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Deuterium nuclear magnetic resonance spectroscopy as a probe for reversed-phase liquid chromatographic bonded phase solvation: methanol and acetonitrile mobile phase components

David M. Bliesner* and Karen B. Sentell

Department of Chemistry, University of Vermont, Burlington, VT 05405-0125 (USA)

ABSTRACT

Using the inversion-recovery method, deuterium longitudinal relaxation times, (T_1), of [$^2H_4$]methanol and [$^2H_3$]acetonitrile in enriched methanol–water and acetonitrile–water mobile phases were measured for the neat binary mixtures, and for the mixtures in contact with two different monomeric C_{18} reversed-phase stationary phases. Changes in the deuterium relaxation times for the organic modifiers in contact with the stationary phase compared to those observed in neat solution give a qualitative understanding of the degree of association of the organic components with the stationary phase. Both bulk solution microstructure and stationary phase C_{18} bonding density play important roles in determining bonded phase solvation.

INTRODUCTION

Reversed-phase liquid chromatography (RPLC) is one of the most widely used methods for separating complex mixtures of compounds with widely varying polarities [1]. However, despite its widespread use, methods development for RPLC separations is often complex and time consuming. This is a manifestation of the overall dearth of understanding about solute retention at the molecular level. Separations in RPLC are thermodynamic processes involving the transfer of solute between the mobile and stationary phases. Originally these transfer processes were modeled in terms of liquid–liquid partitioning processes, such as found in octanol–water systems [2,3]. However, in RPLC this can not be entirely the case, since there is no distinct interfacial boundary between the stationary and mobile phases. The lack of a well defined boundary between the stationary and mobile phases in RPLC is due to the formation of a solvation layer at the surface of the stationary phase, which results from mobile phase interactions with the grafted alkyl chains as well as the silica support. Preferential sorption of mobile phase components by the stationary phase results in a layer whose thickness and composition varies with the distance from the bonded ends of the alkyl chains. The stationary phase alkyl chains are not isotropic; they exhibit ordering due to their covalent attachment to the rigid silica substrate surface [2–4]. Chain ordering also increases with increasing alkyl chain surface coverage [2–4]. Due to varying degrees of chain interaction, mobile phase components will penetrate into the stationary phase in a non-uniform manner [5]. The resulting structure is not a distinct interface but an *interphase* which exhibits an order gradient that varies along the bonded alkyl chain as a function of distance from the silica surface [2–4].

The overall structure of the RPLC solvation layer is a function of several variables. These include: (i)

Correspondence to: K. B. Sentell, Department of Chemistry, University of Vermont, Burlington, VT 05405-0125, USA.

* Present address: ICI Americas, Pharmaceutical Group, NLW2, Wilmington, DE 19897, USA.

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the composition of the bulk mobile phase; (ii) the length of the bonded alkyl chain; (iii) the degree of derivatization of the silica surface or bonding density, which is often expressed in units of micromoles of bonded alkyl ligand per square meter of bonded phase surface (μmol/m^2); (iv) the temperature of the system; and (v) the numbers and types of residual silanols remaining on the silica surface following derivatization. In order to obtain a better understanding of the molecular interactions underlying solute retention in RPLC, it is necessary to develop more accurate models of the composition and structure of the solvation layer as a function of all of these variables [5–9].

In the past, researchers have used various methods to probe the composition of the solvation layer. Some of these have included the chromatographic measurement of adsorption isotherms [6,9–13], gas chromatographic (GC) methods [6–8], optical spectroscopy [14–23], and nuclear magnetic resonance spectroscopy (NMR) [24–40]. NMR offers some interesting possibilities in terms of understanding stationary phase–mobile phase interactions in RPLC systems. The technique permits approaching the problem from the perspective of the stationary phase *or* the mobile phase. It also allows study of these systems under chromatographic conditions with minimal perturbation.

The present paper is a discussion of some of our solution state NMR studies of RPLC stationary phase–mobile phase interactions. This approach is not unique in that several researchers have undertaken such experiments in the past and obtained interesting results [24–40]. However, although many of these studies demonstrated the general validity of the technique, they did so using uncommon RPLC solvents [24–28,32,33,38,40] or they encompassed only a small range of mobile phase compositions [26,28–30,32–37,40]. It was our goal to expand on some of these initial studies in order to obtain a more comprehensive understanding of RPLC stationary phase solvation by commonly used organic modifiers using solution state NMR techniques. This was accomplished by measuring deuterium longitudinal relaxation times, T_1, of deuterated mobile phase components in contact with the stationary phases of interest.

The utility of deuterium longitudinal relaxation time experiments comes about because deuterium is a quadrupolar nucleus with spin quantum number $I = 1$. Therefore its solution state longitudinal relaxation time, T_1, is described by the following equation:

$$\frac{1}{T_1} = 3\pi^2 \frac{2I + 3}{I^2(2I-1)} \chi^2 \tau_c \quad (1)$$

Since I and the quadrupolar coupling constant, χ, are constants for a particular nucleus [41], the inverse of the relaxation time (or the rate of relaxation) is directly proportional to τ_c. The molecular correlation time, τ_c, is given in units of radians per second and is a measure of how long it takes a molecule to rotate through one radian. It is therefore a measure of molecular motion. From eqn. 1 it is evident that as the correlation time increases (*e.g.* molecular motion decreases) the longitudinal relaxation time, T_1, decreases. Specifically, greater molecular association or restriction causes a decrease in T_1.

Our current study is based upon work first conducted by Marshall and McKenna [29] in which they measured the solution state longitudinal relaxation times (T_1) of deuterium in ^2H_2O–acetonitrile mixtures over a composition range of 0% to 50% ^2H_2O as a function of their volume-to-volume (v/v) ratios. These measurements of T_1 *versus* percent ^2H_2O in the mobile phase mixtures were made for the neat binary solutions as well as for samples of the mobile phases combined with various chromatographic supports. By comparing the T_1 values in the neat solutions to those for the same solutions in contact with stationary phases, Marshall and McKenna qualitatively gauged the degree of water association with the stationary phase. Their results are interesting in at least two respects. First, they showed that for the samples they studied, the relative amount of water associated with the stationary phase in acetonitrile–water systems is a function of the amount of water in the bulk mobile phase. Secondly, they did not perform measurements at acetonitrile concentrations less than 50%, since uniform wetting of the stationary phase was not possible at the latter solution compositions when the stationary and mobile phase mixtures were combined at atmospheric pressure. In addition to observing ^2H_2O relaxation times, they also measured the T_1 for [$^2H_3$]acetonitrile under a few of their experimen-

tal conditions. Despite these interesting initial results, to our knowledge no further work of the same sort was undertaken, although Ellison and Marshall [30] have made deuterium and nitrogen-14 longitudinal relaxation time measurements to determine the surface fluidity in RPLC systems.

