture, by

$$v_M = [M] V_M \tag{15}$$

$$v_W = [W] V_W \tag{16}$$

$$v_{MW} = [MW] V_{MW} \tag{17}$$

$$v_{M_2W} = [M_2W] V_{M_2W} \tag{18}$$

where v_M, v_W, v_{MW} and v_{M_2W} are the equilibrium volume of methanol, water and complexes 1 and 2, respectively.

It follows that if the volume change in mixing is measured experimentally, the results can be used to determine K_1, K_2, V_{MW} and V_{M_2W}. This procedure was carried out using eqns. 15–18 and the SIMPLEX algorithm with the aid of a computer. The

TABLE I

EQUILIBRIUM CONSTANTS AND THE MOLAR VOLUME OF THE CORRESPONDING COMPLEXES IN THE BINARY SOLVENT SYSTEMS OF WATER–METHANOL, WATER–ACETONITRILE AND METHANOL–ACETONITRILE

The molar volumes are in ml/mol. The x and y represent the two different solvents.

Mixture	K_1*	K_2*	V_{xy}	V_{x2y}
Water–methanol	$1.944 \cdot 10^{-3}$	1.000	55.38	100.45
Water–acetonitrile	0.25652	–	50.01	–
Methanol–acetonitrile	0.98220	–	63.06	–

* It should be noted that the smaller the K value the stronger the association of the two species would be since, indeed, it represents a dissociation process between the two interacting solvents (see eqn. 1).

particular values for K_1, K_2, V_{MW} and V_{M_2W} are identified as those which provide the minimum error between the calculated volume of any given solvent mixture ($V_M + V_W + V_{MW} + V_{M_2W}$) and that measured experimentally. The final results can be seen in Table I.

(B) Mathematical model for the ternary solvent systems

Since the focus of our study was on ternary solvent systems, it seemed quite appropriate to employ the association model and its characteristics to the ternary system of water, methanol and acetonitrile. In such a treatment one has to take into account the potential association of all components even that of methanol–acetonitrile. This was required, since we were able to show that methanol and acetonitrile do associate as well (see Table I), when the treatment of Katz *et al.*[9] was employed. The degree of association for this pair is, of course, reduced compared to their corresponding aqueous solutions, apparently due to the reduced capabilities for hydrogen bonding.

The moment the three solvents (water–methanol–acetonitrile) are mixed one expects a simultaneous association of different species to take place between different pairs. It would be rather difficult mathematically to evaluate the concentration of each of the components simultaneously. Accordingly, to simplify the derivations, we assume the following order in the association of the solvent pairs:

water–methanol, water–acetonitrile and acetonitrile–methanol

The above order is based on the magnitude of the equilibrium constant value for each solvent pair, found in Table I.

In the following pages, the details of the derivations for a ternary solvent system of water–methanol–acetonitrile are given. According to our assumptions, water and methanol are the first pair to associate through two consecutive associaton processes. The derivations in this part (part 1) are similar to those in section ‘A’, except for the volume fractions of the components, where that of the acetonitrile has to be taken into account appropriately (see eqn. 22, for [W]). Accordingly, from eqns. 25 and 26 those portions of water and methanol which have remained unassociated (free) can be evaluated, respectively. These are the components which would participate in the further interactions with acetonitrile. Association of water and acetonitrile is second in line. Therefore, the free portion of water, W_a, would react with the existing acetonitrile (A) to form complex (A · W) with an association constant of K^A (see eqn. 27). The rest of the derivation can easily be followed through eqn. 35. Once again, the remainder of water which has stayed unassociated and also the free portion of acetonitrile can be evaluated by eqns. 33 and 35, respectively. This portion of acetonitrile (A_1) would react with the remainder of the free methanol (M_1) to form the final and last complex (A · M) with an association constant of K^{AM} (see eqn. 39). Following the derivations through eqn. 48, the unassociated portions of methanol (M_1) and acetonitrile (A_1) can be evaluated from eqns. 46 and 48, respectively.

