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

II. Partitioning vs. adsorption mechanism in monomeric alkyl bonded phase supports

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Abstract

The partitioning and adsorption mechanisms of retention in reversed-phase liquid chromatography (RPLC) were examined based on the free energy of transfer of a methylene group from organic aqueous mixtures to bulk hexadecane. Retention on monomeric bonded phases with octyl chains or longer are dominated by a partitioning-like mechanism. An adsorption-like mechanism contributes to retention in monomeric bonded phases with short bonded chains or with low surface coverage density. The retention mechanism becomes more adsorption-like as the length of the bonded chains decrease. © 1997 Elsevier Science B.V.

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1. Introduction

Despite the great popularity of reversed-phase liquid chromatography, our understanding of the solute retention mechanism at the molecular level is still incomplete. One of the major and long standing issues in the theory of RPLC is whether solute molecules are retained by a partitioning or by an adsorption-like process. Many retention models of partitioning and adsorption mechanisms have been proposed [1–9]; however, even the definitions given for partitioning and adsorption differ.

In this study, we investigate the retention mecha-

nism of nonpolar solutes as per Dill and Dorsey's definitions of partitioning and adsorption given in their simple mean-field lattice model of RPLC [1,10]. According to this model, "partitioning" of a solute takes place when the solute is fully embedded within the stationary phase, i.e. when almost all of its surface is in contact with the bonded alkyl chains that constitute the interphase. On the other hand, a pure "adsorption" model requires the solutes to adsorb onto the nonpolar film established by the nonpolar alkyl chains, i.e. they only make surface contact with the interphase and are not fully embedded. To further illustrate these concepts, imagine a simple lattice model where each solute molecule has z surfaces, that is, its lattice coordination number is z . In the mobile phase, a solute molecule makes z solute–mobile phase contacts. Under the "partition-

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ing” mechanism, all z solute–mobile phase interactions are replaced by solute–stationary phase interactions. The exchange of solute–bulk phase interactions is reflected in the free energy of transfer of a solute from the mobile phase to the stationary phase. Under the “adsorption” mechanism, only a fraction of the solute–mobile phase interactions are replaced by solute–stationary phase interactions. It is difficult to be more quantitative than this without assuming that the mobile phase composition in the interphase region is the same as in the bulk mobile phase. Assuming that this is so indicates that in a simple cubic lattice model where z equals six, only 1/6 of the solute–mobile phase interactions will be replaced as a cubic solute adsorbs onto the bonded phase. It is important to point out that the retention mechanism does not have to be either completely “partitioning” or “adsorption”, it can be both.

Based on the then available experimental results in conjunction with the interphase lattice model, Dill and Dorsey [1,10] indicated three lines of evidence that support partitioning as the principal mechanism in RPLC. First, a partitioning mechanism should be affected by the surface density of the grafted chain phase but the adsorption mechanism should not. Claudy et al. [11] observed a decrease of retention for hexane and benzene upon increasing the grafted chain density. Sentell and Dorsey [12] showed that the partition coefficients of small nonpolar solutes decrease above a critical bonding density of about $3.1 \mu\text{mol}/\text{m}^2$, and k' approximately plateaus because of compensating changes in the partition coefficients and the volume phase ratio. Second, in a wide variety of experiments [3,13–20], the logarithmic capacity factor for a series of solutes is observed to be linearly related to the logarithm of the appropriate oil/water partition coefficient, with a slope of unity. A slope of unity is theoretically expected for a partitioning mechanism. In addition, Lochmuller and Wilder [21] have shown that selectivities are similar for grafted chain phases and in liquid hydrocarbons. Third, Tchaplá et al. [22] showed the existence of a subtle but real discontinuity in plots of $\log k'$ vs. solute carbon number for a homologous series, at a point where the carbon number of the homologous series was about equal to the length of the bonded alkyl chains. The above experimental evidence suggests that a better understanding of the physical

location of the solute molecules could certainly improve the development of molecular retention models, and the construction of quantitative structure retention relationships (QSRR) for the estimation of physicochemical parameters.

In this work, the free energy of transfer of a methylene group in RPLC, i.e. from aqueous organic mixtures into the bonded phase, will be compared to that in a pure partitioning system, i.e. from the same aqueous organic mixtures into bulk hexadecane. Here, bulk hexadecane is used to model the alkyl bonded phase in RPLC. Previous studies have shown that hexadecane is a rather good model of the bonded phase for nonpolar solutes [23]. The approach and results presented here are restricted to nonpolar solutes, and specifically to methylene group. All comparisons will be based on measurable quantities for each type of system, that is, the capacity factors in RPLC and partition coefficients in bulk phase partitioning system.

The chromatographic capacity factor, k' is expressed as the product of $K_{\text{stat}/\text{mob}}$, the corresponding ratio of solute concentrations in the stationary and mobile phase and Φ , the column “phase ratio”.

$$k' = K_{\text{stat}/\text{mob}} \cdot \Phi \quad (1)$$

$K_{\text{stat}/\text{mob}}$ can be expressed in terms of the standard Gibbs free energy of transfer from the mobile phase to the stationary phase ($\Delta G_{\text{stat}/\text{mob}}^\circ$), the gas constant (R) and the absolute temperature (T), as shown below.

$$\ln K_{\text{stat}/\text{mob}} = -\Delta G_{\text{stat}/\text{mob}}^\circ / RT \quad (2)$$

