The mechanism of solute retention in reversed-phase liquid chromatography

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are between 1.4 and 4.4 nm. Thus, the 7 nm of the effective diameter is considered as the maximum value. The persistence lengths (l) estimated were rather insensitive to the effective diameter, or frictional parameter (fp). The l's were between 65 to 150 nm and decreased as the concentrations of NaDNA and/or NaCl increased. The orders of l are quite reasonable if we compare our data with the previous reports.[10,13-20] A theoretical evaluation of the values was also cited in the table using the theoretical equation proposed by Odijk[21,22] and de Breq.[23]

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The Mechanism of Solute Retention in Reversed-Phase Liquid Chromatography

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Theory is developed to describe the retention of small molecules by the grafted chain phases used in reversed-phase liquid chromatography. The affinity of the solute for the grafted phase is determined by the entropy of mixing of the solute, the configurational entropy of the grafted chains, and the contact interactions among solute, solvent, and chains, treated here in the random mixing approximation for single or binary eluents. Retention can be predicted from simple physical quantities, such as the oil–water partition coefficient, but generally not from the solvent surface tension alone, as proposed in the "solvophobic theory". These grafted chains are semiordered at the interface; therefore, (i) solutes should partition less into the grafted phase than into amorphous oillike phases, and (ii) hydrophobic solutes should concentrate nearer the free than the grafted ends. It is shown that retention is principally due to solute partitioning into, rather than adsorption onto, the grafted chains.

Reversed-phase liquid chromatography (RPLC) has rapidly become one of the principal methods for separating molecules in solution. It is estimated that 80–90% of HPLC chromatographic systems currently in use are reversed-phase columns.[1] The most common reversed-phase systems are comprised of a stationary phase in which alkyl chains, ranging in length from 1 to 18 carbons, are grafted onto silica surfaces. Solutes are carried over these surfaces by the flow of mobile-phase solvents, referred to as eluents. Chromatographic separation occurs when there are differences in the degree of binding of different solute molecules to the stationary phase: tightly bound solutes elute after weakly bound solutes since they are less accessible to the flow of the mobile phase. Here we present simple mean-field lattice statistical mechanics theory to describe the nature of this molecular retention.

The quantity which is measured in the chromatography experiment is the retention factor, k', which is the ratio of the number of moles of solute associated with the stationary phase to the number of moles of solute in the mobile phase. For experimental convenience, k' may be expressed as the product of a partition coefficient, K, the ratio of molar concentrations of solute in the stationary and mobile phases, and the "phase ratio", Φ, of the relative volumes of the stationary to mobile phases; i.e., $k' = \Phi K$.

Retention could occur by adsorption of the solute molecules at the ends of the grafted chains, or by partitioning of the solute into the grafted chain phase, or both. The nature of the retention process will depend on the nature of the molecular organization of chains grafted to the stationary phase. Simple descriptions of the alkyl chain packing have previously been proposed;[1] they are shown in Figure 1a. Those models are likely to be oversimplifications in at least two respects. First, they imply that alkyl chains are rigid rods with no internal degrees of freedom. At the temperatures of interest for chromatography, however, alkane molecules are disordered. For example, Sander et al.[2] have shown that the grafted alkyl chains of RPLC stationary phases have significant populations of gauche bonds. Thus, stationary phases are comprised of chains which are more disordered than the models in Figure 1a would imply. Second, the so-called "fur" and "stack" models predict that the grafted chains are fully exposed to the mobile-phase solvent. However, the solvent in reversed-phase experiments is generally water or an aqueous solution. In light of the strength of the hydrophobic effect, it should be prohibitively expensive in free energy terms for the chains to configure themselves to permit such a high degree of exposure. This problem is avoided by the so-called "picket fence" model, shown in Figure 1a, but it represents only the limiting case of maximum possible surface density, a situation seldom achieved in practice.

We propose an alternative model here, shown in Figure 1b. The grafted phase should have the same molecular organization as that of other interfacial phases of chain molecules, herein termed "interphases". Examples of chain molecule interphases include surfactant aggregates such as monolayers, bilayers, micelles, and microemulsions,[3-5] and regions within semicrystalline polymers.[6] Interphases are comprised of chains which have one end anchored at an interface; their surface densities are sufficiently high that configurational constraints of the chains are severe. Often the thickness of an interphase is only a few molecular diameters. Such systems are interfacial in two respects: their surface/volume ratio

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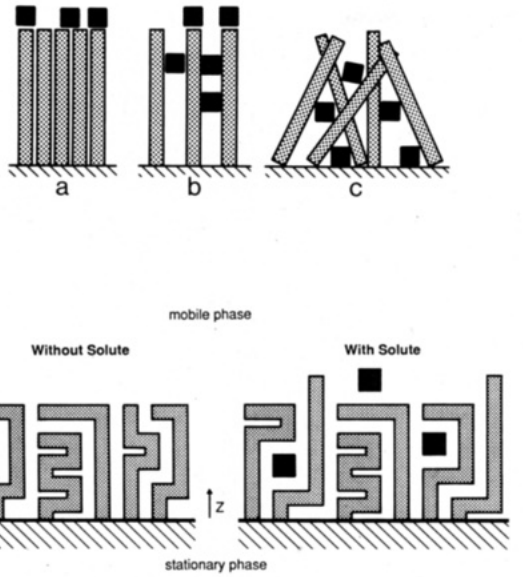

Figure 1. (a, top) Conventional models of molecular organization of stationary-phase chains in RPLC:[1] (a) "picket fence", (b) "fur", (c) "stack". (b, bottom) Interphase model of molecular organization of stationary-phase chains.

is high, and their properties vary with depth from the interface. For example, there is a "disorder gradient" in amphiphilic aggregates: chains have greater orientational order near their head groups than near their methyl ends.[3,5,7] This variation of properties with depth contrasts with bulk phases of matter, whose properties, by definition, are invariant with spatial position.

The molecular organization within the interphase is determined by three factors:[3-6] (i) the constraints imposed by the geometry of the interface and the surface density and lengths of the chains grafted thereon, (ii) in poor solvents (such as for alkyl chains in water), the requirement that the interphase region largely exclude the solvent; this volume is filled principally by chain segments (and solute), and (iii) in conformance with the second law of thermodynamics, the requirement that the chains adopt as much disorder as is consistent with the imposed constraints. The surface densities of the grafted chains in RPLC stationary phases, which typically range from 2 to 4 μmol/m^2,[1] are in the same range as those of other interphases; bilayer membranes of dipalmitoylphosphatidylcholine at their main transition temperature, for example, have surface densities of about 5 μmol/m^2.[3] We consider here only those grafted chain phases with densities greater than about 2.5 μmol/m^2. NMR measurements of cross-polarization[8] and T1 relaxation rates and line widths[9-11] show that the ends of these chains are in more rapid motion than segments near the points of covalent attachment to the silica surfaces; such gradients of motion are characteristic of interphases.[12,13]

We consider separately below partitioning or adsorption as alternative mechanisms of retention. Partitioning is treated by using a lattice approach recently developed for solutes within interphases;[14] adsorption is treated by using the lattice monolayer approximation.[15-20] The principal purposes of the present work are to adapt those treatments to RPLC, to generalize them to account for more than one eluent, most commonly of interest in chromatography, and to estimate the relative importance of partitioning and adsorption in RPLC. The approach presented here permits consideration of any possible geometry of the silica surface onto which the chains are grafted. Although the pores through which the mobile phase flows can be quite small, we assume here that the silica surfaces are approximately planar and are sufficiently separated that chains emanating from one surface do not interact with those of another; these should often be good approximations.[1] The effects of curvature should be small except when radii of curvature are a few molecular chain lengths or less.[14]