To use this model one needs to evaluate the first and second association (equilibrium) constants, K_1^M and K_2^M, in the binary system of water–methanol, as outlined in section A. In addition, the association constants for binary mixtures of water–acetonitrile and acetonitrile–methanol, K^A and K^{AM}, have to be determined, following the approach of Katz *et al.*[9]. Consequently, the need for long and tedious experiments to measure the volume changes in a ternary system which requires very complicated mathematical modeling to evaluate the concentration of each of the existing and the newly formed components is avoided. Hence, this model can be easily used since all the necessary equations are in terms of the original composition of the three solvents.

Derivations on ternary solvent system

(1) Water, methanol association.

$$M + W \rightleftharpoons M \cdot W \qquad K_1^M = \frac{[M][W]}{[MW]} \tag{19}$$

$$M + M \cdot W \rightleftharpoons M_2 \cdot W \qquad K_2^M = \frac{[M][MW]}{[M_2W]} \tag{20}$$

K_1^M and K_2^M are the first and the second association constants between water and methanol, respectively. One can show that

$$[M] = \frac{\alpha_M}{V_M} - [MW] - [M_2W] \tag{21}$$

and

$$[W] = \frac{1 - \alpha_M - \alpha_A}{V_W} - [MW] \tag{22}$$

Using eqns. 19 and 20 in eqns. 21 and 22, we get:

$$[M] = \frac{\alpha_M}{V_M} - \frac{[M][W]}{K_1^M} - \frac{[M]^2[W]}{K_1^M K_2^M} \tag{23}$$

$$[\mathrm{W}] = \frac{1 - \alpha_\mathrm{M} - \alpha_\mathrm{A}}{V_\mathrm{W}} - \frac{[\mathrm{M}][\mathrm{W}]}{K_1^\mathrm{M}} \tag{24}$$

Rearranging eqn. 24, one gets:

$$[\mathrm{W}] = \frac{(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})K_1^\mathrm{M}}{(K_1^\mathrm{M} + [\mathrm{M}])V_\mathrm{W}} \tag{25}$$

Substituting eqn. 25 in eqn. 23, gives

$$[\mathrm{M}] + \frac{[\mathrm{M}]}{K_1^\mathrm{M}} \cdot \frac{(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})K_1^\mathrm{M}}{(K_1^\mathrm{M} + [\mathrm{M}])V_\mathrm{W}} + \frac{[\mathrm{M}]^2}{K_2^\mathrm{M} K_1^\mathrm{M}} \cdot \frac{(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})}{(K_1^\mathrm{M} + [\mathrm{M}])V_\mathrm{W}} - \frac{\alpha_\mathrm{M}}{V_\mathrm{M}} = 0$$

or

$$\frac{\begin{array}{l}[V_\mathrm{M} V_\mathrm{W} K_2^\mathrm{M} (K_1^\mathrm{M} + [\mathrm{M}])[\mathrm{M}] + K_2^\mathrm{M} V_\mathrm{M}(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})[\mathrm{M}] + \\ \qquad + V_\mathrm{M}(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})[\mathrm{M}]^2 - V_\mathrm{W} K_2(K_1 + [\mathrm{M}])\alpha_\mathrm{M}]\end{array}}{V_\mathrm{M} V_\mathrm{W} K_2^\mathrm{M}(K_1^\mathrm{M} + [\mathrm{M}])} = 0$$

or

$$\begin{aligned} &V_\mathrm{W} K_1^\mathrm{M} K_2^\mathrm{M}[\mathrm{M}] + V_\mathrm{W} K_2^\mathrm{M}[\mathrm{M}]^2 + K_2^\mathrm{M}(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})[\mathrm{M}] + \\ &\qquad + (1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})[\mathrm{M}]^2 - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot K_2[\mathrm{M}]\alpha_\mathrm{M} - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot K_2^\mathrm{M} K_1^\mathrm{M} \alpha_\mathrm{M} = 0 \end{aligned}$$