Combining Eq. (1) and Eq. (2), we get Eq. (3) where $\Delta G_{\text{stat}/\text{mob}}^\circ$ is further subdivided into contributions from the methylene group(s) and from the functional and terminal methyl groups, and n_c is the number of methylene group.

$$\begin{aligned} -RT \ln k' &= \Delta G_{\text{stat}/\text{mob}}^\circ - RT \ln \Phi \\ &= n_c \cdot \Delta G_{\text{stat}/\text{mob}}^\circ(\text{CH}_2) + \Delta G_{\text{stat}/\text{mob}}^\circ(\text{functional} \\ &\quad + \text{methyl}) - RT \ln \Phi \end{aligned} \quad (3)$$

Thus, the slope of a $-RT \ln k'$ vs. n_c plot for a homologous series denotes the standard Gibbs free energy of transfer for a methylene group,

$\Delta G^\circ(\text{CH}_2)_{\text{stat/mob}}$, from the mobile phase to the stationary phase. The phase ratio term, Φ , that is so difficult to determine experimentally is not needed to evaluate $\Delta G^\circ(\text{CH}_2)_{\text{stat/mob}}$.

Similarly, the partition coefficient between hexadecane and the mobile phase, $K_{\text{C16/mob}}$, can be related to the free energies of transfer:

$$\begin{aligned} -RT \ln K_{\text{C16/mob}} &= \Delta G_{\text{C16/mob}}^\circ \\ &= n_c \Delta G^\circ(\text{CH}_2)_{\text{C16/mob}} \\ &\quad + \Delta G^\circ(\text{functional} + \text{methyl})_{\text{C16/mob}} \end{aligned} \quad (4)$$

where $\Delta G_{\text{C16/mob}}^\circ$, $\Delta G^\circ(\text{CH}_2)_{\text{C16/mob}}$ and $\Delta G^\circ(\text{functional} + \text{methyl})_{\text{C16/mob}}$ denote the standard free energy of transfer from the mobile phase to bulk hexadecane for a solute, for a methylene group, and for the functional and terminal methyl groups, respectively.

According to the lattice model proposed by Dill and Dorsey [1,10], the ratio of $\Delta G^\circ(\text{CH}_2)_{\text{C16/mob}}$ to $\Delta G^\circ(\text{CH}_2)_{\text{stat/mob}}$, defined here as F , can be used to indicate how closely the RPLC retention resembles the partitioning process.

$$F = \Delta G^\circ(\text{CH}_2)_{\text{C16/mob}} / \Delta G^\circ(\text{CH}_2)_{\text{stat/mob}} \quad (5)$$

In the hexadecane–mobile phase partitioning system, $\Delta G^\circ(\text{CH}_2)_{\text{C16/mob}}$ is associated with the replacement of z methylene–mobile phase interactions with z methylene–hexadecane interactions. A methylene group that is retained by a “partitioning” process in RPLC will have all z methylene–mobile phase interactions replaced with z methylene–bonded phase interactions. If bulk hexadecane is a good model for a bonded phase, the ratio F for a RPLC partitioning process should be close to unity. Conversely, if, in RPLC, a methylene group is retained by an “adsorption” process, only one surface of the solute will be in contact with the bonded phase, and thus F should be considerably larger than unity perhaps as large as z , the lattice coordination number.

Recently, we reported that with methanolic mobile phases and an octadecyl bonded phase, $\Delta G^\circ(\text{CH}_2)_{\text{C16/mob}}$ and $\Delta G^\circ(\text{CH}_2)_{\text{stat/mob}}$ are within 10% of one another over a very wide range in mobile phase composition [23]. This supports the view that

the solute is embedded in the bonded phase chain, and thus supports the partition model. The present work extends the previous study to a series of phases with different chain lengths and surface coverage densities. The ratio F , serves as an indicator as to the relative extent of the contributions of partitioning vs. adsorption mechanism to a methylene group. It is examined here as a function of: (1) sources of bonded phases, (2) the chain length of the bonded phase and (3) the density of the bonded phase chain.

2. Experimental

The standard Gibbs free energies of transfer for a methylene group in RPLC, $\Delta G^\circ(\text{CH}_2)_{\text{stat/mob}}$, were calculated from the capacity factors of alkylbenzenes taken from the work of Czok and Engelhardt [24]. In their work, a unified equation for the natural logarithmic capacity factor was given as:

$$\ln k' = A + Bn_c + C\phi + Dn_c\phi \quad (6)$$

where n_c is the carbon chain length of solute molecule, ϕ is the weight fraction of water in the methanolic mobile phase and A , B , C and D are fitting coefficients. Eq. (6) is based on the k' values of a series of n -alkylbenzenes obtained in mobile phases ranging from 50 to 90% (ϕ) methanol [25]. The fitting coefficients are available for a wide variety of alkyl bonded phases: ten octyl bonded phases and twelve octadecyl bonded phases that were either commercially available or were made by Engelhardt and coworkers [24,25]; and monomeric bonded phases with eight different chain lengths synthesized using the same silica. In general, the stationary phase materials have the following properties: particle size of 10 μm , surface area of 300–350 m^2/g , pore diameter of 90–100 \AA and pore volumes of 1 ml/g . Detailed characteristics of the stationary phase materials can be found in Ref. [24] and references cited therein. The relative standard deviation of the k' values was 15% [24]. Eq. (6) is valid for correlation of retention in mobile phases ranging from 5 to 95% (v/v) water [26].

