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Review

Bonded phase conformation and solvation effects on the stationary phase structure in reversed-phase liquid chromatography

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ABSTRACT

The stationary phase in reversed-phase liquid chromatography on chemically modified silica bonded with alkyl moieties is understood to be a dynamic multicomponent mixture. Through the work of many researchers, an understanding of this near-surface region has been developed as a solvation layer mixture in terms of the length of the bonded hydrocarbon moieties, the surface bonding density, the hydrogen-bonding water adsorption at the silica surface, and the enhanced concentration of organic solvent from the mobile phase. However, the structure and chemical interactions in this near-surface region are not well understood; in fact, some researchers have even considered the stationary phase to be a chemically passive participant in chromatographic separations. Detailed investigation of the thermodynamic and kinetic quantities associated with a temperature-induced conformational change in bonded octadecyl moieties yields information on this interfacial region. The solvation dependence of this surface change as probed by a variety of solutes on different bonding-density octadecyl-bonded silica materials provides a model elucidating the three-dimensional structure and retention interactions of the stationary phase. The general applicability of this model is shown to provide consistent description of solute retention interactions with four different organic modifiers on five octadecyl bonded silicas differing in bonding chemistry, bonding density, and base silica.

CONTENTS

1. Introduction	290
2. Experimental	292
3. Results and discussion	293
3.1. Thermodynamics of chromatography	293
3.2. Solvation interactions	294
3.3. Comparison of bonded-phase materials, LiChrosorb RP-18 and Ultrapack ODS	295
3.4. Conformation change of the bonded octadecylsilane groups	296
3.4.1. Benzene retention	296

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3.4.2. Methoxybenzene and phenol	298
3.4.3. Retention behavior of hydrogen-bond-accepting solutes, amines	299
3.4.4. Relative effects of alkyl and hydrogen-bonding interactions, phenyl alcohols	300
3.5. Bonded phase surface coverage effects	300
3.6. Solvent interactions	303
3.6.1. Acetonitrile–water eluents	303
3.6.2. Structuring solvent contributions, ethanol and THF	307
3.7. Kinetic effects of the bonded octadecyl conformation change	310
3.8. Alternative C ₁₈ -bonded phases	312
4. Conclusions	314
References	315

1. INTRODUCTION

The extensive use of bonded-phase silica packing materials for reversed-phase liquid chromatography (RPLC) in recent years [1–4] has sparked an interest in obtaining more than a working understanding of the stationary phase interactions of these systems. The difficulty in providing a descriptive model of the interactions associated with solute retention on these materials lies in the complexity of the system. A structurally complex surface is presented by the highly porous silica support and the solvation layer has contributions from solvents, the chemically bonded species, and the chemistry of the silica surface itself. All of these provide varying interactions with solutes of widely differing polarity and geometry in chromatographic separations. This review will attempt to address the three-dimensional structure of the surface solvated layer, which acts as the stationary phase, in terms of a temperature- and solvation-dependent change in conformation of the bonded hydrocarbon moieties.

The current understanding of the composition of RPLC stationary phase and its interactions with solute species is based upon three important parameters drawn from the work of many researchers. The stationary phase is a dynamic solvated layer whose structure is determined by (I) the length and type of the bonded hydrocarbon moieties, (II) the surface density of the bonded species, and (III) the types of intermolecular interactions provided by the solvent component(s) imbibed into the stationary phase. Only the effect of linear hydrocarbon chains bonded to the surface will be considered in the current discussions.

RPLC retention cannot be described as a purely adsorptive process because the surface-bonded groups do not present a classical surface [5]. Neither do these groups comprise a liquid phase because of their surface anchoring and sparse population relative to a pure liquid hydrocarbon [6]. It has been shown that solute molecules intercalate themselves between the hydrocarbon chains and that once the entire molecule can be “immersed” in the stationary phase, further increase in bonded chain length has a diminished effect on retention [7]. A plateau in the increase in retention with increasing chain length, as well as a dependence of the critical chain length on the size of the solute molecule has also been observed [8].

Surface coverage of the bonded hydrocarbon moieties has a two-fold effect. As the number of hydrocarbon groups on the surface is increased, the retentive capacity of the material through non-polar interactions increases. However, past a certain point determined by the packing density of the bonded chains, the solute distribution coefficient decreases as a result of restricted access between the bonded hydrocarbon chains [6,9]. A complementary consideration is that, as an increasing number of hydrocarbon groups are bonded to the silica surface through siloxane bonds, the amount of polar silica sites is decreased in both concentration [10] and accessibility [6,11]. The effect of these sites is primarily seen on sparsely derivatized silica in the asymmetric elution band shape of polar solute species. The interactions at these polar sites are usually attributed to silanol groups [12,13] and accompanying adsorbed water [14–18].

The chemical and structural heterogeneity of the base silica [19] can also effect the bonding

density and distribution of alkyl moieties. Surface chemical heterogeneity can produce non-uniformity in the distribution of alkyl moieties during bonded phase synthesis based on the variability in silanol reactivity. The highly complex porous structure of the silica can produce bonding heterogeneity based on both size exclusion of bonding reagent, as well as cooperativity of densely packed alkyl moieties. The resultant heterogeneity in bonded moiety distribution can effect solute and solvent interactions with the stationary phase particularly for polar species which participate in hydrogen-bonding interactions.

A differentiation must be made between densely bonded silica derivatized with monofunctional silanes in which the chain packing density is high and silica with a high carbon loading as provided by di- or trifunctional silanes. These so-called polymeric phases may contain a higher carbon loading, but due to the reactions of the multifunctional silane the interchain packing density may be lower than that obtained with a monofunctional silane at equal carbon loading. The effect of these differences is to provide polymeric bonded phases which have a high retentive capacity, but which may participate in solute and solvation interactions in a manner similar to more lightly bonded monomeric materials [20,21].

Retention of at least non-polar solute species is accompanied by intercalation of the solute molecule into the dynamic solvated stationary phase layer [6,9,22,23]. A statistical mechanical lattice model was developed by Martire and Boehm [24] describing chain organization in the stationary phase as similar to a liquid crystalline material. Refinements to the lattice-interphase model were presented by Dorsey and Dill [6,23] in terms of the surface-anchored chains and their configurational entropy. The model presented by Dorsey and Dill was experimentally supported by bonding density studies of Sentell and Dorsey [9,22]. Evidence that molecular shape selectivity is related to hydrocarbon bonding density, especially on polymeric bonded phases, has been presented by Sander and Wise [20,21] and Sentell and Henderson [25].

The third factor controlling the behavior of the

stationary phase is the type of organic solvent(s) used as the mobile phase modifier(s). The saturated hydrocarbon moieties participate in solvation interactions based upon the relatively weak Van der Waals and dipole-induced dipole forces. However, additional forces (dipole-dipole, hydrogen-bonding) may play a role in the solvent-solvent, solvent-solute [26], and solvent-silica surface interactions. Because of the strength of the specific hydrogen-bonding interactions between the silica surface and water, as evidenced by the difficulty in drying the silica by thermal means [19], it is expected that accessible silica surface is covered by at least one monolayer of adsorbed water [14–17]. The solvation of chemically modified silica by organic solvent-water solutions has been found to possess a positive surface excess of organic solvent on alkyl-modified materials [27–33]. The stationary phase is enriched in the organic solvent component of the mobile phase and this enrichment increases as the chromatographic strength of the solvent increases [$\text{CH}_3\text{OH} < \text{CH}_3\text{CN} < \text{CH}_2\text{CH}_2\text{OH} < \text{tetrahydrofuran (THF)}$]. In addition, the incorporation of water into the solvation layer has been shown to vary significantly with organic solvent component in the order $\text{CH}_3\text{CN} < \text{CH}_3\text{OH} < \text{CH}_2\text{CH}_2\text{OH} \ll \text{THF}$ [30–32].

At this point it is necessary to add a fourth consideration to the structure and interactions of the stationary phase, namely, the conformational shape and mobility of the bonded hydrocarbon chains. Karch *et al.* [34] suggested that the bonded hydrocarbon chains be described as bristles sticking out from the silica surface like a brush. Further research added refinements to this model and Riedo *et al.* [35] showed a phase transition in hydrocarbon bonded to acid-etched silica as detected by a sharp change in wetting angle as a function of temperature and chain length. In comparing liquid-liquid extraction data with RPLC bonded phase selectivity, Lochmuller and Wilder [36] proposed that the hydrocarbon moieties preferentially associate with one another to form pseudo-liquid droplets on the surface. Gilpin and coworkers [37–39] further showed that for intermediate length (C_8 – C_{10}) bonded hydrocarbon materials with pure water mobile phases, an irreversible transition involv-

ing the release of surface organic solvent could be effected as a function of temperature. This transition was shown to be a function of the bonded-alkyl chain length and the type of organic conditioning solvent, but not the type of bonding chemistry [40]. Based upon these data they proposed that hydrocarbon chains held trapped organic solvent until sufficient thermal energy was supplied to extend the chains in pure water and release the trapped solvent. Complementary observations on the kinetics of exchange of stationary phase-entrapped solvent have been presented for gradient elution equilibration [41]. Another type of surface transition was observed by Morel and co-workers [5,42,43] using both GC and LC retention data in which they found a non-linear change in retention as a function of temperature. Similar conformational transitions for well-solvated octadecyl-bonded phases were observed with RPLC on densely bonded materials with non-polar solutes [25,44,45]. Morel *et al.* [43] suggested that it is unfortunate that RPLC phases were not historically developed using C_{16} or shorter bonded hydrocarbons because octadecyl chains form a thermodynamically unstable system over the usual operating temperature range.

The molecular mobility of bonded hydrocarbon chains has also been investigated spectroscopically. Using ^{13}C NMR, Zwier [29] showed that the liquid-like mobility of the chains increased as a function of chain length and solvation, but only minor changes occurred as a function of temperature over the range studied. By selective positional ^{13}C enrichment of hydrocarbon bonding reagents, Gilpin and Gangoda [46] showed with NMR that mobility increases rapidly with distance from the anchoring surface bond along the carbon chain. This is also consistent with the lattice model predictions of Dorsey and Dill [6,23]. Fourier transform (FT) IR spectrometry was employed by Sander *et al.* [47] to show that for dry bonded-phase silica, a phase transition similar to that of a pure liquid hydrocarbon can be thermally induced on long-chain bonded-phases (C_{18} – C_{22}). For well-solvated materials FT-IR measurements indicated a decrease in the number of gauche C–C bonds of

the hydrocarbon moieties relative to unsolvated conditions.

Taken together, the work of these researchers indicates that the conformation and mobility of saturated hydrocarbon chains bonded to silica contribute significantly to chromatographic response. Limiting conformations associated with stiffened chains of limited mobility and bent chains of higher mobility are described. In addition, the average conformation can be altered by solvation, bonding density and/or temperature.

The current review discussion will focus on the equilibrium solvation-dependent thermal transition on octadecyl-bonded silica as detected by the changing retention of a variety of solutes. Investigation of this transition in poorly solvated systems and the response of solutes of differing polarity allows the determination of the nature of the three-dimensional structure of the stationary phase in terms of the location of the interactions responsible for retention of various solute types. The goal is to obtain a general model of stationary phase retention interactions which allows the interpretation of solute retention across a wide variety of ODS-bonded silica differing in bonding chemistry, bonding density and base silica.

2. EXPERIMENTAL

The experimental conditions employed in these chromatographic investigations have been described previously [11,18,32,48–50]. A few key points are reiterated. The column temperature was regulated over the range -15 to $70^{\circ}C$ to within $\pm 0.2^{\circ}C$. All mobile phase solvents were prepared in quantities sufficient to perform an entire temperature study with one batch of mobile phase and were delivered isocratically to avoid variability of pump mixing.

HPLC columns used in these experiments were packed in house (University of Arizona) by a balanced density slurry packing technique described elsewhere [2,3,48]. Studies on a high-bonding-density packing were performed on two different 100×4.6 mm columns packed with $10 \mu m$ LiChrosorb RP-18 obtained from E. Merck (Cherry Hill, NJ, USA; batch numbers 9633245VV1282 or 9620955VV1106). Elemental

analysis of this material showed 19.1 and 19.5% (w/w) carbon, respectively. From the manufacturer's prebonding surface area values, the bonding density was calculated to be $4.3 \mu\text{mol}/\text{m}^2$. This bonding density is consistent with a polymeric bonded phase [20]. Studies on a low-bonding-density packing obtained with monofunctional silane bonding chemistry were conducted with $10 \mu\text{m}$ Ultrapack ODS material obtained from Altex (Fullerton, CA, USA; lot number 81-2). Elemental analysis indicated 10.7% (w/w) carbon loading. Based on the manufacturer's prebonding surface area, the bonding density was calculated to be $2.5 \mu\text{mol}/\text{m}^2$. Studies with aqueous ethanol eluents were performed [32] on a monofunctional silane-derivatized commercial $150 \times 4.6 \text{ mm } 5.5 \mu\text{m}$ Zorbax ODS column (E.I. du Pont de Nemours, Wilmington, DE, USA). Elemental analysis and prebonding surface area indicated an octadecyl bonding density of $2.02 \mu\text{mol}/\text{m}^2$.