Partitioning

We first consider partitioning. The partition coefficient may be computed from the chemical potentials of the solute s, in the mobile-phase mixture of components A and B, and in the grafted chain phase C. We consider only systems in which molecular species s, A, and B are of the same size and are approximately isodiametric. Consistency with the lattice model of the interphase requires that each linear dimension of the solute molecule should be approximately equal to the width of the grafted chains, or about 4–5 Å for alkyl phases. The chemical potential for the solute, s, in the mobile-phase solvent mixture can be derived by using lattice methods.[15] Suppose there are n_x molecules of type x (x = s, A, B), randomly distributed on a space-filling lattice representing the volume occupied by the mobile phase, and that each molecule occupies one and only one lattice site. Complete filling of the lattice requires that

$$N = n_s + n_A + n_B \tag{1}$$

where N is the total number of lattice sites in the mobile phase. In terms of the concentrations, $\phi_x = n_x/N$, the translational entropy of mixing, S_{mix}, of the system is[15]

$$\frac{S_{mix}}{Nk} = -\phi_s \ln \phi_s - \phi_A \ln \phi_A - \phi_B \ln \phi_B \tag{2}$$

where k is Boltzmann's constant. Because solute and solvent are assumed to be of the same size in the lattice theory, mole fraction and volume fraction are identical measures of concentration. When solute and solvent are of different size, a case not treated here, then it follows from Flory–Huggins theory that the appropriate concentration variable is the volume fraction.[21] In addition to the mixing entropy of solute and solvent in the mobile phase, there will be a contact free energy due to the intermolecular interactions of a molecule with its neighbors. The total contact free energy of the mobile phase is the sum of all the free energies of contact

$$F_{contact} = m_{ss}w_{ss} + m_{AA}w_{AA} + m_{BB}w_{BB} + m_{sA}w_{sA} + m_{sB}w_{sB} + m_{AB}w_{AB} \tag{3}$$

where m_{xy} is the number of contacts between molecules of types x and y, and w_{xy} is the local free energy per contact. The latter is the potential of mean force, or the reversible work of bringing the molecules from infinite separation into contact, i.e., the contact energy minus the temperature multiplied by the local entropy. The local entropy includes all but the translational entropy of mixing described above. Let z represent the number of nearest-neighbors of each lattice site. Then the number of contacts is related to the number of molecules of each type by three equations of the form

$$zn_v = 2m_{vv} + m_{vx} + m_{vy} \tag{4}$$

where the subscript for each of the equations is v, x, y = (s, A, B), (A, B, s), and (B, s, A), respectively, for this ternary mixture.

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In the Bragg–Williams approximation, the solution is assumed to be randomly mixed,[15] i.e.

$$m_{xy} \simeq z n_x n_y / N \tag{5}$$

The normalized contact, or exchange, free energy is defined by[15,21]

$$\chi_{xy} = \frac{z}{kT}\left(w_{xy} - \frac{w_{xx} + w_{yy}}{2}\right) \tag{6}$$

where T is the temperature. The total contact free energy is computed from combination of eq 3–6, i.e.

$$\frac{F_{\text{contact}}}{kT} = \left(\frac{zw_{ss}}{2kT}\right)n_s + \left(\frac{zw_{AA}}{2kT}\right)n_A + \left(\frac{zw_{BB}}{2kT}\right)n_B + \chi_{sA}\left(\frac{n_s n_A}{N}\right) + \chi_{sB}\left(\frac{n_s n_B}{N}\right) + \chi_{AB}\left(\frac{n_A n_B}{N}\right) \tag{7}$$

The chemical potential of the solute in this mixed solvent, AB, is

$$\frac{\mu_{AB}(s)}{kT} = (kT)^{-1}\frac{\partial}{\partial n_s}[F_{\text{contact}} - TS_{\text{mix}}]_{n_A,n_B,T} \tag{8}$$

$$= \ln \phi_s + \left(\frac{zw_{ss}}{2kT}\right) + \chi_{sA}\phi_A(1-\phi_s) + \chi_{sB}\phi_B(1-\phi_s) - \chi_{AB}\phi_A\phi_B$$

where S_{mix} and F_{contact} are taken from eq 2 and 7.

Next we consider solute molecules in the interphase, C. In addition to the entropy of mixing and contact free energy, the insertion of solute into the semiordered interphase is accompanied by a decrease in the configurational entropy of the grafted chains.[14] At constant surface density, the increase of volume due to solute uptake must lead to chain extension, and therefore to increased alignment of the chains along the axis normal to the interface. This latter contribution disfavors solute retention. We neglect here the retention of solvents A or B into the grafted phase C; there is evidence that some organic cosolvents may be retained along with the solutes on the stationary phase.[11,22] If there were some uptake of solvent, then the chain ordering would be somewhat greater and it would further disfavor solute retention. Such effects can be fully taken into account using the present theory, but we neglect these minor complications here in order not to obscure the principal features of the theory. The chemical potential for the solute in the interphase is[14]

$$\frac{\mu_i(s)}{kT} = \ln\left(\frac{\phi_i}{q_i}\right) + \frac{zw_{ss}}{2kT} + \chi_{sC}(1-\phi_i)^2 - \frac{g\delta_{i,1}}{kT} \tag{9}$$

where $i = 1, 2, \ldots, L$ denotes the planar layer of the interphase, numbered from the interface; ϕ_i is the fraction of sites in that layer occupied by solute molecules; and q_i is a statistical weight whose value is between 0 and 1; it accounts for the change in conformational entropy of the constrained chains upon uptake of the solute. Values of the q_i's are tabulated elsewhere.[14] The quantity $-g\delta_{i,1}$ accounts only for the layer of solute molecules immediately adjacent to the silica surface within the interphase; it is zero for all but layer $i = 1$ and represents the free energy of replacing a grafted chain segment in contact with the silica surface in layer $i = 1$ by a solute molecule in that layer.[14] This factor takes into account, for example, the effect of interaction of solute with residual silanols underlying the grafted chains.

At equilibrium, the chemical potentials of the solute in the mobile and stationary phases will be equal

$$\mu_i(s) = \mu_{AB}(s) \tag{10}$$

When the solute concentration is small in both mobile and stationary phases ($\phi_s, \phi_i \ll 1$), then combination of eq 1 and 8–10 leads to the partition coefficient:

$$K_i = \frac{\phi_i}{\phi_s} = q_i \exp\left[(\chi_{sA} - \chi_{sC}) + \phi_B(\chi_{sB} - \chi_{sA} - \chi_{AB}) + \phi_B^2\chi_{AB} + \frac{g\delta_{i,1}}{kT}\right] \tag{11}$$

Certain limiting cases of this equation will be useful for the discussion below. First, if the mobile phase contains only a single eluent, pure A and no B, then eq 11 simplifies to

$$\ln\left(\frac{\phi_i}{\phi_s}\right) = \ln q_i + (\chi_{sA} - \chi_{sC}) + \frac{g\delta_{i,1}}{kT} \tag{12}$$

which corresponds to a situation previously considered.[14] The equivalent result obtains, of course, if the mobile-phase eluent is pure B. Second, if C were not an interfacial phase, but were itself comprised of small molecules of the same size and randomly mixed with s, then eq 11 simplifies to

$$\ln K_{C/AB} = \ln\left(\frac{\phi_C}{\phi_s}\right) = (\chi_{sA} - \chi_{sC}) + \phi_B(\chi_{sB} - \chi_{sA} - \chi_{AB}) + \phi_B^2\chi_{AB} \tag{13a}$$

and for the single mobile-phase solvent

$$\ln K_{C/A} = \chi_{sA} - \chi_{sC} \qquad (\phi_B = 0) \tag{13b}$$

since effects of interfacial layers are neglected in that case, and since $q_i = 1$ for amorphous systems with no configurational constraints. The binary interaction parameters, χ, can be obtained from measurements of free energy of transfer or of vapor pressure in the Henry's law region (see below). For example, the transfer of solute molecules of species x from pure x to infinitely dilute solution with y is characterized by a free energy of transfer (divided by kT) of $\chi_{xy} - \chi_{xx} = \chi_{xy}$ (note $\chi_{xx} = 0$; see eq 6).