Because of the demonstrated potential of these T_1 experiments we have expanded on Marshall and McKenna's work. For the mobile phases used in our studies we have been able to encompass the entire binary composition range by overcoming the problem of wetting the stationary phase samples with highly aqueous mobile phases. Since our sample preparation method equilibrates the stationary and mobile phases at pressures comparable to those attained under chromatographic operating conditions, it also much more nearly approximates wetting under real chromatographic conditions. We have also performed our ^2H NMR work using [$^2H_4$]methanol and [$^2H_3$]acetonitrile *in addition to* ^2H_2O. The results obtained with [$^2H_4$]methanol and [$^2H_3$]acetonitrile are reported in this communication; those for ^2H_2O are discussed in a second paper [42]. Finally, we have obtained this information for two well-characterized monomeric C_{18} modified stationary phases with greatly different bonding densities in order to investigate the effect of stationary phase alkyl chain interactions upon solvation layer formation.

EXPERIMENTAL

NMR measurements

T_1 values for the methyl deuterons of [$^2H_4$]methanol–water and [$^2H_3$]acetonitrile–water mobile phase mixtures, both neat and in contact with stationary phase, were measured on a Brüker WM-250 NMR spectrometer operating at a field strength of 5.875 Tesla and a frequency of 38.4 MHz for ^2H. The standard inversion recovery pulse sequence was used (180°–τ–90°–Acquire-Delay)$_n$ where τ ranged from 0.025 to 24 s. The sequence employed no less than ten τ values and each acquisition necessitated from 2 to 32 scans. The relaxation delay was in excess of 5 T_1 to allow the system to return to equilibrium between measurements. Total acquisition times ranged from 10 to 50 min. Care was taken to minimize reflected power for each sample in order to minimize errors in the 180° and 90° pulse angles. Spectra were acquired using automatic field frequency lock. Temperature was kept constant at 30°C (303 K).

T_1 values were calculated by manual measurement of peak height for each τ value followed by a three-parameter least squares exponential fit of the peak height *versus* τ plot. Relative error for replicate measurements was in all cases less than 5%. All T_1 values are stated at confidence level of plus or minus one standard deviation.

Chemicals

[$^2H_4$]Methanol and [$^2H_3$]acetonitrile (Isotec, Miamisburg, OH, USA) were used without further purification. HPLC-grade water was obtained in house using a Nanopure (Sybron, Boston, MA, USA) water purification system. Two silica-based stationary phase materials were used in this work. The first was a monomeric, non-endcapped C_{18} phase of very high alkyl chain bonding density (4.4 μmol/m^2) which was synthesized in our laboratory under conditions which have been previously described [43]. This 10-μm particle diameter stationary phase with a pore diameter of 85 Å is designated herein as LT1. The second stationary phase was a partically endcapped Spherisorb S5-ODS-1 (Phase Separations, Norwalk, CT, USA) monomeric C_{18} phase with a low bonding density (1.5 μmol/m^2). This 5-μm particle diameter stationary phase with a pore diameter of 50 Å is designated herein as ODS-1.

Sample preparation

Approximately 3% (by volume) [$^2H_4$]methanol or [$^2H_3$]acetonitrile was added to its non-labelled analogue in a chromatographic mobile phase reservoir. These solvents and a separate reservoir of HPLC-grade water were degassed by sonication in an ultrasonic bath under vacuum. The desired mobile phase mixture (v/v % ratio of organic co-solvent–water) was delivered into a 10-mm NMR tube by two Waters (Waters, Milford, MA, USA) Model 510 chromatographic pumps controlled by a Waters Model 680 automated gradient controller. Samples were prepared in 10% or smaller volume increments ranging from 100:0 (v/v) to 10:90 (v/v) for [$^2H_4$]methanol–water and [$^2H_3$]acetonitrile–water. ^2H T_1 values for the methyl deuterons of the labeled organic components were then measured for each neat mobile phase sample.

To insure proper wetting of the stationary phase with the labelled mobile phase solutions in the second half of the experiments, mobile–stationary phase mixtures were prepared in the following manner. First, the chromatographic stationary phase was dried under vacuum in excess of 12 h at 110°C to ensure that all physisorbed solvents were removed. The dry stationary phase was hand-packed into an empty 10 cm × 4.6 mm stainless-steel chromatographic column. Mobile phase of the desired volume percent ratio was then pumped through the column at a flow-rate which would generate at least 1500 p.s.i. (10.3 MPa) of backpressure. Approximately 50 ml of solvent was pumped through the column in one direction, at which point the column was reversed and an additional 50 ml was pumped in the opposite direction. Following the wetting procedure, one of the column end fittings was removed from the column, the wetted stationary phase was pumped as a smooth paste into an 8-mm NMR tube and the T_1 experiment was performed as described above for the neat mobile phase samples. It should be noted that at mobile phase compositions containing a very low volume percent of organic solvent, it was necessary to gradually ramp the flow-rate to achieve the desired back pressure and that greater than 100 ml of mobile phase was needed to properly wet the stationary phase. If not properly wetted, the stationary phase would appear either dry or lumpy when pumped from the chromatographic column.

RESULTS AND DISCUSSION

Neat mobile phase ^2H T_1 measurements

The deuterium longitudinal relaxation times, T_1, for the methyl deuterons of [$^2H_3$]acetonitrile–water and [$^2H_4$]methanol–water solvent mixtures *versus* percent organic modifier in the mobile phase are shown in Fig. 1. Recall that a *decrease* in deuterium T_1 implies molecular association or decreased mobility. Over the entire range of [$^2H_3$]acetonitrile–water mixtures, the T_1 values remained essentially constant, averaging approximately 6 s (Fig. 1a). The binary solvent mixture viscosity has also been determined to be approximately constant throughout the composition range [44]. Katz *et al.* [45] have examined the association of methanol–water, acetonitrile–water and tetrahydrofuran–water mixtures both theoretically, using equilibrium equations, and experimentally, by measurement of volume changes upon mixing in these solvent systems. The suggest that these mixtures should be considered as ternary rather than binary; the three components of these mixtures would be free organic solvent (*e.g.* that self-associated rather than associated with water), free water and water–solvent mixed complexes. Their predictions and experiments indicate that in mixtures of acetonitrile and water, the proportion of mixed acetonitrile–water complexes is very small, reaching a maximum volume percentage of *ca.* 5% when the nominal solution volume fraction of acetonitrile is 0.5 [45]. Rowlen and Harris [46] have

