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Revisionist look at solvophobic driving forces in reversed-phase liquid chromatography

III. Comparison of the behavior of nonpolar and polar solutes

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Abstract

Recently we showed that in reversed-phase liquid chromatography (RPLC), solute methylene units are embedded in the bonded alkyl chains of the stationary phase. That is, the ‘partition mechanism’, not the ‘adsorption mechanism’, controls retention of methylene groups. We have also shown that the net interactions of the nonpolar groups of a solute with a nonpolar stationary phase contribute more to the overall free energy of transfer than does the solvophobic interaction in the mobile phase. The present work explores the behavior of several polar functional groups (Cl, OCH₃, CHO, NO₂, CN, and COOCH₃) in RPLC. In contrast to the behavior of methylene groups, the net interactions of more polar functional groups with the mobile phase are larger than are their net interactions with the stationary phase. Further, the data indicate that the free energy of transfer of a polar functional group in bonded phase RPLC is quite different from the free energy of transfer between the mobile phase and a bulk nonpolar liquid used to emulate the bonded phase. This implies that either sorbed mobile phase significantly influences the retention properties of polar groups in the bonded phase, or that the solute functional groups reside at the interface between the mobile and stationary phases.

Keywords: Retention mechanisms; Free energy of transfer; Solvophobic driving forces; Thermodynamic parameters

1. Introduction

Reversed-phase liquid chromatography (RPLC) is the most important mode of liquid chromatography [1]. Despite numerous studies of the theory of retention in RPLC, our understanding of the solute retention process is still quite incomplete [2–10]. In comparison to the situation in normal-phase liquid chromatography there are a number of most fundamental issues still under debate including:

whether the retention mechanism is more ‘partition like’ or more ‘adsorption like’; what is the nature of the driving force for retention; and what is the identity of the phase in which selectivity arises.

Whether the RPLC retention is driven by a partition or adsorption mechanism is closely related to the physical configuration of the bonded alkyl chains. It has been thought that in predominantly aqueous mobile phases the hydrophobic alkyl chains are to assume a highly collapsed or folded configuration and lie flat on the surface in order to minimize their surface contact with the polar mobile phase

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[7,11,12]. The solute molecules are thought not to be able to partition into but adsorb onto the collapsed, rigid chains. On the other hand at high organic modifier concentrations the chains prefer the extended configuration where they are solvated by the mobile phase components, and the solute molecules are allowed to penetrate or partition into them. However, it has been shown that the bonded alkyl chains are not rigid [13–16]. Albert and co-workers showed in their studies of the solid and solution state ^{13}C NMR of the dynamic behavior of monofunctional C_4 – C_{20} alkyl silicas that the alkyl chains of the bonded phases exhibit a liquid-like behavior even in the solid state [13,14]. Schure showed, based on the molecular dynamics simulation of the motions of octyl and octadecyl silane phases attached to a simulated amorphous silica, that at the very low densities in a typical material (2 – $3 \mu\text{mol}/\text{m}^2$) there is a tremendous amount of chain motion. The entire ensemble of chains is fully reorganized within a few nanoseconds [15]. Even in highly aqueous mobile phases the solute could easily penetrate in the so-called collapsed state of the bonded phase [16].

Recently we [17] have shown, based on Dill and Dorsey's definitions of partitioning and adsorption [9,10], that for nonpolar methylene groups, most of the free energy of retention in RPLC arises from net attractive (exoergic) processes in the stationary phase, and not from net repulsive (endoergic) processes in the mobile phase. However, we have also shown [17,18] that variations in retention in RPLC upon changing the mobile phase are dominated by alterations in the net mobile phase processes. Significantly we have shown that the free energy of transfer of a methylene group from the mobile phase to a bonded reversed phase, over a rather wide range in the mobile-phase composition, is almost equal to the free energy of transfer of a methylene group from the mobile phase to bulk hexadecane [17]. This observation strongly supports the partition model of RPLC. That is, it suggests that the methylene group is fully embedded in the layer of bonded phase and is not merely resting on top of the bonded phase. Subsequent studies have shown that as the stationary phase chain length is decreased below about eight carbon units the transfer free energy of a methylene group into a bonded phase becomes increasingly different from that in a pure bulk partition process.

However, even when a trimethylsilane is used to make the bonded phase, the free energies of transfer per methylene group in bonded phase RPLC and in the true bulk phase system only differ by a factor of two [19]. In a recent study of the RPLC behavior of homologous series of solutes with a large range of chain length on bonded phases of a wide range of bonded chain length, Tchaplal and Heron showed that the nonpolar alkyl chains of the solutes are fully inserted into the bonded phase when the solute chain length is equal to or shorter than a critical value but the polar heads of the solutes stay out of the chains of the stationary phase [20].