Whereas the lattice theory above predicts the equilibrium partition coefficient for solute from the mobile phase solvent into layer i of the grafted chain phase, comparison with experiment requires the prediction of the retention factor, k'. This requires that we sum the numbers of molecules over all the layers of the interphase and that we define the phase ratio, Φ. The retention factor is the ratio of the number of solute molecules in the interphase to the number of solute molecules in the mobile phase. Using eq 11, we have

$$k' = \frac{N_0\sum_{i=1}^{L}\phi_i}{N\phi_s} = \left(\frac{N_0}{N}\right)K_{C/AB}\sum_{i=1}^{L}q_i \exp\left(\frac{g\delta_{i,1}}{kT}\right) \tag{14}$$

where N_0 is the number of sites per layer of the interphase and $K_{C/AB}$ is defined in eq 13a. In terms of the net partitioning into the whole interphase (int)

$$K_{\text{int/AB}} = (L\phi_s)^{-1}\sum_{i=1}^{L}\phi_i \tag{15}$$

and the phase ratio must be defined by

$$\Phi = \frac{k'}{K_{\text{int/AB}}} = \frac{N_0 L}{N} = \frac{N_C v_C}{V_0} = \frac{N_C v_C}{N v_s} \tag{16}$$

where the latter equalities follow from the density constraints

$$N_0 L = N_C n = N_C\left(\frac{v_c}{v_s}\right) \tag{17}$$

and where V_0 is the total mobile-phase volume, v_s is the molar solute volume, v_C is the molar volume of the grafted chains, n is the number of lattice segments per chain, and N_C is the number of grafted chains. Thus Φ is just the ratio of the total volume of the grafted chains to the total volume of the mobile phase, all in the absence of solute. This definition of phase ratio applies when the mobile-phase solvent is a pure single component; we do not consider here the experimental problem of definition of the

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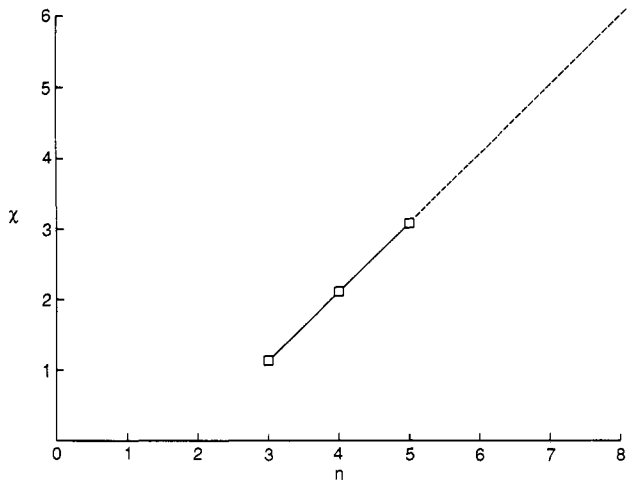

Figure 2. Binary interaction parameter, χ, for methanol with alkanols of other chain lengths, n.[27] We extrapolate this data to $n = 6$ (hexanol) and $n = 8$ (octanol) to obtain the values needed to predict ln k' vs. solvent composition shown in Figure 3.

phase ratio when the mobile phase is a mixture of solvents.

The configurational constraints of the chains become less severe with increasing distance from the silica interface.[3,14] Thus solutes which are alkyl-like, i.e., whose chemical affinity for the silica surface is identical with that of the grafted chains, should be most concentrated near the free ends of the grafted chains and least concentrated near the silica surface (i.e., the q_i are largest near the chain ends; see Figures 2–4 of ref 14). Moreover, the sum of the q_i values determines the total degree of partitioning; this quantity decreases linearly with increasing surface density (see Figure 5 of ref 14). Thus the total solute concentration in interphases is predicted to decrease linearly with increasing surface density of the grafted chains, for a given value of the phase ratio. Solute concentrations in interphases should be lower than in corresponding bulk phases. The theory predicts that partitioning should decrease to zero as the chains reach their maximum possible packing density, approximately 8.1 μmol/m^2 for alkyl chains. In support of this prediction, Claudy et al.[23] have shown that total retention of hexane from the gas phase decreases with increasing surface density of different chromatographic bonded phases.

Many experiments are currently available with which we can test the predicted dependence of retention on solvent composition, eq 14. Comparison of theory with experiments requires knowledge of three constants, χ_{sA}, χ_{sB}, and χ_{AB}. These quantities may be obtained from free energies of transfer as described above and from vapor pressure measurements in the Henry's law region,[15] or they may be estimated from Hildebrand solubility parameters,[24] for example. We compare the predictions of the theory to the experiments of Karger et al.[25] on hexanol and octanol as solutes in water/methanol mixtures. We use the value of $\chi_{AB} = \chi_{MeOH/w} = 0.425$ from the Henry's law measurements of Rytting et al.[26] of the infinite dilution activity coefficient of methanol in water. To obtain χ_{sB}, we have taken the initial slopes of the vapor pressure curves of mixtures of propanol, butanol, and pentanol with methanol;[27,28] the results are shown in Figure 2 as a function of chain length. Inasmuch as these results fall on a straight line, we assume the values for hexanol and octanol may be obtained by linear extrapolation of that data, since values for those systems appear to be otherwise unavailable. Thus $\chi_{sB} = \chi_{hex/MeOH} = 4.05$ and $\chi_{oct/MeOH} = 6.00$. We obtain χ_{sA} from free energies of transfer; these quantities are also linear functions of chain length and may

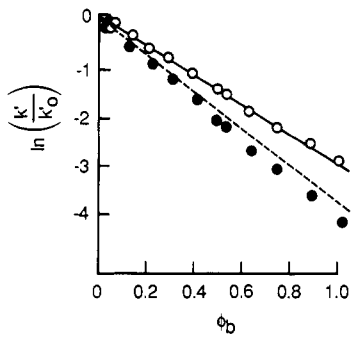

Figure 3. Predictions of the partitioning theory, eq 13a and 14 for ln k' of hexanol (—) or octanol (- - -) as solutes vs. composition in methanol/water mixtures. Values for the binary interaction constants were obtained from data on Henry's law constants or solution measurements of free energies of transfer as described in the text. The data for hexanol (○) and octanol (●) are from Karger et al.[25]

be taken from extrapolations of measurements of the transfer of alkanols from pure alkanol to water;[29] thus, $\chi_{sA} = \chi_{hex/w} = 6.823$ and $\chi_{oct/w} = 9.598$. Substituting these values into eq 14 leads to the predictions shown in Figure 3. With these constants so obtained from independent physical measurements, the theory appears to predict reasonably well the experimental retention data of Karger et al.,[25] also shown in that figure.