or

$$\begin{aligned} &(V_\mathrm{W} K_2^\mathrm{M} + 1 - \alpha_\mathrm{M} - \alpha_\mathrm{A})[\mathrm{M}]^2 + (V_\mathrm{W} K_1^\mathrm{M} K_2^\mathrm{M} + K_2^\mathrm{M} - K_2^\mathrm{M}\alpha_\mathrm{M} - \\ &\qquad K_2^\mathrm{M}\alpha_\mathrm{A} - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot \mathrm{K}_2\alpha_\mathrm{M})[\mathrm{M}] - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot K_2^\mathrm{M} K_1^\mathrm{M} \alpha_\mathrm{M} = 0 \end{aligned}$$

Rearranging the above equation, a second order equation results in term of [M] as

$$\begin{aligned} &[\mathrm{M}]^2 + \frac{K_2^\mathrm{M}(V_\mathrm{W} K_1^\mathrm{M} + 1 - \alpha_\mathrm{M} - \alpha_\mathrm{A} - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot \alpha_\mathrm{M})[\mathrm{M}]}{(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A} + V_\mathrm{W} K_2^\mathrm{M})} - \\ &\qquad \frac{V_\mathrm{W} K_2^\mathrm{M} K_1^\mathrm{M} \alpha_\mathrm{M}}{(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A} + V_\mathrm{W} K_2^\mathrm{M}) V_\mathrm{M}} = 0;\ [\mathrm{M}] = \frac{-\mathrm{b} + \sqrt{b^2 + 4c}}{2} \end{aligned} \tag{26}$$

where

$$b = \frac{K_2^\mathrm{M}(V_\mathrm{W} K_1^\mathrm{M} + 1 - \alpha_\mathrm{M} - \alpha_\mathrm{A} - \frac{V_\mathrm{W}}{V_\mathrm{M}} \cdot \alpha_\mathrm{M})[\mathrm{M}]}{(1 - \alpha_\mathrm{M} - \alpha_\mathrm{A} + V_\mathrm{W} K_2^\mathrm{M})}$$

and

$$c = -\frac{V_W K_2^M K_1^M \alpha_M}{(1 - \alpha_M - \alpha_A + V_W K_2^M) V_M}$$

(2) Water, acetonitrile association

$$A + W_A \rightleftharpoons A \cdot W \qquad K^A = \frac{[A][W_A]}{[AW]} \tag{27}$$

K^A is the association constant resulting from the association of acetonitrile and water. Once again, one can show that

$$[A] = \frac{\alpha_A}{V_A} - [AW] \tag{28}$$

and

$$[W_A] = [W] - [AW] \tag{29}$$

Substituting [AW] from eqn. 27 into eqn. 28 and rearranging it results in:

$$[A] = \alpha_A/(1 + [W_A]/K^A)V_A \tag{30}$$

Substituting [AW] from eqn. 27 into eqn. 29, gives

$$[W_A] + \frac{[A][W_A]}{K^A} = [W] \tag{31}$$

and substituting [A] from eqn. 30 into eqn. 31 and rearranging the terms, we would get a second-order equation in terms of $[W_A]$, as

$$[W_A]^2 + (K^A + \frac{\alpha'_A}{V_A} - [W])[W_A] - K^A[W] = 0 \tag{32}$$

(see Supplement at the end of this section) where $[W_A]$ can be evaluated by

$$[W_A] = \frac{-\mathrm{b} + \sqrt{b^2 + 4c}}{2} \tag{33}$$

where

$$b = K^A + \frac{\alpha'_A}{V_A} - [W]$$

$$c = -K^A[W]$$

We can also show that:

$$[\mathrm{AW}] = [\mathrm{W}] - [\mathrm{W_A}] \tag{34}$$

$$[\mathrm{A}] = \frac{\alpha'_{\mathrm{A}}}{V_{\mathrm{A}}} - [\mathrm{AW}] \tag{35}$$