Because large amounts of organic modifier and water are sorbed into the stationary phase, the above observation and the use of bulk hexadecane as a model for the bonded phase are only likely to be valid for nonpolar solutes whose interactions with sorbed mobile phase components are minimal. It is not likely to be valid for polar solutes which we feel will inevitably interact with the sorbed polar solvents through dipole–dipole and hydrogen bonding interactions. This possibility is supported by the data of Tsukahara et al. [21]. These workers examined the RPLC capacity factors (k') of a large number of nonpolar and polar solutes on an ODS column and plotted them against their partition coefficients (K) between dodecane and aqueous methanol mixtures of widely varying compositions. The polar solutes, those with smaller K values, deviate in a positive direction from the line established by the following relationship:

$$\log k' = \log K + \log \Phi \quad (1)$$

where Φ is the column phase ratio. Their observation clearly implies that polar solutes prefer ODS to dodecane when being transferred from an aqueous methanol mobile phase.

The use of bulk phase alkanes as models for bonded phases has been investigated [22]. Hexadecane that is equilibrated with hydro-organic mixtures actually dissolves very little water or organic modifier. For methanol–water mixtures the amount of methanol dissolved in hexadecane varies from zero up to $2.8 \cdot 10^{-5} \text{ mol/l}$ and for acetonitrile–water mixtures the amount of acetonitrile that dissolves varies from zero up to $3.5 \cdot 10^{-6} \text{ mol/l}$ as the organic modifier is increased to 50% by volume [22]. The

amount of water that dissolves is even smaller. We have shown that dissolved modifier has almost no effect on the partial molar free energy of alkyl-benzenes when dissolved in hexadecane [18,22,23]. Finally solvatochromic studies of hexadecane equilibrated with various hydro-organic mixtures show only minor variations in properties in contrast to the very high polarity observed in solvatochromic studies of bonded phase RPLC supports [24]. Thus it seems that bulk hexadecane when equilibrated with a hydro-organic mixture may behave differently than a bonded phase towards nonpolar and polar functional groups.

In this work we investigated the retention mechanism of polar solutes in RPLC. Specifically this is done by comparing the free energies of transfer of a number of relatively more polar functional groups (Cl, OCH₃, CHO, NO₂, CN, and COOCH₃) from hydro-organic mixtures to the bonded phase to those from the same hydro-organic mixtures to bulk hexadecane. We determined the free energies of transfer of benzene and a monofunctional benzene from the hydro-organic solvent mixture to hexadecane by measuring the partition coefficients of the solutes at infinite dilution. The free energy of transfer of a functional group from the hydro-organic solvent mixture to hexadecane is calculated by subtracting the free energy of benzene from that of the substituted benzene. Similarly, the free energy of transfer of a functional group from the hydro-organic solvent mixture to a bonded stationary phase is obtained from the retention factor measured using the hydro-organic mobile phases of the same compositions (see below). Thus,

$$\Delta G^\circ(X) \equiv \Delta G^\circ(\text{PhX}) - \Delta G^\circ(\text{PhH}) \quad (2)$$

where $\Delta G^\circ(\text{PhX})$ and $\Delta G^\circ(\text{PhH})$ denote the free energy of transfer of benzene with a polar functional group and benzene, respectively. $\Delta G^\circ(X)$ is our definition of the free energy of transfer of a functional group. The free energies are computed as:

$$\Delta G^\circ_{\text{RPLC}} = -RT \ln(k'/\Phi) \quad (3)$$

$$\Delta G^\circ_{\text{bulk}} = -RT \ln K \quad (4)$$

where k' and Φ are the capacity factor and phase ratio in bonded phase RPLC, respectively, and K is

the partition coefficient between hexadecane and a hydro-organic phase.

2. Experimental

Partition coefficients of benzene, chlorobenzene, anisole, methyl benzoate, benzaldehyde, benzonitrile and nitrobenzene between hexadecane and hydro-organic mixtures were measured by headspace gas chromatography (HSGC). The compositions of the hydro-organic mixtures studied are methanol–water (10–50%, v/v) and acetonitrile–water (20–50%, v/v) at 10% intervals. The ranges in compositions studied were established by the ability to get reproducible and meaningful k' data and not by the HSGC measurements. The details of the HSGC system have been presented elsewhere [25]. All measurements were made at $25.00 \pm 0.02^\circ\text{C}$ with the solutes at infinite dilution in the solvents of interest. The solutions containing the solutes at a single concentration in these solvent mixtures (or hexadecane) were prepared by adding the solutes gravimetrically. They were maintained in an infinitely dilute concentration range. An aliquot of this solution was added to a water-jacketed cell. Three replicate measurements of the equilibrated gas phase above these solutions were made. A known amount of hexadecane (or hydro-organic mixture) was added to the solution in the cell via a computer-controlled burette. Six increments of hexadecane (or hydro-organic mixture) were added to the cell. After each addition, the solution was allowed to equilibrate for 60 min and then three replicate analyses of the headspace were made. From the area counts before and after the addition, hexadecane–hydro-organic partition coefficients can be calculated [23,26]. A detailed discussion of the data analysis for the calculation of the hexadecane–hydro-organic partition coefficients is given elsewhere [26]. Previously we found excellent agreement between the gas–hydro-organic partition coefficients (and infinite dilution activity coefficients) obtained in this fashion and those obtained in a completely independent method which does not involve partitioning into hexadecane [18,23]. Such agreement can only take place if the water and organic modifier which dissolve in the hexadecane phase have minimal effects on the phase

properties. In the present work we assume that such is true even for the polar solutes.