For chromatographic measurements the column was equilibrated to the desired temperature with at least 100 column volumes of mobile phase solvent prior to use. Initial equilibration at high temperature with each mobile phase composition was done to ensure stationary phase solvation equilibrium. All temperature studies were performed in sequence from high to low temperature. This procedure eliminated potential difficulties with stationary phase solvent entrapment as has been documented by other workers [39,41]. For every solute the average retention volume was obtained from between three and eight measurements. Retention volumes were corrected for flow-rate deviations from the nominal instrument setting of $1.00 \text{ ml}/\text{min}$ by volumetrically determining the flow-rate under every set of conditions. Retention volumes were also corrected for extra column volume prior to calculation of capacity factors.

Mobile phase volumes, V_m , were determined by the method of linearization of the retention of an homologous series of n -alcohols ($n = 3$ to 9) by minimization of the χ^2 function [48]. The V_m values were determined under all conditions and it was found that no determinate variation in V_m with temperature was detectable. Based upon this, average V_m values and their variances were

calculated for each column as the mean and variance of all values at a given mobile phase composition. This resulted in an increase in V_m relative standard deviation by roughly a factor of six relative to individual V_m measurements.

The precision of individual $\ln k'$ values were determined by propagation of error of the retention volume, V_r , and V_m variances through the capacity factor calculation. Error bars at 90% confidence representative of each set of conditions are shown for the highest temperature data point in each accompanying figure. Nominal variance was observed to be 0.0074 with an average of four repeat measurements.

Linear segments of Van 't Hoff plot data were determined by least squares linear regression. Individual $\ln k'$ data points were assigned to particular linear segments based upon their contribution to non-determinate variation in subsequent temperature data points. In some plots additional linear segments are indicated as a guide for the reader in following the data trends. Only the deviation from the first high temperature segment was considered for interpretation of solute retention interactions.

Efficiency calculations were based upon a gaussian peak shape model using the peak width at half height [51].

3. RESULTS AND DISCUSSION

3.1. Thermodynamics of chromatography

The basis of separation in RPLC is the distribution of solute molecules between the flowing mobile phase and the stationary phase on the support particle surfaces. Thermodynamic quantities of the enthalpy (ΔH) and entropy (ΔS) of the retention process can be related to experimentally measured quantities by the Van 't Hoff equation.

$$\begin{aligned} \ln k' &= \ln\left(\frac{V_r - V_m}{V_m}\right) \\ &= -\left(\frac{\Delta H}{R}\right) \frac{1}{T} + \left[\frac{\Delta S}{R} + \ln\left(\frac{V_s}{V_m}\right)\right] \end{aligned}$$

The logarithm of the solute capacity factor k' is thus linearly related to ΔH and ΔS by the

reciprocal absolute temperature T , where R is the gas constant and V_r , V_m , and V_s are the chromatographic retention volume, mobile phase volume and stationary phase volume, respectively. Unfortunately, the important quantity of the entropy change of the retention process is convolved with a quantity whose value is difficult to determine accurately, namely the volume of the stationary phase, V_s . No attempt was made to estimate V_s to extract ΔS for the studies described in this review.

In order to make comparisons of RPLC thermodynamic data, the determination of accurate mobile phase volume is critical. The major difficulty in the determination of V_m in RPLC results not so much from the method employed, as it does from a difficulty in defining the value being sought. This arises from the fact that in RPLC the interface between the mobile and stationary phases is not clearly defined. The stationary phase composition and volume vary with the type and length of the bonded alkyl moiety, and the type and concentration of the solvents used as the mobile phase [29–31], and thus V_m varies as well. In addition, since accurate thermodynamic data are desired, consideration must be given to the measurement of V_m under conditions which do not perturb the system in a manner different from that of the rest of the experimental probe solutes. A method must be chosen which determines the appropriate V_m for the system under study based upon the assumptions of the model being employed. For these reasons the method of linearization of retention data of an homologous series was chosen for V_m determination in these studies.

As an alternative example, Sentell and Dorsey [9] used the maximum mobile phase volume (V_{\max}) determined gravimetrically for calculation of RPLC phase ratio (V_s/V_m). This was appropriate for their experimental investigations of the relationship between solute distribution coefficient and surface bonded phase density relative to Dorsey and Dill's [6,23] lattice model. That is, V_{\max} is consistent with the assumptions of the stationary phase lattice model. This model dependence is recognized in their temperature studies [44,45] which employed V_m determined

with $2\text{H}_2\text{O}$ [17]. The V_{\max} value would overestimate V_m with the different assumptions of the Van 't Hoff equation used in the experiments described in the current review.

As the Van 't Hoff equation states, if the type of retention interactions are constant over a given temperature range (*i.e.*, the equilibrium distribution of a retained solute can be described by a single distribution coefficient, K , and $\Delta G = -RT \ln K$ applies), then a linear plot of $\ln k'$ versus $1/T$ will be obtained. However, if a change in retention interactions occurs in the temperature range measured, then non-linearity will be observed. This non-linearity may range from smooth curvature [44,45] to a discontinuity between two linear segments [25]. This type of behavior has been used extensively in gas chromatography to study phase transitions [52]. A form of this behavior was discussed by Nahum and Horváth [13] in which two constant retention interactions were assumed to exist over a temperature range, but with their relative proportion of contribution to retention changing as a function of temperature. This effect however can produce either positive curvature or linearity, but not negative deviations as are conventionally observed in phase transition behavior [52].

3.2. Solvation interactions

When a solute molecule is solvated by a bulk liquid solvent, a solvation sphere of solvent molecules forms around the solute molecule [53,54]. That is, the molecules arrange themselves with a degree of order which provides a minimum in the total potential energy of the system. The degree of organization and long-range order of this solvation sphere depends upon both the strength and the directionality of the intermolecular interactions. When the solute moves from the mobile phase solvent to the stationary phase, the formation of a classical solvation sphere is no longer possible because the surface solvated layer is not a classical liquid with all three degrees of freedom. The formation of a different arrangement of solvent molecules and bonded hydrocarbon chains induced by the presence of the solute molecule would nonethe-

less occur [55] because of the change in the position of the minimum in the potential energy of the collective molecular interactions.

3.3. Comparison of bonded-phase materials, LiChrosorb RP-18 and Ultrapack ODS

Prior to consideration of the thermal response of chromatographic retention on the 4.3 and 2.5 $\mu\text{mol}/\text{m}^2$ octadecylsilane (ODS) bonded silicas, a comparison of the isothermal retention and selectivity is appropriate with methanol–water or acetonitrile–water mobile phases. For comparison purposes, retention data is described at 50°C, well above any changes in the stationary phase. Based upon the comparison of k' values, no difference in these materials was detected within experimental precision, regardless of solvent type and concentration or solute. The selectivity values with respect to benzene ($\alpha = k'_X/k'_{\phi H}$), shown in Table 1, indicate a slight enhancement in selectivity of polar solutes relative to benzene on the 2.5 $\mu\text{mol}/\text{m}^2$ ODS material.

A more informative difference between the two materials was found if the ΔG per methylene unit for the homologous series of n -alcohols was considered. Fig. 1 shows a plot of ΔG_{CH_2} versus percentage organic solvent (v/v) in the mobile phase. These plots indicated that methanol (MeOH) can alter the free energy of interactions

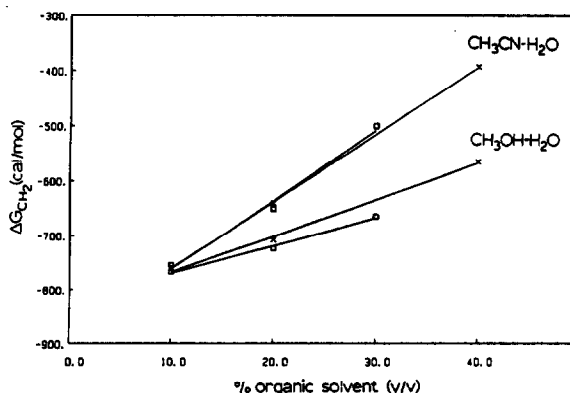


Fig. 1. Free energy of retention per methylene unit as a function of mobile phase composition for aqueous MeOH and MeCN mobile phases on (\square) 4.3 $\mu\text{mol}/\text{m}^2$ and (\times) 2.5 $\mu\text{mol}/\text{m}^2$ ODS-bonded silicas.

associated with a hydrocarbon chain less on the lightly loaded silica and less than in acetonitrile (MeCN). MeCN showed no difference in its effects on these interactions as a function of the surface-bonding density on these materials. These effects are related to the relative interaction of a hydrogen-bond-donating solvent and a dipolar solvent with the silica surface and with the bonded hydrocarbon [55]. In general, the slight differences between these packings was related to both the bonded-hydrocarbon density and the difference in accessibility of the silica surface to both solutes and solvent.

TABLE 1

SOLUTE SELECTIVITY $\alpha = k'_X/k'_{\phi H}$ ON 4.3 $\mu\text{mol}/\text{m}^2$ ODS BONDED SILICA AT $T = 50^\circ\text{C}$ ($\phi = \text{C}_6\text{H}_6$)

Mobile phase	ODS ($\mu\text{mol}/\text{m}^2$)	ϕOCH_3	ϕOH	$\phi\text{CH}_2\text{OH}$	$\phi(\text{CH}_2)_3\text{OH}$	ϕNH_2
<i>Methanol–water</i>						
10:90	4.3	1.41	0.21	0.27	1.73	0.19
	2.5	1.43	0.21	0.28	1.84	0.18
20:80	4.3	1.34	0.19	0.26	1.36	0.21
	2.5	1.19	0.19	0.23	1.25	0.17
<i>Acetonitrile–water</i>						
10:90	4.3	1.26	0.20	0.20	1.10	0.20
	2.5	1.24	0.19	0.18	1.03	0.15
20:80	4.3	1.05	0.18	0.15	0.60	0.18
	2.5	1.03	0.17	0.13	0.54	0.15

3.4. Conformation change of the bonded octadecylsilane groups

Different molecular probes should provide different sensitivity to a change in the thermally induced conformation and solvation changes in the stationary phase depending upon their ability to participate in the interactions associated with the change. In the experimental studies described, the effect of altering the conformation and mobility of the octadecyl-bonded groups on the retention interactions of various solutes was examined. If the stationary phase was well solvated by a high concentration of an organic solvent, then these variable interactions would be expected to be damped out by the mobility provided by solvation. These experiments were therefore carried out at low percentage organic modifier in order to provide relatively poor bonded-phase solvation and improve the interactions of probe solutes with the stationary phase changes. For well-solvated monomeric ODS-bonded silica, thermally induced non-linear changes in non-polar solute retention have been observed only on densely bonded materials [25,44,45]. This is consistent with the expected decreased ability of both solvents and solutes to intercalate between the increasingly ordered bonded chains with increasing surface-bonding density [6,23].

3.4.1. Benzene retention

Under conditions of poor solvation on a silica material with high bonded-ODS coverage ($4.3 \mu\text{mol}/\text{m}^2$), a non-polar probe would be expected to provide the best sensitivity to any change in the stationary phase hydrocarbon moieties. Therefore, the chromatographic response of benzene is considered first, as shown in Fig. 2. At high temperature the linear behavior predicted by the Van 't Hoff equation was observed; but, as the thermal energy of the system was decreased, a deviation in the response to lower than expected retention was observed.