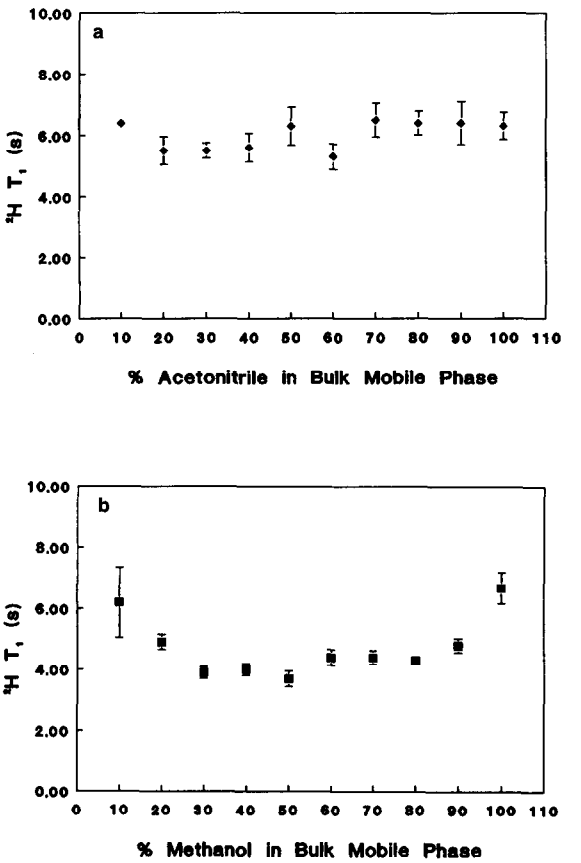

Fig. 1. ^2H longitudinal relaxation time (T_1) for deuterated organic modifier *versus* volume percent organic modifier in bulk organic modifier–water mixture for (a) [$^2H_3$]acetonitrile (◆) and (b) [$^2H_4$]methanol (■).

reported Raman spectroscopic studies that provide additional experimental evidence that there is a concentration-dependent equilibrium between forms of acetonitrile in aqueous mixtures. Their measurements on the concentration dependence of the CN stretching band area and bandwidth also indicate that at acetonitrile mole fractions (χ_{ACN}) greater than 0.3 (*ca.* 0.55 volume fraction), large numbers of self-associated acetonitrile species are present in solution, and that at $\chi_{ACN} > 0.55$ (*ca.* 0.78 volume fraction) little to no water–acetonitrile mixed species exist [46]. Alvarez-Zedpeda *et al.* [47] have also described the concentration-dependent microstructure of acetonitrile–water solutions. Upon addition of acetonitrile to pure water, it can initially enter cavities in the water structure. However, once these sites are occupied, acetonitrile becomes increasingly self-associated in aggregates or loosely defined clusters, resulting in bulk *solution* microheterogeneity [47]. The acetonitrile species experience a relatively *homogeneous* solution environment over a large nominal binary composition range, due to their extensive self-association. It is therefore quite reasonable that, as illustrated in Fig. 1a, the [$^2H_3$]acetonitrile T_1 values are approximately constant throughout the composition range.

The plot of T_1 *versus* percent organic modifier in the mobile phase for the methyl deuterons of [$^2H_4$]methanol–water (Fig. 1b) exhibits different trends than that of the [$^2H_3$]acetonitrile–water system. In this case, the [$^2H_4$]methanol T_1 values are more or less constant from approximately 30% to 80% methanol in the mobile phase. However, at both lower and higher concentrations of methanol, a marked change to higher overall longitudinal relaxation times is noted. The T_1 behavior follows that for viscosity in methanol–water mixtures [44]. There is a much greater degree of hydrogen bonding in methanol–water systems than that exhibited in acetonitrile–water systems, which is primarily due to the presence of an additional pair of unpaired electrons on the methanol oxygen [48]. Katz *et al.* [45,49] have also investigated methanol–water mixtures and have found that they are essentially ternary mixtures with three distinct composition-dependent distributions. Their extensive studies indicate that as the nominal methanol volume fraction increases from zero to *ca.* 0.4, there is a linear increase in water–methanol associated species that corresponds with a commensurate decrease in free water species; there is little to no free methanol present in solution. As the nominal volume fraction of methanol increases from *ca.* 0.4 to *ca.* 0.8, the amount of free water decreases drastically, that of the water–methanol associated species goes through a maximum at a nominal methanol volume fraction of *ca.* 0.6, and the volume fraction of free methanol increases rapidly. At nominal methanol volume fractions above *ca.* 0.8, the volume fraction of free methanol increases linearly, commensurately with the decrease in water–methanol associated species [45,49]. As seen in Fig. 1b, the measured T_1 values for the [$^2H_4$]methanol maintain a value of approximately 4 s from *ca.* 30 to 80% methanol in the mobile phase, which corresponds to the region in which water–methanol associated species predominate. The greatly decreased T_1 values measured in this composition region (*ca.* 4 s) compared to those measured in the acetonitrile–water solutions (*ca.* 6 s) are indicative of restricted mobility of the methanol species due to the greater extent of hydrogen bonding in water–methanol associated species than in self-associated methanol. At nominal methanol volume percentages less than 30% and greater than 80%, the larger [$^2H_4$]methanol T_1 values indicate that methanol experiences a lesser degree of association than in the intermediate composition region. For the latter region, it is plausible that this is due to the increased number of self-associated methanol species in this composition range [45,49], in which hydrogen bonding is expected to be considerably less strong than in the water–methanol mixed species.

The relaxation time behavior for the composition range from 0–30% methanol is less facile to model. The measured T_1 values for methanol at less than 30% methanol are comparable to those measured for the same nominal volume compositions of the acetonitrile mixtures. One possible explanation for this similarity is that at low nominal concentrations, methanol acts much the way that acetonitrile does, entering cavities in the water structure until these sites are occupied. It is reasonable that methanol in such cavities would have a much larger degree of contact with other methanol molecules in the cavity than with aqueous species in the water structure, resulting in T_1 values comparable to those measured for high methanol content mix-

tures. Once these cavities in the water structure are occupied, methanol will be more actively incorporated into the water structure via the formation of water–methanol associated species.