Liquid chromatographic measurements were made on a Zorbax SB-C₁₈ column (Rockland Technologies; pore size, 80 Å) at 25.00±0.05°C in the methanol–water and acetonitrile–water mobile phase of the same compositions as used in the HSGC measurements. The capacity factors, given elsewhere [27], were averages of at least triplicate determinations. The void-volume of the system was taken as the peak produced by ²H₂O. All measurements were made with a Hewlett-Packard 1090 liquid chromatograph. A UV detector set at a wavelength of 254 nm was used. Retention times were taken at the peak maximum reported by a Hewlett-Packard 9153 data system. Samples were prepared in the mobile phase under study. Typically the column was flushed with 50 column volumes of mobile phase per each percentage change in composition from pure modifier to the analytical composition.

HPLC-grade water, methanol and acetonitrile (Fisher Scientific) were used throughout this work. Hexadecane (99%, Aldrich) was used without further purification. All solutes used were obtained commercially and were analytical grade in the highest purity available.

3. Results and discussion

3.1. Comparison of present data to literature data

The measured hexadecane–hydro-organic mixture partition coefficients for benzene, chlorobenzene, anisole, methyl benzoate, benzaldehyde, benzonitrile and nitrobenzene are given in Table 1. The logarithm of the hexadecane–hydro-organic mixture partition coefficient for each species was linearly extrapolated vs. volume fraction to 100% water to obtain an estimate of its hexadecane–100% water partition coefficient (Table 2). These plots are actually nicely linear. The agreement between the two extrapolated partition coefficients for each solute is very impressive. This argues for a high degree of internal consistency in the data and that the properties of the bulk hexadecane phase are not seriously effected by the dissolved modifier. The extrapolated values for all solutes are consistently higher than the literature

Table 1

Logarithmic hexadecane–hydro-organic partition coefficients at 25°C

Solute	MeOH				
	10%	20%	30%	40%	50%
Benzene	2.05	1.95	1.82	1.58	1.30
Chlorobenzene	2.63	2.49	2.27	1.97	1.61
Anisole	1.89	1.77	1.59	1.35	1.05
Methyl benzoate	1.6	1.53	1.3	1.06	0.65
Benzaldehyde	0.87	0.74	0.63	0.40	0.17
Benzonitrile	0.82	0.70	0.55	0.31	−0.13
Nitrobenzene	1.32	1.19	1.05	0.79	0.44
	MeCN				
	10%	20%	30%	40%	50%
Benzene	ND ^a	1.74	1.38	1.03	0.75
Chlorobenzene	ND	2.18	1.73	1.31	0.85
Anisole	ND	1.46	1.07	0.70	0.38
Methyl benzoate	ND	1.16	0.78	0.44	0.06
Benzaldehyde	ND	0.47	0.13	−0.19	−0.45
Benzonitrile	ND	0.47	0.12	−0.34	−0.62
Nitrobenzene	ND	0.89	0.50	0.03	−0.25

^a No data.

value (see Table 2). However, the differences between our extrapolation-based estimates and the literature, except for methyl benzoate, are so small that in view of the very different experimental methods used we feel that no further discussion is needed.

3.2. Examination of the bulk phase partition process

According to the solvophobic model of RPLC [2,3], solutes are driven into the stationary phase due to their dislike of the aqueous mobile phase, rather than attracted into the nonpolar phase by their intrinsic affinity for the nonpolar stationary phase. This view is in agreement with the classical concept of the hydrophobic effect as espoused by Tanford [28] and is the usual explanation for such phenomena as the formation of micelles, the folding of proteins, etc. The stationary phase is considered as a more or less passive receptor. In contradistinction to the above, but in good agreement with the ideas of Abraham [29], we have recently shown that for methylene groups the net interaction with a nonpolar stationary phase contributes more to the overall

Table 2

Logarithmic hexadecane–water partition coefficients obtained by extrapolation to pure water of hexadecane–hydro-organic partition coefficients at 25°C^a

Solute	$\log K_{C_{16}/w}(\text{ext})^b$	$\log K_{C_{16}/w}(\text{ext})^c$	$\log K_{C_{16}/w}(\text{lit})^d$
Benzene	2.31	2.39	2.17
Chlorobenzene	2.96	2.98	2.84
Anisole	2.16	2.17	2.13
Methyl benzoate	1.99	1.89	1.49
Benzaldehyde	1.08	1.08	1.03
Benzonitrile	1.14	1.22	0.99
Nitrobenzene	1.61	1.65	1.44

^a Hexadecane–hydro-organic partition coefficient data are from Table 1.

^b Based on linear extrapolation of the logarithm of the hexadecane–aqueous methanol partition coefficients vs. Φ .

^c Based on linear extrapolation of the logarithm of the hexadecane–aqueous acetonitrile partition coefficients vs. Φ .

^d Experimental values from compilation of Abraham et al. [30].

retention processes in RPLC [17]. In order to see whether the retention process in RPLC is driven by the mobile phase or the stationary phase, it is important to compare the absolute free energy of the solute in the mobile phase and stationary phase separately.