A smooth curvature in Van 't Hoff plot data for benzene retention was observed by Cole *et al.* [45] on densely bonded ODS silica with poor solvation in *n*-propanol–water (5:95, v/v) eluent. It was postulated that this retention non-linearity

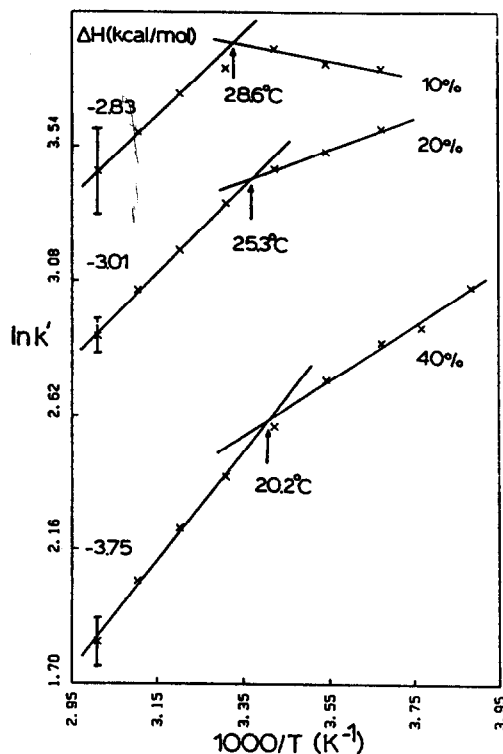


Fig. 2. Chromatographic retention of benzene as a function of temperature in 10, 20 and 40% (v/v) MeOH in water mobile phases on $4.3 \mu\text{mol}/\text{m}^2$ ODS-bonded silica. From ref. 49.

was associated with the minimum in aqueous solubility for benzene in pure water observed at 20°C [56]. This does not, however, explain the decrease in slope change and shift to lower temperature of the retention maximum with increasing ODS bonding density also observed [45]. Neither does the hydrophobic solubility argument account for the enhanced shape selectivity with decreasing temperature observed for polycyclic aromatic hydrocarbons on well-solvated ODS-bonded silica [21,25]. An alternative explanation of non-linear RPLC Van 't Hoff plot data will be presented in the current discussion which also takes into account the retention non-linearity of a variety of solutes of differing polarity and varying solvation effects.

It is postulated [25,43,44,47] that the non-linearity observed in Van 't Hoff plots is related to an alteration in the retention interactions with temperature. This change is produced by the

changing equilibrium surface conditions associated with a change in molecular conformation and mobility of the bonded ODS moieties. Therefore, the Van 't Hoff equation actually fails at the onset of this thermally induced conformational change. In gas chromatographic studies, equilibrium stationary phase conditions may be obtained at both high and low temperatures [52]. These regions are separated by a nonequilibrium transition region in which retention interactions are changing with temperature. Due to the limited temperature range available to RPLC systems, imposed by the freezing point of the mobile phase, a second equilibrium condition is not attainable at low temperature. For this reason it is not appropriate to attempt to calculate the enthalpy of retention at temperatures below the transition point. This was clearly

evidenced by the data points below 28°C in MeOH–water (10:90) eluent in Fig. 2, which would provide a positive ΔH .

For comparison purposes, a deviation onset temperature was assigned to the non-linear Van 't Hoff plots by calculating the best two linear segments for the high- and low-temperature branches. As the concentration of organic solvent in the mobile phase was increased, two effects on the non-linear behavior were observed. The temperature of the deviation onset decreased, indicating a dependence upon the composition of the surface solvation layer. And the magnitude of the deviation as measured by the change in slope ($-R\Delta$ slope) decreased (Table 2) indicating that better solvation attenuated the effect of the surface change with respect to solute interactions.

TABLE 2

VAN 'T HOFF PLOT PARAMETERS FOR SOLUTES ON 4.3 $\mu\text{mol}/\text{m}^2$ ODS BONDED SILICA

Mobile phase		ϕH	ϕOCH_3	ϕOH	$\phi\text{CH}_2\text{OH}$	$\phi(\text{CH}_2)_2\text{OH}$	$\phi(\text{CH}_2)_3\text{OH}$	$\phi(\text{CH}_2)_4\text{OH}$	ϕNH_2
<i>Methanol–water</i>									
10:90	T (°C)	28.6	32.8	22.1	35.3	35.0	35.5		
	ΔH (kcal/mol)	-2.83	-4.15	-3.78	-3.50	-3.91	-4.80		-3.72
20:80	$-R\Delta$ slope	-3.35	-1.84	-1.71	-1.33	-1.67	-1.72		+0.11
	T (°C)	25.3	26.3	16.8	26.5	25.5	25.9	28.5	None
40:60	ΔH (kcal/mol)	-3.01	-4.36	-3.65	-3.60	-4.08	-4.98	-5.63	-3.96
	$-R\Delta$ slope	-1.88	-1.15	-1.19	-1.16	-1.70	-1.74	-1.71	0.0
40:60	T (°C)	20.2	15.6	10.3	43	42	43	41	30.5
	ΔH (kcal/mol)	-3.75	-4.46	-3.78	-5.50	-5.44	-5.94	-6.52	-4.82
40:60	$-R\Delta$ slope	-1.96	-0.64	-0.89	-1.15	-2.18	-2.04	-2.19	+0.42
	<i>Acetonitrile–water</i>								
10:90	T (°C)	32.2	34.6	36.5	30.3		34.2		None
	ΔH (kcal/mol)	-3.35	-4.21	-4.10	-3.41		-4.49		-3.81
20:80	$-R\Delta$ slope	-2.57	-1.80	-1.01	-2.02		-1.67		0.0
	T (°C)	31.7	35.7	29.6	30.6		27.3		None
40:60	ΔH (kcal/mol)	-3.66	-3.89	-3.49	-2.59		-3.59		-3.05
	$-R\Delta$ slope	-1.86	-0.94	-0.80	-1.12		-1.33		0.0
40:60	T (°C)	31.8	35.7	None	35		35		None
	ΔH (kcal/mol)	-2.79	-2.94	-2.68	-2.19		-2.11		-2.53
40:60	$-R\Delta$ slope	-0.79	-0.63	0.0	-0.59		-0.82		0.0

3.4.2. Methoxybenzene and phenol

Probing the stationary phase with a slightly more polar methyl ether solute, methoxybenzene, displayed similar behavior to benzene in that negative deviations were observed (Fig. 3A). The magnitude of the deviation was less than was observed for benzene, as would be expected for a more polar probe sensing a change in the non-polar interactions of the stationary phase. The deviation temperature also decreased with increasing percentage organic solvent, but at a greater rate than for benzene due to the greater participation of the ether with the changing solvation interactions. The still more polar solute phenol, which is a hydrogen-bond donor, gave different results (Fig. 3B). Again the deviation magnitude was less than for benzene, but similar to methoxybenzene. The deviation temperature was shifted to lower values than benzene by 7 to 10°C, but the rate of change with mobile phase concentration was similar to benzene. The fact that phenol, which is completely soluble in both mobile phase components, exhibited similar Van 't Hoff non-linearity to benzene presents strong evidence

against the hydrophobic model of temperature-dependent retention changes [45].

At this point the response of these three solute probes in terms of the previously established models of a solvated surface is considered. As the temperature is decreased, the molecular flexibility of long-chain hydrocarbons decreases, such that the molecules become elongated and rigid, having fewer bends and kinks [47]. Also, the carbon atoms with the least mobility are those close to the anchoring site at the silica surface [29,57]. As the solute molecules intercalate themselves between the solvated bonded hydrocarbon chains in the retention process [7,8], they reside, on the average, in the region of the solvated layer which provides the most favorable interactions. The retention location of the three solutes can be discussed in a simplified picture of the stationary phase. Consider first the behavior of the system at high temperature. Benzene finds its most favorable interactions through Van der Waals and dipole-induced dipole forces [26] near the central region of the layer where a minimum in polarity should exist. Further penetration to near the silica surface would

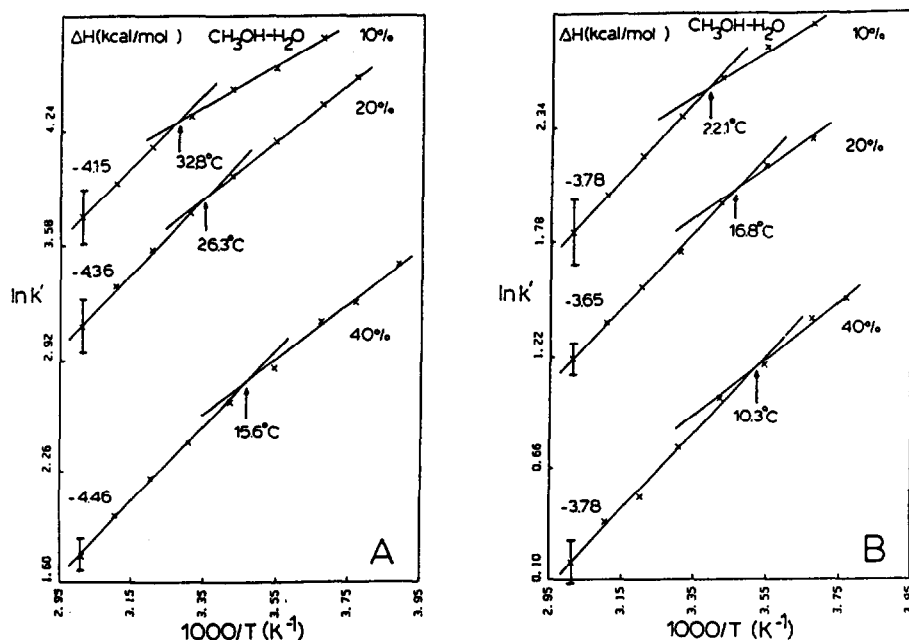


Fig. 3. Chromatographic retention of (A) methoxybenzene and (B) phenol as a function of temperature under the same conditions as Fig. 2. From ref. 49.

be enthalpically less favorable because of the increased polarity near the adsorbed water layer and entropically less favorable due to increased bonded chain ordering [6,23,46]. Methoxybenzene resides in nearly the same region as benzene, but will be oriented to keep its polar ether group nearer a higher polarity region. The location of phenol molecules will be on the average in the higher regions of the solvated layer, with the hydroxyl group oriented toward the mobile phase. In this location, phenol can find both non-polar interactions with the solvated hydrocarbon chains and more favorable hydrogen-bonding interactions with either methanol or water in a less restricted environment than near the silica surface. Although better hydrogen-bonding interactions may be available nearer the adsorbed water layer, competition with the methanol solvent molecules, which are in much higher concentration, would restrict the availability of these interactions.

Upon reducing the thermal energy of the bonded hydrocarbon groups, the chains lose mobility and become more rigid [23,47]. This restricted motion is expected to move up from the anchored end of the molecule as the temperature decreases [23,29,37]. Thus, as the restricted region extends into the location of retention of a particular solute, the retention behavior of that solute changes. As the temperature of the system is reduced and restriction in chain mobility moves up from the silica surface, the retention of solutes retained deep in the stationary phase, such as benzene, is affected first. It is not until more thermal energy is removed and nearly the entire length of the hydrocarbon chain is restricted, that the retention of solutes retained higher in the solvated layer, such as phenol, are affected. This description will be refined and supported by consideration of solute retention under additional RPLC conditions.

In the same manner that solute access is restricted by the reduced mobility of the hydrocarbon chains, solvent access and mobility between the chains must also be restricted. The polarity gradient between the chains, *i.e.* the solvation layer composition [33], is therefore expected to be altered by this restriction. It will

be shown, when the retention data for MeCN solvated systems are considered, that it is this change in interchain polarity gradient that is associated with the mobile phase concentration dependence of the deviation temperature. This polarity gradient is also responsible for the difference in the rate of change of the deviation temperature with solvent concentration for two solutes which are retained in similar regions of the stationary phase, such as benzene and methoxybenzene.

3.4.3. Retention behavior of hydrogen-bond-accepting solutes, amines

In order to determine the effect on the accessibility of the near silica surface accompanying the bonded-hydrocarbon conformation and solvation-layer changes, hydrogen-bond-accepting solutes, aniline and N-methylaniline, were used as stationary phase probes. It has been proposed that the retention of this type of solute is greatly affected by the availability of the silica surface, because of strong hydrogen-bonding interactions with acidic silica sites [12]. Quite different Van 't Hoff plot behavior was observed for all conditions in the retention of these solutes (Fig. 4). At MeOH–water (10:90), a deviation between two lines of nearly equal slope was barely discernable between 30 and 40°C. Linear behavior was observed at 20% MeOH with a good deal of scatter in the data due to asymmetric elution profiles. Under these conditions aniline was unaffected by the surface change detected by other solutes.