It should be noted that in this part of the derivations, α'_{A} has been used instead of α_{A}, the reason being that the volume fraction of acetonitrile no longer has the same value as the starting value as the original mixture and this is due to the fact that two new components have been added into the mixture arising from the association of water and methanol, as shown in the first part. The value of α'_{A} can be given as:

$$\alpha'_{\mathrm{A}} = \frac{\alpha_{\mathrm{A}}/V_{\mathrm{A}}}{[\mathrm{M}] + [\mathrm{W}] + [\mathrm{MW}] + [\mathrm{M_2W}]}$$

(2a) Supplement

From eqns. 30 and 29 we have

$$[\mathrm{W_A}] + \frac{\alpha_{\mathrm{A}}}{(1 + [\mathrm{W_A}]/K^{\mathrm{A}})V_{\mathrm{A}}} \cdot \frac{[\mathrm{W_A}]}{K^{\mathrm{A}}} = [\mathrm{W}] \tag{36}$$

or

$$[\mathrm{W_A}] + \frac{\alpha_{\mathrm{A}}[\mathrm{W_A}]}{(K^{\mathrm{A}} + [\mathrm{W_A}])V_{\mathrm{A}}} = [\mathrm{W}] \tag{37}$$

$$[\mathrm{W_A}](K^{\mathrm{A}} + [\mathrm{W_A}])V_{\mathrm{A}} + \alpha_{\mathrm{A}}[\mathrm{W_A}] - [\mathrm{W}](K^{\mathrm{A}} + [\mathrm{W_A}])V_{\mathrm{A}} = 0$$

$$[\mathrm{W_A}]K^{\mathrm{A}}V_{\mathrm{A}} + [\mathrm{W_A}]^2V_{\mathrm{A}} + \alpha_{\mathrm{A}}[\mathrm{W_A}] - [\mathrm{W}]K^{\mathrm{A}}V_{\mathrm{A}} - [\mathrm{W}][\mathrm{W_A}]V_{\mathrm{A}} = 0$$

$$[\mathrm{W_A}]^2V_{\mathrm{A}} + (K^{\mathrm{A}}V_{\mathrm{A}} + \alpha_{\mathrm{A}} - [\mathrm{W}]V_{\mathrm{A}})[\mathrm{W_A}] - [\mathrm{W}]K^{\mathrm{A}}V_{\mathrm{A}} = 0$$

or

$$[\mathrm{W_A}]^2 + (K^{\mathrm{A}} + \frac{\alpha_{\mathrm{A}}}{V_{\mathrm{A}}}[\mathrm{W}])[\mathrm{W_A}] - [\mathrm{W}]K^{\mathrm{A}} = 0 \tag{38}$$

$$b_1 = K^{\mathrm{A}} + \frac{\alpha_{\mathrm{A}}}{V_{\mathrm{A}}} - [\mathrm{W}]$$

$$c_1 = -[\mathrm{W}]K^{\mathrm{A}}$$

(3) Acetonitrile, methanol association

$$A + M \rightleftharpoons A \cdot M \qquad K^{AM} = \frac{[A_1]\,[M_1]}{[AM]} \tag{39}$$

K^{AM} is the association constant resulting from methanol and acetonitrile association. $[A_1]$ and $[M_1]$ are the amounts of acetonitrile and methanol not being associated, respectively:

$$[A_1] = [A] - [AM] \tag{40}$$

$$[M_1] = [M] - [AM] \tag{41}$$

Substituting the [AM] value from eqn. 39 into eqn. 40, we get

$$[A_1] + [M_1][A_1]/K^{AM} = [A] \tag{42}$$

or

$$[A_1] = [A]/(1 + [M_1]/K^{AM}) \tag{43}$$

Substituting the [AM] value from eqn. 39 into eqn. 41 and rearranging it, one gets:

$$[M_1] + \frac{[A_1][M_1]}{K^{AM}} = [M] \tag{44}$$

Substituting the $[A_1]$ value from eqn. 42 into eqn. 44 and rearranging it, results in