Hexadecane–hydro-organic mobile phase partition coefficients ($K_{C_{16}/\text{mob}}$) are related to hexadecane–gas ($K_{C_{16}/\text{gas}}$) and hydro-organic mobile phase–gas partition coefficient ($K_{\text{mob}/\text{gas}}$) in the following fashion:

$$K_{C_{16}/\text{mob}} = [A]_{C_{16}}/[A]_{\text{mob}} = K_{C_{16}/\text{gas}}/K_{\text{mob}/\text{gas}} \quad (5)$$

$$K_{C_{16}/\text{gas}} = [A]_{C_{16}}/[A]_{\text{gas}} \quad (6)$$

$$K_{\text{mob}/\text{gas}} = [A]_{\text{mob}}/[A]_{\text{gas}} \quad (7)$$

where the subscripts C16, mob, and gas denote bulk liquid hexadecane, the hydro-organic mobile phase and the gas phase, respectively. $[A]$ is the solute concentration (mol/l) in the respective phase. One can compute $K_{\text{mob}/\text{gas}}$ using Eq. 5 from measured $K_{C_{16}/\text{mob}}$ and $K_{C_{16}/\text{gas}}$ values [30] assuming that the dissolved organic cosolvent in hexadecane does not influence the thermodynamic properties of hexadecane. Fig. 1 shows the variation of the free energies of transfer from gas to hexadecane and to the hydro-organic mobile phases of the various functional groups for the 10% methanol–water system. For a nonpolar methylene group the free energy of transfer from the gas to the mobile phase is positive (unfavorable) and much smaller in magnitude than is the negative (favorable) free energy of transfer from the

gas phase to hexadecane. This indicates that the net attractive interaction of a nonpolar group of a solute with a nonpolar stationary phase contributes more to the overall free energy of transfer than does the net repulsive solvophobic interaction in the mobile phase. As the amount of organic cosolvent in the hydro-organic mobile phase is increased the free energy of transfer of a nonpolar methylene group from the gas to the mobile phase becomes more negative but its magnitude remains smaller than that from the gas phase to hexadecane [17]. Hence for nonpolar solutes retention is not mobile phase-driven, as indicated by the solvophobic theory [2,3], but rather is stationary phase-driven.

In complete contrast to the behavior of nonpolar

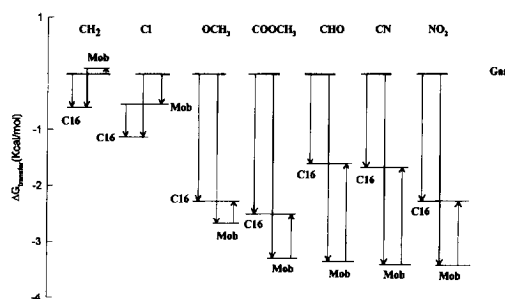


Fig. 1. Free energy level diagram for polar solutes. Data for free energy of transfer from gas to hexadecane and from gas to 10% methanol–water. The gas phase, shown as the bold line, is taken as the reference phase and assigned a zero solute–phase interaction energy. The arrow denotes the direction of the transfer process. Free energy data for the methylene group is for the alkylbenzenes and is taken from Carr et al. [17]. Symbols: C₁₆=hexadecane, Mob=10% methanol–water.

groups (CH_2 and Cl), the interactions of the highly polar groups (OCH_3 , CHO , NO_2 , CN , and COOCH_3) with the mobile phase are both favorable and much greater than are their interactions with hexadecane (see Fig. 1). This is fully expected because the additional dipolar (dipole–dipole and dipole-induced dipole) and hydrogen bonding interactions take place between the polar functional group and the polar mobile phase components. A similar result is also observed with the acetonitrile–water systems (see Fig. 2). Based on this we can state that the decreased retention of a polar benzene derivative relative to benzene comes about primarily due to the very favorable net free energy of the polar group with the hydro-organic phase. This observation may imply that the retention process for polar solutes is in fact mobile phase-driven. However, while the mobile phase interactions are more important for the polar functional groups than are the interactions in the hexadecane phase, the interactions are in a direction so as to decrease retention and thus we can conclude that the solvophobic mechanism is not dominant for either nonpolar methylene groups or for polar functional groups such as OCH_3 , CHO , NO_2 , CN , and COOCH_3 . It is our view that the solvophobic theory is fundamentally incorrect and is not able to explain the free energy of the transfer process for either a nonpolar or polar moiety from a hydro-organic phase to bulk hexadecane.

It should be noted that our studies of the relative stationary phase activity coefficients of alkylbenzenes in bonded phase RPLC indicate that the

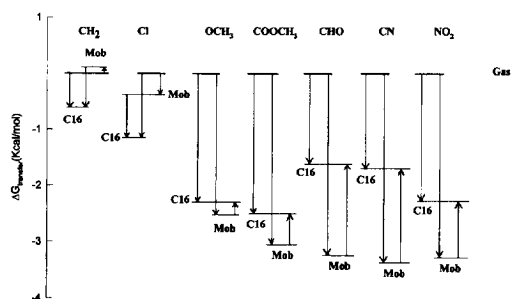


Fig. 2. Free energy level diagram for polar solutes. Data is for free energy of transfer from gas to hexadecane and from gas to 20% acetonitrile-water. Free energy data for the methylene group is for the alkylbenzenes and taken from Carr et al. [17]. Symbols: C_{16} = hexadecane, Mob = 20% acetonitrile–water.