The Gibbs standard free energies of transfer of a methylene group in a hexadecane–organic aqueous partitioning system, $\Delta G^\circ(\text{CH}_2)_{\text{C16/mob}}$, were taken

from the work of Li [27]. These free energy values were also based on the use of alkylbenzene homologs. At the lowest methanol concentration, the mutual solubilities between the organic aqueous phase and hexadecane are insignificant [28]. Thus, $K_{16/mob}$ values for 0–50% methanol aqueous mixtures were acquired through the direct measurement of solute concentrations in bulk hexadecane and organic aqueous phases by head space gas chromatographic (HSGC) measurements [27]. On the other hand, the $K_{C16/mob}$ values for 60–100% methanol aqueous mixtures were calculated from two separate measurements of partition coefficients: K_{mob} (partition coefficient between aqueous organic mixture and the gas phase) and K_{C16} (the partition coefficient between hexadecane and the gas phase) values based on Eq. (7).

$$K_{C16/mob} = K_{mob} / K_{C16} \quad (7)$$

Both the RPLC and bulk phase partitioning measurements mentioned above were made at 25°C.

3. Results and discussion

3.1. Sources of bonded phases

First, we examined the retention mechanism of a series of monomeric octyl bonded phases that differ in their silica support characteristics and silanization processes. The bonded phases are either commercially available or homemade [24]. Fig. 1 shows the free energy ratios, F , of the octyl phases vs. methanol concentration (% volume) in the mobile phase. For ease of discussion, the figure is divided into two regions: (I) 0–70 (% volume) and (II) 70–100 (% volume) methanol concentrations.

In region I, the free energy ratios are close to unity, varying from 1.1 to 1.6. Clearly, the ratios are nearly independent of the mobile phase composition. At most there is a slight upward trend. It is crucial to point out that there is no significant difference in F among the octyl phases of different sources. According to the Dill–Dorsey lattice model, a free energy ratio close to unity indicates that the partition mechanism is dominant. Therefore, in the mobile phase composition range from 0 to 70% methanol, a

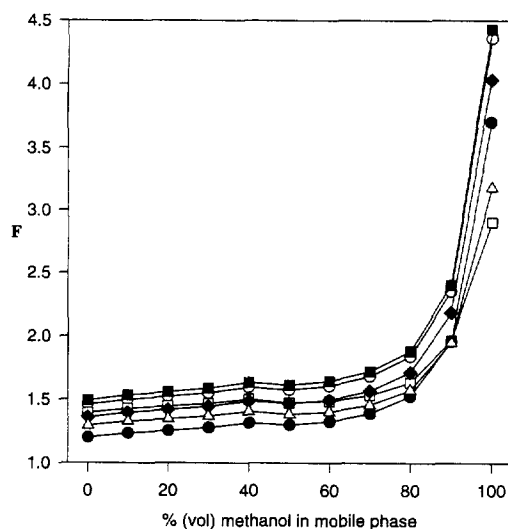
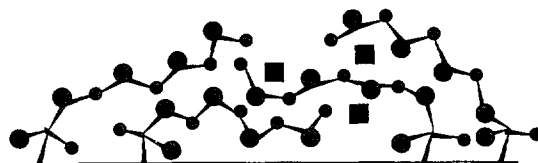


Fig. 1. Plots of F vs. mobile phase composition for octyl bonded phases Lichrospher (Δ), LiChrosorb (\circ), LiChrosorb Select B (\square), Nucleosil (\blacksquare), Hypersil (\bullet) and Spherisorb (\blacklozenge). Data were taken from the work of Jungheim in Ref. [24].

methylene group shows a partition-like retention mechanism on all the octyl bonded phases studied here. Although these octyl phases differ in their silica support characteristics (pore size, surface chemistry, etc.) and silanization processes, their retention mechanisms, at least for a methylene group, are essentially the same. As shown in Scheme 1, in the 0–70% methanol range, the bonded chains are expected to take on a folded configuration due to the favorable dispersive (London) interactions among the chains, and the unfavorable interactions of the chains with the “hostile” mobile phase [21]. The “hydrophobic clustering” of bonded chains provides a liquid-like environment for a methylene solute to be retained via a partitioning mechanism.

In this study, we assume that methylene groups interact only with the nonpolar bonded alkyl chains,



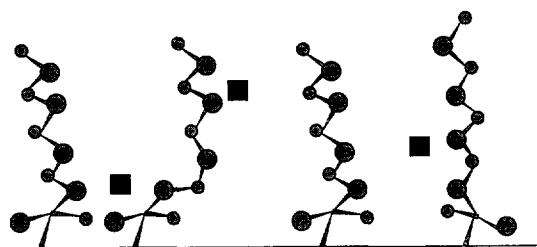
Scheme 1. Bonded chains assume a folded configuration in highly aqueous mobile phase.