The most interesting behavior occurred at 40% MeOH for aniline and N-methylaniline (Fig. 4B). Linear high-temperature response was followed by a sharp positive deviation. This indicated that more favorable interactions were available at low temperature. These data can be used to improve the detail in the stationary phase retention model developed thus far. If the strong hydrogen-bonding interactions of basic solutes dominate retention interactions, then these solutes should be retained deep in the stationary phase near the adsorbed water layer on the silica surface. Upon lowering the temperature and inducing a change in conformation and mobility of the bonded hydrocarbon chains,

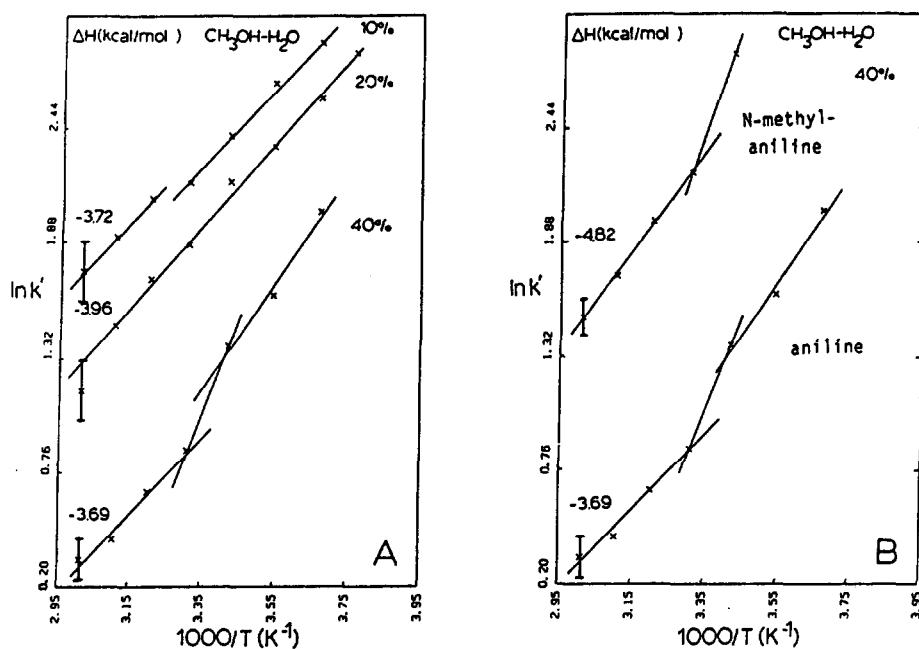


Fig. 4. Chromatographic retention of (A) aniline and (B) comparison with N-methylaniline as a function of temperature under the same conditions as Fig. 2. From ref. 49.

the interchain polarity gradient of the solvation layer should decrease. Since these solutes are still attracted to the silica surface by strong hydrogen-bonding interactions, the local solvation environment should become enthalpically more favorable for solvation of the phenyl ring. That is, unlike the previous solutes, the interaction location does not change significantly with the stationary phase mobility change. However, because of the increasingly restricted motion near the silica surface, the entropic contribution is less favorable. Thus at 10% MeOH, the data indicate little solvent polarity change, but a restriction in surface accessibility. At 20% no change was detectable for aniline and at 40% both a decrease in interchain solvent polarity and a restriction in access occurred.

3.4.4. Relative effects of alkyl and hydrogen-bonding interactions, phenyl alcohols

The effect of incremental change in the hydrocarbon character of probe solutes was investigated through the comparison of a series of *n*-phenyl alcohols, benzyl alcohol to 4-phenyl-1-butanol (Fig. 5). As seen in the data of Table 2, for each mobile phase concentration the

transition temperature and slope change were relatively constant for all but benzyl alcohol, despite observation of the expected increase in magnitude of the high-temperature ΔH with increasing number of methylene units. This indicated that the *n*-phenyl alcohols are all retained at similar depth of penetration into the stationary phase. With 40% MeOH eluent the retention behavior of all of these solutes differed from that observed at lower solvent concentration. The non-linear deviation was shifted to higher temperature. This shift in deviation temperature to higher values is consistent with the deeper penetration of the solutes into the stationary phase associated with improved solvation and their ability to participate in both non-polar interactions with the ODS chains and hydrogen-bonding interactions with solvent components. The model of retention interactions of these solutes will be refined when kinetic data is considered.

3.5. Bonded phase surface coverage effects

The contribution of the average surface coverage of the bonded ODS groups on the silica surface is considered next. With the commercial-

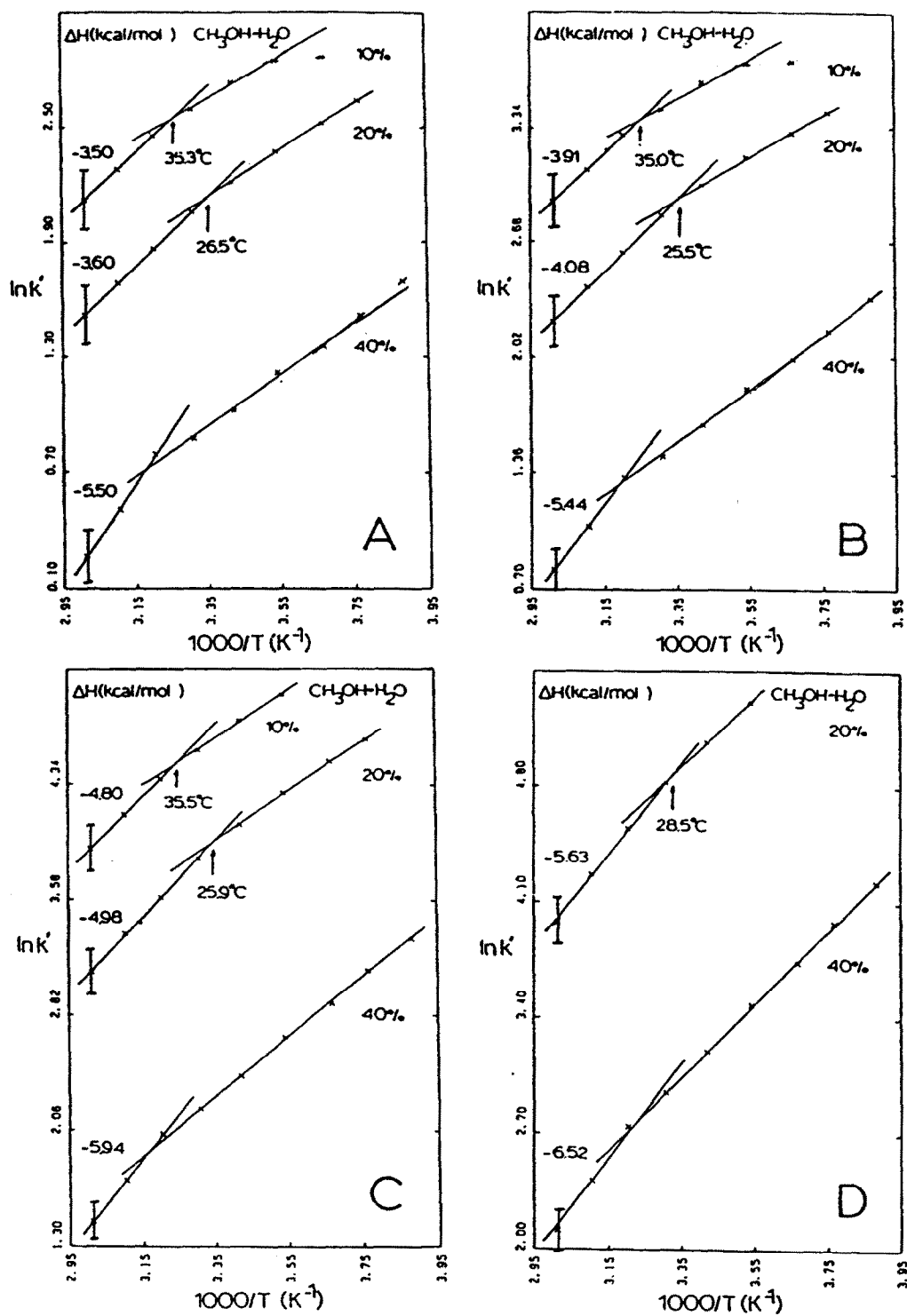


Fig. 5. Chromatographic retention of (A) benzyl alcohol, (B) phenethyl alcohol, (C) 3-phenyl-1-propanol and (D) 4-phenyl-1-butanol as a function of temperature under the same conditions as Fig. 2.

ly prepared ODS-bonded materials employed, this factor includes the complementary contributions to the stationary phase structure of average spacing between bonded groups, retentive capacity of the total hydrocarbon loading, and accessibility of hydrogen-bonding interactions near the silica surface. In these experiments the use of a more efficient column with $2.5 \mu\text{mol}/\text{m}^2$ ODS also allowed some investigation of the kinetic ramifications of the stationary phase conformational change, as will be discussed later.

For non-polar solutes the dependence of retention behavior on the surface reorganization for a $2.5 \mu\text{mol}/\text{m}^2$ ODS-bonded silica (Fig. 6A) was as would be predicted from the model established on the $4.3 \mu\text{mol}/\text{m}^2$ ODS material. The rate of increase in ΔH of retention with increasing percent MeOH was similar to that observed on the more heavily bonded silica. However, the increased access of benzene penetration into the more lightly bonded stationary phase provided marginally more favorable enthalpic interactions. In addition, the rate of decrease in deviation temperature with increas-

ing percent MeOH was more than twice that observed on the more heavily bonded silica. This is consistent with the expected increased mobility and solute penetration on the more lightly bonded surface.

For the hydrogen-bond-donating solute phenol, the response to the surface reorganization changed as a function of ODS surface coverage (Fig. 6B). On the silica material having fewer bonded groups, the same temperature range affected the retention of both phenol and benzene. This indicated that, as the average distance between chains was increased, phenol could penetrate deeper into the stationary phase, finding its strong hydrogen-bonding interactions in a less restricted environment.

Major differences in the retention behavior of hydrogen-bond-accepting solutes were observed as a function of ODS surface coverage. A reduction in the entropic retention deviation for aniline was observed (Fig. 7A). At the lower percentages of MeOH, aniline displayed a slope change similar in magnitude to phenol. At higher percent MeOH, a negative deviation from linearity was observed, unlike the trend toward

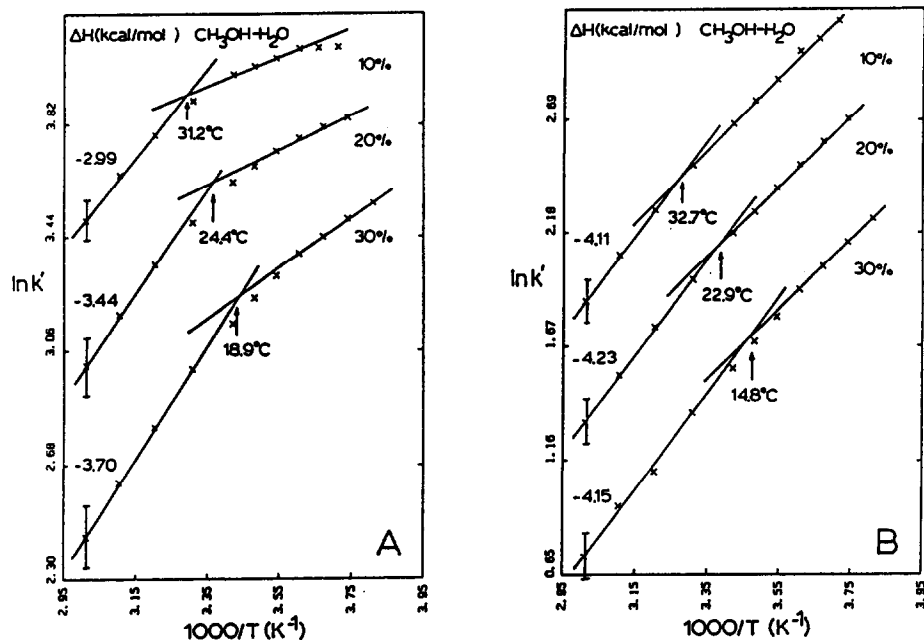


Fig. 6. Chromatographic retention of (A) benzene and (B) phenol as a function of temperature in 10, 20 and 30% (v/v) MeOH in water mobile phases on $2.5 \mu\text{mol}/\text{m}^2$ ODS-bonded silica.