Mobile phase ^2H T_1 measurements for contact with the stationary phase

If stationary phase is combined with the neat solutions in the manner described in the experimental section, and the longitudinal relaxation times are re-measured, it becomes useful to compare the *change* in the methyl deuteron T_1 values in contact with the stationary phase *versus* those observed in neat solution. Although it may be expected that relaxation time measurements at the bonded phase–mobile phase interface might suffer from interface-induced inhomogeneities in the magnetic field, in practice T_1 measurements are relatively insensitive to magnetic field inhomogeneities, although this is not the case for T_2 [50]. Again, by eqn. 1, which states that a decrease in deuterium T_1 values implies restriction of molecular motion or association, it can be concluded that a decrease in T_1 for the methyl deuterons of [$^2H_4$]methanol or [$^2H_3$]acetonitrile when combined with the stationary phase compared to that for the neat mobile phase mixture implies a reduction of motional freedom of these species relative to the bulk solution due to association of methanol or acetonitrile with the stationary phase [29].

In Fig. 2a, the relaxation time of [$^2H_4$]methanol in the neat mobile phase *minus* the relaxation time of [$^2H_4$]methanol when the same mobile phase is in contact with stationary phase, which will be denoted as ΔT_1, is plotted *versus* percent methanol in the bulk mobile phase for the ODS-1 stationary phase. For this low C_{18} bonding density phase (1.5 μmol/m^2) there is relatively little disparity in the deuterium ΔT_1 values for bulk mobile phase compositions ranging from *ca.* 30 to 80% methanol. Although there is association of methanol with the stationary phase, which is reflected by a positive value for ΔT_1, the *degree* of association of methanol with the stationary phase is constant throughout this composition range, and is also less than for any other range of bulk methanol–water compositions. This is not surprising in light of the proposed solution structure of methanol–water mixtures in this composition range, as well as our neat mobile phase T_1 measurements. Recall that this region corresponds to that in which water–methanol associated species predominate in Katz *et al.*'s model [45,49]. There is a greater degree of hydrogen bonding in water–methanol associated species than in self-associated methanol species. Moreover, the methanol–water hydrogen bonding interactions predominant over this composition range are stronger and more directed than the weaker, predominantly dispersive interactions that take place between methanol and C_{18} stationary phases. Doubtless accessible silanol groups on the silica support could also participate in hydrogen bonding with any of the solution species. However, the octadecyl ligands bound to the

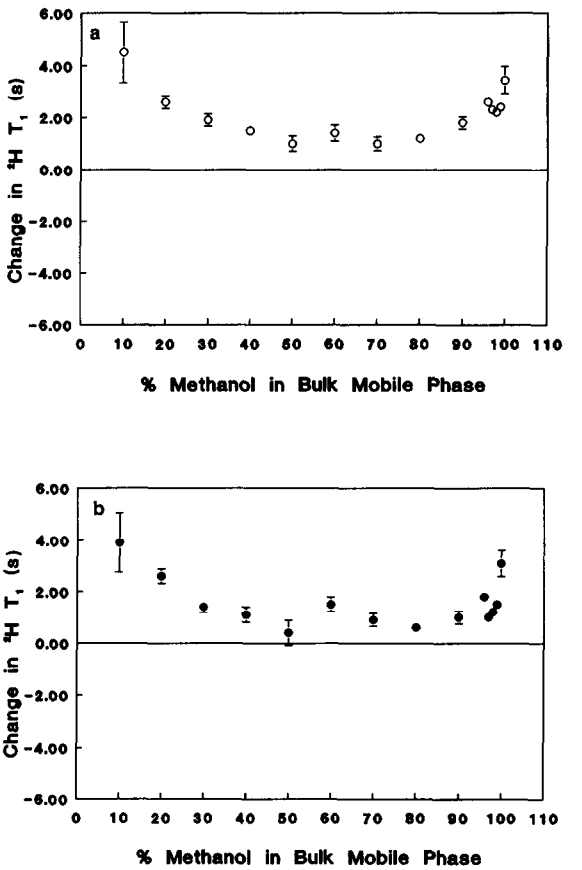

Fig. 2. Change in ^2H longitudinal relaxation time (T_1) for [$^2H_4$]methanol in contact with monomeric C_{18} stationary phases *versus* volume percent methanol in bulk methanol–water mobile phase. (a) Low bonding density phase (ODS-1; 1.5 μmol/m^2) (○). (b) High bonding density phase (LT1; 4.4 μmol/m^2) (●).

silica support are likely to be only partially extended [22] due to limited methanol uptake over this composition range and therefore would provide at least some degree of shielding of mobile phase components from the support surface. Consequently, little if any association of methanol with the stationary phase is expected over this mobile phase composition range, and from the bulk solution behavior, this degree of association would be expected to remain constant. The expected behavior is observed.

At compositions with more than 80% methanol in the mobile phase, the methanol deuterium ΔT_1 values increase with the volume percent of methanol in the bulk mobile phase. This implies a greater degree of association for methanol with the stationary phase than is exhibited in the intermediate composition region. Recall that at nominal methanol volume fractions above *ca.* 0.8, the volume fraction of self-associated methanol complexes increases linearly, commensurately with the decrease in water–methanol associated species [45,49]. Since the self-associated solution species are not strongly hydrogen bonded with water, they are more likely to experience dispersive interactions with the C_{18} chains of the stationary phase due to the hydrophobic character of the methyl group [6–8]. Therefore, because of the bulk solution composition of the mobile phase at these higher methanol concentrations, uptake of methanol self-associated species should enable the bonded alkyl chains to assume a more extended configuration [5,15]. Once the stationary phase chains assume a more extended configuration, there is more accessible chain volume for further methanol partitioning and the hydroxyl moiety of the alcohol would also be better able to participate in hydrogen bonding with residual silanol groups on the silica support surface. Although other NMR experiments in our laboratory have indicated that the latter effect is negligible compared to the former [42], the overall effect of methanol uptake is to allow greater penetration and corresponding association of methanol with the stationary phase.

At mobile phase compositions with less than 30% methanol, a marked increase in ΔT_1 is again observed, implying a larger degree of association of methanol with the stationary phase than observed in the intermediate composition range. It is well known that in highly aqueous mobile phases, the hydrophobic alkyl stationary phase chains will assume a collapsed or folded configuration in order to minimize their surface contact with the polar mobile phase [3,5,15,51]. This would cause methanol to become entrapped within the collapsed chain structure (most likely within narrow-necked pores in the silica support), and thereby result in a distinct decrease in the [$^2H_4$]methanol ^2H T_1 values. The analogous ΔT_1 *versus* volume percent methanol plot (Fig. 2b) for the high bonding density stationary phase (4.4 μmol/m^2); LT1 illustrates that the same general trends are observed as for the low bonding density phase (ODS-1).