but not with the polar sorbed mobile phase components [23]. This assumption is expected to hold in mobile phases of low methanol content, where solvent sorption into the bonded phase is rather low [29,30]; but not at high methanol content where solvent sorption into the bonded phase is significant [29,30]. If the probe solutes interact with the sorbed modifier, this will act to decrease $\Delta G^\circ(\text{CH}_2)_{\text{stat}/\text{mob}}$. That should result because sorption of modifier should produce behavior which is intermediate between that of a neat bonded phase and the mobile phase. We say this because $\Delta G^\circ(\text{CH}_2)_{\text{gas}/\text{alcohol}}$ varies monotonically as the alcohol phase is varied from *n*-methanol to *n*-octanol, and there is little difference in $\Delta G^\circ(\text{CH}_2)_{\text{gas}/\text{hexadecane}}$ and $\Delta G^\circ(\text{CH}_2)_{\text{gas}/\text{octanol}}$ [31]. A smaller $\Delta G^\circ(\text{CH}_2)_{\text{stat}/\text{mob}}$ value should lead to larger **F** values because $\Delta G^\circ(\text{CH}_2)_{\text{C}_{16}/\text{mob}}$ is independent of the mobile phase composition. Thus, a large value of **F** may reflect either a greater contribution to retention by the adsorption mechanism and/or sorption of solvent into the bonded phase. However, **F** values close to unity indicate that the entire solute surface is in contact with the bonded alkyl chains and thus must suggest a partitioning mechanism. The solubility of methanol and water in bulk hexadecane is extremely low [28,32]. Previous work in this laboratory has demonstrated that the minute amount of methanol and water dissolved in bulk hexadecane has very little effect on the measured gas–liquid partition coefficients for hexadecane equilibrated with water–methanol mixtures [28,33].

In region II [$>70\%$ (v/v) methanol), the trend in the free energy ratio is much more complicated. As seen in Fig. 1, the free energy ratios increase as the amount of methanol in the mobile phase increases. At 100% methanol, the free energy ratio varies over a wide range, from 2.9 to 4.5. The results in region II are subject to considerable uncertainty. First, the amount of mobile phase sorbed into the bonded phase in this region is substantial [29,30,34]. Thus, as discussed above, the large $\Delta G^\circ(\text{CH}_2)_{\text{stat}/\text{mob}}$ value may indicate sorption of mobile phase components into the bonded phase, instead of reflecting an increased contribution from the adsorption mechanism of a methylene group. Second, sorption of mobile phase components induces the *trans* conformation of the bonded chains and causes them to

become more extended [35]. As a result, the bonded chains may no longer provide an environment such that a methylene group can be totally surrounded by the bonded chains (see Scheme 2). Consequently only a fraction of the solute surface can be in contact with the bonded chains and **F** increases. In addition, variations in the base silica and silanization properties may have substantial effects on the chain configurations and lead to a large range of **F** values at 100% methanol. Third, solutes are only very weakly retained (most *k'* values were much smaller than unity) in the highly organic mobile phases and thus the results are subject to considerable experimental error due to problems in determining the system dead volume. The interpretation of the results in region II is somewhat ambiguous. Hence we are reluctant to draw any conclusions as to the solute retention mechanism in region II.

We next examined a series of monomeric octadecyl bonded phases of different silica support characteristics and silanization processes, where the free energy ratios are shown in Fig. 2. The results for the octadecyl phases are very similar to those for the octyl phases. In the mobile phase region of 0 to 70% methanol, the **F** values are only slightly dependent on the mobile phase composition. Within this range, the free energy ratios are close to unity, i.e. the values range from 1.1 to 1.4. These values are only slightly lower than those for octyl phases (this will be discussed below). In addition, there is no significant difference among the **F** values for various octadecyl phases, just as we observed for the octyl phases. Again, based on Dill's lattice model, a methylene group shows a partition-like retention mechanism on all the octadecyl bonded phases studied. These results confirm our previous observa-



Scheme 2. Bonded chains assume an extended configuration in highly organic mobile phase.

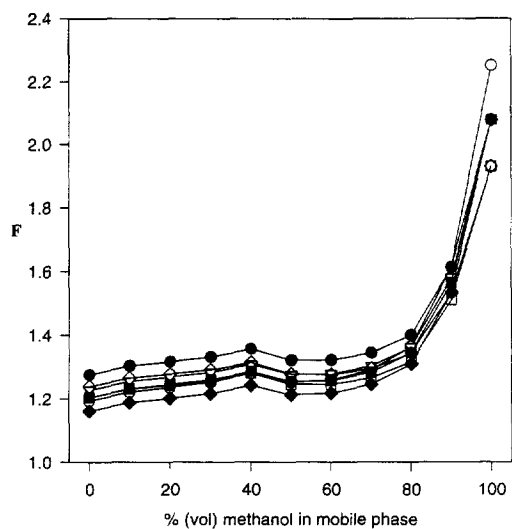


Fig. 2. Plots of F vs. mobile phase composition for octadecyl bonded phases 37-Lichrospher (\circ), 16-LiChrosorb (∇), 29-Nucleosil (\diamond), 31-Zorbax (\blacklozenge), 21-Hypersil (\square), 23-Spherisorb (\blacksquare) and 25-Whatman 15% (\bullet). Data were taken from the work of Jungheim in Ref. [24].

tions based on a Zorbax ODS phase [23] and thus indicate that the partitioning mechanism for methylene groups is universal and fundamentally significant for a wide variety of octadecyl bonded phases.

The results in region II for the octadecyl phases are more complicated, as also observed for the octyl phases. In Fig. 2, the F values for most of the octadecyl phases increase with the amount of methanol in the mobile phase. At 100% methanol, the ratio for some phases exceeds 2. Overall, the F values for octadecyl phases in region II are lower than those for octyl phases. In other words, the retention mechanism for a methylene group is somewhat more partition-like in octadecyl phases than in octyl phases in organic rich mobile phases. The results are consistent with the view that octadecyl chains are still somewhat folded at high methanol concentration. This is not unreasonable because methanol is considered to be a relatively weak mobile phase modifier in RPLC. However, the results in region II are subjected to many uncertainties and should not be considered as final. Discussion of the results obtained in the range of 70–100% will be excluded from the remaining studies.