$$[M_1]^2 + (K^{AM} + [A] - [M])[M_1] - [M]K^{AM} = 0 \tag{45}$$

which is a second-order equation in terms of $[M_1]$, and can be solved as

$$[M_1] = \frac{-b + \sqrt{b^2 + 4c}}{2} \tag{46}$$

where

$$b = K^{AM} + [A] - [M]$$

$$c = -[M]K^{AM}$$

Combining eqns. 40, 41 and 46, one gets:

$$[AM] = [M] - [M_1] \tag{47}$$

$$[A_1] = [A] - [AM] \tag{48}$$

RESULTS

Utilizing the procedure outlined in section A for a binary solvent system of water—methanol and the experimental data on the volume change upon mixing from ref. 9, the first and second equilibrium constants, K_1^M,K_2^M, and the molar volume of the two complexes ($M \cdot W$ and $M_2 \cdot W$) were determined. The results are given in Table I. As can be seen from these values $K_1^M < K_2^M$, as expected, *i.e.*, that the first association is much stronger than the second, but the introduction of a second equilibrium constant in the model indicates an improvement in the prediction of the volume changes when compared to the experimental values. The experimental data on binary mixtures of acetonitrtile–water were also taken from Scott's paper employing our SIMPLEX driven, non-linear least squares program, the equilibrium constant, K^A, and the molar volume of the complex ($A \cdot W$) were determined (see Table I). No data are available on volume change on mixing of methanol–acetonitrile, but there are density data on such mixtures in the literature[13] which can be used to calculate the volume change for such mixtures. Using those data, Scott's approach and our SIMPLEX program, the equilibrium constant for methanol–acetonitrile system, K^{AM}, and the molar volume of the corresponding complex ($M \cdot A$) were determined, as shown in Table I. Accordingly, having all the equilibrium constants evaluated, a computer program was written based on the ternary solvent system model (section B) to determine the final concentration of the three solvents (unassociated) in a ternary mixture of water–methanol–acetonitrile at any desired starting composition.

The capacity factor *vs.* volume fraction of acetonitrile or THF organic modifiers for three different solutes have been plotted in Fig. 1a and b, respectively. In

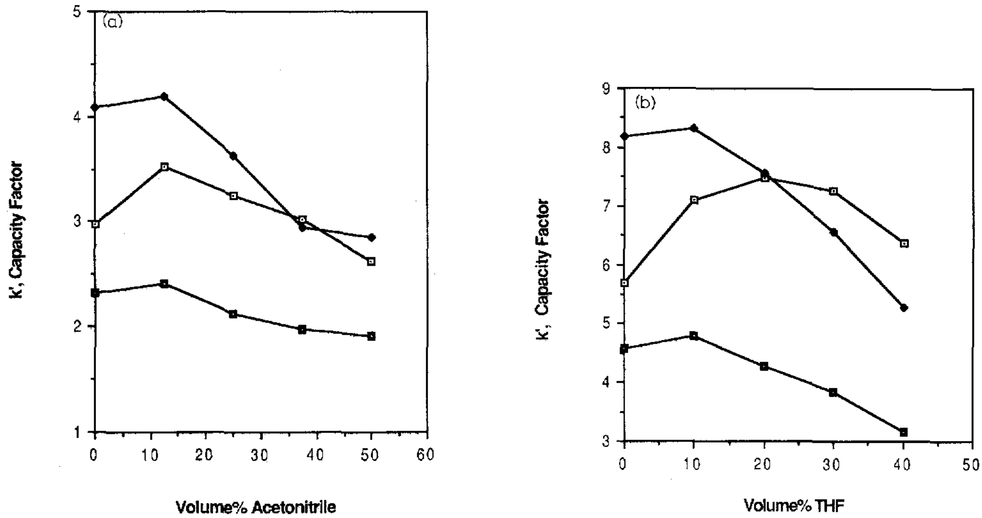

Fig. 1. The capacity factor behavior of three different solutes in ternary mixtures of water, methanol, acetonitrile and water, methanol and THF as a function of % (v/v) acetonitrile (a) or THF (b), respectively. Solutes: *p*-fluoronitrobenzene (◆); *p*-dinitrobenzene (⊡); *p*-nitrobenzaldehyde (□). For more examples on the above physical properties, contact the principal investigator directly.