The same types of experiments were also performed using the same stationary phase materials and [$^2H_3$]acetonitrile–water mobile phase mixtures. In Fig. 3a and b the changes in [$^2H_3$]acetonitrile deuterium T_1 values in contact with ODS-1 and LT1 are shown. Just as exhibited in the bulk solution measurements (Fig. 1a), the [$^2H_3$]acetonitrile–water mobile phase system demonstrates distinctly different behavior than the [$^2H_4$]methanol–water system. For both the LT1 and ODS-1 stationary phases, the ΔT_1 values for acetonitrile in contact with stationary phase are essentially constant over the *entire* concentration range. This reflects the bulk solution properties of [$^2H_3$]acetonitrile–water mixtures in the same manner as was seen with [$^2H_4$]methanol–water mixtures. Recall that the microstructure of acetonitrile–water solutions is concentration-dependent. In the bulk solution model, acetonitrile enter cavities in the water structure until these sites are occupied. With further increase in the amount of acetonitrile, it becomes increasingly self-associated in aggregates or loosely defined clusters, resulting in bulk solution microheterogeneity [47]. However, the acetonitrile species experience a relatively homogeneous solution environment over a large nominal binary composition range, due to their extensive self-association [45–47]. It is therefore quite reasonable that, as illustrated in Fig. 3, [$^2H_3$]acetonitrile exhibits a relatively monotonic degree of association behavior with both stationary phases throughout the composition range. It is interesting to note that the compositon at which both the bulk solution (Fig. 1a) and the high chain density stationary phase (Fig. 3b) exhibit their minimum T_1 value is 60% acetonitrile; this is approximately the same composition at which Rowlen and Har-

ris's Raman measurements first indicate large numbers of self-associated acetonitrile species present in solution [45]. The ΔT_1 values for [$^2H_3$]acetonitrile–water systems are maximized for the 10% acetonitrile bulk solution, indicating that acetonitrile is most highly associated with the stationary phase at this composition. Just as is the case for comparable methanol compositions, acetonitrile is likely entrapped within silica pores beneath the collapsed chain structure that the hydrophobic alkyl stationary phase chains will assume in order to minimize their surface contact with highly aqueous mobile phases [15].

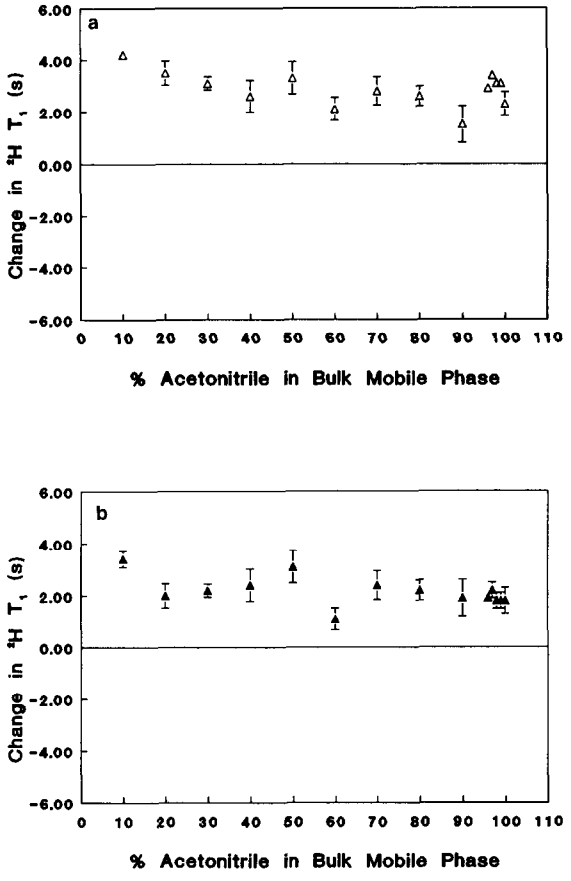

Fig. 3. Change in ^2H longitudinal relaxation time (T_1) for [$^2H_3$]acetonitrile in contact with monomeric C_{18} stationary phases *versus* volume percent acetonitrile in bulk acetonitrile–water mobile phase. (a) Low bonding density phase (ODS-1; 1.5 μmol/m^2) △). (b) High bonding density phase (LT1; 4.4 μmol/m^2) (▲).

If at this point a comparison of the results for ODS-1 (1.5 μmol C_{18} ligand/m^2 of bonded phase surface) with those obtained for LT1 (4.4 μmol/m^2) is made by superimposing the ΔT_1 plots (Fig. 4), some subtle but interesting results emerge. Recall that the primary difference between ODS-1 and LT1 is their large difference in the degree of surface derivatization (octadecyl bonding density). Yet as shown in Fig. 4a, the degree of association of methanol with the stationary phase for both chromatographic supports is virtually the same, although upon close inspection, it can be noticed that there is a *slightly* larger degree of methanol association with the ODS-1 stationary phase than for LT1 at several mobile phase compositions. Although the difference for these compositions is small, it is statistical-

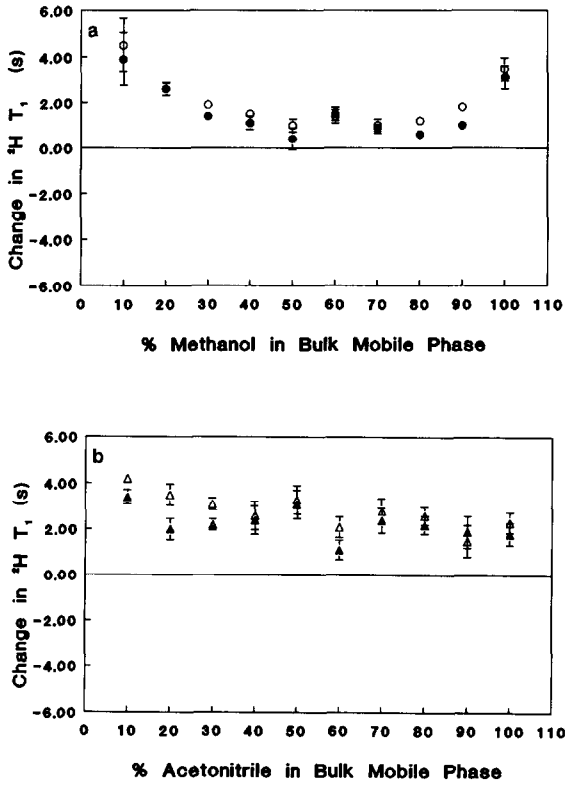

Fig. 4. Comparison of the change in ^2H longitudinal relaxation time (T_1) for the organic modifier methyl deuterons in contact with low *versus* high density monomeric C_{18} stationary phases. (a) [$^2H_4$]Methanol with ODS-1 (○) *versus* LT1 (●). (b) [$^2H_3$]Acetonitrile with ODS-1 (△) *versus* LT1 (▲).