In addition to the above results taken from a single

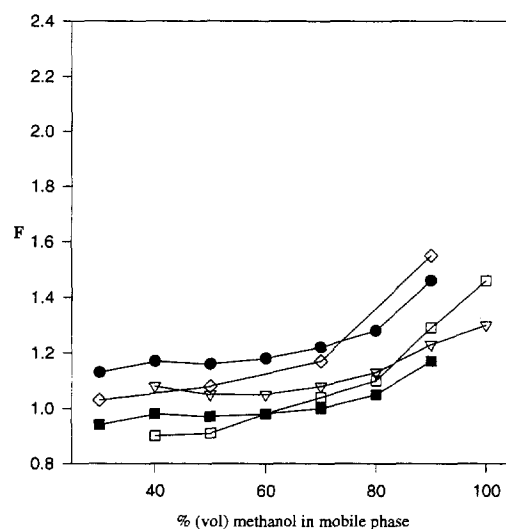


Fig. 3. Plots of F vs. mobile phase composition for octadecyl bonded phases taken from different sources: Zorbax (∇), Bondapak (\blacksquare), Hypersil (\bullet), Lichrosorb (\diamond) and μ -Bondapak (\square).

source, we also investigated reversed-phase data taken from other research groups. Fig. 3 shows the free energy ratio plots for five different octadecyl bonded phases taken from different sources [36–40]. The results are virtually the same as those observed in Fig. 2. We thus conclude that the partitioning mechanism is generally valid and must be of fundamental significance.

Overall, the retention mechanism of a nonpolar moiety resembles a partitioning system more than an adsorption system, on both octadecyl and octyl bonded phases. The results hold for many types of bonded phases that vary in their silica support characteristics and silanization processes. However, we must caution that we are speaking in very broad terms. Chromatography is so selective that extremely small differences in the free energy of transfer have easily measured chromatographic consequences. There are real differences in the behavior of the materials described thus far.

3.2. Chain length of the bonded phase

We next investigated the retention mechanism of a methylene group as a function of bonded phase chain length. Fig. 4 shows the F plots of eight monomeric bonded phases with different bonded chain lengths,

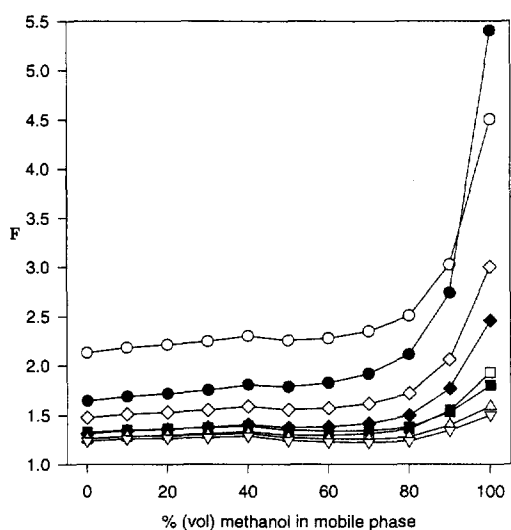
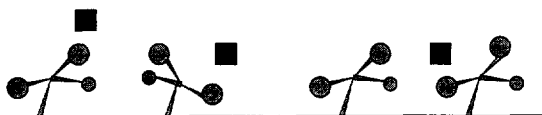


Fig. 4. Plots of F vs. mobile phase composition for monomeric alkyl bonded phases: RP-1 (\circ), RP-3 (\bullet), RP-6 (\diamond), RP-8 (\blacklozenge), RP-10 (\square), RP-12 (\blacksquare), RP-16 (\triangle) and RP-18 (∇).

i.e. RP-1, RP-3, RP-6, RP-8, RP-10, RP-12, RP-16 and RP-18. These bonded phases were prepared on the same silica support [24].

Fig. 4 clearly shows that at any given mobile phase composition, the free energy ratio decreases as the bonded phase chain length increases. Over the entire range of 0 to 70% methanol, the F values for the RP-1 phase are larger than 2; that for the RP-3 phase varies from 1.7 to 1.9; and that for the RP-6 phase, from 1.5 to 1.6. The F values for bonded phases with chain length RP-8 or longer are virtually the same, that is, they vary from 1.2 to 1.4. Applying Dill's model to interpret this: the retention mechanism of a methylene group becomes somewhat less partition-like as the bonded phase chain length decreases. This is in accord with chemical intuition. For a RP-1 bonded phase, there is only a thin layer of alkyl chain deposited on the silica base. A methylene moiety cannot be fully embedded in the interphase (see Scheme 3). The bonded chains are



Scheme 3. Bonded phase with short alkyl chains (RP-1).

not long enough to contact all the surface of a solute molecule, nor can they provide a liquid-like environment for a partitioning mechanism. A free energy ratio of 2 indicates that about half of the surface of a methylene group can be in contact with the bound trimethyl silyl groups in an RP-1 material. As the bonded phase chain length increases, more solute surface can interact with the bonded phase molecules and the free energy ratio drops. The results agree with reports that methylene selectivity increases as a function of bonded phase chain length [41–44]. If adsorption were the sole retention governing mechanism, the methylene selectivity for all bonded phases, regardless of chain length, would not be affected by the bonded phase chain length.