stationary phase appears to be about as polar as isopropanol [18]. It can then be argued that since the bonded phase has the same polarity as isopropanol hexadecane cannot be a useful model of the bonded phase. However, the free energy of transfer of a CH_2 group from gas to hexadecane is quite similar in magnitude to that from gas to isopropanol [$-685(\pm 13)$ vs. $-604(\pm 5)$ cal/mol] [25]. This indicates that the use of isopropanol instead of hexadecane will not change the preceding conclusions of the behavior of CH_2 groups. The free energies of transfer of the polar groups from gas to hexadecane estimated by the UNIFAC activity coefficient method [31] are also similar in magnitude to those from gas to isopropanol (-1292 vs. -1796 cal/mol for OCH_3 , -2162 vs. -2808 cal/mol for COOCH_3 , -2199 vs. -2332 cal/mol for CHO and -2302 vs. -2978 cal/mol for NO_2 , etc.). Thus the use of isopropanol instead of hexadecane will not change our preceding conclusions of the behavior of polar groups.

As the data in Table 1 show, all the liquid–liquid partition coefficients, as expected, decrease very substantially with increasing amount of methanol or acetonitrile in the hydro-organic phase. The changes in the partition coefficients relative to those of benzene ($K_{\text{PhX/PhH}}$) are shown in Fig. 3. The direction of the changes relative to benzene indicates that all of the more polar solutes, except benzaldehyde, increasingly prefer the hydro-organic phase as the amount of organic modifier is increased. It is im-

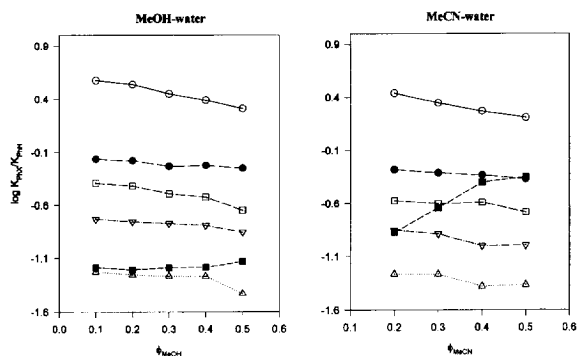


Fig. 3. Plots of ratios of hexadecane–hydro-organic partition coefficients of polar solutes to that of benzene vs. mobile phase composition (Φ). Symbols: (\circ) chlorobenzene, (\bullet) anisole, (\square) methyl benzoate, (\blacksquare) benzaldehyde, (\triangle) benzonitrile, (∇) nitrobenzene.

portant to note that these relative changes are rather small compared to the absolute changes in the partition coefficients.

These results can be rationalized as follows. First, we assume that there are only minimal or no changes in the hexadecane phase as the hydro-organic composition is varied. Thus, all changes are attributed to the net interactions in the hydro-organic phase. Second, the dipolar and hydrogen bonding interactions in the hydro-organic phase diminish as the volume fraction of organic cosolvent is increased. This has been thoroughly established in a number of detailed solvatochromic studies of the dipolarity (π^*), HB donor acidity (α) and HB acceptor basicity (β) of hydro-organic phases [32–36]. Thus we must conclude that the increasing preference of the polar solutes for the hydro-organic phase as the volume fraction of organic solvent is increased must be due to a diminution in the magnitude of the unfavorable solvophobic interaction upon change in composition. We must point out that we are now talking of a small effect. Further we should not be misunderstood as having dismissed entirely the significance of net repulsive effects in the water phase. Rather for non-polar solutes and functional groups we believe that attractive interactions with the nonpolar stationary phase are the greater contributor to retention than are interactions (net unfavorable) of the same moieties in the mobile phase. We also believe that changes in retention as the mobile phase is varied are due predominantly to changes in solute–mobile phase interactions.

The above interpretation that the increasing preference of polar solutes for the hydro-organic phases as the amount of organic modifier is increased is due to a diminution in the strength of solvophobic interactions was substantiated by computing the partition coefficients of the polar solutes relative to hypothetical alkylbenzenes of the same size. This approach of using an equal-sized homomorph is well established [37–39]. By reference to previously measured $K_{C16/mob}$ values for a series of alkylbenzenes [23] and use of the solute hard core volume computed according to McGowan [40,41] to scale the solute size (see Table 3) a new set of relative K values were computed and are given in Fig. 4. By comparison to the previous results (i.e., compare Fig. 4 to Fig. 3) we see that as organic modifier is added the inter-

Table 3
Physical properties of the solutes^a

Solute	$V_x/100$	$\sum \pi_2^*$	R_2	$\sum \alpha_2^H$	$\sum \beta_2^H$
Benzene	0.716	0.52	0.610	0	0.14
Chlorobenzene	0.839	0.65	0.718	0	0.07
Anisole	0.916	0.75	0.708	0	0.29
Methyl benzoate	1.073	0.85	0.733	0	0.46
Benzaldehyde	0.873	1.00	0.820	0	0.39
Benzonitrile	0.871	1.11	0.742	0	0.33
Nitrobenzene	0.891	1.11	0.871	0	0.28

^a Values of V_x were computed according to McGowan [40,41], while values of $\sum \pi_2^*$, R_2 , $\sum \alpha_2^H$ and $\sum \beta_2^H$ were obtained from Ref. [50].

action free energies now generally diminish as the amount of organic cosolvent increases. Thus once the size-dependent solvophobic factors are canceled by referencing behavior to a hypothetical nonpolar molecule of the same size the expected trend with composition is observed.

