Modeling the effect of solvation on solute retention in reversed-phase liquid chromatography

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Abstract

The retention of a homologous series of alkylbenzenes was determined on octyl and octadecyl reversed-phase columns in several polar organic liquids. Free energies of transfer were calculated by the SM5.0R classical solvation model for each organic liquid tested and for several alkanes. The relationships between the measured retention factors and the calculated free energies of transfer were then investigated. Although the natural logarithms of the retention factor and the calculated free energies of transfer were linearly correlated, the obtained free energies of transfer of the solutes did not completely explain the retention behavior of the solutes. Nonetheless, even in these pure organic liquids, the energetics of RPLC retention behaved very similarly to those of partitioning. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Reversed-phase liquid chromatography (RPLC) is widely used (e.g., Ref. [1]), yet RPLC retention is one of the most challenging phenomena to model at the molecular level. While numerous studies have attempted to elucidate the precise origin of RPLC retention [2], even the question of whether the process should be likened to adsorption or partitioning is by no means unambiguously answered [3]. Regardless of whether the operative retention mechanism in RPLC is adsorption, partitioning, a combination of both, or neither, there is considerable experimental evidence that RPLC retention shares some significant similarities with partitioning.

For example, Lochmuller and Wilder concluded that retention of simple aromatics on dodecyl and longer bonded phases follows a partitioning-like behavior which they speculate is due to the formation of liquid-like 'clumps' of bonded phase moieties [4].

Recently, Tan and Carr measured

$$F = \Delta G^\circ(CH_2)_{C_{16}/mob} / \Delta G^\circ(CH_2)_{stat/mob} \tag{1}$$

where $\Delta G^\circ(CH_2)_{C_{16}/mob}$ is the standard free energy of transfer for a methylene group from the mobile phase to liquid hexadecane and $\Delta G^\circ(CH_2)_{stat/mob}$ is the standard free energy of transfer for a methylene group from the mobile phase to the stationary phase [5]. For methanol–water mobile phases of 0–70% methanol, $1.1 < F < 1.6$ on a number of octyl and octadecyl columns. In other words, the energetics of retention are very similar to the energetics of partitioning.

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However, above 70% methanol on octyl columns, F increased to greater than 2.5. To quote Tan and Carr, ''The interpretation of the results in region II (above 70%) is somewhat ambiguous. Hence we are reluctant to draw any conclusions as to the solute retention mechanism in region II.'' Suggested explanations included the effect of organic modifier sorption, an increased contribution from methylene group adsorption, or possibly due to experimental error [5].

Vailaya and Horváth included pure organics as part of a similar study and likewise found significant deviations from unity in the above-mentioned ratio [3].

In addition to these (and other) experimental investigations, a variety of computational approaches have been employed to investigate the retention mechanism. For example, Beck and co-workers employed classical molecular dynamics (MD to model the RP stationary phase [6,7] and to model solute transfer in RPLC [8]. MD simulations involve treating the molecular system as a collection of particles, with each particle obeying Newton's laws of motion. Additional assumptions made in performing an MD simulation involve the nature of the forces each particle experiences during the simulated time. In an MD study, a partitioning or adsorption mechanism need not be assumed a priori and solute interactions with the stationary and mobile phases can be examined, including conformational effects.

Quantitative structure–activity relationships (QSARs) have also been employed to gain insight into the retention mechanism. For example, Breneman and Rhem used solute descriptors obtained from transferable atom equivalent-derived surface property indices to predict retention factors for a set of high-energy materials [9]. In general, QSAR methods involve the use of multivariate statistical methods to build linear or nonlinear models relating chemical structure to a property such as retention.

Similarly, Carr and co-workers have employed linear solvation energy relationships (LSERs) to predict retention behavior [10–12]. The LSER approach considers the free energy of retention to be the sum of weighted solute descriptors, with the weighting factors representing the difference of the mobile and stationary phase contributions. Mechanistic information has been derived from the magnitude of the obtained weighting factors [11].