For bonded phase RP-8, the chains are long enough to interact with almost all the solute surface, as indicated by the free energy ratio near unity. Once this situation prevails, further elongation of the bonded chain no longer affects the free energy ratio. In other words, for bonded phases with chain length RP-8 or longer, "partitioning" becomes the principal retention mechanism for a methylene group. This is in accord with the prediction of Dill's theory [1,10] in which solute adsorption is the dominant mechanism of retention for short chain phases, whereas solute partitioning is the dominant mechanism of longer chain phases. The results are consistent with previous findings where further increases in the chain length of the bonded phase chains no longer increases the methylene selectivity [45,46]. A certain critical chain length is required to provide a liquid-like environment for the partitioning of a methylene group. Once this is achieved, further elongation of the bonded chain is no longer very effective.

In an independent study, Eikens measured the gas-liquid partition coefficients of *n*-alkane homolog in a series of *n*-alkane solvents varying from *n*-pentane to *n*-hexadecane [31]. The change in the slope of the free energy vs. solute carbon number, i.e. the methylene selectivity, is very small (see Table 1). In a separate liquid-liquid extraction study, no change in methylene selectivity was observed with increasing chain length for the nonpolar phase [47]. The above results indicate that pure partitioning is not sensitive to solvent chain length, which is opposite to what we observed on RPLC bonded phases of chain length shorter than eight carbon.

Table 1
The slopes of \ln (gas–liquid) partition coefficients versus carbon number for *n*-alkane homolog^a

Solvent	Slope ^b
<i>n</i> -Pentane	1.159(0.023)
<i>n</i> -Hexane	1.182(0.019)
<i>n</i> -Heptane	1.174(0.002)
<i>n</i> -Octane	1.171(0.002)
<i>n</i> -Nonane	1.149(0.012)
<i>n</i> -Decane	1.132(0.013)
<i>n</i> -Dodecane	1.153(0.009)
<i>n</i> -Hexadecane	1.156(0.003)

^a Data taken from Ref. [46].

^b The value in parentheses denotes the standard deviation.

This suggests that although a methylene group is embedded in the bonded phase, as indicated by the free energy ratio, the partitioning mechanism involved is different from a bulk phase partitioning system. The partitioning system in RPLC is complicated by the packing constraints resulting from the lateral interactions among neighboring chains [1]. In addition, alkyl chains are anchored on the fixed silica support in RPLC and thus the entropy effect is greater for solutes to reside near the grafted ends. Consequently, solutes accumulate on the mobile phase-bonded phase boundary region instead of distributing uniformly in the bonded phase. Our terminology of “partitioning” simply emphasizes that almost all the surface of a solute is in contact with the bonded chains, but does not imply the uniform distribution of solute as in a bulk phase partitioning system.

In addition, the fact that pure partitioning is not sensitive to solvent chain length [31] suggests that bulk hexadecane is a legitimate model for bonded phase of various chain lengths. We do not need to use a bulk alkane whose chain length is comparable to its bonded phase counterpart in this study.

3.3. Density of bonded phase chain

Fig. 5 shows results obtained with Whatman Partisil octadecyl phases of different carbon loading, i.e. 5, 10 and 15%. Because these bonded phases are based on the same silica supports, their carbon loadings correlate with the chain bonding density. According to the information provided by the manufacturer, the 5, 10 and 15% carbon loading materials

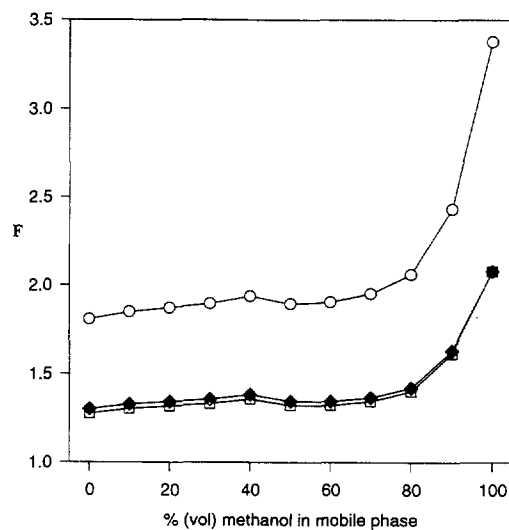
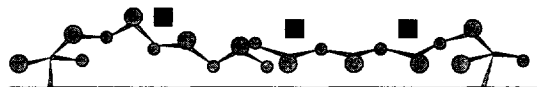


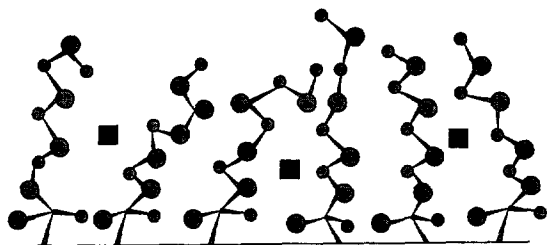
Fig. 5. Plots of F vs. mobile phase composition for Whatman Partisil RP-18 phases of different chain densities: $0.6 \mu\text{mol}/\text{m}^2$ or 5% carbon (\circ), $1.4 \mu\text{mol}/\text{m}^2$ or 10% carbon (\blacklozenge) and $2.3 \mu\text{mol}/\text{m}^2$ or 15% carbon (\square).

correspond to bonded chain density of 0.6, 1.4 and $2.3 \mu\text{mol}/\text{m}^2$, respectively. The F values for the $0.6 \mu\text{mol}/\text{m}^2$ phase are significantly larger than the other two phases of higher carbon loading, at all mobile phase composition. The F values for the 1.4 and $2.3 \mu\text{mol}/\text{m}^2$ phases are virtually the same, and close to unity in 0–70 (% vol.) methanol in the mobile phase.

