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Comparative Study of Hydrocarbon, Fluorocarbon, and Aromatic Bonded RP-HPLC Stationary Phases by Linear Solvation Energy Relationships

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The retention properties of eight alkyl, aromatic, and fluorinated reversed-phase high-performance liquid chromatography bonded phases were characterized through the use of linear solvation energy relationships (LSERs). The stationary phases were investigated in a series of methanol/water mobile phases. LSER results show that solute molecular size and hydrogen bond acceptor basicity under all conditions are the two dominant retention controlling factors and that these two factors are linearly correlated when either different stationary phases at a fixed mobile-phase composition or different mobile-phase compositions at a fixed stationary phase are considered. The large variation in the dependence of retention on solute molecular volume as only the stationary phase is changed indicates that the dispersive interactions between nonpolar solutes and the stationary phase are quite significant relative to the energy of the mobile-phase cavity formation process. PCA results indicate that one PCA factor is required to explain the data when stationary phases of the same chemical nature (alkyl, aromatic, and fluoroalkyl phases) are individually considered. However, three PCA factors are not quite sufficient to explain the whole data set for the three classes of stationary phases. Despite this, the average standard deviation obtained by the use of these principal component factors are significantly smaller than the average standard deviation obtained by the LSER approach. In addition, selectivities predicted through the LSER equation are not in complete agreement with experimental results. These results show that the LSER model does not properly account for all molecular interactions involved in RP-HPLC. The failure could reside in the V_2 solute parameter used to account for both dispersive and cohesive interactions since "shape selectivity" predictions for a pair of structural isomers are very bad.

There have been many studies of retention on RP-HPLC stationary phases by selectivity studies,^{1,2} chemometric

procedures,^{3–7} and the linear solvation free energy relationship approach (LSER).^{8–14} In this work, we apply the LSER method to a series of aliphatic, aromatic, and highly fluorinated phases. Previously, Sadek et al.¹⁵ studied the retention properties of three fluorinated RP-HPLC bonded phases and a decyl hydrocarbon phase using two homologous series of solutes. In later work, they¹⁶ studied the same phases in a more detailed fashion. They found that fluorinated bonded phases are significantly less retentive than are the analogous hydrocarbon materials. Fluorinated bonded phases are particularly useful for the separation of proteins.¹⁷ Their lower polarity decreases retention times, allowing the use of water-rich, less denaturing mobile phases.

The fluorocarbon solvents that are most closely related to fluorinated bonded phases are very nonpolar, as reflected in their Hildebrand solubility parameters (δ_H), which are considerably smaller than those of the analogous hydrocarbon liquids.^{18,19} In addition, fluorocarbon solvents have very low Kamlet–Taft dipo-

- (1) Poole, C. F.; Poole, S. K. *Chromatography Today*; Elsevier: Amsterdam, 1991.
- (2) Jandera, P. J. *J. Chromatogr., A* **1993**, 656, 381.
- (3) Schmitz, S. J.; Zwanger, H.; Engelhardt, H. *J. Chromatogr., A* **1991**, 544, 381.
- (4) Righezza, M.; Chrétien, J. R. *J. Chromatogr.* **1991**, 544, 393.
- (5) Turowski, M.; Kaliszan, R.; Lüllman, C.; Genieser, H. G.; Jastorff, B. *J. Chromatogr., A* **1996**, 728, 201.
- (6) Cruz, E.; Eureby, M. R.; Johnson, C. M.; Jaccett, C. A. *Chromatographia* **1997**, 44, 3/4, 151.
- (7) Coenegracht, P. M. J.; Smilde, A. K.; Benak, H.; Bruins, C. H. P.; Metting, H. J.; De Vries, H.; Doornbos, D. A. *J. Chromatogr.* **1991**, 550, 397.
- (8) Park, J. H.; Carr, P. W.; Abraham, M. H.; Taft, R. W.; Doherty, R. M.; Kamlet, M. J. *Chromatographia* **1988**, 25, 369.
- (9) Abraham, M. H.; Rosés, M.; Poole, C. F.; Poole, S. K. *J. Phys. Org. Chem.* **1997**, 10, 358.
- (10) Buszewski, B.; Gadzata-Kopciuch, R. M.; Markuszewski, M.; Kaliszan, R. *Anal. Chem.* **1997**, 69, 3277.
- (11) Hsieh, M.-M.; Dorsey, J. G. *J. Chromatogr.* **1993**, 631, 63.
- (12) Chen, N.; Zhang, Y.; Lu, P. *J. Chromatogr.* **1993**, 633, 31.
- (13) Abraham, M. H.; Rosés, M. *J. Phys. Org. Chem.* **1994**, 7, 672.
- (14) Leahy, D. E.; Carr, P. W.; Pearlman, S.; Taft, R. W.; Kamlet, M. J. *Chromatographia* **1986**, 21, 473.
- (15) Sadek, P. C.; Carr, P. W. *J. Chromatogr.* **1984**, 288, 25.
- (16) Sadek, P. C.; Carr, P. W.; Ruggio, M. J. *Anal. Chem.* **1987**, 59, 1032.
- (17) Xindu, G.; Carr, P. W. *J. Chromatogr.* **1983**, 269, 96.
- (18) Hildebrand, J. H.; Prausnitz, J. M.; Scott, R. C. *Regular and Related Solutions*; Van Nostrand-Reinhold: New York, 1970.