Cramer and co-workers have developed a series of highly successful methods for calculating the free energy of solvation [13–15]. These SM5.x solvation energy models require only the solute geometry, which can be calculated by any of a variety of well-established ab initio or semi-empirical molecular orbital calculations. While the SM5.x solvation energy models can only be applied to pure solvents, the agreement between the calculated standard free energies of solvation and the experimental values is excellent [13,14]. This suggested that the SM5.x models might be suitable for the prediction of retention in nonaqueous RPLC, since, to the extent that nonaqueous RPLC retention can be represented as partitioning, the free energy of retention will be proportional to the solute's free energy of transfer, i.e., the difference between the standard free energy of solvation in the stationary phase and the standard free energy of solvation in the mobile phase.

As part of a study on the thermodynamics of nonaqueous RPLC, we also made measurements of the retention of alkylbenzenes in pure organic liquids on octadecyl and octyl columns. The retention factors were highly correlated to free energies of transfer from the organic liquid to hexadecane, as calculated using AM1 gas-phase optimized geometries and the SM5.0R solvation model. This study represents the first application of the SM5.0R solvation energy model to the study of RPLC retention.

2. Experimental

The HPLC system was assembled from a LS 3200 autosampler (SGE, Austin, TX, USA), an AC6U valve (VICI Valco Instruments, Houston, TX, USA), a Waters 501 pump (Waters, Milford, MA, USA), a Model 450 UV detector (Alltech, Deerfield, IL, USA), and a Model 3393A integrator (Hewlett-Packard, Avondale, PA, USA). The Model 7315 column inlet filter (Rheodyne, Rohnert Park, CA, USA) and column were thermostated by means of a column water jacket and a circulating water bath (VWR, West Chester, PA, USA). The temperature was measured to the nearest 0.1° with a NIST traceable digital thermometer (VWR).

The columns used in this study were a Pinnacle ODS 5 μm d_{p}, 12-nm pore size, 150×4.6 mm (length by diameter), and a Pinnacle Octyl 5 μm d_{p},

12-nm pore size, 150×4.6 mm column, both from Restek (Bellefont, PA, USA). According to the manufacturer, the bonding density of the ODS column is 2.8 $\mu mol/m^2$ while that of the octyl column is 3.8 $\mu mol/m^2$.

The alkylbenzenes were obtained from Aldrich (Milwaukee, WI, USA) and were as follows: benzene, toluene, *n*-ethylbenzene, *n*-propylbenzene, *n*-butylbenzene, *n*-pentylbenzene, *n*-hexylbenzene, *n*-heptylbenzene, *n*-octylbenzene, *n*-nonylbenzene, *n*-decylbenzene, *n*-undecylbenzene, *n*-dodecylbenzene, and *n*-tridecylbenzene. The mobile phases were ACS reagent-grade methanol, 1-propanol, and acetonitrile (ACN) (Fisher Scientific, NJ, USA), and 200 proof ethanol (Aaper Alcohol and Chemical, Shelbyville, KY, USA).

The alkylbenzene samples were prepared by mixing 2 μl of the alkylbenzene and 10 ml of the appropriate mobile phase. The injection volume was 13 μl. All samples were run in triplicate with some exceptions as noted in Table 1.

When changing mobile phases, the column was flushed with at least 100 column volumes of the new mobile phase to ensure that it was completely equilibrated. When changing from either the 75% or the 80% methanol in water test mobile phases to a miscible organic mobile phase, the column was flushed with at least 200 column volumes of the new

mobile phase. In each case, the mobile phases were obtained from freshly opened bottles, although no special effort was made to exclude water from the mobile phases.

The octadecyl column was tested before and after experimental data were obtained by measuring the retention and efficiency of uracil, benzene, and naphthalene in methanol–water (80:20, v/v) at 25.0°C and 1.0 ml/min, following the manufacturer's recommendations. The octyl column was similarly evaluated, using instead methanol–water (75:25). While such tests are not the most sensitive to changes in the chromatographic surface, the chromatographic behavior of the alkylbenzenes used in this study should not be affected by any change not detected by these tests.