The results suggest that the retention mechanism becomes more “partition-like” as the coverage density increases. However, once a critical surface coverage for the bonded phase is achieved, a methylene group is retained via a “partition-like” mechanism. At low coverage density, i.e. $0.6 \mu\text{mol}/\text{m}^2$ in this case, the bonded chains only manage to form a thin nonpolar film on the silica base (see Scheme 4). The bonded phase is not thick enough to interact with the entire surface of a methylene group. F values of 1.8 indicate that only about half of the solute surface is in contact with the alkyl chains. As



Scheme 4. Alkyl bonded phase of low surface coverage density.



Scheme 5. Alkyl bonded phase of high surface coverage density. The solute molecules are shown as small cubes.

the chain density increases, the bonded phase becomes denser and thus interacts with more of the solute surface (see Scheme 5). In other words, the bonded phase is dense enough to create a liquid-like medium that a methylene group can partition into. Once the critical limit of surface coverage is reached, further increase in surface coverage has very little effect on the free energy ratio. Our results are consistent with Engelhardt and Jungheim's [48] report on the effect of bonded chain coverage on hydrophobic/methylene selectivity (relative retention of ethylbenzene to toluene). For Whatman Partisil octadecyl phases of 5, 10 and 15% carbon loading, they reported hydrophobic/methylene selectivity of 1.54, 1.82 and 1.91, respectively. Again, because these bonded phases are based on the same silica supports, their carbon loading correlates with the chain bonding density. Obviously, there is a significant difference in selectivity between the low and the medium density phase, but a rather small difference between the medium and the high density phase. Nevertheless, we expect that the chain density of the $2.3 \mu\text{mol}/\text{m}^2$ phase we discuss here has not yet reached the critical value where entropy dominates and causes repulsion of solutes from the bonded phase, as predicted by Dill [1,10] and experimentally shown by Sentell and Dorsey [12].

3.4. Comparison with previous RPLC models

In simple terms, factor F is the ratio of the free energy per methylene group involved in the hexadecane/mobile phase partitioning process ($\Delta G^\circ_{\text{partitioning}}$) to the free energy involved in RPLC ($\Delta G^\circ_{\text{RPLC}}$). $\Delta G^\circ_{\text{partitioning}}$ is comprised of the free energy involved in both the C16 phase and the

mobile phase, while $\Delta G^\circ_{\text{RPLC}}$ results from free energy involved in both the bonded stationary phase and the mobile phase:

$$F \equiv \Delta G^\circ_{\text{partitioning}} / \Delta G^\circ_{\text{RPLC}} \approx (\Delta G^\circ_{\text{C16}} + \Delta G^\circ_{\text{mob}}) / (\Delta G^\circ_{\text{stat}} + \Delta G^\circ_{\text{mob}}) \quad (8)$$

The free energy terms for the mobile phase in both the bulk partitioning process and RPLC are the same and therefore are assigned the same symbol, $\Delta G^\circ_{\text{mob}}$.

Consequently in order for F to be equal or close to unity,

$$\Delta G^\circ_{\text{stat}} \approx \Delta G^\circ_{\text{C16}} \quad (9)$$

Because the transfer into the bulk C16 phase must be a partitioning process, we conclude that RPLC is much closer to being a partitioning than an adsorption process.

The traditional RPLC model of solvophobic theory [49–51] argues that retention of a nonpolar solute is largely controlled by the free energy liberated by the formation of water–water contacts in the mobile phase upon the removal of the nonpolar solute, which implies that the net free energy of interaction is much greater in the mobile phase than in the hexadecane phase or the stationary phase:

$$\Delta G^\circ_{\text{mob}} \gg \Delta G^\circ_{\text{C16}}, \text{ and } \Delta G^\circ_{\text{mob}} \gg \Delta G^\circ_{\text{stat}} \quad (10)$$

These theoretical assumptions of solvophobic theory also lead to values of F near unity. However, *these assumptions were proven not true in our previous work which is based on direct experimental measurement* [23]. Our previous work has shown that the net free energy of interactions of a methylene group with the hexadecane is larger than the net free energy of interactions of a methylene group with the mobile phase, or $\Delta G^\circ_{\text{C16}} > \Delta G^\circ_{\text{mob}}$. Using a series of alkylbenzene homologs, $\Delta G^\circ(\text{CH}_2)_{\text{mob/gas}}$ ranges from +141 cal/mol to –4.59 cal/mol as methanol content in the mobile phase changes from 0 to 100%. In comparison, $\Delta G^\circ(\text{CH}_2)_{\text{C16/gas}}$ is –606 cal/mol for the alkylbenzenes. Therefore, *we emphasize that a value of F close to unity does not prove the solvophobic theory.*

Although the above studies were based solely on free energy values derived from the alkylbenzene

homologs, we expect similar results if other homologous series were to be used. Tchaplal et al. [22] showed that the slope of $\log k'$ vs. solute carbon number, from which the above free energies were derived, is essentially independent of the homolog series. In addition, Carr et al. showed that the free energies of transfer of a methylene group from water to hexadecane are similar for a series of aliphatic and aromatic solutes [23]. However, the above results on the partitioning vs. adsorption mechanism are restricted to methylene groups, and probably nonpolar solutes in general. We anticipate a different retention mechanism for polar solutes whose polar headgroups tend to extend into the mobile phase [35,52,53].