3.3. Analysis of bonded phase RPLC

Based on the above observations with bulk phases it is not at all clear that the retention mechanism (partitioning) and driving force for retention (attraction into a nonpolar phase) which apply to methylene and chloro groups in bonded phase RPLC will be applicable to polar groups. First, based on the above it is clear that polar functional groups interact with the mobile phase components much more strongly than they do with the nonpolar parts of the stationary

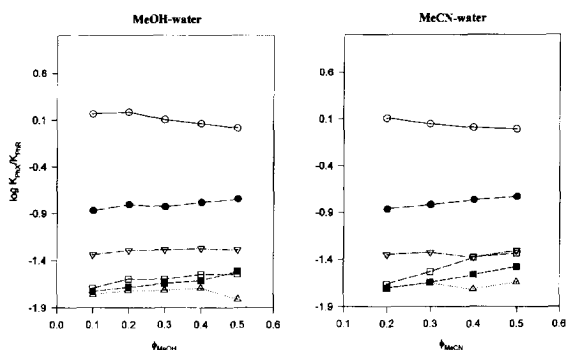


Fig. 4. Plots of ratios of hexadecane–hydro-organic partition coefficients of polar solutes to that of alkylbenzene having the same hard core volume as the polar solutes vs. mobile phase composition (Φ). Symbols are the same as in Fig. 3.

phase. Second, it is well known [42–46] that a large amount of solvent is sorbed into the bonded stationary phase and polar solutes will very likely interact with the sorbed polar solvents through dipolar and hydrogen bonding interactions. Third, solvatochromic investigations of the bonded phase in RPLC using polar probes show that the bonded phase is really rather polar ($\pi^*=0.80$) [47]. Finally, our studies of the relative stationary phase activity coefficients of alkylbenzenes in bonded phase RPLC indicate that the stationary phase appears to be about as polar as isopropanol [18]. The importance of polar interactions in the stationary phase will be discussed below.

According to our interpretation [17] of Dill and Dorsey's lattice model of RPLC [9,10], the ratio (F) of $\Delta G_{\text{bulk}}^{\circ}$ to $\Delta G_{\text{RPLC}}^{\circ}$ can be used to indicate how closely retention in bonded phase RPLC resembles a bulk phase partition process.

$$F = \Delta G_{\text{bulk}}^{\circ} / \Delta G_{\text{RPLC}}^{\circ} \quad (8)$$

The analysis in our previous work [17] is based on Dill's ideas of the number of points of contact that a solute makes when it is adsorbed on top of the phase vs. number of points of contact it makes when it penetrates the phase [10]. In the hexadecane–mobile phase partitioning system, $\Delta G_{\text{bulk}}^{\circ}(X)$ arises from replacement of z functional group–mobile phase interactions with z functional group–hexadecane interactions, where z is the solute's lattice coordination number. For a simple cubic lattice model, z is equal to six. A functional group that is retained by a partition process will have its z functional group–mobile phase interactions replaced with z functional group–bonded phase interactions. If bulk hexadecane is a good model for a bonded phase, then F should be close to unity. In contrast, when an adsorption mechanism is dominant, only one face of the solute will be in contact with the bonded phase and thus F should be about z . Thus the free energy of transfer of a functional group for a partition process, assuming a cubic lattice, will be about 6-fold that for an adsorption process. For a methylene group with an aqueous methanol mobile phase and an octadecyl bonded phase it has been shown that $\Delta G_{\text{bulk}}^{\circ}(X)$ and $\Delta G_{\text{RPLC}}^{\circ}(X)$ are equal to within 10–20% over a wide

range in mobile phase composition. This indicates that nonpolar solutes are almost fully embedded in the bonded phase chains and hence the partition model of RPLC is valid. This also implies that bulk hexadecane is a good model of nonpolar RPLC bonded phases for nonpolar solutes [17].

Fig. 5 shows plots of F for several polar functional groups vs. the volume fraction of organic cosolvent in the methanol–water and the acetonitrile–water system. In marked contrast to what we have observed for methylene groups the free energies of transfer of polar functional groups in RPLC are quite different from the free energies of transfer between the bulk phases. The trends observed in the variation of the ratio (F) with organic volume fraction for polar groups are also very different from what is seen for methylene groups. For some polar groups F increases as the volume fraction of organic modifier increases, while for the other groups it becomes smaller. For a nonpolar methylene group the F ratio is about 1.1 to 1.2 and increases with organic volume fraction over the composition range studied although the extent of increase is small [17]. In some cases with aqueous methanol mobile phases the F values for polar groups are less than unity and negative, but in most cases the F values are much greater than unity. This clearly indicates that bulk hexadecane is not a good model of the bonded phase for polar groups.