Equations 13a and 14 predict that the dependence of the logarithm of the retention factor on solvent composition should be linear whenever the two solvents are highly compatible, $\chi_{AB} \simeq 0$. Some eluent pairs are not as compatible as methanol and water considered above; acetonitrile in water is an example. Unlike the situation for methanol/water described above, not all of the binary interaction parameters for hexanol or octanol in acetonitrile/water are available in the literature or through extrapolation, and without the physical chemical parameters, we cannot attempt a priori prediction of retention vs. composition. However, the procedure can be reversed: the interaction parameters can be obtained from the retention experiments. At the same time we can determine whether any deviation from linearity of ln k' vs. solvent composition is due to just strong antipathy, χ_{AB} large, or is due to failure of the random mixing approximation. According to eq 13a and 14

$$\left(\frac{1}{\phi_B}\right) \ln\left(\frac{k'}{k_0'}\right) = (\chi_{sB} - \chi_{sA} - \chi_{AB}) + \phi_B\chi_{AB}$$

where k'_0 is the value of k' when $\phi_B = 0$. Thus a plot of $(1/\phi_B)$ ln (k'/k'_0) vs. ϕ_B should be linear for any value of χ_{AB}, provided the random mixing approximation holds, but it should be nonlinear when that approximation fails. When such a plot is linear, its slope will be χ_{AB}, independent of the solute, and its intercept at $\phi_B = 1$ will be $\chi_{sB} - \chi_{sA}$. By definition, for the other intercept at $\phi_B = 0$, ln $(k'/k'_0) = 0$; k'_0 is dependent on the phase ratio, the partition coefficient, and the molecular organization of the interphase. Using eq 13a and 14

$$k'_0 = \Phi K_{C/A}\langle q\rangle \qquad (17.5)$$

where

$$\langle q\rangle = L^{-1}\sum_{i=1}^{L} q_i e^{g\delta_{i,1}/kT}$$

(Note that as $\phi_B \to 0$, $1/\phi_B \to \infty$ and ln $(k'/k_0) \to 0$, but the product of these factors will be finite and equal to $\chi_{sB} - \chi_{sA} - \chi_{AB}$. Errors in the data may be magnified in the extrapolation toward $\phi_B = 0$).

The data of Karger et al.[25] for hexanol and octanol as solutes in mixtures of acetonitrile in water are plotted in this fashion and shown in Figure 4. These plots are approximately linear, implying that the random mixing approximation is suitable here. The slopes of the curves for these two solutes are not identical, as the theory

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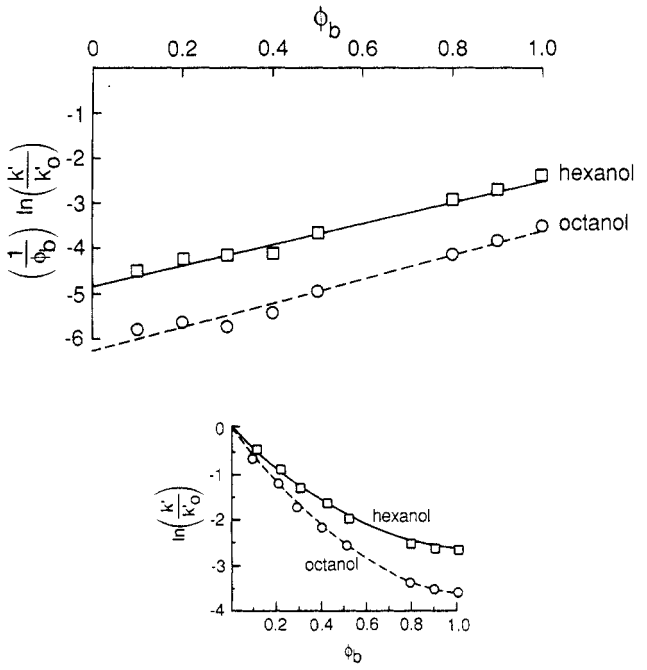

Figure 4. Retention vs. solvent composition for hexanol and octanol as solutes in acetonitrile/water mixtures. The top figure should be linear if the random mixing approximation holds; see eq 13a and 14 and subsequent discussion; the slope should be independent of the solute. The binary interaction constants can be obtained from the slope and intercept. From these curves we find $1/\phi_B \ln (k'/k'_0) = -4.823 + 2.323\phi_B$ for hexanol and $1/\phi_B \ln (k'/k'_0) = -6.295 + 2.691\phi_B$ for octanol. Using these interaction constants, the more common retention plot is shown in the bottom figure.

implies they should be; nevertheless, they differ by only about 15%, being 2.323 for hexanol and 2.691 for octanol. Moreover, the slopes compare favorably with the corresponding quantity ($\simeq$2.21) obtained by vapor pressure measurements in the Henry's law region for acetonitrile in water.[28,30,31] In plots of this type, the slope indicates the incompatibility of the solvents; for methanol/water, the slope is very small. A principal value of a plot of this type is that it permits determination of the binary exchange free energies directly from measurements of retention vs. solvent composition.

The quadratic dependence of ln k' on ϕ_B, given by eq 13a and 14 has previously been noted by Schoenmakers et al.[32,33] They found the quadratic dependence to be a useful predictor of retention behavior in methanol, ethanol, and 1-propanol as coeluents with water, but they also found that the interaction strengths were not well predicted by simple Hildebrand solubility parameters. Their approach makes use of a further approximation than that of random mixing which we have adopted here. Through their use of the Hildebrand solubility parameter of regular solution theory,[24] they have assumed that the binary interaction parameters, χ, are further factorable into unitary interaction constants (i.e., $\chi_{AB} = (\bar{v}/RT)(\delta_A - \delta_B)^2$); this is the so-called "geometric mean" approximation. In the geometric mean approximation, the binary interaction of species A with B is assumed to be equal to the geometric mean of the interactions of AA and BB. This approximation is known to be poor when there is local ordering of one molecule in the presence of the other, for example, nonpolar molecules in hydrogen-bonding solvents such as water. In that regard, binary interaction parameters in eq 13a and 14 for the predictions in Figures 3 and 4 appear to be somewhat more successful than the Hildebrand solubility parameters, perhaps because of the avoidance of the geometric mean approximation.

Nevertheless, even with the binary interaction constants, we cannot expect to predict the temperature dependence of retention in the absence of knowledge of the temperature dependence of the χ's, for in such solvents, $\chi(T)$ describes the local free energy, whose temperature dependence may be complex. If the temperature dependence of χ is not known, then retention can only be predicted at the temperature for which χ has been measured.