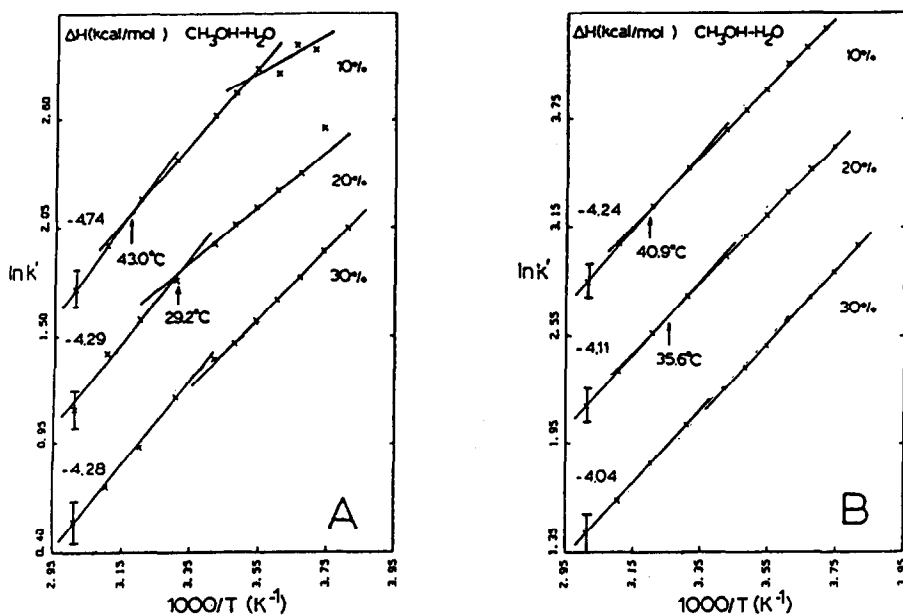


Fig. 7. Chromatographic retention of (A) aniline and (B) benzylamine as a function of temperature under the same conditions as Fig. 6.

positive deviation on the $4.3 \mu\text{mol}/\text{m}^2$ ODS-bonded silica. This was indicative of the change in accessibility accompanying the ODS conformation change on the more lightly bonded silica. Benzylamine provided a perspective on the contribution of hydrocarbon interactions in the region of the stationary phase sampled by amines (Fig. 7B). Deviations from linearity were so small as to be within the precision of the data. Apparently, the addition of one methylene unit produced a probe solute which found stable interactions despite the conformation change, while still probing a sterically restricted region. The major difference accompanying a change in bonded-group coverage was in the amount of restriction between the bonded chains and not necessarily the interactions of the ODS groups themselves. This behavior will be examined further in other solvent systems.

3.6. Solvent interactions

3.6.1. Acetonitrile–water eluents

In order to determine the effect of the type of solvation interactions available from the organic solvent component of the mobile phase, the

same ODS-bonded silicas and solute probes were studied with acetonitrile as well as methanol. MeOH is a dipolar solvent with good hydrogen-bond-donating capability, whereas MeCN is a dipolar solvent with only weak hydrogen-bond-accepting ability [58]. A useful comparison between these two solvents is the high-temperature ΔH of retention. Since the retention of a solute decreases with increasing concentration of organic solvent in the mobile phase, its free energy of retention must also become less negative (assuming that the phase ratio is relatively constant or increases). In general, the ΔH of retention also followed this trend for MeCN mobile phases. With MeOH mobile phases however, the opposite trend was observed with ΔH becoming more negative with increasing concentration (Tables 2 and 3). Notable exceptions were amines on the more lightly bonded packing which showed less negative ΔH values and phenol on both packings which showed relatively constant ΔH . These exceptions will be discussed in detail later. In general, these observations indicated that for poorly solvated systems, the entropic contribution to retention must be more important in MeOH than in MeCN solvated

TABLE 3

VAN 'T HOFF PLOT PARAMETERS FOR SOLUTES ON 2.5 $\mu\text{mol}/\text{m}^2$ ODS BONDED SILICA

Mobile phase		ϕH	ϕOCH_3	ϕOH	$\phi\text{CH}_2\text{OH}$	$\phi(\text{CH}_2)_2\text{OH}$	$\phi(\text{CH}_2)_3\text{OH}$	ϕNH_2	$\phi\text{CH}_2\text{NH}_2$
<i>Methanol-water</i>									
10:90	T ($^{\circ}\text{C}$)	31.2	27.3	32.7	27.3	29.6	30.4	43.0	40.9
	ΔH (kcal/mol)	-2.99	-3.89	-4.11	-3.55	-3.90	-5.00	-4.74	-4.24
	$-R\Delta$ slope	-2.06	-1.20	-1.06	-1.12	-1.19	-1.77	-0.74	-0.32
20:80	T ($^{\circ}\text{C}$)	24.4	22.2	22.9	25.0	24.7	27.8	29.2	35.6
	ΔH (kcal/mol)	-3.44	-4.04	-4.23	-3.62	-3.97	-5.03	-4.29	-4.11
	$-R\Delta$ slope	-2.33	-0.90	-1.09	-1.07	-1.37	-1.56	-1.42	-0.25
30:70	T ($^{\circ}\text{C}$)	18.9	20.0	14.8	25.5	19.7	25.0	35	25
	ΔH (kcal/mol)	-3.70	-4.42	-4.15	-3.89	-4.07	-4.97	-4.28	-4.04
	$-R\Delta$ slope	-2.03	-1.20	-1.03	-1.25	-1.38	-1.14	-0.78	+0.02
<i>Acetonitrile-water</i>									
10:90	T ($^{\circ}\text{C}$)	26.2	29.3	25.1	31.5	28.7	30.3	26.9	31.1
	ΔH (kcal/mol)	-3.33	-4.00	-4.01	-3.01	-3.37	-4.15	-3.24	-3.61
	$-R\Delta$ slope	-2.58	-1.71	-1.36	-1.28	-1.70	-1.71	-1.18	-1.28
20:80	T ($^{\circ}\text{C}$)	25.6	29.9	25.4	32.7	25.8	24.1	22.7	25.6
	ΔH (kcal/mol)	-3.52	-3.83	-3.60	-2.27	-2.55	-2.99	-2.73	-3.09
	$-R\Delta$ slope	-1.62	-1.01	-1.16	-0.96	-1.43	-1.71	+0.19	-0.63
30:70	T ($^{\circ}\text{C}$)	25.4	31.9	45	30	29.0	22.2	35	20
	ΔH (kcal/mol)	-2.94	-3.25	-3.44	-1.71	-1.72	-1.89	-2.37	-2.76
	$-R\Delta$ slope	-0.57	-0.53	-0.28	-1.16	-1.21	-1.09	-0.01	+0.17

stationary phases. The relative entropic contribution between these two solvents has also been shown to be quite variable over wide eluent composition ranges at elevated temperatures [55].

In general, the dependence of retention behavior on the temperature-induced stationary phase conformation change was very similar for MeCN and MeOH mobile phases. There were, however, certain notable exceptions. For benzene on the 4.3 $\mu\text{mol}/\text{m}^2$ ODS-bonded silica (Fig. 8A), negative deviations with the slope change decreasing in magnitude were observed as the percentage of organic solvent was increased (Table 2). However, the deviation temperature was nearly constant. It appears that the deviation temperature was a function of the change in the solvation layer polarity gradient of the stationary phase accompanying the con-

formational change, the magnitude of which appeared to be much greater for MeOH than for MeCN solvated systems.

With MeCN modifier, the hydrogen-bond-donating solute phenol (Fig. 8B) showed a deviation temperature dependence similar to non-polar solutes. However, at 40% MeCN no discernable deviation from linearity was observed. Unlike its behavior in MeOH, phenol did not have to compete with MeCN in the same manner for hydrogen-bonding sites and the amount of water in the stationary phase solvation layer was greatly reduced [28,30]. Phenol could thus be retained deeper in the stationary phase. This resulted in its retention being affected by the hydrocarbon conformation change at the same temperature as the non-polar solutes.

For the retention of aniline with MeCN mobile phases (Fig. 8C) regardless of the mobile phase

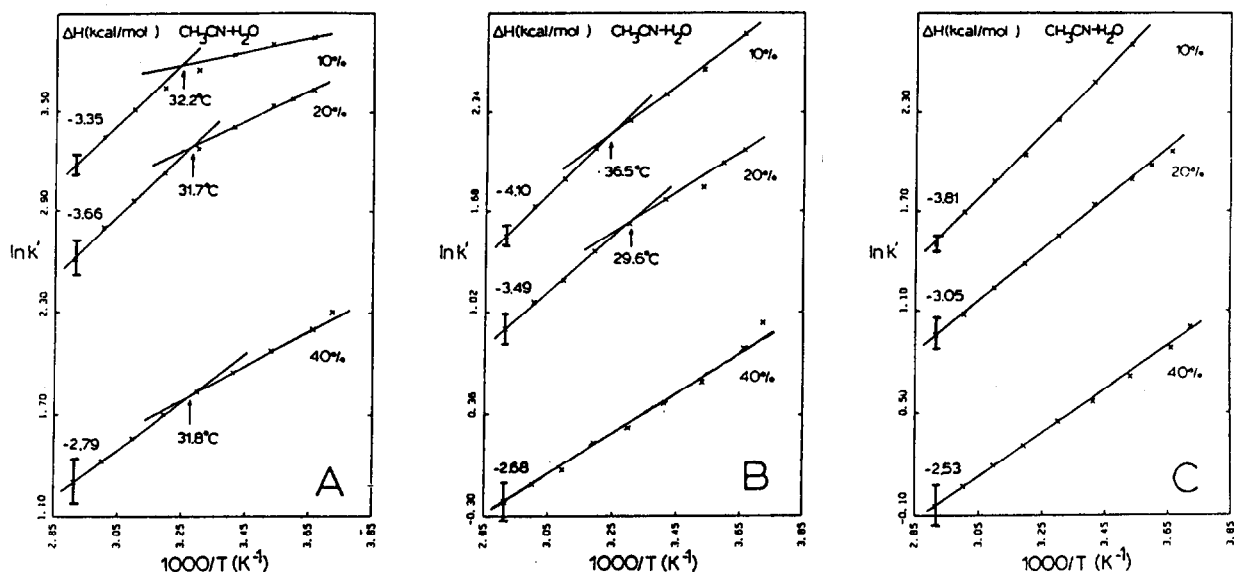


Fig. 8. Chromatographic retention of (A) benzene, (B) phenol and (C) aniline as a function of temperature in 10, 20 and 40% (v/v) MeCN in water mobile phases on $4.3 \mu\text{mol}/\text{m}^2$ ODS-bonded silica.

concentration or temperature, linear Van 't Hoff plots were obtained. That is, aniline did not probe the change in the bonded-hydrocarbon conformation at all. This indicated that the region probed by aniline was stable. The solvation layer composition must remain fairly constant in polarity and degree of organization near the surface regardless of the changes in the stationary phase higher up the hydrocarbon-bonded chains. In general, more favorable enthalpy values were measured for MeOH mobile phases. Amines thus probed a region of less favorable polarity and higher organization when MeCN was used to solvate the stationary phase.