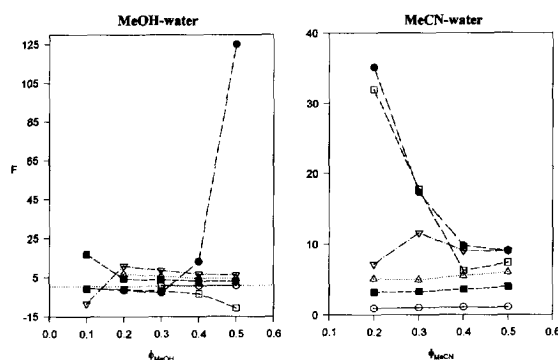


Fig. 5. Plots of ratios (F) of free energies of transfer of functional groups from mobile phase to hexadecane to that from mobile phase to ODS bonded phase vs. mobile phase composition (Φ). Symbols: (○) Cl, (●) OCH₃, (□) COOCH₃, (■) CHO, (△) CN, (▽) NO₂.

3.4. Effect of mobile phase on ratio of K to k'

The effect of changes in the composition of the hydro-organic phase on the relative retention processes is shown in Fig. 6. Here we show plots of the ratio of the bulk phase partition coefficient (K) to the capacity factor (k') vs. composition. For any specific solute these changes are really rather small but not so small as to be negligible. To understand these plots we point out that the free energies of interactions in the hydro-organic phase completely cancel out when we take this ratio. We will not consider the meaning of the absolute numbers here since this would require measurement of the phase ratio Φ , a notoriously difficult parameter to define for bonded phase RPLC. Rather we confine our attention to the change in the ratio (K/k'). As the volume fraction of organic modifier is increased, the ratio generally decreases in the acetonitrile–water systems. This means that the free energy of transfer from bulk hexadecane to the bonded phase becomes more favorable as acetonitrile is added to the hydro-organic phase. However, in the methanol–water systems the ratio generally increases as methanol is added to the hydro-organic phase. This indicates that the stationary phase formed by sorption in the acetonitrile–water systems is different from that formed in the methanol–water systems. It is well known that the relative amount of water and organic modifier sorbed onto the bonded phase in the acetonitrile–water system is different from that in the methanol–water systems [42–46]. With different

amounts of water and organic modifier sorbed on the bonded phase the strength of dipolar and HB interactions of the stationary phase with polar and hydrogen bonding solutes (see Table 3) may vary differently in these two mobile phase systems as the composition is varied. The amount of HB acidic surface silanol groups being blocked by the sorbed solvents will also vary differently.

3.5. Use of linear solvation energy relationships to understand differences in RPLC and bulk partitioning

In order to understand in more detail which types of interactions between polar solutes and the stationary phase might make an RPLC bonded phase chemically different from bulk hexadecane we compared the linear solvation energy relationships for hexadecane–water partition coefficients ($K_{C16/w}$) [48] to those for RPLC k' values. Estimated k' values for an octadecyl bonded stationary phase in pure water mobile phase [27] were analyzed. These k' values were estimated by linearly extrapolating data obtained in methanol–water mixtures vs. volume fraction to pure water. We must point out that the k'_w values obtained by extrapolation from different organic modifier can be considerably different [49]; however, this will not alter our qualitative conclusion. These results are shown in Eqs. 9 and 10.

$$\begin{aligned} \log k'_w = & -0.56 + 4.01V_x/100 - 0.55 \sum \pi_2^* \\ & + 0.30R_2 - 0.57 \sum \alpha_2^H - 2.15 \sum \beta_2^H \\ n = & 52, \text{ S.D.} = 0.12, r = 0.9920 \end{aligned} \quad (9)$$

$$\begin{aligned} \log K_{C16/w} = & 0.13 + 4.39V_x/100 - 1.60 \sum \pi_2^* \\ & + 0.65R_2 - 3.55 \sum \alpha_2^H - 4.91 \sum \beta_2^H \\ n = & 374, \text{ S.D.} = 0.13, r = 0.9980 \end{aligned} \quad (10)$$

The symbol V_x denotes the solute hard core volume computed according to McGowan [40,41], $\sum \pi_2^*$ is the solute dipolarity/polarizability, R_2 the excess molar refraction, $\sum \alpha_2^H$ is the solute hydrogen bonding (HB) acidity and $\sum \beta_2^H$ is the solute HB basicity [50]. The various coefficients in Eqs. 9 and 10 are related to the difference in the corresponding

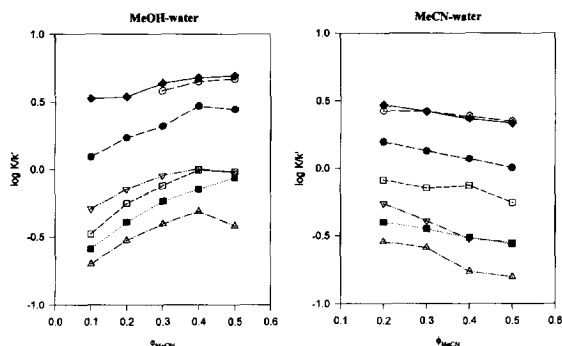


Fig. 6. Plots of ratios of the bulk partition coefficient (K) to the capacity factor (k') vs. mobile phase composition (Φ). Symbols: (\diamond) benzene, (\circ) chlorobenzene, (\bullet) anisole, (\square) methyl benzoate, (\blacksquare) benzaldehyde, (\triangle) benzonitrile, (∇) nitrobenzene.

complementary property of the mobile and stationary phases. Thus if the mobile and stationary phase are equally dipolar then there should be no dependence of k' or K on solute dipolarity. Similarly if the stationary and mobile phases are equally good HB donors there should be no dependence on solute HB basicity.