larity/polarizability parameters (π^*). Values of π^* as low as -0.41 for perfluorooctane and -0.39 for perfluoroheptane compared to much higher values for the corresponding hydrocarbons have been reported.²⁰ Fluorinated compounds are also less polarizable than their hydrocarbon homologues as indicated by the refractive indexes of the corresponding liquids.^{21,22} These values are often less than 1.3 (e.g., $n\text{-C}_6\text{F}_{14}$, $n_D^{20} = 1.2515$).²³

The utility of aromatic bonded phases is related to their differential selectivity, mainly for aromatic compounds, compared to alkyl-bonded silicas. This can facilitate the separation of solutes with similar structures.^{24–26} Their differential selectivity has been attributed mainly to π – π interactions that augment the fundamentally hydrophobic process that is responsible for retention in RPLC.^{27–29}

A great deal of research using LSERs has been carried out to better understand the intermolecular processes in RPLC stationary phases. Carr et al.³⁰ employed the LSER approach and used Abraham's solute parameters to study the retention of 87 highly variegated aliphatic and aromatic solutes on different octyl and octadecyl phases. In more recent work, Zhao and Carr³¹ studied the retention characteristics of both silica- and zirconia-based aromatic and aliphatic phases in a series of acetonitrile–water mobile phases through LSERs.

Based on the LSER approach^{32,33} the free energy of retention in RPLC can be correlated with various fundamental solute properties through eq 1. Here K' is the retention factor and the

$$\log K' = \log K_0 + vV_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + rR_2 \quad (1)$$

explanatory variables (solute descriptors) are as follows:^{34–36} R_2 is the excess molar refraction, π_2^H is the solute dipolarity/polarizability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are the solute “overall” or “effective” hydrogen bond acidity and basicity, and V_2 is McGowan's characteristic volume.³⁷ The rR_2 term compensates for the inadequacy of lumping solute polarity and polarizability into a single parameter (π_2^H) and reflects the tendency of the system (mobile and stationary phases) to interact with solutes through

π - and n-electron pairs (dispersion interactions).³⁸ The intercept, $\log K_0$, and the regression coefficients r , v , s , a , and b are determined by the stationary and mobile phases and are obtained by multivariable, simultaneous, least-squares regressions.³⁸ Each coefficient reflects the *difference* in the complementary property of the mobile phase and stationary phase as follows:

$$\log K = c + \mathbf{V}(\psi_s - \psi_m)V_2 + \mathbf{S}(\pi_s^* - \pi_m^*)\pi_2^H + \mathbf{A}(\beta_s - \beta_m)\Sigma\alpha_2^H + \mathbf{B}(\alpha_s - \alpha_m)\Sigma\beta_2^H + \mathbf{R}(R_s - R_m)R_2 \quad (2)$$

where the bold characters represent coefficients for the global model and the subscripts s and m denote the stationary- and mobile-phase properties, respectively. The coefficients \mathbf{V} , \mathbf{S} , \mathbf{A} , \mathbf{B} , and \mathbf{R} are fitting parameters, which ought to be independent of both the solute and the chromatographic phases used if the LSER formalism were rigorously correct.^{39,40} Each term in eq 2 is more completely set forth in ref 41. However, to explain the results of this work, it is important to more closely consider the $\mathbf{V}(\psi_s - \psi_m)V_2$ term. Originally, this term was called the “cavity term” and was viewed solely as a measure of the endoergic (unfavorable) process of separating solvent or stationary phase molecules to provide an adequate sized cavity for the solute.^{39,41,42} Thus, it should only reflect the difference in the cohesivity between the mobile and stationary phases. More recently it has been suggested that this term arises from a combination of cavity effects and dispersive interactions between the solute and the solvent.⁴³ Recent work by Carr et al.⁴⁴ has shown that the absolute magnitude of overall free energy of transfer of a methylene group from a bulk mobile-phase mixture, such as methanol–water, to bulk hexadecane *is controlled more by the net attractive interactions in the nonpolar phase and is not dominated by the net unfavorable interactions in the mobile phase*. This indicates that dispersive interactions between the nonpolar segments of the solute and the stationary phase are important to both the retention and selectivity in RP-HPLC. Therefore it may be useful to formally dissect the $\mathbf{V}(\psi_s - \psi_m)V_2$ term into two terms, a cavity term and a dispersive term:

$$vV_2 = \mathbf{V}(\psi_s - \psi_m)V_2 = \mathbf{V}_1(\sigma_m - \sigma_s)V_2 + \mathbf{V}_2(\lambda_s - \lambda_m)V_2 \quad (3)$$

Here σ denotes some measure of the energy required to form a “hole” in a solvent (related to the cohesive energy density) and λ is a dispersion parameter representing the strength or susceptibility of the solvent to engage in dispersion interactions. When solubility parameter theory holds, σ can be taken as the square of the solvent's Hildebrand solubility parameter (δ_H^2). We see from eq 3 that as the cohesivity of the mobile phase is increased (i.e.,