The "group selectivity" is defined as the ratio of the retention factor for one molecule containing a particular subunit to that of a reference molecule which does not contain the subunit. The selectivity is a measure of the partition coefficient for the added molecular subunit, provided the free energies of partitioning of the individual groups are additive. Use of eq 14 above leads to the prediction that the logarithm of the selectivity should be a linear function of coeluent concentration (provided the interphase ordering is little affected by the added subgroup)

$$\ln\left(\frac{k'_2}{k'_1}\right) = (\Delta\chi_{sA} - \Delta\chi_{sC}) + \phi_B(\Delta\chi_{sB} - \Delta\chi_{sA}) \quad (18)$$

where k'_2 represents retention of the molecule with the added subgroup, and k'_1 is retention for the molecule without the subgroup, and

$$\Delta\chi_{xy} = \chi_{x_2y} - \chi_{x_1y}$$

Thus a plot of the logarithm of selectivity vs. coeluent concentration is predicted to have as its intercept (at $\phi_B = 0$) the selectivity between eluent A and bulk hydrocarbon, and as its slope the selectivity between eluent A and eluent B. For homologous series which differ only in alkyl chain length of the solute, for example, the intercept will be the free energy of transfer (divided by kT) of a methylene group from water to pure amorphous hydrocarbon. We find that the intercepts in two recent studies of selectivity of methylene groups are 821 cal/mol[25] and 759 cal/mol;[34] these should be compared to 825 cal/mol from classical shake-flask experiments.[29] The experimental intercepts are observed to be independent of coeluent B, as expected in accordance with eq 18 for $\phi_B = 0$. In some cases, the selectivity is observed to have a nonlinear dependence on solvent composition;[35-37] the Bragg–Williams approximation is not adequate in these cases.

The observations above suggest that RPLC is a useful alternative to shake-flask experiments for measurement of free energies of transfer. Used as described above, RPLC can be of advantage when a partition coefficient is required between water and a pure hydrocarbon; these may often be difficult to obtain otherwise since solute concentrations are often very small in one phase or the other. The use of the second eluent in the chromatography experiment can reduce the partition coefficient to a readily measurable range, and these selectivities may be extrapolated to the composition of pure water.

One important difference between retention and selectivity is that retention will reflect effects of chain ordering whereas selectivity will not. The solute will be excluded from a layer of the interphase relative to the equivalent amorphous hydrocarbon phase by a factor of $q_i \leq 1$ (see eq 14). Thus the retention will be smaller by this factor than predicted by the oil/water partition coefficient. Selectivities, however, will be unaffected by this factor (see eq 18); thus, selectivities in RPLC should be the same as oil/water selectivities. In agreement with this prediction, it is observed that RPLC selectivities are approximately the same as those in oil/water systems and that retention in RPLC is smaller than in bulk oil.[1,35,38] A previous alternative hypothesis was that the factor of reduction observed in retention experiments arises because solute adsorbs rather than partitions.[1,35] In the discussion below, however, we describe evidence that supports the view that partitioning, rather than adsorption, is the principal mechanism of retention and that the solute exclusion relative to amorphous alkane solvents

(30) Blackford, D. S.; York, R. *J. Chem. Eng. Data* **1965**, *10*, 313.
(31) Maslan, F. D.; Stoddard, E. A. *J. Phys. Chem.* **1956**, *60*, 1146.
(32) Schoenmakers, P. J. et al. *J. Chromatogr.* **1978**, *149*, 519.
(33) Schoenmakers, P. J.; Billiet, H. A. H.; de Galan, L. *Chromatographia* **1982**, *15*, 205.
(34) Tanaka, N.; Thornton, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 7300.
(35) Colin, H.; Guiochon, G. *J. Chromatogr.* **1978**, *158*, 183.
(36) Nahum, A.; Horvath, Cs. *J. Chromatogr.* **1981**, *203*, 53.
(37) Bij, K. E.; Horvath, Cs.; Melander, W. R.; Nahum, A. *J. Chromatogr.* **1981**, *203*, 65.
(38) Lochmuller, C. H.; Wilder, D. R. *J. Chromatogr. Sci.* **1979**, *17*, 574.

is due to the partial alignment of the grafted chains normal to the interface.

Adsorption

Next we consider adsorption as a possible mechanism of retention. For this situation, we assume there is a single layer of N_m lattice sites at the interface between the stationary and mobile phases. This approach has previously been developed by others;[15-20] one application has been to various forms of chromatography. Although the lattice monolayer approximation is less suitable for adsorption at liquidlike interfaces than for adsorption to solid surfaces because of its disregard for fluctuations, nevertheless qualitative predictions should not be seriously in error for the purposes at hand. This layer may have any possible geometry, provided it does not overlap or become adjacent to itself; it need not be completely contiguous, and it may include a layer of solute adsorbed onto the methyl end-capped silica as well as a layer of solute at the ends of the grafted chains. However, for present purposes, we assume the surface of adsorption has only a single chemical character, that of hydrocarbon chains; we do not consider the effects of bare silica surface or exposed silanols. Let the fraction of sites occupied by x (=s, A, B) be θ_x; then complete volume filling requires that

$$\theta_s + \theta_A + \theta_B = 1 \tag{19}$$

The entropy of mixing, S_m, within the monolayer is

$$\frac{S_m}{N_m k} = -\theta_s \ln \theta_s - \theta_A \ln \theta_A - \theta_B \ln \theta_B \tag{20}$$

The total contact free energy is

$$\frac{F_{m,cont}}{N_m kT} = \alpha\left[\left(\frac{zw_{AA}}{2kT}\right)\theta_A + \left(\frac{zw_{BB}}{2kT}\right)\theta_B + \left(\frac{zw_{ss}}{2kT}\right)\theta_s + \chi_{sA}\theta_s\theta_A + \chi_{sB}\theta_s\theta_B + \chi_{AB}\theta_A\theta_B\right] + \left(\frac{w_{sC}}{kT}\right)\theta_s + \left(\frac{w_{AC}}{kT}\right)\theta_A + \left(\frac{w_{BC}}{kT}\right)\theta_B \tag{21}$$

where the same derivation and definitions apply as for partitioning and $\alpha = (z-1)/z$ ($\alpha = 5/6$ for the simple cubic lattice) is the factor which accounts for the single contact of each molecule in the monolayer with the hydrocarbon surface of the stationary phase, C, and the last terms on the right-hand side account for the surface contacts of s, A, and B, with C. Inasmuch as the process of adsorption of dn moles of solute into the monolayer involves a corresponding removal of dn moles of A and B, in any relative proportion, then the two conditions of equilibrium are

$$\mu_m(s) - \mu_{bulk}(s) = \mu_m(A) - \mu_{bulk}(A) = \mu_m(B) - \mu_{bulk}(B) \tag{22}$$

The chemical potentials are given by the appropriate derivatives of eq 20 and 21; the result is three pairs of equations of the form

$$\frac{\mu_{bulk}(x)}{kT} = \ln \phi_x + \frac{zw_{xx}}{2kT} + \chi_{xy}\phi_y(1-\phi_x) + \chi_{vx}\phi_v(1-\phi_x) - \chi_{vy}\phi_v\phi_y \tag{23}$$

$$\frac{\mu_m(x)}{kT} = \ln \theta_x + \alpha\left[\frac{zw_{xx}}{2kT} + \chi_{xy}\theta_y(1-\theta_x) + \chi_{vx}\theta_v(1-\theta_x) - \chi_{vy}\theta_v\theta_y\right] + \left(\frac{w_{xC}}{kT}\right) \tag{24}$$

where (v, x, y) = (s, A, B), (A, B, s), and (B, s, A). Substitution of eq 23 and 24 into the equilibrium eq 22 and use of eq 19 permit us to solve for the adsorbed concentrations, θ_x, in terms of the given mobile-phase solvent concentrations, ϕ_x. Consider the case in which the solute concentration is small in the mobile phase and in the adsorbed layer, ϕ_s, $\theta_s \ll 1$. We can express the binding isotherms in terms of the binding constants of A and B to the surface C, $K_A^{ads} = \theta_A/\phi_A$, and $K_B^{ads} = \theta_B/\phi_B$, respectively

$$\ln(\theta_s/\phi_s) = \ln K_A^{ads} + (1/z)(\chi_{AC} - \chi_{sC} + \chi_{sA}) + \phi_B(\alpha K_B^{ads} - 1)(\chi_{sA} - \chi_{sB} + \chi_{AB}) \tag{25}$$

$$= \ln K_B^{ads} + (1/z)(\chi_{BC} - \chi_{sC} - \chi_{AB} + \chi_{sA}) + \phi_B(\alpha K_B^{ads} - 1)(\chi_{sA} - \chi_{sB} - \chi_{AB})$$

and

$$\ln\left(\frac{K_A^{ads}}{K_B^{ads}}\right) = (1/z)(\chi_{BC} - \chi_{AC} - \chi_{AB}) - 2\phi_B(\alpha K_B^{ads} - 1)\chi_{AB} \tag{26}$$