The retention and efficiency of standards on each column before and after the experiments showed no significant change in either characteristic (data not shown). Likewise, no noticeable change in pressure drop was observed (data not shown). Thus, it is unlikely that the columns deteriorated significantly during the course of these measurements.

3. Calculations and data analysis

Statistical data analysis was generally performed

Table 1
Retention times of alkylbenzenes in pure organic mobile phases and alkyl-modified stationary phases

	Retention time (SD) (min)						
	ACN/C_{18}	MeOH/C_{18}	EtOH/C_{18}	PrOH/C_{18}	ACN/C_8	MeOH/C_8	EtOH/C_8
Benzene	1.860 (0.000)	1.853 (0.006)	1.810 (0.000)	1.810 (0.000)	1.920 (0.000)	1.883 (0.012)	1.860 (0.000)
Toluene	1.950 (0.000)	1.940 (0.000)	1.850 (0.014)	1.840[b]	1.970 (0.000)	1.940 (0.000)	1.880 (0.000)
Ethylbenzene	2.037 (0.006)	2.010 (0.000)	1.873 (0.007)	1.840 (0.000)	2.020 (0.000)	1.970 (0.000)	1.890 (0.000)
n-Propylbenzene	2.163 (0.006)	2.100 (0.000)	1.910 (0.007)	1.855 (0.006)	2.090 (0.000)	2.023 (0.006)	1.900 (0.000)
n-Butylbenzene	2.320 (0.000)	2.220 (0.000)	1.947 (0.007)	1.870 (0.000)	2.177 (0.006)	2.073 (0.006)	1.917 (0.006)
n-Pentylbenzene	2.520 (0.000)	2.350 (0.000)	1.990 (0.014)	1.887 (0.006)	2.260 (0.000)	2.123 (0.006)	1.933 (0.006)
n-Hexylbenzene	2.770 (0.000)	2.520 (0.000)	2.030 (0.014)	1.903 (0.005)	2.370 (0.000)	2.187 (0.006)	1.950 (0.000)
n-Heptylbenzene	3.090 (0.000)	2.730 (0.000)	2.080 (0.007)	1.927 (0.006)	2.500 (0.000)	2.250[b]	1.970 (0.000)
1-Phenyloctane	3.490 (0.000)	2.980 (0.000)	2.140 (0.014)	1.947 (0.012)	2.640 (0.000)	2.330 (0.000)	1.980 (0.000)
1-Phenylnonane	4.000 (0.000)	3.260 (0.000)	2.200 (0.007)	1.960 (0.010)	2.803 (0.006)	2.420 (0.000)	2.000 (0.000)
1-Phenyldecane	4.630 (0.000)	3.620 (0.000)	2.260 (0.007)	2.010 (0.023)	2.997 (0.006)	2.507 (0.006)	2.020 (0.000)
1-Phenylundecane	5.417 (0.006)	4.027 (0.006)	2.333 (0.007)	2.013 (0.006)	3.210 (0.000)	2.620 (0.000)	2.037 (0.006)
1-Phenyldodecane	6.403 (0.006)	4.510 (0.000)	2.410 (0.021)	2.040 (0.000)	3.473 (0.006)	2.733 (0.006)	2.050 (0.000)
1-Phenyltridecane	7.635 (0.007)	5.083 (0.006)	2.490 (0.000)	2.070 (0.000)	3.770 (0.010)	2.857 (0.006)	2.070 (0.000)
Hold-up time[a]	1.5359	1.4974	1.5129	1.6463	1.5875	1.5806	1.4348

[a] Best-fit value by nonlinear regression to the Martin equation.
[b] Only one retention measurement is available.

using the routines provided in Axum 5.0 (Mathsoft, Cambridge, MA, USA).

Given the relatively short retention times and the extreme sensitivity of small values of the retention factor (k) to errors in t_0, proper choice of t_0 is important in determining k for these solutes. Thus, t_0 was taken as the value that gave the best least-squares fit to the Martin equation, as has been previously recommended [16]. Rather than fit ln k to the carbon number (η), t_0 was determined by fitting the retention data for toluene through n-tridecylbenzene to exp(η), according to

$$t_R = be^{a\eta} + t_o \tag{2}$$

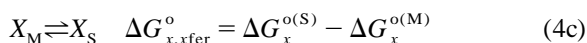The resultant t_0 values are also given in Table 1.