- (19) Schoenmakers, P. J.; Billiet, H. A. H.; de Galan, L. *Chromatographia* **1982**, *15*, 205.
- (20) Brady, L. E.; Carr, P. W. *Anal. Chem.* **1982**, *54*, 1751.
- (21) Aldrich Catalog, 1997.
- (22) Riddick, J. A.; Bunger, W. B.; Sakano, T. K. *Organic Solvents: Physical Properties and Methods of Purification*, 4th ed.; Wiley-Interscience: New York, 1986.
- (23) Banks, R. E. *Fluorocarbons and their derivatives*; Oldbrune Press: London, 1965.
- (24) Kaibara, A.; Hirose, M.; Nakawa, T. *Chromatographia* **1990**, *30*, 1/2, 99.
- (25) Nasuto, R.; Kwietniewski, L. *Chromatographia* **1990**, *29* (11/12), 597.
- (26) Kahie, Y. D.; Pietrogrande, C.; Medina Mendez, M. I.; Reschiglian, P.; Dondi, F. *Chromatographia* **1990**, *30*, 477.
- (27) Héron, S.; Tchapla, A. *J. Chromatogr., A* **1996**, *725*, 205.
- (28) Wielandt, W.; Ellwanger, A.; Albert, K.; Lindner, E. *J. Chromatogr., A* **1998**, *805*, 71.
- (29) Jinno, K.; Nakagawa, K.; Saito, Y.; Ohta, H.; Nagashima, H.; Itoh, K.; Archer, J.; Chen, Y.-L. *J. Chromatogr., A* **1995**, *691*, 91.
- (30) Tan, L. C.; Carr, P. W.; Abraham, M. H. *J. Chromatogr., A* **1996**, *752*, 1.
- (31) Zhao, J.; Carr, P. W. *Anal. Chem.* **1998**, *70*, 3619.
- (32) Kamlet, M. J.; Taft, R. W. *J. Am. Chem. Soc.* **1976**, *98*, 377.
- (33) Kamlet, M. J.; Taft, R. W. *J. Am. Chem. Soc.* **1976**, *98*, 2886.
- (34) Abraham, M. H. *Chem. Soc. Rev.* **1993**, *22*, 73.
- (35) Abraham, M. H. *J. Phys. Org. Chem.* **1993**, *6*, 660.
- (36) Abraham, M. H.; Whiting, G. S.; Doherty, R. M.; Shuely, W. J. *J. Chromatogr.* **1991**, *587*, 213.
- (37) Abraham, M. H.; McGowan, J. C. *Chromatographia* **1987**, *23*, 243.

- (38) Carr, P. W.; Doherty, R. M.; Kamlet, M. J.; Taft, R. W.; Melander, W.; Horvath, Cs. *Anal. Chem.* **1986**, *58*, 2674.
- (39) Kamlet, M. J.; Abraham, M. H.; Carr, P. W.; Doherty, R. M.; Taft, R. W. *J. Chem. Soc., Perkin Trans. 2* **1988**, 2087.
- (40) Li, J.; Zhang, Y.; Ouyang, H.; Carr, P. W. *J. Am. Chem. Soc.* **1992**, *114*, 9813.
- (41) Tan, L. C.; Carr, P. W. *J. Chromatogr., A* **1998**, *799*, 1.
- (42) Carr, P. W.; Doherty, R. M.; Kamlet, M. J.; Taft, R. W.; Melander, W.; Horvath, Cs. *Anal. Chem.* **1986**, *58*, 2674.
- (43) Abraham, M. H.; Whiting, G. S.; Fuchs, R.; Chambers, E. J. *J. Chem. Soc., Perkin Trans 2* **1990**, 291.
- (44) Carr, P. W.; Li, J.; Dallas, A. J.; Eikens, D. I.; Tan, L. C. *J. Chromatogr., A* **1993**, *656*, 113.

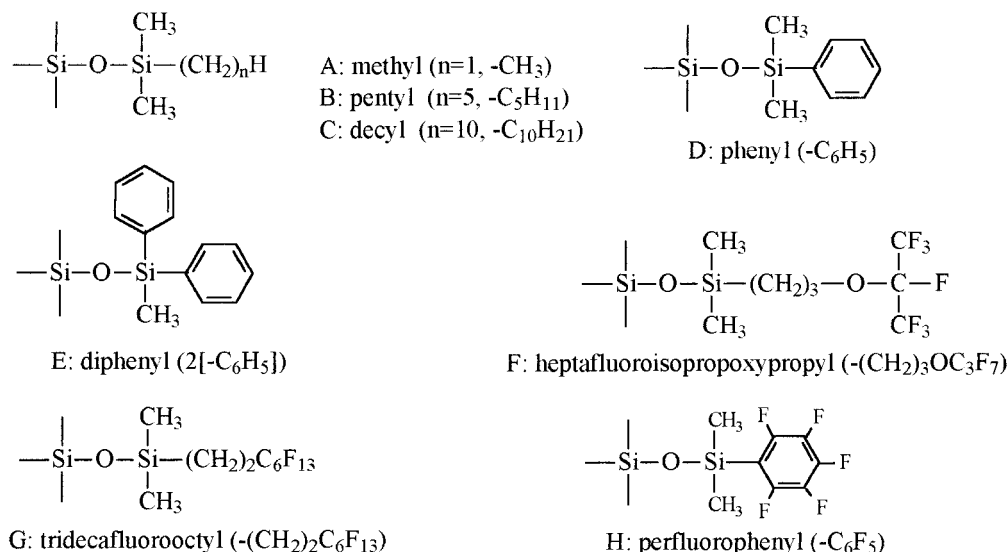


Figure 1. Stationary phases chemical structures used in this work.