ly significant. Upon initial considerations, this observation might appear somewhat surprising. Based strictly on the degree of carbon loading, a qualitative prediction might have been made that there would be a *significantly* greater degree of methanol association with the more hydrophobic high bonding density phase (LT1) than with the low bonding density phase (ODS-1). Yet if anything, the reverse is indicated by our experimental measurements. This apparent anomaly is readily explained if a more comprehensive model of reversed-phase stationary phase structure, which takes into account the effect of stationary phase chain organization on solute partitioning, is considered.

As previously discussed, RPLC stationary phases cannot be thought of strictly as bulk phases. The stationary and mobile phase interact significantly, resulting in the formation of the solvation layer. Dill has predicted [2–4] and Sentell and Dorsey have shown experimentally [52] that the degree of bonded phase alkyl chain interaction is a function of bonding density, and that the degree of chain interaction strongly affects the ability of any solute, including mobile phase components, to penetrate the chain structure. At low bonding densities, there are relatively few interactions between neighboring C_{18} ligands; mobile phase access to the alkyl ligands as well as to residual surface silanols is relatively facile. Methanol species should therefore be able to participate in dispersive interactions with the stationary phase alkyl chains as well as associate with any accessible surface silanols through hydrogen bonding. As alkyl chain surface coverage increases, the latter effect is expected to decrease. Additionally, for monomeric bonded phases there is a critical bonding density at about 3.0 μmol/m^2 wherein the alkyl chains are in close enough proximity for interactions with neighboring chains to become important. Above this value, the degree of chain interaction becomes a significant factor in determining the extent of solute partitioning, since any solute partitioning *into* the stationary phase must overcome these interactions [2–4,52]. As bonding density further increases, the free energy required to overcome these chain interactions becomes increasingly prohibitive, leading to commensurate decreases in solute partitioning [2–4,52]. This should result in a decreasing degree of penetration of mobile phase components into the stationary phase for bonded phase surface densities of *ca.* 3 μmol/m^2 or greater. Cole and Dorsey [53] have confirmed this experimentally by observing the effects of bonding density on mobile phase re-equilibration volumes following gradient elution.

The comparison between the ODS-1 and LT1 phases in Fig. 4 can be reasonably made using this interphase model for solute partitioning. When the large disparity in bonding densities between LT1 and ODS-1 is considered, the experimental results are logical and it is expected that methanol will be able to participate in a greater degree of association with the ODS-1 stationary phase than with the LT1 phase (Fig. 4a). This is because in the former, there should be much greater accessibility of methanol to both the hydrophobic C_{18} stationary phase chains and to residual silanols. This provides dual mechanisms for the larger decrease in the [$^2H_4$]methanol T_1 values via association with the ODS-1 phase. In contrast, the very high bonding density of the LT1 phase should lead to at least partial exclusion of methanol from the interphase structure due to the high degree of chain cooperativity as well as decreased access to surface silanols. Therefore it is both expected and observed that [$^2H_4$]methanol undergoes less association with the high density LT1 stationary phase than with the low bonding density ODS-1 phase. The same behavior is observed for [$^2H_3$]acetonitrile (Fig. 4b).

It is more interesting to compare and contrast the ΔT_1 data between methanol and acetonitrile for both stationary phases. In Fig. 5, the ΔT_1 plots for each of the stationary phases in contact with the two different mobile phase systems are overlaid. The results show that over the bulk mobile phase composition range from *ca.* 30 to 80% organic modifier, acetonitrile displays a greater degree of association with both stationary phases than methanol does. This is doubtless due to their differences in bulk solution microstructure. Since in this general composition range acetonitrile–water solution chemistry is dominated by the formation of relatively hydrophobic self-associated acetonitrile microphases [45–47] and methanol–water solution behavior is dominated by the formation of much more polar water–methanol associated species [45,49], it is reasonable that acetonitrile would be more highly associated with both stationary phases. At bulk mobile phase compositions with 20% or less organic

modifier, the ΔT_1 values, and thus the degree of association of the acetonitrile and methanol components of the mobile phase, are comparable. This is doubtless due to the collapsed chain structure assumed by hydrophobic alkyl stationary phase chains in highly aqueous mobile phases. Finally, for purely organic bulk mobile phases, methanol displays a larger ΔT_1 value than acetonitrile. Since in pure methanol solutions the methanol species are not hydrogen bonded with water, they are much more available to participate in dispersive interactions with the C_{18} chains of the stationary phase than when water is present. This should bring about a more extended configuration of the bonded alkyl chains, which further increases methanol accessibility to the bonded phase surface. These two effects allow a greater degree of association of methanol with the stationary phase when water is not present. As discussed previously, acetonitrile exists in bulk solution primarily as self-associated species throughout the binary composition range, enabling its accessibility to interact with the stationary phase to remain more or constant [47].

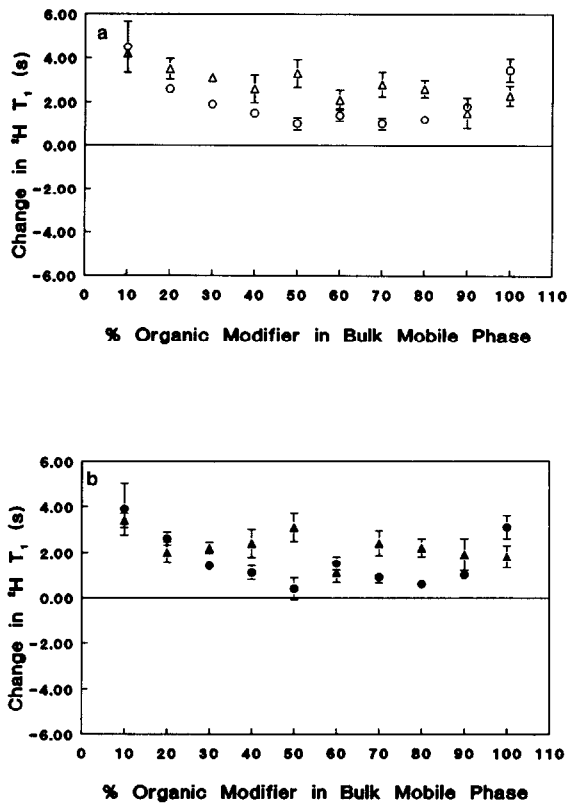

Fig. 5. Comparison of the change in ^2H longitudinal relaxation time (T_1) for [$^2H_3$]acetonitrile *versus* [$^2H_4$]methanol in contact with monomeric C_{18} stationary phases. (a) [$^2H_4$]Methanol (○) *versus* [$^2H_3$]acetonitrile (△) with ODS-1. (b) [$^2H_4$]Methanol (●) *versus* [$^2H_3$]acetonitrile (▲) with LT1.