When the mobile-phase eluent is pure A, (i.e., $\phi_B = 0$), or when A and B are chemically similar to each other ($\chi_{AB} \simeq 0$ and $\chi_{sA} - \chi_{sB} \simeq 0$), then eq 25 predicts the adsorption isotherm

$$\ln\left(\frac{\theta_s}{\phi_s}\right) = (1/z)(\chi_{AC} - \chi_{sC} + \chi_{sA}) = \frac{w_{sA} + w_{AC} - w_{AA} - w_{sC}}{kT} \tag{27}$$

If the retention mechanism is purely adsorption, and the random mixing approximation is valid, then the group selectivity is predicted to be

$$\ln\left(\frac{k'_2}{k'_1}\right) = (1/z)(\Delta\chi_{sA} - \Delta\chi_{sC}) + \phi_B(\alpha K_B^{ads} - 1)(\Delta\chi_{sA} - \Delta\chi_{sB}) \tag{28}$$

where the $\Delta\chi$'s are defined as for eq 18.

If the solute distribution is governed by both partitioning and adsorption in accord with the mechanisms specified by eq 14 and 27, then the fraction of bound molecules which are adsorbed from a single eluent A, f_{ads}, is

$$f_{ads} = \frac{N_m\theta_s}{N_m\theta_s + N_0\sum_{i=1}^{L}\phi_i} = \left[1 + \left(\frac{N_0}{N_m}\right)K_{C/A}{}^{\alpha}e^{-(\chi_{Ac}/z)}\sum_{i=1}^{L}q_i e^{g\delta_{i,1}/kT}\right]^{-1} \tag{29}$$

The fraction of solute molecules adsorbed will depend on the surface density and chain lengths of the grafted chains, the interaction free energies, and the relative areas accessible for adsorption or partitioning, N_0/N_m. Increasing the surface density of the chains will lead to less partitioning and more adsorption; in the limit of maximum packing, $\sum q_i \exp(g\delta_{i,1}/kT) \to 0$, and the fraction adsorbed will approach 1. As the lengths of the grafted chains decrease, L decreases, and adsorption becomes increasingly important. Solutes with greater tendencies to partition into oil will favor partitioning to adsorption, since the interior of the interphase is a more favorable environment for the solute than is the interface between chains and water. Because the adsorbed region will be subject to significant fluctuations of the mobile-phase/chain interface, neglected here, and because adsorption in real solutions may involve more than a single monolayer, it is difficult to estimate N_m. Nevertheless, except for the factor of $K_{C/A}{}^{\alpha}$, the product of the other factors on the right-hand side within the brackets of eq 29 should be typically of the order of one. Therefore, for typical stationary phases (C8 or longer), for molecules with oil/water partition coefficients greater than one, partitioning should be dominant relative to adsorption. Thus even though the theory predicts hydrophobic solute concentrations to be highest near the free ends of the grafted chains, nevertheless most of the molecules should be embedded within, rather than adsorbed onto, the grafted chain phase. Evidence supporting this view is given below.

For a single eluent, a concept which follows from eq 27 is that of the "eluotropic strength" of a solvent. Originated for other

forms of adsorption chromatography,[39] it has more recently been applied to RPLC.[1,35] When solute binding is strong and the bulk interactions are negligible relative to adsorption, and when the interactions are principally due to dispersion forces, then $|w_{AA}|$, $|w_{sA}| \ll |w_{AC}|$, $|w_{sC}|$ and eq 27 becomes[39]

$$\ln\left(\frac{\theta_s}{\phi_s}\right) = \frac{w_{AC} - w_{sC}}{kT} \tag{30}$$

For a given solute, s, and adsorption surface, C, the relative adsorption from two different eluents would then be predicted to be

$$\ln \frac{[\theta_s/\phi_s]_2}{[\theta_s/\phi_s]_1} = \frac{w_{A_2C} - w_{A_1C}}{kT} = a_s(\epsilon_2 - \epsilon_1) \tag{31}$$

where ϵ_j is defined as the eluotropic strength of solvent j, and a_s is the area per solute molecule in contact with the surface. The value of eq 31 is that it provides a method for comparing relative strengths of solvents (for a given adsorption surface) in a manner that is independent of the chemical character of the solute.

Note that these underlying assumptions do not apply, however, when the solvent is water, or when binding is weak, situations which are common in RPLC. If we therefore do not make the approximation noted above, and we use eq 27 to compare two different solvents, for a given solute and surface, then we have

$$\ln \frac{[\theta_s/\phi_s]_2}{[\theta_s/\phi_s]_1} = (kT)^{-1}(w_{A_2s} + w_{A_2c} - w_{A_2A_2} - w_{A_1s} - w_{A_1C} + w_{A_1A_1}) \tag{32}$$

and the more general definition of eluotropic strength is given by

$$a_s\epsilon_j = (kT)^{-1}(w_{sA} + w_{AC} - w_{AA}) \tag{33}$$

The eluotropic strength is thus the affinity (free energy divided by kT per unit area of the solute) of the solute to adsorb to the surface C and displace a molecule of eluent A. If the mechanism of retention is principally partitioning rather than adsorption, then it follows that the eluotropic strength would instead be given by (see eq 13b)

$$a_s\epsilon_j = \left(\frac{z}{kT}\right)\left(w_{sA} - \frac{w_{AA}}{2}\right) \tag{34}$$

where a_s now represents the total area of the solute in contact with surrounding neighbors in the grafted chain phase. If retention has a component of adsorption, and if the solvent is water or the binding is weak, these arguments suggest that the eluotropic strength will not be independent of the solute or the stationary phase. If retention is dominated by partitioning, then the eluotropic strength should only be independent of C. Consistent with these arguments, experimental evidence shows that the eluotropic strength is not completely independent of these factors,[1,35] and in that regard, this concept of eluotropic strength will not be as useful for RPLC as for other forms of chromatography.

Comparison of Retention Mechanisms

Use of the models presented above, in conjunction with available experimental evidence, strongly supports the view that the principal mechanism of retention is partitioning. This follows from two lines of evidence. First, partitioning should be affected by the surface density of the grafted chain phase but adsorption should not. If partitioning dominates, then retention should decrease with increasing surface density of the grafted chains. The data of Claudy et al.[23] show that retention of hexane and benzene decrease with increasing surface density of the grafted chains.