when the amount of water in the mobile phase is increased), the v term and retention increase. In the same way, when dispersive interactions in the stationary phase increase (e.g., longer alkyl-bonded chains, alkylphenyl-bonded chains), the v term and retention increase. However, we cannot effectively separate these terms using regression methods because solute size descriptors (volume, area) and dispersion interactions descriptors (molar refraction, polarizability) covary too strongly.³⁰

It is well-known that RP-type stationary phases also contain a significant amount of sorbed organic solvent and water.⁴⁵ This means that π_s^* , α_s , and β_s ought not to be zero as they are for purely aliphatic bulk phases. Both chromatographic and spectroscopic measurements show that the π_s^* , α_s , and β_s values of bonded stationary phases are significantly greater than zero.^{46–53} Typical values for π_s^* range from 0.7 to 1.1, between 0.8 and 1.1 for α_s , and between 0.35 and 0.8 for β_s , depending on the mobile-phase composition and type of organic solvent.^{46,52}

All the above cited studies suggest that the stationary phase plays an important role in governing retention in RPLC. Thus, a better understanding and quantification of the various chemical properties of the bonded phase should facilitate prediction of retention in RPLC. The purpose of the present work is to compare the chromatographic characteristics of different alkyl, phenyl, fluorophenyl, and fluoroalkyl stationary phases in methanol–water mixtures through the use of the LSER approach. In this work, a set of 17 test solutes and the Abraham's current values of the solvatochromic solute parameters⁵⁴ are used. We attempt to assess

Table 1. Solvatochromic Solute Parameters for the Probe Solute^a

	probe solute	$V_2/100$	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	R_2
1	phenol	0.775	0.89	0.60	0.30	0.805
2	benzyl alcohol	0.916	0.87	0.33	0.56	0.803
3	<i>p</i> -nitrophenol	0.949	1.72	0.82	0.26	1.070
4	2-phenylethanol	1.057	0.91	0.30	0.64	0.811
5	benzaldehyde	0.873	1.00	0.00	0.39	0.820
6	benzonitrile	0.871	1.11	0.00	0.33	0.742
7	<i>p</i> -chlorophenol	0.897	1.08	0.67	0.20	0.915
8	benzene	0.716	0.52	0.00	0.14	0.610
9	nitrobenzene	0.891	1.11	0.00	0.28	0.871
10	anisole	0.916	0.75	0.00	0.29	0.708
11	fluorobenzene	0.734	0.57	0.00	0.10	0.477
12	iodobenzene	0.975	0.82	0.00	0.12	1.188
13	biphenyl	1.324	0.99	0.00	0.22	1.360
14	mesitylene	1.139	0.52	0.00	0.19	0.649
15	anthracene	1.454	1.34	0.00	0.26	2.290
16	<i>tert</i> -butylbenzene	1.280	0.49	0.00	0.16	0.619
17	<i>n</i> -butylbenzene	1.280	0.51	0.00	0.15	0.600

^a Taken from ref 54.

the chemical origin of the differences in retention characteristics between the above stationary phases and determine the origin of any difference in the selectivity between these materials.

EXPERIMENTAL SECTION

The 17 probe solutes studied along with the values for the Abraham's solute descriptors⁵⁴ are listed in Table 1. The eight stationary phases investigated are shown in Figure 1. The fluorinated stationary phases were prepared on 5- μm Hypersil, and the other supports were Amicon 10- μm silica. The procedures for preparing the stationary phases and the packing of the columns have been given previously.¹⁵ Uracil was used to measure the void volume. The instruments used to make all the chromatographic measurements were described previously.⁵⁵

PCA studies were performed by using the MATLAB 4.1c.v for Windows.

- (45) Brown, P. R.; Hartwick, R. A. *High Performance Liquid Chromatography*; Wiley: New York, 1989; Vol. 98, Chapter 2.
 (46) (a) Lu, H.; Rutan, S. C. *Anal. Chem.* **1996**, *68*, 1387. (b) Dallas, A. J. Ph.D. Thesis, University of Minnesota, 1995.
 (47) Cheong, W. J.; Carr, P. W. *J. Chromatogr.* **1990**, *499*, 373.
 (48) Jones, L. J.; Rutan, S. C. *Anal. Chem.* **1991**, *63*, 1318.
 (49) Carr, J. W.; Harris, J. M. *Anal. Chem.* **1986**, *58*, 626.
 (50) Carr, J. W.; Harris, J. M. *Anal. Chem.* **1987**, *59*, 2546.
 (51) Lochmüller, C. H.; Marshall, D. B.; Wilder, D. R. *Anal. Chim. Acta* **1980**, *130*, 31.
 (52) Helburn, R. S.; Rutan, S. C.; Pompano, J.; Mitchem, A.; Patterson, W. T. *Anal. Chem.* **1994**, *66*, 610.
 (53) Men, Y. D.; Marshall, D. B. *Anal. Chem.* **1990**, *62*, 2606.
 (54) Abraham, M. H. *J. Pharm. Sci.* **1994**, *83*, 1085.