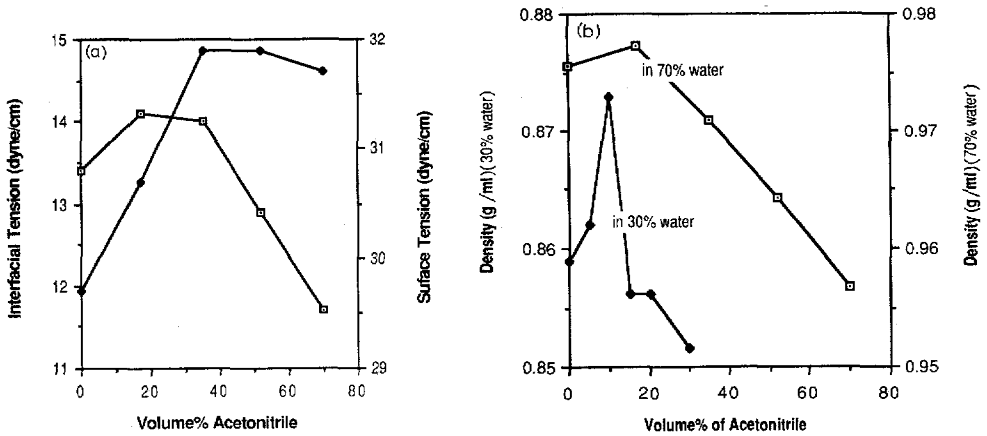

Fig. 2. (a) The surface (◆) and interfacial tension (☐) behavior of a 30% fixed water and 70% methanol and acetonitrile mixture, as a function of acetonitrile % (v/v) change. (b) The density behavior at two different mixtures of fixed 30% and 70% water (the rest methanol and acetonitrile mixture) as a function of acetonitrile % (v/v) change. For more examples on the above physical properties, contact the principal investigator directly.

Fig. 2a the surface tension and interfacial tension for a mixture containing 30% (v/v) water have been plotted against volume percent of acetonitrile replacing 70% methanol. In Fig. 2b the density for two mixtures of 30 and 70% water have been plotted. Fig. 3 represents the behavior of the distribution coefficients for three different solutes in a ternary solvent system of 30% water and 70% methanol and acetonitrile combined. In Fig. 4a–c the concentration of free (unassociated) water, acetonitrile and methanol at three different starting water compositions (30, 40 and 70%) are plotted against their corresponding acetonitrile volume fraction in the ternary mixtures, respectively.

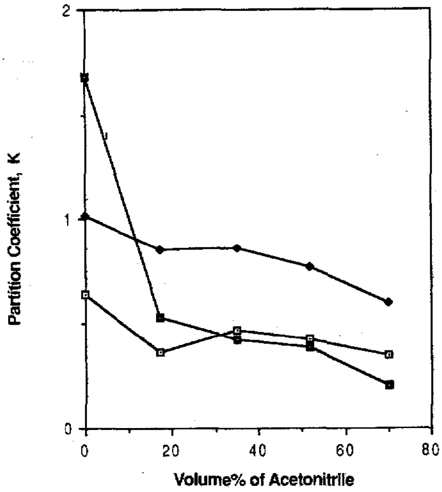

Fig. 3. The partition coefficient behavior of three different solutes between a 30% fixed water, 70% methanol and acetonitrile and a *n*-hexadecane paraffinic phase, as a function of acetonitrile % (v/v) change. Solutes: benzene (☐); benzonitrile (◆); nitrobenzene (☐).