For the $2.5 \mu\text{mol}/\text{m}^2$ ODS-bonded silica, as with the heavily loaded silica, a comparison of the high-temperature enthalpy of the retention process indicated a larger entropy contribution to retention in MeOH than in MeCN solvated systems. The non-polar solutes behaved in a similar manner on both of the ODS-bonded silicas. With MeCN mobile phases on the $2.5 \mu\text{mol}/\text{m}^2$ ODS-bonded silica, constancy in the deviation temperature with changing mobile phase concentration indicated a similar change in stationary phase solvation regardless of the background level (Fig. 9A). Phenol, however, presented a very informative response to the surface

conformation change at higher concentrations of MeCN on the two bonded phase materials (Figs. 8B and 9B). On the $4.3 \mu\text{mol}/\text{m}^2$ ODS material in 40% MeCN, no deviation was discernable. However, for phenol at 30% MeCN on the lightly loaded material, a large entropic change occurred. It has been seen so far that a decrease in entropic effects accompanied the change to the $2.5 \mu\text{mol}/\text{m}^2$ material. It is proposed that phenol penetrates deeply into the stationary phase in MeCN solvated systems in the absence of hydrogen-bonding solvent competition. Previously, no information was available on whether or not phenol oriented itself to hydrogen-bond with the adsorbed-water layer, deep in the stationary phase. These additional data on the more lightly covered silica now appear to indicate that, in the absence of hydrogen-bonding solvent competition, when the steric restriction of closely spaced octadecyl chains was reduced, phenol could find the most favorable hydrogen-bonding interactions near the highly organized adsorbed-water layer. On silica with more tightly packed octadecyl chains, though hydrogen-bonding interactions were more favorable deeper in the stationary phase, both steric restriction and interactions with the bonded chains reduced the degree of orientation necessary for phenol to

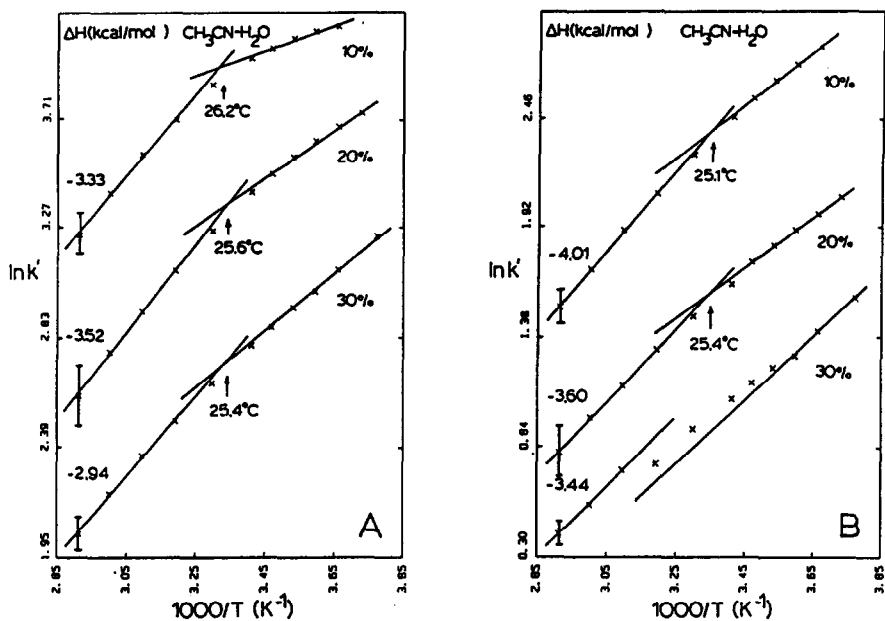


Fig. 9. Chromatographic retention of (A) benzene and (B) phenol as a function of temperature in 10, 20 and 30% (v/v) MeCN in water mobile phases on $2.5 \mu\text{mol}/\text{m}^2$ ODS-bonded silica.

find its most energetically favorable solvation interactions.

The behavior of aniline was unique on the $4.3 \mu\text{mol}/\text{m}^2$ ODS material in that complete lineari-

ty with no deviations was observed in all MeCN mobile phases studied. This behavior was interpreted in terms of a stable solvation layer in the near surface region with MeCN solvated

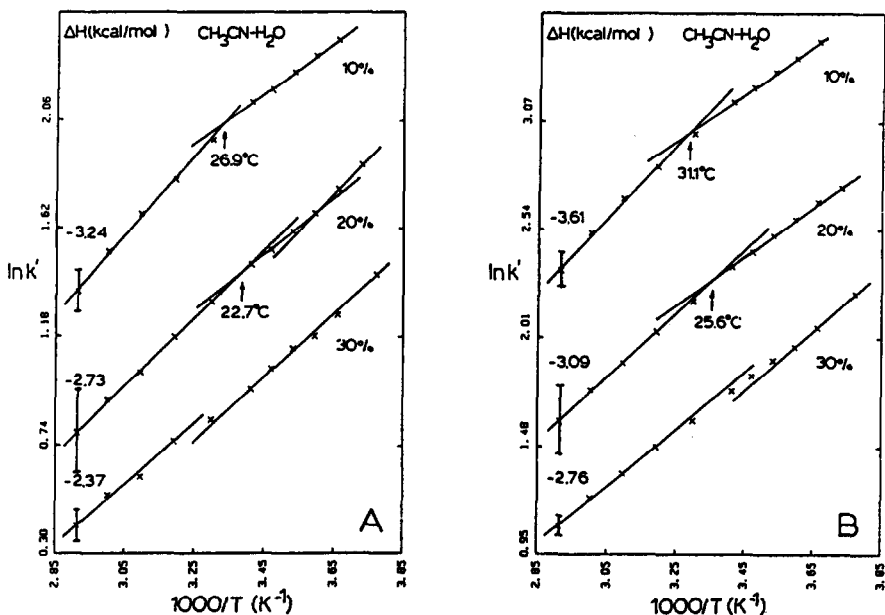


Fig. 10. Chromatographic retention of (A) aniline and (B) benzylamine as a function of temperature under the same conditions as Fig. 9.

systems. With these mobile phases, on the more lightly loaded silica, aniline and benzylamine (Fig. 10A and B) exhibit responses similar to those observed in MeOH mobile phases on the same material. An increase in entropic discontinuity deviations were found relative to the MeOH data. Benzylamine demonstrated an enhancement in both the slope-change magnitudes and the entropic effect at 30% MeCN. Overall, it appeared that in MeCN solvated systems the amines still probed a well-organized solvated region and the addition of alkyl character to the amine provided better interactions with the bonded chains. However, reduced octadecyl surface coverage provided for more variation in the solvation-layer composition very close to the silica surface accompanying the octadecyl conformation change.

3.6.2. Structuring solvent contributions, ethanol and THF

The effects on retention interactions and temperature-dependent stationary phase changes were investigated with ethanol–water mobile phases using a commercial ODS-bonded silica of $2.02 \mu\text{mol}/\text{m}^2$ coverage [32]. The Van 't Hoff plot data for these systems are summarized in Table 4. Temperature-dependent retention behavior differed in this solvent system in that, in

general, the enthalpy of retention passed through a maximum near 20% ethanol (EtOH). In addition, positive Van 't Hoff plot deviations were common when solvation was improved for all solutes participating in hydrogen-bonding interactions. Previously, positive deviations were only observed for amines. These positive deviations were interpreted in terms of the improved solvation and degraded entropic interactions associated with the stationary phase conformation changes. For this EtOH solvated lightly bonded system, apparently the organization in the stationary phase was increased with increasing EtOH concentration. This was most effectively probed by solutes which participated in hydrogen-bonding interactions. This was consistent with stationary phase isotherm studies of EtOH–water solvated ODS-bonded silica [32] carried out in a similar manner to that of Yonker and co-workers [28,30,31]. The amount of water incorporated into the stationary phase solvation layer was higher in EtOH–water mobile phases than when MeOH–water or MeCN–water was used and reached a plateau at approximately EtOH–water (30:70).

More dramatic positive Van 't Hoff plot deviation behavior was observed for every probe solute when aqueous THF mobile phases were employed (Figs. 11 and 12). Table 5 lists the Van

TABLE 4

VAN 'T HOFF PLOT PARAMETERS FOR SOLUTES IN EtOH–WATER ON ZORBAX ODS [32]

Mobile phase		ϕH	ϕCH_3	ϕOCH_3	ϕOH	$\phi\text{CH}_2\text{OH}$	$\phi(\text{CH}_2)_2\text{OH}$	ϕNH_2
<i>Ethanol–water</i>								
10:90	T (°C)	36.4	34.0	40.8	21.5	40.1	41.6	23.1
	ΔH (kcal/mol)	-3.5	-4.0	-4.5	-4.0	-3.9	-4.3	-3.2
	$-R\Delta$ slope	-2.6	-2.1	-1.2	-0.8	-1.2	-1.4	+1.0
20:80	T (°C)	23.6	24.9	None	None	23	None	18
	ΔH (kcal/mol)	-2.0	-4.8	-4.6	-4.3	-3.6	-4.0	-4.0
	$-R\Delta$ slope	-1.9	-2.3	0.0	0.0	-0.8	0.0	+0.3
40:60	T (°C)	6	None	None	25.1	38.4	16.8	39.2
	ΔH (kcal/mol)	-3.7	-4.5	-4.4	-3.2	-2.9	-3.4	-2.4
	$-R\Delta$ slope	-1.0	0.0	0.0	+1.6	+0.9	+1.6	+1.5

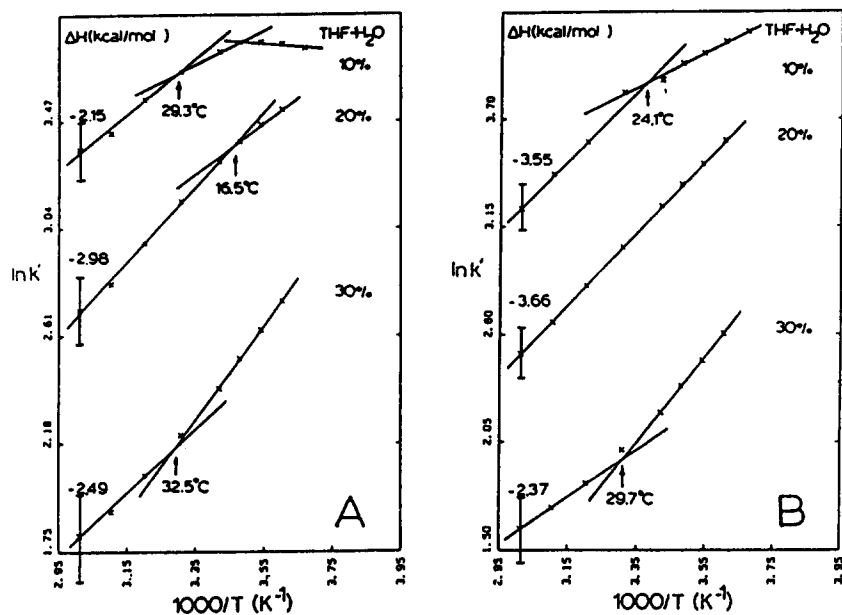


Fig. 11. Chromatographic retention of (A) benzene and (B) methoxybenzene as a function of temperature in 10, 20 and 30% (v/v) THF in water mobile phases on 4.3 $\mu\text{mol}/\text{m}^2$ ODS-bonded silica.

't Hoff plot data obtained with THF-water on the 4.3 $\mu\text{mol}/\text{m}^2$ ODS-bonded silica employed previously. THF differs from the other solvents considered in these discussions in that it is a

good solvent for the hydrocarbon-bonded moieties, whereas MeOH, EtOH, MeCN, and especially water are non solvents. In addition, Yonker and co-workers [28,30,31] showed that THF-sol-

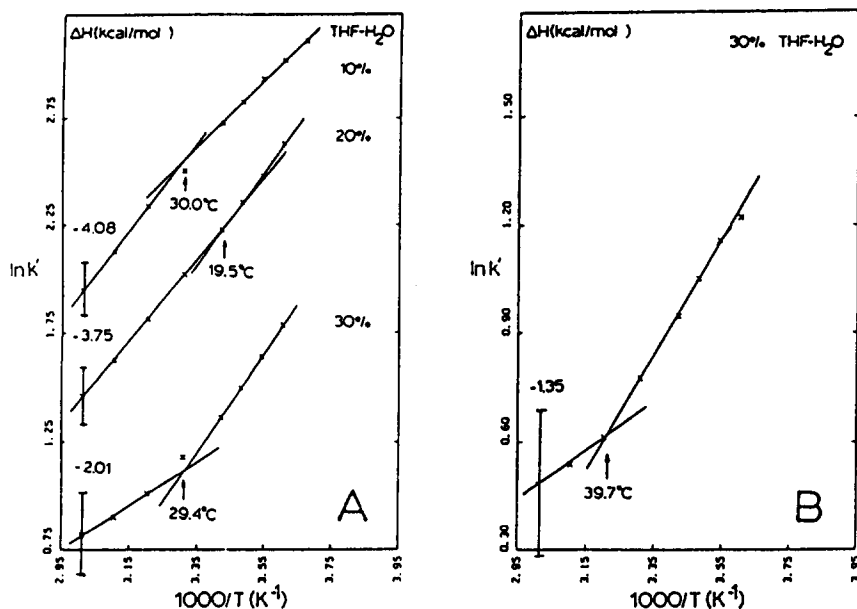


Fig. 12. Chromatographic retention of (A) phenol and (B) aniline as a function of temperature under the same conditions as Fig. 11.