Based on the surface coverage of bonded alkyl chains, which usually varies from 2.0 to 4.0 $\mu\text{mol}/\text{m}^2$, RPLC bonded phases are significantly less dense than in bulk hexadecane. However, values of F near unity indicate that both the bonded phase and bulk hexadecane behave rather similarly despite the difference in density. This suggests that the bonded alkyl chains may be present in a dense liquid form as proposed in the Lochmuller and Wilder liquid–droplet model of the bonded phase [21]. According to this model, alkyl chains collapse and form patches of alkyl droplets that permit three-dimensional interactions with solute molecules. Thus the local density of bonded chains, that is as they exist in patches, is greater and therefore more liquid-like than is the overall average density. A recent molecular dynamics simulation of bonded alkyl phases by Klatt and Beck [54] come to very similar conclusions. Based on topographical maps of the interphase's outer surfaces, they suggest that the bonded chains agglomerate into liquid-like patches which they call "hills", due to energy-favorable chain–chain interactions. These hilly agglomerates exist in a state of "dynamic equilibrium", i.e. the ordering persists over long times, but locally the agglomerated chains constantly undergo relatively rapid liquid-like motions. These oscillatory density profiles arise even in vacuum and suggest that the chain–chain attractive interactions are the main driving force for the chains to maximize their contacts and form the agglomerates. Klatt and Beck's work concludes that the interphase will show extensive disorder, roughness and fluidity.

Based on the results and discussion presented

above, we emphasize two models for bonded phase liquid chromatography. The first is the partition model where the solute is essentially fully embedded in the stationary phase, and the other is the adsorption model in which the sorbed solute is not fully immersed within the bonded phase chains, but rather it is, at most, in only partial contact with the bonded phase chains. Below, the term "sorption" is used to describe the process of transfer of a solute from its equilibrium mobile phase environment to its equilibrium stationary phase environment without any implication as to whether the solute partitions within or adsorbs on top of the stationary phase medium. Similarly the solute in its equilibrium stationary phase state will be called the "sorbed" solute. Based on the chemical and physical differences in the stationary phase, and how the solute senses the stationary phase, the partition model can be further subdivided into the bulk partition model and the surface partition model.

In the *bulk partition* model, the stationary phase is chemically and physically similar to a low surface area, bulk liquid-like phase such as *n*-hexadecane. It does not have much water or organic modifier associated or dissolved in it. The surface of the support particle does not have much effect on the chemical potential of any solutes sorbed within the phase either chemically through the surface silanol groups, or electrostatically via the action of the potential field at the boundary of the solid. Furthermore the highly curved radius of the pore does not influence the chemical environment. We note that direct measurements of the water and organic modifier present in bulk hexadecane equilibrated with aqueous–organic mixtures indicates that vastly less water and methanol or acetonitrile are present compared to the amount that becomes associated with a bonded phase [28].

In the *surface partition* model, the solute is still extensively embedded within the bonded phase interphase layer but it senses an environment that is chemically and physically different from a simple bulk fluid analog such as *n*-hexadecane. In this case the chemical potential of the solute is influenced by the water and organic solvent associated with the surface bonded ligands or with the solid surface per se or its silanol groups. This perturbation takes place through the action of the very high surface area of

the highly dispersed thin surface phase or through the electrostatic potential due to the proximity to the solid surface phase boundary. The solute's chemical potential could also be perturbed by the high curvature of the small pores in which it is found when in the stationary phase.

A common feature of all liquid phase adsorption models is the necessity of exchange of adsorbing solute for pre-adsorbed mobile phase species. Based on how the solute interacts with the pre-adsorbed mobile phase species, the adsorption model is subdivided into the modified substrate model and multi-layer model. The extent of solute adsorption, that is, the fractional amount of solute that adsorbs, under linear chromatographic conditions, will vary in proportion to the area of the adsorbent and not the volume of the bonded phase that modifies the substrate.

In the *modified substrate* model, the bonded phase chains chemically modify the underlying silica surface and transform it from a polar to a nonpolar or distinctly less polar surface. Components of the eluent then differentially adsorb onto and perhaps within the bonded phase chains. Solute molecules sorb by partially interacting with the new surface thereby displacing or exchanging with some of the initially adsorbed eluent species.

In the *multi-layer* model, a multi-layer of eluent adsorbs strongly on top of the bonded phase chains. The first layer adsorbs so strongly, compactly and completely that it is not displaced when the solute adsorbs. The locus of solute adsorption is the less strongly adsorbed, partially filled (mezzanine) layer of solute. Some groups indicate that the mezzanine layer is so sparsely populated that solute can adsorb within it without displacing pre-adsorbed eluent species, however, this is unlikely in liquid processes but it may take place in supercritical fluids or in gases.

4. Conclusions

The above thermodynamic analysis shows that a methylene group is retained via a partition-like mechanism on monomeric bonded phases with octyl chains or longer. We have no evidence for a partitioning mechanism for monomeric bonded phase

with short bonded chains (shorter than hexyl) or with low surface coverage density (less than $1.4 \mu\text{mol}/\text{m}^2$) as may be indicated by $F > 1$. A F ratio greater than unity suggests the possibility of an adsorption-like process for these systems, but as pointed out above, other interpretations are possible. A monomeric bonded phase has to reach a threshold value of some minimal bonded chain length or surface coverage in order to provide a "liquid-like" environment for the partitioning of a methylene group. For bonded chain lengths shorter than octyl, the retention mechanism becomes less bulk partition-like as the length of the bonded chains decreases. Thus, for practical RPLC purposes, bonded phases of moderate chain length and bonding density are desired so as to generate sufficient methylene selectivity. In addition, since partitioning is the dominant mechanism for nonpolar solutes, the solute size (volume), instead of the solute surface area is crucial in determining solute retention.

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