- (55) Sadek, P. C.; Carr, P. W. *J. Chromatogr. Sci.* **1983**, *21*, 314.

Table 2. log *K'* Values in MeOH–Water

solute		2(C ₆ H ₅) column ^a				CH ₃ column ^a			
		60	55	50	45	60	55	50	45
1	phenol	−0.527	−0.407	−0.298	−0.187	−0.732	−0.653	−0.552	−0.432
2	benzyl alcohol	−0.475	−0.355	−0.260	−0.148	−0.698	−0.607	−0.525	−0.415
3	<i>p</i> -nitrophenol	−0.397	−0.255	−0.132	0.011	−0.697	−0.546	−0.418	−0.272
4	2-phenylethanol	−0.320	−0.201	−0.086	0.038	−0.565	−0.464	−0.369	−0.251
5	benzaldehyde	−0.191	−0.063	0.055	0.183	−0.534	−0.427	−0.329	−0.202
6	benzonitrile	−0.131	0.002	0.128	0.264	−0.475	−0.371	−0.261	−0.125
7	<i>p</i> -chlorophenol	−0.280	−0.128	0.005	0.153	−0.492	−0.358	−0.227	−0.086
8	benzene	−0.162	−0.042	0.074	0.183	−0.408	−0.316	−0.216	−0.117
9	nitrobenzene	−0.039	0.092	0.215	0.343	−0.411	−0.302	−0.200	−0.078
10	anisole	−0.069	0.041	0.186	0.316	−0.395	−0.285	−0.178	−0.058
11	fluorobenzene	−0.135	−0.026	0.118	0.242	−0.363	−0.259	−0.152	−0.036
12	iodobenzene	0.188	0.359	0.524	0.706	−0.186	−0.043	0.108	0.259
13	biphenyl	0.455	0.672	0.881	1.121	−0.003	0.172	0.359	0.558
14	mesitylene	0.292	0.483	0.667	0.854	0.008	0.173	0.331	0.499
15	anthracene	0.630	0.875	1.123	1.400	0.095	0.293	0.507	0.740
16	<i>tert</i> -butylbenzene	0.371	0.581	0.784	1.002	0.086	0.266	0.446	0.634
17	<i>n</i> -butylbenzene	0.466	0.691	0.906	1.132	0.181	0.370	0.566	0.770

solute		C ₆ F ₅ column ^a				C ₅ H ₁₁ column ^a			
		60	55	50	45	60	55	50	45
1	phenol	−0.573	−0.438	−0.323	−0.213	−0.310	−0.187	−0.066	0.059
2	benzyl alcohol	−0.551	−0.431	−0.314	−0.211	−0.285	−0.168	−0.055	0.069
3	<i>p</i> -nitrophenol	−0.372	−0.238	−0.092	0.051	−0.179	−0.049	0.088	0.251
4	2-phenylethanol	−0.402	−0.262	−0.133	−0.008	−0.126	0.011	0.138	0.278
5	benzaldehyde	−0.303	−0.176	−0.038	0.090	−0.111	0.021	0.145	0.287
6	benzonitrile	−0.254	−0.117	0.018	0.157	−0.073	0.070	0.210	0.358
7	<i>p</i> -chlorophenol	−0.262	−0.106	0.048	0.198	0.012	0.172	0.329	0.494
8	benzene	−0.185	−0.060	0.065	0.177	0.108	0.241	0.369	0.498
9	nitrobenzene	−0.139	−0.001	0.129	0.262	0.037	0.179	0.314	0.453
10	anisole	−0.106	0.029	0.164	0.297	0.111	0.256	0.397	0.543
11	fluorobenzene	−0.120	0.021	0.152	0.280	0.143	0.290	0.405	0.573
12	iodobenzene	0.090	0.274	0.446	0.620	0.454	0.642	0.832	1.027
13	biphenyl	0.382	0.594	0.808	1.025	0.687	0.919	1.161	1.410
14	mesitylene	0.416	0.605	0.788	0.974	0.723	0.936	1.155	1.365
15	anthracene	0.580	0.823	1.067	1.318	0.814	1.068	1.346	1.625
16	<i>tert</i> -butylbenzene	0.414	0.625	0.827	1.029	0.852	1.086	1.330	1.571
17	<i>n</i> -butylbenzene	0.504	0.726	0.941	1.159	0.964	1.215	1.477	1.735