The standard free energy change for transporting solute x from liquid M to liquid S, $\Delta G^o_{x,x\text{fer}}$ can be written as

$$\Delta G^o_{x,x\text{fer}} = \Delta G^{o(S)}_x - \Delta G^{o(M)}_x \tag{3}$$

This can be obtained by combining two processes,

$$X_G \rightleftharpoons X_M \quad \Delta G^{o(M)}_x \tag{4a}$$

$$X_G \rightleftharpoons X_S \quad \Delta G^{o(S)}_x \tag{4b}$$

to give

$$X_M \rightleftharpoons X_S \quad \Delta G^o_{x,x\text{fer}} = \Delta G^{o(S)}_x - \Delta G^{o(M)}_x \tag{4c}$$

where X_G (X_M, X_S) is the solute of interest in the gas phase (solvent M, solvent S), respectively. $\Delta G^{o(M)}_x$ and $\Delta G^{o(S)}_x$ are the standard free energies of solvation of X in solvent M and in solvent S, respectively.

In this study, $\Delta G^{o(M)}_x$ and $\Delta G^{o(S)}_x$ were calculated using the SM5.0R solvation model developed by Cramer and co-workers [13,14]. This model is particularly suitable for the present purpose as it is parameterized for many different organic solvents. The SM5.0R model employs atomic surface tensions and electrostatic effects are included only implicitly [13,14]. It achieves a mean unsigned error (MUE) in the calculated standard free energy of solvation of about 0.4 kcal/mol (1.7 kJ/mol) using gas phase solute geometries from either Hartree–Fock level calculations or from semi-empirical molecular orbital calculations [14]. This MUE is reported from the calculations of 227 neutral solutes in 90 organic

solvents for a total of 1836 data points [14]. Our calculations were checked against the input and output test data provided by OMNISOL [15], with which the SM5.0R model is distributed.

For this work, the gas phase geometry of each alkylbenzene was calculated using the AM1 geometry optimization routine in PC Spartan Plus (Wavefunction, Irvine, CA, USA). For longer-chain solutes, the final optimized geometry in the AM1 calculation tends to depend on the initial geometry constructed by the user. This is because the calculation converges to a local minimum. When several different optimized geometries for a solute were compared, the calculated free energy of solvation deviated within the mean unsigned error value given above.

4. Results and discussion

The use of pure organic liquids resulted in relatively short retention times (Table 1). Nonetheless, the precision of the measurements was good, as evidenced by the standard deviations of the retention times, also shown in Table 1. Propanol was used only with the octadecyl column due to the extremely short retention times that resulted; tetrahydrofuran (THF) was also tested, but gave essentially no retention on either column for any of the tested solutes (data not shown). Successively larger alkyl-benzenes gave measurable increases in retention even using pure 1-propanol as the mobile phase. Interestingly, while acetonitrile is typically considered a stronger mobile phase than methanol (when mobile phases are aqueous mixtures), the retention factors as well as the retention *times* of the larger alkylbenzenes were longer in acetonitrile than in methanol on both columns. Although at least one reference indicates that *pure* acetonitrile is iso-eluotropic with *pure* methanol [16], that fact still does not explain the longer retention time for the longer alkylbenzenes in acetonitrile.

Given the relatively short retention times and the extreme sensitivity of small values of the retention factor (k) to errors in t_0, proper choice of t_0 is important in determining k for these solutes. The value used here is that which gives the best fit, in the least-squares sense, to the Martin equation, as has

been recommended previously [17]. Tchapla et al. have noted that, in comparison to other homologous series, alkylbenzenes behave somewhat anomalously and act as though the phenyl group corresponds to approximately three methylene groups [18]. While the results discussed below are based on the best-fit t_0 values given in Table 1, the trends in k were essentially unchanged when these 'additional' methylenes were added prior to performing the nonlinear regression (data not shown). In other words, while the experimental k values are sensitive to the choice of t_0, the observed behaviors are qualitatively the same regardless of t_0 values precise value.