The coefficients of the volume terms in Eqs. 9 and 10 are in very good agreement. This is a most important observation given that Eq. 9 applies to bonded phase RPLC and Eq. 10 applies to bulk phase partitioning. To interpret the meaning of this agreement we should recognize that within a homologous series of solutes there is almost no variation in dipolarity, HB acidity or HB basicity. Thus except for a small effect due to changes in R_2 all differences are due to V_x . It is evident that V_x (solute size) is a linear function of carbon number. The slope of this relationship is 14.08 (cc/mol CH_2). We can now convert the slopes of Eqs. 9 and 10 to free energies per CH_2 by assuming that only V_x varies in a homolog series. We find -565 and -618 cal/mol CH_2 for bonded phase RPLC and for bulk hexadecane, respectively. These free energies are in good agreement with one another. In view of the fact that these free energy values are obtained from data for a large number of nonhomologous solutes they are in reasonably good agreement with the free energy obtained by examining the transfer of a series of n -alkylbenzenes from water to hexadecane (-774 cal/mol CH_2). Furthermore the ratio of the slopes of Eqs. 9 and 10 and therefore of the free energies per CH_2 is 1.09; this is in exceedingly good agreement with the results we reported previously as obtained with a homologous series of n -alkylbenzenes [17].

We turn now to a rationalization of the differences in behavior of polar solutes in a bulk partitioning system and in bonded phase RPLC. The largest differences between Eqs. 9 and 10 are in the coefficients of the HB basicity ($\Sigma\beta_2$) and acidity ($\Sigma\alpha_2$). Water and the ODS phase are HB acidic as well as HB basic. The hydrogen bonding properties of the ODS stationary phase are due to the presence of unreacted surface silanol groups and sorbed polar solvents. In contrast hexadecane can not act as either an HB donor or acceptor. Thus the net difference in HB acidity between hexadecane and water, which is reflected in the magnitude of the coefficient for HB

basicity (-4.91), is greater than the net difference in the HB acidity between the ODS bonded phase and water (-2.15). For the same reason the net difference in the HB basicity between hexadecane and water is also greater than the net difference in the HB basicity between the ODS bonded phase and water. The coefficient for the dipolarity/polarizability term ($\Sigma\pi_2^*$) is also quite different since hexadecane is much less dipolar than is the ODS phase when modified by sorbed solvent. Thus, according to Eqs. 9 and 10, polar and hydrogen bonding functional groups should prefer the ODS phase to the hexadecane phase. Since the dipolarity and hydrogen bonding properties of the aqueous mobile phases when modified with up to 50% methanol and acetonitrile are not very different from those of pure water [32–36] the above explanation also holds for other mobile phase systems studied. On the other hand, nonpolar solutes which do not hydrogen bond, can not differentiate between the ODS and hexadecane phases. This leads to the conclusion that while bulk hexadecane can be used to model the behavior of methylene groups in RPLC bonded phases it is definitely not a good model for polar solutes.

An alternative explanation for the deviation of F from unity for polar solutes is that the polar groups, in contrast to methylene groups, are not embedded in the bonded phase but rather they sit at the interface between the bonded and mobile phases. We offer in support of this the following observation. A common indicator dye for the measurement of a solvent's HB acidity, 2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridino)-phenolate, is dipolar and strongly HB basic. This molecule is essentially insoluble in hexadecane equilibrated with either aqueous methanol or aqueous acetonitrile of the same compositions as those studied in this work [22]. The dye is, however, strongly retained on an ODS stationary phase. The capacity factor for this dye is greater than 10 with pure acetonitrile mobile phase [47]. This striking contrast between the abnormally high retention on the ODS phase and negligible solubility of the dye in the hexadecane phase can hardly be explained solely by differences in interaction properties (dipolarity and HB strength) between the two phases. We ascribe the abnormally high retention on the ODS phase to a huge difference in interfacial surface area between the ODS bonded phase and the hexadecane phase, in

addition to the differences in the interaction properties between them.

It is also possible that upon placing a polar solute in the bonded phase, solute-induced solvent sorting takes place [36,51–54]. The polar solute is preferentially solvated by polar components sorbed in the bonded phase and thus retained strongly by the polar environment which the solute induced in the bonded phase.

4. Conclusions

In contrast to methylene units, the interactions of polar functional groups with the mobile phase are often larger than are their interactions with the stationary phase. Further, the free energy of transfer of a polar functional group in RPLC is quite different from the free energy of transfer between the mobile phase and a bulk nonpolar liquid bonded phase emulsator. This implies that either sorbed mobile phase significantly influences the retention properties of polar groups, or that polar functional groups reside at the interface between the mobile and stationary phases.

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