solute		C ₆ H ₅ column ^a				(CH ₂) ₃ OC ₃ F ₇ column ^a			
		60	55	50	45	60	55	50	45
1	phenol	−0.785	−0.690	−0.580	−0.473	−0.675	−0.545	−0.417	−0.297
2	benzyl alcohol	−0.740	−0.630	−0.537	−0.437	−0.618	−0.501	−0.373	−0.258
3	<i>p</i> -nitrophenol	−0.682	−0.573	−0.442	−0.330	−0.462	−0.311	−0.169	−0.036
4	2-phenylethanol	−0.611	−0.501	−0.393	−0.280	−0.504	−0.359	−0.229	−0.087
5	benzaldehyde	−0.480	−0.387	−0.270	−0.162	−0.367	−0.246	−0.095	0.036
6	benzonitrile	−0.428	−0.316	−0.201	−0.080	−0.300	−0.158	−0.007	0.134
7	<i>p</i> -chlorophenol	−0.553	−0.422	−0.302	−0.161	−0.421	−0.255	−0.095	0.057
8	benzene	−0.444	−0.347	−0.249	−0.150	−0.132	0.004	0.134	0.255
9	nitrobenzene	−0.359	−0.259	−0.149	−0.039	−0.185	−0.044	0.096	0.227
10	anisole	−0.382	−0.279	−0.165	−0.048	−0.174	−0.025	0.117	0.228
11	fluorobenzene	−0.399	−0.302	−0.183	−0.074	−0.069	0.083	0.227	0.362
12	iodobenzene	−0.184	−0.043	0.104	0.250	−0.017	0.173	0.351	0.526
13	biphenyl	0.005	0.179	0.364	0.544	0.193	0.426	0.647	0.869
14	mesitylene	−0.056	0.081	0.235	0.388	0.340	0.545	0.739	0.934
15	anthracene	0.1338	0.328	0.538	0.738	0.296	0.551	0.809	1.064
16	<i>tert</i> -butylbenzene	−0.011	0.172	0.330	0.501	0.424	0.643	0.859	1.070
17	<i>n</i> -butylbenzene	0.074	0.249	0.434	0.600	0.493	0.728	0.960	1.186

solute		C ₁₀ H ₂₁ column ^a				(CH ₂) ₂ C ₆ F ₁₃ column ^a			
		60	55	50	45	60	55	50	45
1	phenol	−0.732	−0.612	−0.478	−0.374	−0.692	−0.555	−0.423	−0.285
2	benzyl alcohol	−0.640	−0.518	−0.406	−0.301	−0.605	−0.476	−0.354	−0.215
3	<i>p</i> -nitrophenol	−0.536	−0.396	−0.268	−0.156	−0.477	−0.330	−0.179	−0.018
4	2-phenylethanol	−0.443	−0.335	−0.216	−0.102	−0.489	−0.346	−0.200	−0.053
5	benzaldehyde	−0.413	−0.288	−0.162	−0.048	−0.322	−0.171	−0.030	0.116
6	benzonitrile	−0.392	−0.251	−0.119	0.005	−0.255	−0.101	0.052	0.209
7	<i>p</i> -chlorophenol	−0.374	−0.217	−0.071	0.057	−0.445	−0.271	−0.094	0.067
8	benzene	−0.260	−0.132	−0.014	0.082	−0.118	0.024	0.160	0.298
9	nitrobenzene	−0.258	−0.129	−0.003	0.105	−0.155	−0.002	0.144	0.296
10	anisole	−0.236	−0.101	0.024	0.139	−0.178	−0.019	0.139	0.284
11	fluorobenzene	−0.236	−0.091	0.034	0.145	−0.113	0.131	0.283	0.433
12	iodobenzene	0.121	0.279	0.444	0.601	−0.009	0.192	0.386	0.577
13	biphenyl	0.324	0.52	0.726	0.929	0.196	0.433	0.671	0.915
14	mesitylene	0.346	0.526	0.705	0.869	0.401	0.619	0.829	1.041
15	anthracene	0.510	0.733	0.966	1.204	0.218	0.473	0.740	1.018
16	<i>tert</i> -butylbenzene	0.396	0.594	0.791	0.990	0.531	0.766	0.997	1.231
17	<i>n</i> -butylbenzene	0.522	0.740	0.954	1.166	0.599	0.849	1.058	1.343

^a With 60, 55, 50, and 45% MeOH mobile phases.

Table 3. LSER Coefficients (Eq 1)