TABLE 5

VAN 'T HOFF PLOT PARAMETERS FOR SOLUTES IN THF-WATER ON 4.3 $\mu\text{mol}/\text{m}^2$ ODS BONDED SILICA

Mobile phase		ϕH	ϕOCH_3	ϕOH	$\phi\text{CH}_2\text{OH}$	$\phi(\text{CH}_2)_3\text{OH}$	ϕNH_2
<i>THF-water</i>							
10:90	<i>T</i> (°C)	29.3	24.1	30.0	26.2	24.5	
	ΔH (kcal/mol)	-2.15	-3.55	-4.08	-2.47	-3.94	
	- <i>R</i> Δ slope	-2.41	-1.83	-0.92	-0.51	-0.89	
20:80	<i>T</i> (°C)	16.5	None	19.5	None	25.5	
	ΔH (kcal/mol)	-2.98	-3.66	-3.75	-2.45	-3.74	
	- <i>R</i> Δ slope	-0.96	0	+0.52	0	+0.60	
30:70	<i>T</i> (°C)	32.5	29.7	29.4	30.7	25.2	39.7
	ΔH (kcal/mol)	-2.49	-2.37	-2.01	-1.10	-2.64	-1.35
	- <i>R</i> Δ slope	+1.23	+1.96	+2.53	+2.00	+1.86	+1.84

vated ODS stationary phases contained much higher amounts of water than with either aqueous MeOH or MeCN eluents. The amount of water reached a maximum at approximately THF-water (40:60).

THF provided a new type of behavior which was described as a structuring solvent effect. Such a solvent appeared to enhance the entropic effects associated with the stationary phase change by forcing a highly organized structuring of the water molecules in the stationary phase. These chromatographic observations were supported by the freezing behavior of homogeneous aqueous solutions of THF. For aqueous MeOH or MeCN mixtures, the freezing point showed a monotonic decrease with increasing organic solvent concentration. For THF-water solutions, however, the freezing point passed through a minimum at approximately 7% THF and then increased to plateau at 4°C for solutions in excess of 15% THF [48].

It has been noted that the entropic contribution to retention was greater for MeOH than for MeCN mobile phase modifiers. This was concluded from the trend of the high-temperature ΔH of retention becoming more negative with increasing solvent concentration of MeOH in opposition to the trend of decreasing retention. Although this was a general trend for MeOH

mobile phases, only the more non-polar solutes demonstrated this behavior in THF mobile phases (Table 5). This placed the relative importance of entropic contributions to retention for THF solvated systems intermediate between MeOH and MeCN at high temperatures. The general retention behavior of THF mobile phases also indicated greater contributions of surface polar interactions. Unlike in MeOH and MeCN mobile phases, in which almost every solute showed a decrease in selectivity relative to benzene with increasing mobile phase concentration, THF-water mobile phases showed increasing selectivity values for phenol and benzyl alcohol (Table 6). The only solute with such behavior previously was aniline in MeOH-water mobile phases. In addition, aniline showed a very strong dependence of retention and enthalpy of retention on THF concentration along with highly asymmetric elution profiles.

The fact that benzene (Fig. 11A) displayed a positive retention deviation with decreasing temperature (*i.e.*, an improvement in stationary phase interactions) indicated a large dependence of non-polar interactions on the change in surface solvation accompanying the ODS conformation change in the THF solvated stationary phase. Aniline in THF-water (30:70) mobile phase (Fig. 12B) displayed a sharp positive

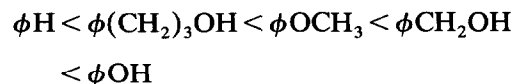
TABLE 6

SELECTIVITY ON THF-WATER SOLVATED 4.3 $\mu\text{mol}/\text{m}^2$ ODS BONDED SILICA AT $T = 50^\circ\text{C}$, $\alpha = k'_x/k'_{\text{H}}$

Mobile phase	ϕOCH_3	ϕOH	$\phi\text{CH}_2\text{OH}$	$\phi(\text{CH}_2)_3\text{OH}$	ϕNH_2
<i>THF-water</i>					
10:90	0.99	0.27	0.13	0.58	
20:80	0.86	0.30	0.13	0.39	0.70 (40°)
30:70	0.82	0.37	0.18	0.34	0.25

deviation starting at 39.7°C. This deviation temperature was approximately 10°C higher than that of the other solutes, consistent with the earlier models of near-surface hydrogen-bonding retention of aniline and its observed high peak asymmetry.

Conclusions can be made about the source of the positive retention deviations on THF-water solvated ODS-bonded silica by comparing the solvent concentration dependence of the inversion from negative to positive Van 't Hoff plot deviations. For all solutes, there appeared to be a correlation between solute polarity and the inversion of the Van 't Hoff plot deviation. Comparing the data at THF-water (20:80), benzene showed the expected negative deviation, but a slight increase in polarity to methoxybenzene displayed no deviation (Fig. 11B). Further increase in polarity to phenol showed a positive deviation (Fig. 12A). The same polarity trend in terms of the magnitude of the slope change of the positive deviation was observed for solutes in 30% THF mobile phase. The solutes can be placed in an order of relative contribution of hydrogen-bond-donating interactions and non-polar hydrocarbon content consistent with the positive magnitude of the slope change.



Based upon these observations, it would appear that the increase in favorable interactions in the stationary phase was based upon a balance of both non-polar interactions (alkyl chains) and interactions with hydrogen-bond-donating character (hydroxyl groups). Most simply stated

in terms of the model for stationary phase interactions developed thus far, these non-linear Van 't Hoff plot responses were associated with the depth of penetration of each solute into the organized solvation layer provided by THF-water solvation. The retention behavior of aniline was once again consistent with strong hydrogen-bond-accepting interactions deep in the stationary phase near the silica surface.

It is concluded that these retention responses were a result of the formation of a highly structured solvation layer. In concert with the bonded ODS conformation change at lower temperatures, the THF-water mixture in the stationary phase apparently formed a highly organized system. This organized layer provided both improvement in the enthalpy of retention interactions and a more negative entropy of retention. That is, although the overall intermolecular interactions became energetically more favorable, the transfer of a solute from the mobile to the stationary phase became entropically more difficult. The behavior of THF was particularly significant in light of its use in ternary mobile phases to provide highly selective separations [59]. This structuring solvent behavior deserves further investigation to elucidate the source and extent of the intermolecular interactions responsible.

3.7. Kinetic effects of the bonded octadecyl conformation change

On a theoretical basis, no dependence of efficiency on the type of solute retention interactions is predicted, except as a function of relative retention [60]. Based upon the previous thermo-

dynamic investigations of the octadecyl conformation change and associated solvation layer changes, significant differences in retention interactions of solutes were observed to accompany these stationary phase changes. These differences were postulated to result primarily from changes in the location of average intermolecular interactions in the stationary phase solvated layer. Thus, any relative temperature dependence of efficiency on solute intermolecular interactions would be expected to be associated with relative changes in solute mobility in the stationary phase based upon their average retention location.

Due to the complexity of solute transport, only relative changes in column efficiency should be considered as informative of kinetic variations associated with solute interactions. Fig. 13 shows the variation in column efficiency with temperature for benzene with MeOH-water (10:90) mobile phase on the $2.5 \mu\text{mol}/\text{m}^2$ ODS silica. A smooth variation with decreasing efficiency at lower temperature was observed. This does not seem unusual given the expected decrease in diffusion coefficient of all components with decreasing temperature [61]. However, relative changes for solutes of similar size would be indicative of the solutes probing different molecular mobility portions of the stationary phase. Because of the tendency of plate height (H) plots to attenuate differences at high efficiencies, discussion of the data will center on plots of the number of theoretical plates, N , versus temperature, keeping in mind the relationship, $H = L/N$, where L is column length. A good deal of scatter was observed in the data primarily from the error associated with measuring peak width.

The relative changes in efficiency as a function of temperature for benzene and benzylamine are shown in Fig. 13. Within the scatter of the data, similar efficiency was observed at high temperatures. At lower temperatures, upon stationary phase reorganization, both solutes showed a decrease in efficiency. A much more rapid decrease occurred for the amine. It has been proposed that amine solutes were retained deep in the stationary phase, near the silica surface, regardless of temperature. This efficiency response is consistent with that hypothesis. As the

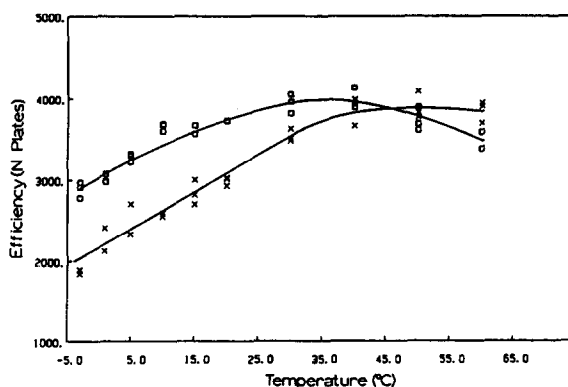


Fig. 13. Comparison of efficiency variation with temperature in theoretical plates for benzene (□) and benzylamine (×) in MeOH-water (10:90, v/v) mobile phase on $2.5 \mu\text{mol}/\text{m}^2$ ODS-bonded silica.

mobility of the ODS chains decreased, the diffusion coefficient of solutes in the stationary phase decreased. Thus, if the average retention location of a solute remained constant in this region of the stationary phase, a more significant drop in efficiency should accompany the surface mobility change relative to a solute which changed retention location with temperature (benzene).

Based on the similarity in the efficiency response of benzene and 3-phenyl-1-propanol, it would appear that their retention locations possessed similar mobility. The comparison of the efficiency response of 3-phenyl-1-propanol and phenethyl alcohol provided more detailed information about the relative retention location of these very similar solutes. With MeOH-water (10:90) solvation, the difference of one methylene unit gave a relative increase in efficiency for phenethyl alcohol at high temperatures with identical behavior at lower temperatures (Fig. 14). In considering the structure of the stationary phase solvated layer, the molecular mobility gradually decreased upon moving from near the mobile phase to deep in the solvated layer near the silica surface [23,29,37]. The model of solute retention in the solvated stationary phase based on thermodynamic data indicated that both phenyl alcohols were oriented in the upper portion of the stationary phase in a manner similar to what has been proposed for phenol retention in MeOH solvated

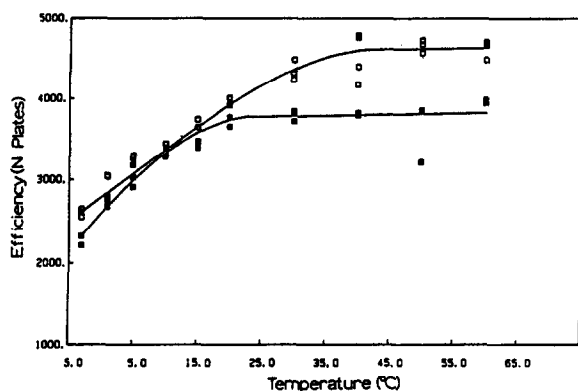


Fig. 14. Comparison of efficiency variation with temperature for phenethyl alcohol (□) and 3-phenyl-1-propanol (■) under the same conditions as Fig. 13.

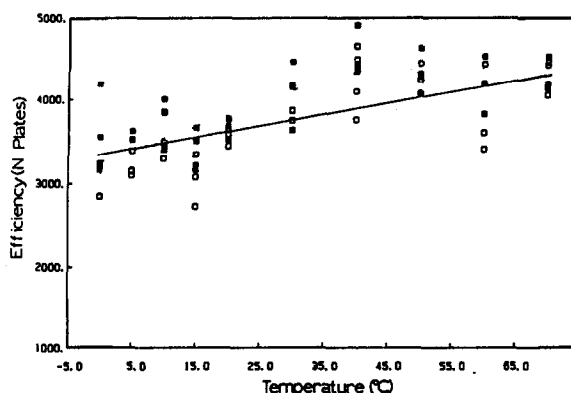


Fig. 15. Comparison of efficiency variation with temperature for phenethyl alcohol (□) and 3-phenyl-1-propanol (■) in MeCN-water (10:90, v/v) mobile phase on 2.5 $\mu\text{mol}/\text{m}^2$ ODS-bonded silica.

systems. The difference of one methylene unit improved nonpolar interactions and moved the phenyl ring of 3-phenyl-1-propanol deeper into the stationary phase. This represents a move to a region of lower molecular mobility, thus causing a decrease in solute mobility. Accompanying the surface reorganization, both solutes moved to an average retention location of equivalent but decreasing mobility.