Comparisons to other techniques

Before the results of this study are compared to those obtained by other methods, it must again be stressed that the ΔT_1 measurements made here are not *quantitative* measurements of the *amount* of sorbed organic modifier in the stationary phase systems, but rather are *qualitative* measurements of how strongly these components of the mobile phase are *associated* with the stationary phase under these conditions. Extremely precise quantitative measurements via integration of these NMR peak areas are very difficult (if not impossible) to make. Additionally, T_1 measurements for solvents in contact with stationary phases will of necessity be a weighted average of the bulk solvent and associated solvent T_1 values, due to exchange between these two sites that is fast relative to the time frame of the NMR experiment [29]. However, because the T_1 values for the bound solvents are typically much shorter than those for the bulk solution solvents and the rate of exchange occurs on a much faster time scale, the T_1 values measured for the paste samples are dominated by the relaxation time of the bound solvent species [29]. Our examination of the *difference* between the bulk solution T_1 values and those measured for the solution in contact with the stationary phase should also help to correct for the contributions from the relaxation of the species of interest in the bulk solution. Finally, it should be noted that any spectroscopic measurement of the interactions of a species of interest with an RPLC bonded phase will be representative of the *average* interaction of that species with the bonded phase surface. For NMR T_1 studies on any solute, it is expected that a distribution of relaxation times would result from the varying degrees of solute association with such a heteroenergetic surface [30].

Despite the above restrictions, our ^2H NMR T_1 studies provide both comparable and complimentary information to previous studies. Lochmüller

and Hunnicutt [15] have stated "...local *n*-alkyl density effects as well as specific solvent–hydrocarbon interactions influence the ultimate conformation of monomeric *n*-octadecyl bonded phases." In acetonitrile mobile phases, our study indicates that the acetonitrile species exhibit a relatively monotonic degree of association behavior with both C_{18} stationary phases throughout most of the composition range. Analogous results have been reported from adsorption isotherm studies [6,8,13,54]. A number of spectroscopic studies have also indicated that *changes* in stationary phase polarity as a function of bulk acetonitrile composition in these systems (due to intercalated mobile phase components and/or stationary phase chain extension) are also small [18,21,23]. In contrast, changes in stationary phase polarity have been found to be much more composition dependent for methanol–water mobile phase systems [19,20,23]. Upon inspection, these polarity measurements correspond well with both the bulk solution composition characteristics reported by Katz *et al.* [45,49] and the results of our [$^2H_4$]methanol studies reported here. Accordingly, the cooperative solvation layer formation model for C_{18} stationary phases in methanol–water mobile phase systems proposed by Yonker *et al.* [7] is further corroborated by our present studies.

At highly aqueous mobile phase compositions, our present studies indicate that acetonitrile and methanol are highly associated with the stationary phase. These species are likely entrapped in narrow-necked ("ink bottle") pores beneath the collapsed C_{18} chain structure that Lochmüller and Hunnicutt [15] have described; the hydrophobic alkyl stationary phase chains will assume this minimum free energy configuration in order to minimize their surface contact with highly aqueous mobile phases. Convincing chromatographic evidence for solvent entrapment under these conditions has been presented by Gilpin and Squires [51] from thermal studies on reversed-phase materials. This description is also consistent with models for alkyl chain conformations at various stages of stationary phase wetting derived from the work of Maciel and Zeigler [31–33], who used CP/MAS solid state ^{13}C NMR and ^2H solid state quadrupole echo NMR measurements of selectively deuterated C_{18} alkyl chain positions to study the mobility of these bonded phases for a variety of contact solvents. From the results of these studies, they maintain that at high water content the C_{18} chains will be associated and collapsed due to hydrophobic interactions. Bayer *et al.* [34–36] and McNally and Rogers [37] have also found significant reductions in ^{13}C T_1 measurements (*i.e.* reduced mobility) for bonded phase carbons in contact with highly aqueous systems.

Our experiments indicate that acetonitrile is for the most part more associated with either stationary phase than methanol. Spin probe studies [55] as well as numerous adsorption isotherm measurements [6,8,10–13] support this conclusion. Furthermore, we have shown that the degree of organic modifier association with the stationary phase is a function of alkyl chain surface density, which correlates well with previous ^{13}C NMR [25,33], spin probe [55] and chromatographic [53] measurements. In summary, our deuterium NMR studies exhibit very good correlations with studies of mobile and stationary phase interactions carried out via a number of disparate experimental techniques.

CONCLUSIONS

The work described here confirms the usefulness of using solution state deuterium NMR measurements for studying microenvironments in reversed-phase chromatographic systems. Moreover, it has also been shown that by using the sample preparation method described herein, this technique is applicable to studying a full range of binary mobile phase compositions. The results obtained here give a qualitative picture of the extent of organic mobile phase components' interactions with the stationary phase as a function of bulk mobile phase composition and demonstrate that the degree of this interaction is strongly related to bulk solution structure. They further support a partitioning model for describing solute interactions with the stationary phase in reversed-phase chromatographic systems [2–4] by demonstrating that stationary phase bonding density is an important factor in solvation layer formation. It will be interesting to extend these experiments to include additional stationary phases of intermediate bonding densities, as well as to observe the behavior of other mobile phase systems. Moreover, a complimentary communication forthcoming from our laboratory reports and contrasts the changes we have observed in ^2H_2O T_1 values as a

function of composition for the same mobile and stationary phase systems discussed in this work [42].