% MeOH	$\log k_0$	v	s	a	b	r	R^2	SD
CH ₃								
45	-0.92 ± 0.06	1.45 ± 0.05	-0.13 ± 0.04	-0.24 ± 0.05	-1.08 ± 0.08	<i>a</i>	0.9901	0.046
50	-0.91 ± 0.06	1.25 ± 0.05	-0.12 ± 0.04	-0.26 ± 0.04	-0.99 ± 0.07	<i>a</i>	0.9894	0.041
55	-0.91 ± 0.06	1.13 ± 0.05	-0.15 ± 0.04	-0.25 ± 0.05	-0.87 ± 0.07	<i>a</i>	0.9879	0.042
60	-0.91 ± 0.06	0.97 ± 0.04	-0.16 ± 0.03	-0.27 ± 0.04	-0.76 ± 0.07	<i>a</i>	0.9882	0.037
C ₅ H ₁₁								
45	-0.63 ± 0.08	2.10 ± 0.09	-0.30 ± 0.05	-0.22 ± 0.06	-1.56 ± 0.10	<i>a</i>	0.9929	0.056
50	-0.63 ± 0.07	1.85 ± 0.09	-0.28 ± 0.05	-0.23 ± 0.06	-1.41 ± 0.09	<i>a</i>	0.9918	0.051
55	-0.62 ± 0.07	1.66 ± 0.08	-0.29 ± 0.05	-0.21 ± 0.06	-1.30 ± 0.09	<i>a</i>	0.9914	0.051
60	-0.65 ± 0.07	1.47 ± 0.08	-0.27 ± 0.04	-0.21 ± 0.05	-1.18 ± 0.08	<i>a</i>	0.9906	0.048
C ₁₀ H ₂₁								
45	-0.99 ± 0.09	1.87 ± 0.08	-0.15 ± 0.06	-0.36 ± 0.07	-1.36 ± 0.11	<i>a</i>	0.9885	0.065
50	-0.97 ± 0.09	1.65 ± 0.08	-0.13 ± 0.06	-0.36 ± 0.07	-1.26 ± 0.11	<i>a</i>	0.9854	0.064
55	-1.00 ± 0.09	1.52 ± 0.08	-0.15 ± 0.06	-0.35 ± 0.07	-1.18 ± 0.11	<i>a</i>	0.9849	0.063
60	-1.05 ± 0.09	1.39 ± 0.08	-0.14 ± 0.06	-0.35 ± 0.07	-1.07 ± 0.11	<i>a</i>	0.9848	0.077
C ₆ H ₅								
45	-0.92 ± 0.05	1.27 ± 0.05	<i>a</i>	-0.43 ± 0.04	-0.97 ± 0.07	<i>a</i>	0.9916	0.040
50	-0.94 ± 0.05	1.13 ± 0.04	<i>a</i>	-0.43 ± 0.04	-0.88 ± 0.06	<i>a</i>	0.9921	0.035
55	-0.95 ± 0.04	1.02 ± 0.04	<i>a</i>	-0.42 ± 0.04	-0.80 ± 0.05	<i>a</i>	0.9921	0.033
60	-0.96 ± 0.04	0.87 ± 0.04	<i>a</i>	-0.43 ± 0.04	-0.74 ± 0.06	<i>a</i>	0.9906	0.033
2(C ₆ H ₅)								
45	-0.83 ± 0.06	1.58 ± 0.06	<i>a</i>	-0.51 ± 0.05	-1.16 ± 0.07	0.11 ± 0.04	0.9954	0.039
50	-0.79 ± 0.06	1.34 ± 0.06	<i>a</i>	-0.51 ± 0.04	-1.03 ± 0.06	0.11 ± 0.04	0.9954	0.035
55	-0.84 ± 0.06	1.24 ± 0.06	<i>a</i>	-0.48 ± 0.04	-0.94 ± 0.07	0.06 ± 0.04	0.9938	0.037
60	-0.84 ± 0.05	1.07 ± 0.05	<i>a</i>	-0.49 ± 0.04	-0.84 ± 0.06	0.05 ± 0.03	0.9942	0.032
(CH ₂) ₃ OC ₃ F ₇								
45	-0.67 ± 0.10	1.68 ± 0.10	<i>a</i>	-0.43 ± 0.08	-1.45 ± 0.12	-0.13 ± 0.07	0.9867	0.067
50	-0.67 ± 0.11	1.47 ± 0.12	<i>a</i>	-0.43 ± 0.08	-1.35 ± 0.12	-0.13 ± 0.07	0.9916	0.065
55	-0.72 ± 0.10	1.36 ± 0.10	<i>a</i>	-0.42 ± 0.07	-1.27 ± 0.11	-0.16 ± 0.07	0.9831	0.064
60	-0.77 ± 0.09	1.20 ± 0.09	<i>a</i>	-0.43 ± 0.07	-1.15 ± 0.11^a	-0.18 ± 0.07	0.9801	0.062
(CH ₂) ₂ C ₆ F ₁₃								
45	-0.66 ± 0.10	1.84 ± 0.10	<i>a</i>	-0.50 ± 0.08	-1.51 ± 0.12	-0.28 ± 0.07	0.9884	0.066
50	-0.67 ± 0.10	1.64 ± 0.10	<i>a</i>	-0.49 ± 0.07	-1.40 ± 0.11	-0.28 ± 0.07	0.9874	0.062
55	-0.72 ± 0.10	1.50 ± 0.10	<i>a</i>	-0.49 ± 0.07	-1.30 ± 0.11	-0.31 ± 0.07	0.9851	0.063
60	-0.84 ± 0.10	1.37 ± 0.10	<i>a</i>	-0.48 ± 0.07	-1.15 ± 0.11	-0.32 ± 0.07	0.9815	0.063
C ₆ F ₅								
45	-0.85 ± 0.08	1.74 ± 0.07	<i>a</i>	-0.36 ± 0.07	-1.36 ± 0.11	<i>a</i>	0.9885	0.061
50	-0.85 ± 0.08	1.56 ± 0.08	<i>a</i>	-0.35 ± 0.07	-1.25 ± 0.11	<i>a</i>	0.9856	0.060
55	-0.86 ± 0.08	1.40 ± 0.07	<i>a</i>	-0.34 ± 0.06	-1.15 ± 0.10	<i>a</i>	0.9861	0.056
60	-0.90 ± 0.08	1.24 ± 0.07	<i>a</i>	-0.34 ± 0.06	-1.06 ± 0.10	<i>a</i>	0.9832	0.056

^a Coefficient values are statistically zero based on the standard deviation.