It has been observed that there is less retention by grafted chain stationary phases than by bulk alkane phases; this has previously been interpreted as favoring an adsorption mechanism.[1,35] However, the alternative explanation offered here is that the partial chain ordering in the stationary phase leads to less retention than in a corresponding alkane liquid. Lochmuller and Wilder[38] have shown that selectivities are the same in grafted chain phases as in liquid hydrocarbons. This is consistent with the present interpretation, inasmuch as selectivities, unlike retention factors, should be unaffected by the molecular organization of the grafted chains (compare eq 14 and 18).

A second line of evidence also favors partitioning as the dominant mechanism of retention. In brief, in a wide variety of experiments, described in more detail below, $\ln k'$ for a series of solutes is observed to be a linear function of the logarithm of the appropriate oil/water partition coefficient, with a slope of 1. A slope of 1 is expected for a partitioning mechanism, whereas a slope of $1/z$ (approximately $^1/_6$) is expected for adsorption, since only a small fraction of the surface of the solute would contact the hydrocarbon phase in the latter case, and hence the driving force would be smaller. Two classes of experiments can be interpreted in this manner; they are described below.

First, we compare two experiments: (i) solute is transferred into the interphase, C, from a mobile-phase mixed solvent AB, and (ii) solute is transferred into an amorphous alkane otherwise identical with C, from the same mixed-phase solvent, AB. The latter, the bulk-phase partition process, will be described by the partition coefficient, $K_{C/AB}$ given by eq 13a. If partitioning is the dominant mechanism of retention, then comparison of eq 13a and 14 shows that $\ln k'$ will be a linear function of $\ln K_{C/AB}$ with a slope of 1 and that the intercept will depend on the phase ratio and the surface density of the chains. If adsorption is the dominant mechanism of retention, then there are two possibilities. First, if adsorption is weak, then according to eq 25, $\ln k'$ will be a linear function of $\ln K_{C/AB}$ but with slope equal to $1/z$. Although the slope is more ambiguous if the adsorption is not weak, nevertheless one limiting circumstance is unambiguous. In pure eluent A, partitioning predicts a slope of 1 and adsorption predicts a slope of $1/z$. Experiments on a wide range of solutes, including those in, or extrapolated to, a pure mobile-phase eluent show that this dependence is linear with a slope of 1.[1,40-47] Thus this evidence strongly supports a partitioning mechanism.

An alternative test is to compare the following two experiments: (i) solute partitions into the interphase, C, from a mobile-phase mixed solvent AB, and (ii) solute partitions into the alkane equivalent of C from pure solvent A (water, for example). Thus here we compare the transfer from mixed solvent into the interphase with the simple oil/water partitioning process, whereas above we compared partitioning into the oil or the interphase from the same mixed solvent AB. The predictions from the two comparisons are different, and they are revealing. We now require the slope of $\ln k'$ vs. the logarithm of the oil/water partition coefficient, $\ln K_{C/A}$. Using eq 13a, 14, and 17.5, the retention factor can be expressed as

$$\ln k' = \ln \Phi\langle q\rangle + \ln K_{C/AB} \tag{35}$$

$$= \ln \Phi\langle q\rangle + \ln K_{C/A} + b\phi + c\phi^2$$

where $b = \chi_{sB} - \chi_{sA} - \chi_{AB}$ and $c = \chi_{AB}$. It is observed[48] that when $\ln K_{C/A} = 0$, the corresponding value of k', denoted here as k^*, is independent of ϕ; therefore

$$\ln k^* = \ln \Phi\langle q\rangle \tag{36}$$

Thus the slope of a plot of retention vs. partition coefficient is

$$\frac{\Delta \ln k'}{\Delta \ln K_{C/A}} = \frac{\ln k' - \ln k^*}{\ln K_{C/A}} = 1 + \frac{b\phi + c\phi^2}{\ln K_{C/A}} \tag{37}$$

(39) Snyder, L. R. *Principles of Adsorption Chromatography*; Dekker: New York, 1968.

(40) Butte, et al., W. *J. Chromatogr.* **1981**, *214*, 59.
(41) Unger, S. H.; Chiang, G. H. *J. Med. Chem.* **1981**, *24*, 262.
(42) Colin, H.; Krstulovic, A.; Guiochon, G. *J. Chromatogr.* **1983**, *255*, 295.
(43) Miyake, K.; Terada, H. *J. Chromatogr.* **1978**, *157*, 386.
(44) Nahum, A.; Horvath, Cs. *J. Chromatogr.* **1980**, *192*, 315.
(45) Mirrlees, M. S. et al. *J. Med. Chem.* **1976**, *19*, 615.
(46) Baker, J. K. *Anal. Chem.* **1979**, *51*, 1693.
(47) Molnar, I.; Horvath, Cs. *J. Chromatogr.* **1977**, *142*, 623.

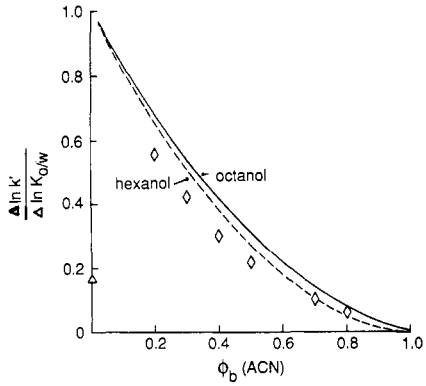

Figure 5. The slope of ln k' vs. ln $K_{C/A}$, retention vs. oil/water partition coefficient, depends on solvent composition. The lines are predicted from the partitioning theory eq 35 for hexanol or octanol as solutes in acetonitrile/water mixtures. The data of D'Amboise and Hanai[48] (◊) for many different solutes tend toward a slope of 1 in pure water; this supports the view that partitioning is the principal mechanism of retention. If adsorption were the retention mechanism, the slope would tend toward approximately $^1/_6$, indicated by (Δ) on the figure.

In this case, a partitioning mechanism predicts that ln k' will be a linear function of ln $K_{C/A}$ with slope equal to 1 when the eluent is pure A (water), but now the slope of the line will depend on the solvent composition. In particular, inasmuch as b is generally negative and larger in magnitude than c, these slopes should decrease toward zero with increasing concentration of the nonaqueous eluent. Any model for adsorption would predict different behavior; the slope would approach $1/z$ as the concentration of the organic eluent approaches zero.

These predictions are compared to the data compiled by D'Amboise and Hanai[48] in Figure 5. We use the values for the interaction parameters for hexanol and octanol in acetonitrile and water which we derived from the plot in Figure 4, substitute them into eq 37, and use the approximation that $\chi_{sC} = \chi_{sB}$ in the absence of an available experimental value. Figure 5 shows these slopes as a function of solvent composition. If partitioning is the dominant mechanism of retention, then these slopes should approach 1 in the limit as the solvent becomes pure water; if adsorption is the mechanism of retention, then these slopes should approach $1/z$ in the limit as the solvent becomes pure water. The compilation of D'Amboise and Hanai shows that these slopes approach 1; this supports the view that retention is dominated by partitioning. Furthermore, the functional dependence of retention on solvent composition appears to be relatively well predicted by the partitioning theory.