The variation in efficiency as a function of temperature for solutes with MeCN-water (10:90) mobile phase was much less than was observed with 10% MeOH. No determinate variation outside of the scatter in the data points was seen for benzene and benzylamine. This is consistent with the more stable MeCN solvated composition effects which have been observed thermodynamically. Phenethyl alcohol and 3-phenyl-1-propanol showed a decrease in efficiency with decreasing temperature (Fig. 15) in a similar manner to that observed with MeOH solvation. There was, however, no difference in the efficiency between the two solutes at any temperature. This indicates that both of these phenyl alcohols probe similar stationary phase regions regardless of temperature. Relative to the efficiency data with MeOH solvation, this allows a further refinement in the stationary phase model for these solutes. In a similar manner to the proposed retention behavior of phenol with MeCN solvation, when hydrogen-bonding site competition was reduced, molecular

orientation with the hydroxyl group of the phenyl alcohols near the adsorbed water layer on the silica surface became favored in MeCN relative to MeOH solvation.

3.8. Alternative C_{18} -bonded phases

Golding and Burke [11,18] synthesized octadecylchlorosilane in order to minimize steric constraints and increase packing density of the ODS-bonded moieties. This allowed the silica bonding density of this monomeric species to be increased to 3.9 $\mu\text{mol}/\text{m}^2$. In addition, a percentage of the chemically labile silicon-hydrogen bonds could be readily oxidized to silanols. This provided a bonded phase with high packing density, as well as high near-surface silanol concentration. These materials were evaluated for retentivity and selectivity under both normal- and reversed-phase LC conditions. The results of these studies, as well as Van 't Hoff plot data, were interpreted in terms of a constrained near-surface region due to enhanced water adsorption at the silanol-enriched near-surface region. Consistent with the results of Sentell and Dorsey [9], Golding and Burke observed a decrease in the retentive ability of this densely bonded phase for non-polar hydrocarbons. Polar solutes such as aniline, however, showed an increase in selectivity relative to benzene. The Van 't Hoff plot data for oxidized octadecylsilane was com-

pared with that from an octadecyldimethylsilane bonded silica of similar high bonding-densities (3.9 and 3.1 $\mu\text{mol}/\text{m}^2$, respectively). The primary difference in this data was in the retention behavior of aniline. The dimethyl phase showed a negative low-temperature retention deviation and the oxidized dihydro phase showed a positive deviation. These data indicated that the primary difference was in the near surface region where a significant difference in silanol concentration was present. An increase in near-surface stationary phase structure was proposed to be associated with the increased hydrogen bonding interactions with adsorbed water on the oxidized octadecyldihydrosilane bonded phase.

In an effort to further elucidate the significance of ODS-bonded group conformation on the RPLC retention process, Palmer [50] synthesized oleyldimethylchlorosilane (9-*cis*-octadecenyldimethylchlorosilane). The presence of a double bond between the C-9 and C-10 carbon atoms along the C₁₈ chain placed a permanent kink in the chain. This is analogous to the gauche bonds produced by free rotation of carbon-carbon bonds in the octadecyl backbone as observed by Sander *et al.* [47] using FT-IR spectrometry. The motivation to prepare this model RPLC stationary phase was based upon the observed effects of the oleyl structure on the formation of lipid bilayers and Langmuir films [62]. Lipid bilayers exhibit a thermal transition temperature increasing by 17°C for each increase in alkyl chain length by two methylene units. However, a drop of 80°C is observed in the transition temperature upon the addition of the double bond on going from dioctadecylphosphatidylcholine to dioleylphosphatidylcholine bilayers. In addition, Langmuir films of oleic acid show a 50% decrease in packing density relative to that of octadecanoic acid. These steric constraints of the bent molecular structure of the oleyl chain also affected the silica derivatization reaction to yield low surface coverage (1.6 $\mu\text{mol}/\text{m}^2$) [50].

Similar Van 't Hoff plot data was obtained for the oleyldimethylsilane bonded phase in both aqueous MeOH and MeCN mobile phases to that observed previously on ODS-bonded silica. Consistent with the stationary phase solvation-

layer model, the thermally induced retention changes of non-polar solutes differed from those of hydrogen-bonding solutes. The Van 't Hoff plot data are summarized in Table 7 [50]. The trends in transition temperature, high-temperature ΔH , and deviation magnitude were consistent with the lower bonding density of the oleyldimethyl bonded phase. Two differences were noted. In MeCN-water mobile phases, larger entropic contributions were apparent in the correlation of high-temperature ΔH with percent organic solvent and also in the more positive retention deviations of aniline. These were consistent with the more restricted motion of the oleyl phase. The expected major shift in deviation temperature associated with the oleyl moieties was not observed.

These results emphasize the differences between lipid bilayer structures and the RPLC stationary phase consisting of solvated covalently anchored hydrocarbons chains [6]. The considerably increased interchain spacing on lightly bonded silica, as compared to lipid bilayers, as

TABLE 7

VAN 'T HOFF PLOT PARAMETERS FOR SOLUTES ON 1.60 $\mu\text{mol}/\text{m}^2$ OLEYLDIMETHYLSILANE BONDED SILICA [50]

Mobile phase		ϕH	ϕOH	ϕNH_2
<i>Methanol-water</i>				
10:90	<i>T</i> (°C)	32.6	33.1	29.8
	ΔH (kcal/mol)	-2.38	-3.29	-3.63
	- <i>RA</i> slope	-2.17	-1.37	-0.58
20:80	<i>T</i> (°C)	31.2	35.6	None
	ΔH (kcal/mol)	-2.54	-3.32	-3.50
	- <i>RA</i> slope	-1.41	-0.69	0.0
30:70	<i>T</i> (°C)	26.4	None	None
	ΔH (kcal/mol)	-3.01	-3.18	-3.03
	- <i>RA</i> slope	-0.96	0.0	0.0
<i>Acetonitrile-water</i>				
10:90	<i>T</i> (°C)	16.6	16.8	15.5
	ΔH (kcal/mol)	-2.31	-2.98	-3.58
	- <i>RA</i> slope	-1.72	-0.77	-0.38
20:80	<i>T</i> (°C)	18.5	None	16
	ΔH (kcal/mol)	-2.70	-2.52	-2.86
	- <i>RA</i> slope	-0.50	0.0	+0.42
30:70	<i>T</i> (°C)	None	None	18.0
	ΔH (kcal/mol)	-2.95	-2.80	-3.12
	- <i>RA</i> slope	0.0	0.0	+0.82

well as the structure of the solvation layer significantly alter the hydrocarbon chain mobility. Comparison of transition temperatures for 1,2-dioctadecenoyl-*sn*-glycero-3-phosphorylcholine bilayers differing in the position of the double bond along the octadecyl chain show an increase of roughly 30°C on moving the double bond from C-9–C-10 to C-4–C-5 [63]. Spectroscopic studies have shown that the bonded hydrocarbon chain mobility is greatly restricted for 25% of the chain nearest the silica anchoring bond [29,37]. This effectively placed the oleyl *cis* double bond much lower in the freely moving portion of the bonded chain, thus limiting its effects on thermally induced stationary phase changes [50,62].

4. CONCLUSIONS

The stationary phase in RPLC with chemically modified silica was previously understood as a dynamic multicomponent mixture in the approximately 30 Å thick region nearest the silica surface. Through the work of many researchers, an understanding had been developed of this near surface region as a complex solvation layer in terms of the length of the bonded hydrocarbon moieties, their bonding density, adsorbed water at the silica surface, and the enhanced concentration of imbibed organic solvent from the mobile phase. However, the structure and chemical interactions in this near-surface region were not understood. By detailed investigation of the thermodynamic and kinetic quantities associated with a thermally induced conformational change in the bonded ODS moieties, the work described in this review has further elucidated the nature of the three-dimensional structure and interactions of this interfacial region in terms of its solvation, and the location and orientation of retention interactions of molecular solutes of differing polarity. The general applicability of the solute retention interaction model derived from these studies has been shown to provide consistent description with four different organic modifiers on five octadecyl bonded silicas differing in bonding chemistry, bonding density, and base silica.

The chromatographic investigation of the bonded ODS conformation change has led to

refinements in the model of the solvated layer stationary phase in RPLC. For organic solvents with hydrogen-bond-donating capability, effective competition occurs with hydrogen-bond-donating solutes, causing these solutes to be retained in the upper portion of the stationary phase. Regardless of solvent interactions, hydrogen-bond-accepting solutes find their most favorable interactions deep in the stationary phase near the silica surface. Low polarity and non-polar solutes whose retention interactions are primarily through Van der Waals and dipole-induced dipole forces are on average retained in the central region of the stationary phase where a minimum in polarity exists and the most favorable non-polar solvation interactions occur.

When thermal energy is removed from the system, the bonded ODS groups lose mobility and become more organized. This conformation change is accompanied by a restriction in the interchain access of both solvent and solute molecules and an alteration in the composition of the solvation layer. In general, a net decrease in retention occurs with this surface reorganization. Overall, a change in relative concentration of the solvent components of the stationary phase was determined to be responsible for the temperature dependence and magnitude of the observed deviation from retention linearity. The proposed polarity profile of the solvated layer is shown diagrammatically in Fig. 16 as a function of distance from the silica surface and interchain distance. It has been proposed that the sensitivity of probe solutes to the change in the solvated layer polarity is based upon the interchain polarity gradient of solvent molecules interacting with the bonded-hydrocarbon and silica surface. The restricted access accompanying the conformational change in the bonded groups is overcome only by solutes with strong hydrogen-bond-accepting ability for the water-rich near-surface region.

The same changes in the conformation and mobility of the chemically attached hydrocarbon groups were observed to occur on five different C₁₈-bonded materials differing in bonding chemistry, bonding density, and base silica. However, all probe solutes found increased access into the surface solvated layer and im-

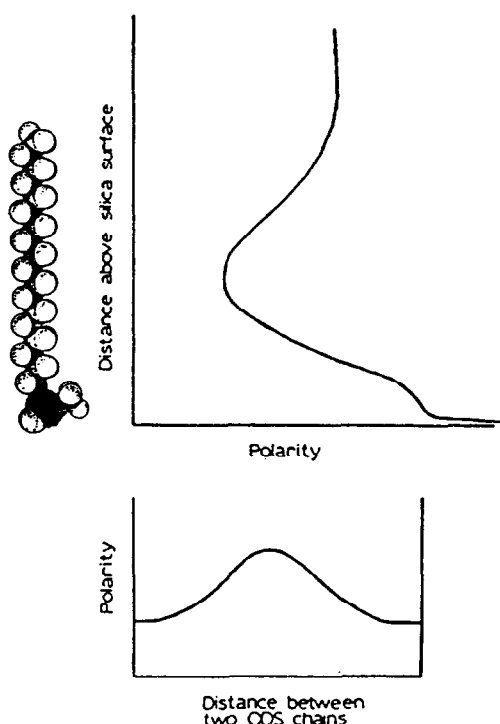


Fig. 16. Schematic representation of the postulated solvation-layer polarity gradients in the ODS-bonded silica stationary phase. From ref. 49.

proved interactions with the silica surface adsorbed-water layer when the bonding density was decreased. In addition, the average solvation layer was found to undergo less drastic changes in relative interactions on less densely covered silicas. The kinetic behavior of molecular interactions in the interfacial region was found to support the conclusions arrived at through the evaluation of thermodynamic data. However, additional information on the orientation of hydrogen-bond-donating solutes with significant alkyl character was obtained from their sensitivity to local mobility variations within the stationary phase. These molecular probes were shown to orient with their hydroxyl groups near the mobile phase when in competition with a hydrogen-bond-donating solvent and to reverse this orientation in the absence of hydrogen-bonding competition.

Finally, some solvents (EtOH and THF) were observed to produce an enhanced organization of the stationary phase solvation-layer. At low

percentages of THF, chromatographic thermal behavior resembled that of MeOH with enhanced surface interactions for polar solutes, especially hydrogen-bond-accepting solutes. However, as the concentration of THF in the mobile phase was increased, preferentially enhanced retention of polar solutes was observed to accompany the ODS conformation change. This enhancement in enthalpy and degradation in entropy of retention was interpreted in terms of a rigid organization of the stationary phase solvent components.

It must be stressed that the detailed models of chemical interactions in the solvated stationary phase layer on octadecylsilane bonded silica which have been developed are an attempt to represent the average interactions which are available. These refinements of the RPLC stationary phase model provide a more detailed and accurate description of the intermolecular interactions responsible for retention and selectivity than was previously available. However, it must be remembered that, when a highly porous silica surface is chemically modified with an alkyl silane, a surface which is heterogeneous in both physical structure and chemical activity is obtained, so that any average picture of the system is not complete in itself. Further considerations such as the nature of the original surface, the intermolecular interactions of the solvent components of the contacting mobile phase, and the chemical nature of the bonded species must be taken into account in order to understand the behavior of widely varying systems.

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