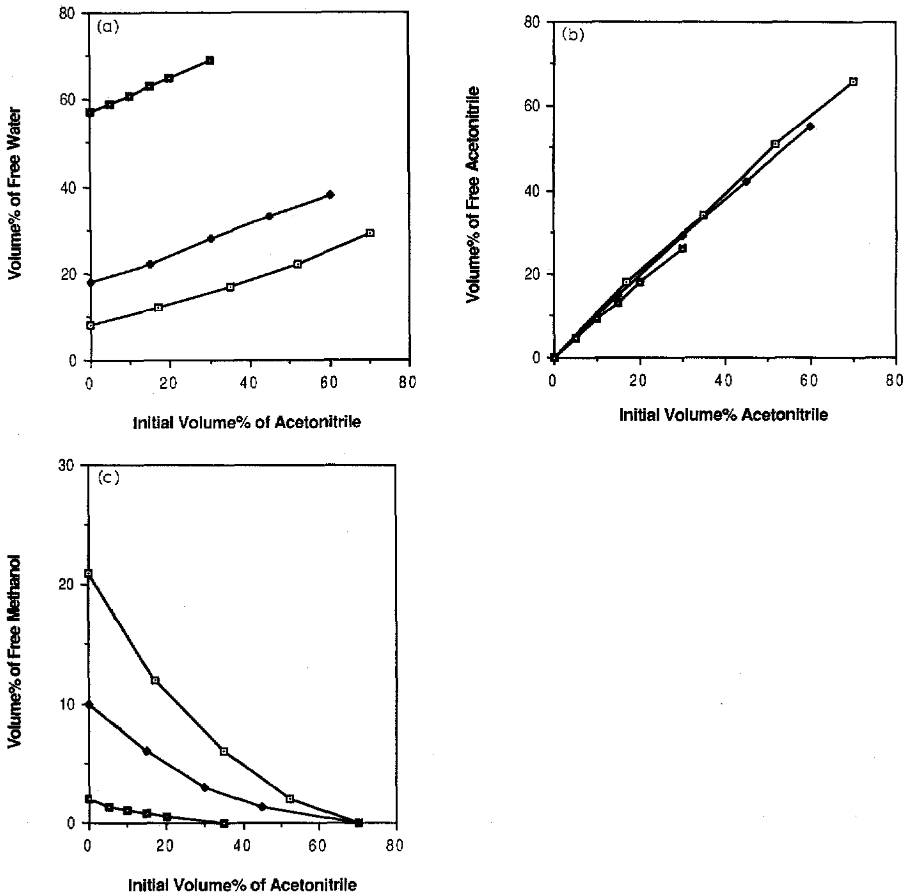

Fig. 4. The volume % of "free" (unassociated) water (a), acetonitrile (b) and methanol (c) as a function of experimental acetonitrile % (v/v) in three different ternary mixtures of the above solvents, respectively. Water contents: 30 (□); 40 (◆); 70% (□).

DISCUSSION

In a ternary solvent system when going from a high polar to a much less polar solvent mixture one anticipates a relatively smooth and monotonic decrease in the retention time in RPLC. Contrary to this expectation, a very irregular trend appears in the behavior of the capacity factor, when at a fixed value of water content methanol is being replaced by a much less polar organic modifier such as acetonitrile or THF, as can be seen in Fig. 1a and b. In the search for an answer to what causes such an anomaly in retention behavior, one needs to know more about the nature of the eluent, specifically, and the structure of the bonded phase which, indeed, is directly affected by the mobile phase properties.

In RPLC, surface tension of the mobile phase, γ_s, and the interfacial tension, γ, between the mobile phase and the bonded phase are considered to be important factors in interactions of a solute in the column[14,15]. Accordingly, these two parameters were measured in a few ternary solvent mixtures where unusual results were observed in these cases also (see Fig. 2a). At a constant water composition, as methanol is replaced by acetonitrile the surface and the interfacial tensions tend to go to a maxima then start dropping continuously. In determination of the interfacial tension values, one needs to know the density of each participating phase; the mixture and the paraffin. Once these values were measured, it seemed appropriate to look at the behavior of the density as a function of solvent composition. It was astonishing to see that the density follows more or less the same trend as the surface or interfacial tension. Again, as the amount of acetonitrile is increased in the ternary mixture, the density reaches a maxima and then tends to descend continuously, as seen in Fig. 2b. This trend seems very peculiar, since at a constant concentration of water with the highest density (0.9982 g/ml at 20°C), as acetonitrile with the lowest density (0.7857 g/ml at 20°C) is replacing methanol with a density between water and acetonitrile, one simply expects a continuous decrease in the density of the mixture.