The appropriateness of these t_0 values is demonstrated in Fig. 1, which shows α values for successive members of the series, i.e., the methylene group selectivity. Each mobile phase yielded an essentially horizontal line, with little scatter in the α values. As expected, α values for the smallest members of the series generally have more scatter. The general independence of α and carbon number is consistent with the observations of Tchapla et al. [18]. Irregularities in these plots are observed for larger ranges of homologs than were tested here. The data shown are for the octadecyl column; those for the octyl column are qualitatively the same. While the regression procedure used forces these α values to be the most consistent possible, this regularity indicates that the resulting k values are reliable.
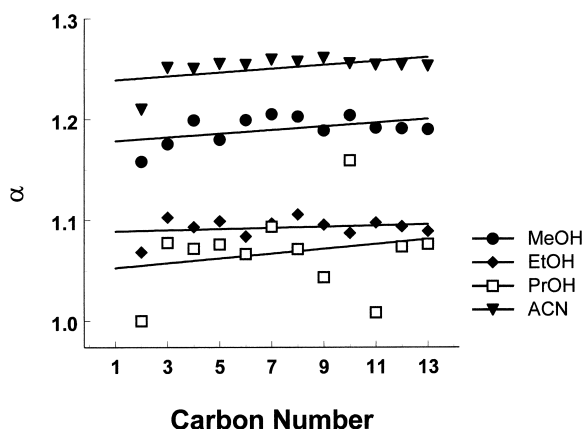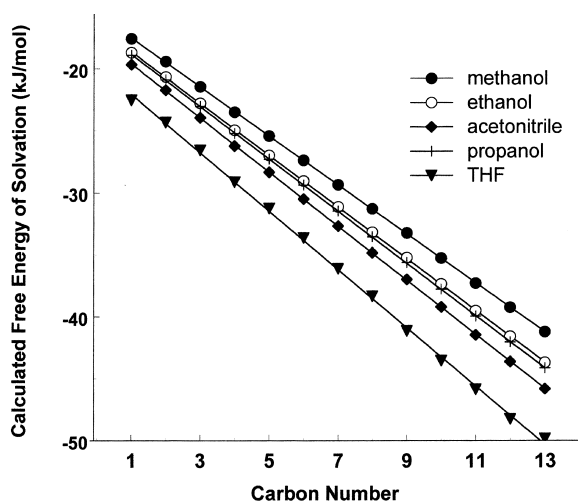
Fig. 2 shows the calculated free energies of

Fig. 2. Calculated free energies of solvation of alkylbenzenes in pure organic liquids as a function of carbon number on the alkyl chain.

solvation of the alkylbenzenes as a function of carbon number for the tested organic liquids. As expected, the more negative (favorable) solvation energies are associated with less polar liquids, with the general trend being methanol>ethanol> propanol>acetonitrile>tetrahydrofuran; This general trend in solvation energies did not completely follow the RPLC solvent strength trend found in the literature, e.g., methanol (weakest)<acetonitrile< ethanol<tetrahydrofuran<propanol (strongest) [17]. Less obvious, but still apparent, is that the slopes of these lines also vary slightly with the steeper slope again corresponding to the least polar liquid. The slope corresponds to the free energy of solvation of a methylene group and, again as expected, the less polar liquids exhibit more favorable solvation.

A central issue in the use of partitioning models for RPLC is the choice of the appropriate liquid to represent the stationary phase. Hexadecane [19,20] and dodecanol [21] have been used previously as model liquids for octadecyl stationary phases. Carr et al. present computational and experimental evidence that hexadecane is a suitable model liquid for octadecyl RPLC stationary phases for nonpolar solutes [19]. They also point out that this is probably not true for polar solutes.