A related experiment is that of Colin et al.[49] who measured ln k' vs. the chain length, n_c, of solute molecules with alkyl "tails" of different lengths; they did so for a series of different solvent compositions, for a given grafted phase. They observed that ln k' is a linear function of n_c, and, as in the experiments described above, that the slopes of these curves decrease with increasing concentration of the nonaqueous eluent. They observed that these lines approximately all intersect at a single point represented by the (x,y) coordinates denoted $(-n_c^*, \ln k^*)$. These data can be interpreted by using eq 35. Molecules in homologous series can be considered to be comprised of a tail whose length changes within the series and a fixed head. If the free energies of transfer among bulk phase solvents of these subunits are additive, then each χ term can be expressed as a sum of a head group free energy of transfer plus n_c multiplied by a free energy of transfer per tail segment; i.e., eq 35 becomes

$$\ln k' = \ln \Phi\langle q\rangle + (a_h + n_c a_t) + (b_h + n_c b_t)\phi + (c_h + n_c c_t)\phi^2 = \ln \Phi\langle q\rangle + \Delta\chi_h + n_c\Delta\chi_t \quad (38)$$

where $\Delta\chi_h = a_h + b_h\phi + c_h\phi^2$ represents the transfer of the head segment from the AB mixture to C, and $\Delta\chi_t = a_t + b_t\phi + c_t\phi^2$ represents the transfer of a single tail segment from the AB mixture to C. This equation predicts that ln k' vs. n_c should be linear with slope equal to $\partial \ln k'/\partial n_c = \Delta\chi_t$; the slope should equal the free energy of transfer from mixed solvent AB to amorphous C of an individual monomer which comprises the tail. This equation also predicts that $-n_c^*$, the value of n_c for which ln k' becomes independent of ϕ, is equal to $-\Delta\chi_h/\Delta\chi_t$. This quantity is interpreted as the number of tail monomers whose transfer would have the same free energy as the transfer of the head. In support of this interpretation, Colin et al. have observed that this quantity depends significantly on the solute, but not on the solvent. The quantitative values of n_c^* observed by Colin et al. are also consistent with this interpretation. For example, for alkane solutes, the two methyl end groups should be considered the head, for they must occur in all homologues in the series, the tail being comprised of the internal methylenes. Inasmuch as the free energy of transfer of two methyls (from water to pure alkane) is 4204 cal/mol, and for a methylene group is 884 cal/mol,[29] the theory predicts $n_c^* = 4.75$. This is close to the value of $n_c^* = 4.0$ for alkanes observed by Colin et al. They have also observed $n_c^* = 0$ for methyl esters, and 6 for *n*-alkylbenzenes. The common intercept of these plots at n_c^* is predicted to be

$$\ln k'(-n_c^*) = \ln k^* = \ln \Phi\langle q\rangle \quad (39)$$

where the latter equality follows from eq 36. This intercept should depend only on the phase ratio and the surface density of the chains; in agreement with this prediction, Colin et al. have observed that this intercept is independent of the solute.

The evidence cited above thus offers strong support for the view that retention is dominated by partitioning into a semiordered phase of the grafted chains. Moreover, Tchapla et al.[50] have shown that selectivities change markedly when the lengths of alkyl chain solutes are approximately equal to those of the grafted chains. This observation is difficult to reconcile with an adsorption mechanism. Other evidence also bears on the distribution of solute, but it is less direct than that discussed above.[51,52]

Comparison with Other Models of Retention

Other models have previously been proposed for the molecular mechanism of retention in RPLC.[1,18,53-55] Of those efforts, only Martire and Boehm[55] have explicitly considered a partitioning process into a phase of disordered alkyl chains. Their treatment is similar in some regards to that presented here; for example, their exchange free energies and the mixing component of the entropy are similar and follow from similar lattice methods. Their treatment differs in that it does not attempt to account for the interfacial character of the grafted chain phase, a consideration which is essential for the questions addressed here.

A widely adopted model for the molecular mechanism of retention in RPLC has been the "solvophobic theory".[1,53] Retention is therein assumed to depend on the free energy of creation of a solute-sized cavity in the mobile-phase solvent. That model derives from theory of Sinanoglu and others[56,57] which treats the effects of solvation on bimolecular binding processes, processes in which a cavity is formed for the complex from a merging of the reactant cavities, in a given solvent.

Bimolecular binding, however, is not an appropriate model for chromatographic retention processes. Bimolecular binding simply involves the conversion of two smaller cavities into one larger one, in a given solvent. Chromatographic retention involves the transfer of solute from one solvent, the mobile phase, to another, the stationary phase. (The complexity of the "solvent" is not an issue

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here; the transfer process can involve either partitioning or adsorption, the "solvent" being a surface in the latter case.) The principal solvation process in chromatographic separation is the creation of an acceptor cavity in (or on) the stationary phase and the destruction of the donor cavity in the mobile phase.

Thus binding processes and chromatographic retention processes are quite different: the former requires only the change of cavity size in a single solvent; the latter requires creation of a cavity in one solvent and the destruction of a cavity in another solvent. The solvophobic theory is based on the premise that the only cavity which is relevant to retention is that in the mobile-phase solvent; it neglects the acceptor cavity in the stationary phase. Consequently, it predicts that retention should depend only on the surface tension of the mobile-phase solvent and not on the surface tension or other physical properties of the grafted stationary phase. In a partitioning theory such as the present one, cavities are described through the binary interaction constants, χ; their differences account for the driving force for retention. The solvophobic theory therefore errs in important respects. For example: (i) it does not rationalize the general observation[40-48] that ln k' should be a simple function of a relevant partition coefficient, and (ii) it specifies that retention should be independent of the nature of the grafted chain phase. There is much evidence that retention does depend on the grafted chain phase, some of which unambiguously cannot be interpreted in terms of effects of the phase ratio.[35,58-61]

On the other hand, some predictions of the solvophobic theory will resemble those of the present treatment in some circumstances. Inasmuch as retention is increased by unfavorable solute/eluent interaction or by favorable solute/grafted-phase interaction, then when the former contribution is dominant, predictions of the solvophobic theory will be qualitatively similar to the present treatment; the following are examples. Increased salt concentration in aqueous solvents decreases the solubility of hydrophobic molecules;[29] to approximately the same degree, increasing salt increases the retention factor.[1] Increasing the pH for acidic solutes increases their net charge and decreases their affinities for the hydrocarbon stationary phase; the retention factor is correspondingly reduced.[1] Moreover, both the free energy of transfer and the free energy of creation of a cavity in the mobile phase depend approximately linearly on the surface area of the solute. Thus the solvophobic theory should also resemble any partitioning theory in predicting the widely observed linear dependence of ln k' on surface area of the solute.[1]

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Dynamics of Two-Dimensional Diffusional Barrier Crossing

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The time-dependent diffusion equation is solved directly, by a new numerical algorithm, for a two-dimensional potential energy surface. The dynamics gives a qualitative description of isomerization reactions in solution. Along the reaction coordinate, the potential has a double-well shape, and the diffusion coefficient is inversely proportional to solvent viscosity. Along the perpendicular coordinate there are also two wells, the barrier height varies and the diffusion coefficient is assumed independent of viscosity. Under these conditions, in the high-viscosity regime, the diffusional flux can bypass the reaction-coordinate barrier, achieving a higher reaction rate. The calculated rate coefficient shows a non-Kramers, fractional viscosity dependence, in agreement with the isomerization experiments.

Introduction

The Kramers problem[1] of diffusional barrier crossing has attracted much attention recently.[2-21] In the low viscosity regime, there has been an effort to detect the maximum in the viscosity (η) dependence of the rate coefficient (k), known as the "Kramers'

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