As can be seen in Fig. 4a–c, in a ternary solvent system, at a constant water composition, as the methanol is replaced by acetonitrile the amount of free acetonitrile increases gradually, as expected (Fig. 4b), and free methanol content decreases very rapidly, indeed, much faster than appearance of added acetonitrile (Fig. 4c). The result is an unexpected increase in the concentration of free water as acetonitrile is added, and almost at the same rate as acetonitrile is introduced into the mixture. This behavior can be explained by the strength of the association between each pair. As discussed before (see Table I), water and methanol show the highest degree of association compared to water–acetonitrile and methanol–acetonitrile pairs. Naturally, as methanol is being replaced by acetonitrile, the acetonitrile concentration would increase rapidly since it has a very weak association capability with either water or methanol. Methanol tends to disappear at a much faster rate than the appearance of acetonitrile since first, it is being replaced, and second because it has a strong tendency to associate with water. Hence, a fair amount of methanol would be used up to associate with water, and as a result not much free methanol can be expected to remain in the mixture. In the case of water, even though its starting concentration is kept constant, its free concentration tends to increase almost at the same rate as the disappearance of methanol. This is the case, since the water–acetonitrile pair do not associate very strongly (see Table I), on one hand, and no matter how much acetonitrile is added a very small fraction of water would associate with acetonitrile and the rest remains free or complexed with the methanol in the mixture. On the other hand, since methanol is being replaced by acetonitrile, continously lesser and lesser methanol is going to be available to associate with water and consequently more free water is going to be released into the mixture (see Fig. 4a).

Considering the above findings, the content in terms of free water seems very important, since one can correlate the behavior of the surface tension, interfacial tension, density, partition coefficient and the capacity factor to it in various mixtures. The density, surface tension and interfacial tension tend to increase at first, when acetonitrile starts replacing methanol in the mixture of water–methanol (while the starting composition of water is kept fixed). This replacement results in the release of some water due to the existence of a lesser amount of methanol to associate with water, on one hand and a much lower degree of association of water with acetonitrile, on the other. The result would be the addition of more free water into the solution.

Water with the highest value in density, surface and interfacial tensions, compared to methanol and acetonitrile, would force these quantities to rise, as seen in Fig. 2a and b. As the replacement of methanol with acetonitrile continues, the amount of free acetonitrile with a lower value in density, surface and interfacial tensions, compared to water, increases high enough to overcome the rate of release of water in the mixture. This would make acetonitrile to become the dominant component in determining the physical properties of the mixture and the descending path of the above quantities would be a direct result of this change, as seen in Fig. 2a and b). The same argument applies in the cases of partition coefficient and capacity factor (since they behave relatively similar to density and surface tension, see Fig. 1a and b and 3). One observes a lag or even an increase in the capacity factor (retention time) and partition coefficient, depending on the solute, at the beginning when acetonitrile starts to replace methanol (in a fixed starting composition for water) in the mixture of water–methanol. This, again, seems to be the case because more free water gets released into the mixture. Introduction of more water into the mixture tends to reduce or even cancel the effect of added acetonitrile, which is a better organic solvent, and consequently resulting in a lag or even an increase in the retention time (capacity factor) and partition coefficient. As this process continues the acetonitrile concentration increases enough to overcome the undesired effect of the released free water and that is when the retention time starts to descend.

It appears then that the anomalous behaviour of solute retention found experimentally can be explained by a judicious extension of the approach by Katz *et al.*[9] to the competitive equilibria occurring in ternary mixtures. The measurement of other physical parameters such as density and surface tension supports the model.

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