Quinones et al. used the slightly more polar dodecanol to model the adsorption isotherms of

Fig. 1. Methylene group selectivity α of alkylbenzenes in neat organic liquids on a C_{18} column.

simple aromatic compounds on an octadecyl column [21]. While successful in that application, it is not particularly relevant to this problem. The central issue in modeling the sorption isotherms is finding a liquid which mimics the way the stationary phase is modified by the presence of sorbed *solute* as a function of that solute's concentration. The present study is more concerned with modeling the relative magnitude of interactions between the stationary phase and a virtually infinitely dilute concentration of solute.

Fig. 3 shows the calculated free energies of solvation of the alkylbenzenes as a function of carbon number for a variety of compounds that might be considered as representative of the stationary phase. Changing from hexane to hexadecane has very little effect on the solvation energy. Thus, the exact choice of alkane is unlikely to be important, to the extent that an alkane is the appropriate model liquid. Consequently, hexadecane was used as the model liquid in all subsequent calculations. Note that in both Figs. 2 and 3, no additional correction is applied to the calculated free energies of solvation for the 'volume entropy' [22]. While such a correction has been used by others [19], OMNISOL is parameterized to provide free energies of solvation including the effects of volume entropy. Furthermore, the experimental k values must necessarily also include these same effects. Since the calculated

free energies will be compared to the experimental k values, such a correction is inappropriate.

A plot of $\ln k$ versus the negative of the free energy of transfer from the mobile phase to the model stationary phase divided by RT should be linear and have a slope of 1 if the retention process is partitioning. Note that this convention is consistent with Dill's original scheme [23], although it is the reciprocal of the F as defined by Tan and Carr [5] and Vailaya and Horvath [3].

Fig. 4 shows such a plot for the experimental k values obtained on the octadecyl column vs. the calculated free energies of transfer from the appropriate organic liquid to hexadecane. Similar plots for k values obtained on the octyl column are likewise straight. The slopes, intercepts, and correlation coefficients are shown in Table 2. The large departure of the slopes for acetonitrile mobile phase from the unity or from those for alcohol mobile phases was not due to the errors specific to acetonitrile in using the SM5.0R model. The MUE for two nitriles, which includes acetonitrile, as a solvent class is reported to be 0.47 kcal/mol (2.0 kJ/mol) and the MUE for 12 aliphatic alcohols as a solvent class is reported to be 0.45 kcal/mol (1.9 kJ/mol), when AM1 optimized gas-phase geometries for test solutes are used [14].

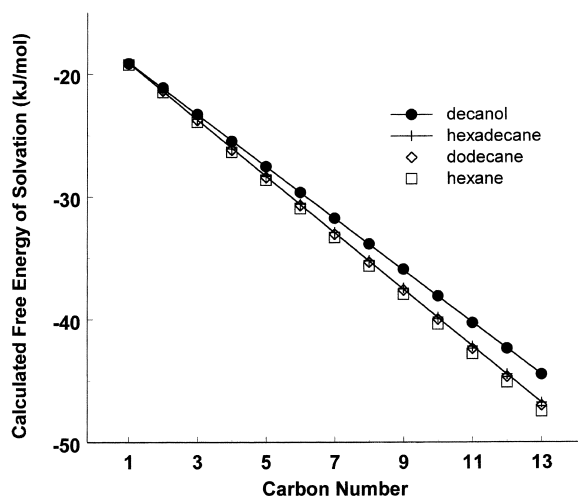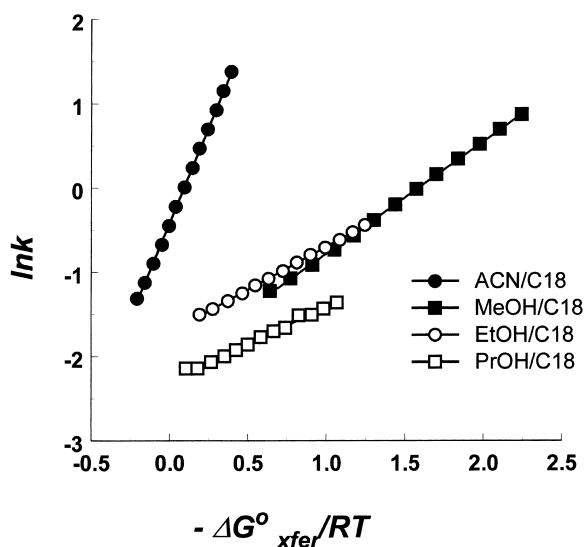Given the nature of the approximations made, it seems reasonable to say that the k values of the

Fig. 3. Calculated free energies of solvation of alkylbenzenes in model stationary phase liquids as a function of carbon number on the alkyl chain.