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REFERENCES

1 R. E. Majors, *LC · GC*, 10 (1991) 686.
2 J. A. Marqusee and K. A. Dill, *J. Chem. Phys.*, 85 (1986) 434.
3 K. A. Dill, *J. Phys. Chem.*, 91 (1987) 1980.
4 J. G. Dorsey and K. A. Dill, *Chem. Rev.*, 89 (1989) 331.
5 D. E. Martire and R. E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
6 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249.
7 C. R. Yonker, T. A. Zwier and M. F. Burke, *J. Chromatogr.*, 241 (1982) 257.
8 C. R. Yonker, T. A. Zwier and M. F. Burke, *J. Chromatogr.*, 241 (1982) 269.
9 R. P. W. Scott and C. F. Simpson, *Faraday Symp. Chem. Soc.*, 15 (1980) 69.
10 E. H. Slaats, E. Markovski, J. Fekete and J. Poppe, *J. Chromatogr.*, 207 (1981) 299.
11 G. Foti, C. Martinez and E. Kovats, *J. Chromatogr.*, 461 (1989) 269.
12 G. Foti, C. deReyff and E. Kovats, *Langmuir*, 6 (1990) 759.
13 R. K. Gilpin, M. Jaroniec and S. Lin, *Anal. Chem.*, 62 (1990) 2092.
14 L. C. Sander, J. B. Callis and L. R. Field, *Anal. Chem.*, 55 (1983) 1068.
15 C. H. Lochmüller and M. L. Hunnicutt, *J. Phys. Chem.*, 90 (1986) 4318.
16 J. Stahlberg and M. Almgren, *Anal. Chem.*, 57 (1985) 817.
17 J. Stahlberg, M. Almgren and J. Alsins, *Anal. Chem.*, 60 (1988) 2487.
18 J. W. Carr and J. M. Harris, *Anal. Chem.*, 58 (1986) 626.
19 J. W. Carr and J. M. Harris, *Anal. Chem.*, 59 (1987) 2546.
20 A. L. Wong, M. L. Hunnicutt and J. M. Harris, *Anal. Chem.*, 63 (1991) 1076.
21 J. L. Jones and S. C. Rutan, *Anal. Chem.*, 63 (1991) 1318.
22 M. E. Montgomery Jr., M. A. Green and M. J. Wirth, *Anal. Chem.*, 64 (1992) 1170.
23 Y.-D. Men and D. B. Marshall, *Anal. Chem.*, 62 (1990) 2606.
24 R. K. Gilpin and M. E. Gangoda, *J. Chromatogr. Sci.*, 21 (1983) 352.
25 R. K. Gilpin and M. E. Gangoda, *Anal. Chem.*, 56 (1984) 1470.
26 R. K. Gilpin and M. E. Gangoda, *J. Magn. Reson.*, 64 (1985) 408.
27 M. Gangoda and R. K. Gilpin, *J. Magn. Reson.*, 74 (1987) 134.
28 M. E. Gangoda and R. K. Gilpin, *Langmuir*, 6 (1990) 941.
29 D. B. Marshall and W. P. McKenna, *Anal. Chem.*, 56 (1984) 2090.
30 E. H. Ellison and D. B. Marshall, *J. Phys. Chem.*, 95 (1991) 808.
31 G. E. Maciel, R. C. Zeigler and R. K. Taft, in D. E. Leyden (Editor), *Silanes, Surfaces and Interfaces (Chemically Modified Surfaces Series*, Vol. 1), Gordon and Breach, New York, 1986, p. 413.
32 R. C. Zeigler and G. E. Maciel, in D. E. Leyden (Editor), *Chemically Modified Surfaces in Science and Industry (Chemically Modified Surfaces Series*, Vol. 2), Gordon and Breach, New York, 1988, p. 319.
33 R. C. Zeigler and G. E. Maciel, *J. Am. Chem. Soc.*, 113 (1991) 6349.
34 K. Albert, B. Evers and E. Bayer, *J. Magn. Res.*, 62 (1985) 428.
35 E. Bayer, A. Paulus, B. Peters, G. Laupp, J. Reiners and A. Klaus, *J. Chromatogr.*, 364 (1986) 25.
36 K. Albert, B. Pfleiderer and E. Bayer, in D. E. Leyden (Editor), *Chemically Modified Surfaces in Science and Industry (Chemically Modified Surfaces Series*, Vol. 2). Gordon and Breach, New York, 1988, p. 287.
37 M. E. McNally and L. B. Rogers, *J. Chromatogr.*, 331 (1985) 23.
38 P. Shah, L. B. Rogers and J. C. Fetzer, *J. Chromatogr.*, 388 (1987) 411.
39 H. A. Claessens, L. van de Ven, J. de Haan, C. A. Cramers and N. Vonk, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 433.
40 E. C. Kelusky and C. A. Fyfe, *J. Am. Chem. Soc.*, 108 (1986) 1746.
41 R. K. Harris, *Nuclear Magnetic Resonance Spectroscopy: A Physicochemical View*, Pitman Books, London, 1983.
42 D. M. Bliesner and K. B. Sentell, *Anal. Chem.*, submitted for publication.
43 K. B. Sentell, K. W. Barnes and J. G. Dorsey, *J. Chromatogr.*, 455 (1988) 95.

44 J. Timmermans, *The Physico-Chemical Constants of Binary Systems in Concentrated Solutions Vol. 4*, Interscience, New York, 1960.
45 E. D. Katz, K. Ogan and R. P. W. Scott, *J. Chromatogr.*, 352 (1986) 67.
46 K. L. Rowlen and J. M. Harris, *Anal. Chem.*, 63 (1991) 964.
47 A. Alvarez-Zepeda, B. N. Barman and D. E. Martire, *Anal. Chem.*, 64 (1992) 1978.
48 F. Franks and D. J. G. Ives, *Quart. Rev.*, 20 (1) (1966) 1.
49 E. D. Katz, C. H. Lochmüller and R. P. W. Scott, *Anal. Chem.*, 61 (1989) 349.
50 J. A. Glasel and K. H. Lee, *J. Am. Chem. Soc.*, 96 (1974) 970.
51 R. K. Gilpin and J. A. Squires, *J. Chromatogr. Sci.*, 19 (1981) 195.
52 K. B. Sentell and J. G. Dorsey, *Anal. Chem.*, 61 (1989) 2373.
53 L. A. Cole and J. G. Dorsey, *Anal. Chem.*, 62 (1990) 16.
54 A. Alvarez-Zepeda and D. E. Martire, *J. Chromatogr.*, 550 (1991) 285.
55 P. B. Wright, E. Lamb, J. G. Dorsey and R. G. Kooser, *Anal. Chem.*, 64 (1992) 785.