Fig. 4. Experimental $\ln k_x$ versus calculated $\Delta G^{\circ}_{x,\text{xfer}}/RT$.

Table 2

Statistics describing the linear regressions of measured $\ln k$ onto the calculated free energies of transfer from solvent to hexadecane for the alkylbenzenes[a]

Column	Methanol	Ethanol	Propanol	Acetonitrile
Octadecyl				
Slope	1.3	1.0	0.87	4.5
SD[b] of slope	0.0087	0.0058	0.024	0.032
Intercept	−2.1	−1.7	−2.3	−0.42
SD of intercept	0.013	0.0046	0.015	0.0066
r^2	0.9995	0.9996	0.9920	0.9995
Octyl				
Slope	0.80	0.34	–	2.9
SD of slope	0.0039	0.0048	–	0.017
Intercept	−2.0	−1.2	–	−0.84
SD of intercept	0.0060	0.0038	–	0.0035
r^2	0.9997	0.9978	–	0.9996

[a] The regression equation is $\ln k_x = \text{(slope)} \, (-\Delta G^{o}_{x,\text{xfer}}/RT) + \text{intercept}$, where k_x is the measured retention factor of solute x and $\Delta G^{o}_{x,\text{xfer}}$ is the calculated standard free energy of transfer, R is the gas constant and T is the absolute temperature.

[b] SD, standard deviation.

alkylbenzenes on the octadecyl column in the alcohol mobile phases are consistent with partitioning energetics. This is also true for methanol on the octyl column, but not for ethanol.

The k values obtained in acetonitrile are not consistent with partitioning energetics on either column, at least when hexadecane is used as a model for the stationary phase.

We note, although we cannot explain, that the ratio we observe with acetonitrile on both columns is greater than 1 while, Vailaya and Horváth report an F ratio of approximately 1 for alkylbenzenes in pure acetonitrile [3].

Dill's interphase model indicates that the ratio approaches $1/z$ when adsorption occurs, where z is the number of nearest neighbors to the adsorption site in the lattice [23]. Thus, slopes less than 1 (such as for ethanol on the octyl column) can be explained by invoking an increased contribution from adsorption. However, an explanation for a slope greater than 1 is less obvious. The simplest such is that, in neat acetonitrile, hexadecane is not a suitable model for the stationary phase, since the retention energetics exceed the partitioning energetics. It is well known that the degree of sorption of the solvent (organic modifier) molecules by the alkyl-bonded stationary phases differs from one solvent to another.

It is thus tempting to speculate that the sorbed acetonitrile on the stationary phases alters the stationary phases in a significantly different fashion from that of the sorbed alcohols, thus altering the 'mechanism' with which the solutes interact with the stationary phase. However, it is beyond the scope of this study to examine this aspect.

In our study, the standard free energy of transfer of solutes from a *bulk* mobile phase to a *bulk* model stationary phase was calculated using the SM5.0R solvation model and the solutes' AM1 gas-phase geometries. We then investigated the correlation between the experimental retention factor and the calculated standard free energy of transfer, thus investigating the relationship between retention energetics and partitioning energetics. Our results indicate that the energetics of retention on octadecyl columns are very similar to the energetics of partitioning for pure alcohol mobile phases, but not for acetonitrile. For octyl columns, the energetics of retention in methanol are similar to the energetics of partitioning, but not those of ethanol or acetonitrile. Even in those cases where the energetics are not similar, they are highly correlated.

Acknowledgements

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