Table 4. Correlation Coefficient Matrix of Solute Descriptors

	V_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	R_2
V_2	1				
π_2^H	0.038	1			
$\Sigma\alpha_2^H$	-0.270	0.539	1		
$\Sigma\beta_2^H$	-0.074	0.292	0.257	1	
R_2	0.565	0.597	0.014	0.026	1

RESULTS AND DISCUSSION

I. LSER Studies. In Table 2 we report the $\log K$ values for the 17 solutes on the 8 different stationary phases at 4 mobile-phase compositions. The LSER coefficients using eq 1 are given in Table 3. These coefficients were obtained from multivariable linear regression of $\log K$ against the solute descriptors. The variance–covariance matrix of these parameters is shown in Table 4. It indicates that the covariances among the variables are weak and only π_2^H and R_2 , R_2 and V_2 show some slight correlation. This is expected since both π_2^H and R_2 reflect the polarizability characteristics of the solute and no aliphatic solutes are present in the solute set to break the covariance. In general, the regression coefficients obtained for our data set based on eq 1 were all higher than 0.98 and the average standard deviations of the fits were all

smaller than 0.07. The stability of the LSER coefficients were tested by removing anthracene and *p*-nitrophenol from the solute set in two different steps. These solutes were chosen because anthracene has the biggest V_2 and R_2 values and *p*-nitrophenol has the biggest π_2^H and $\Sigma\alpha_2^H$ values of any solutes used. The qualities of the fits remained equally good and the LSER coefficients were statistically unaltered. These fit qualities mean that Abraham's parameters in conjunction with the LSER methodology can be used to model the retention process in this heterogeneous set of bonded reversed phases. As shown in Figure 2, using the results in 60% methanol as a representative example, the residuals of the fit are consistent from column to column. Similar plots are observed at the other mobile-phase compositions. This indicates that the LSER model does not completely account for the retention variations among the different solutes. We will discuss this problem at some length below. However, none of the solutes appear to be significant outliers and the coefficients are stable, so we believed the chemical interpretations below are justified.

As is commonly observed in RPLC,^{8,9,15,16,30,39,42} the cavity formation process (the v coefficient) and the solute hydrogen bond basicity interactions (the b coefficient) are the most important interactions governing retention. In Figure 3 we show the LSER coefficients at 45 and 60% methanol. We see that both plots are

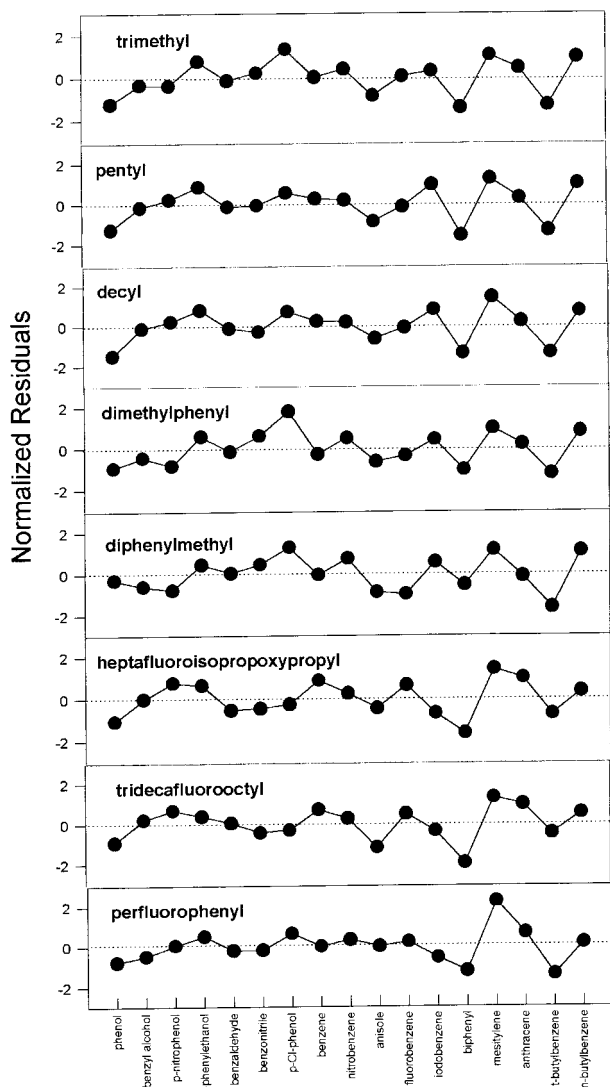


Figure 2. Normalized residuals (residuals divided by the standard deviation) corresponding to the LSER regression eq 1 (see text) at 60% methanol–water.

very similar. The relative magnitudes of the coefficients for the different stationary phases are all similar at the four mobile-phase compositions studied. Only the larger LSER coefficients b and v change significantly with mobile-phase composition. More detailed discussions about the chemical meaning of the sign and magnitude of the different coefficients and their variation with mobile-phase composition have been given previously.^{30,31,41} In addition, we must be cautious in our interpretation of these results since the different surface coverages of these bonded phases due to steric shielding during the bonding procedure can complicate the comparisons. In Table 5 we show, for reference purposes, the solvatochromic and physical properties of bulk solvents that are analogous to the mobile and stationary phases.

The s Coefficient. Only the alkyl phases have s coefficients that are statistically significant. The magnitude of s is independent of the mobile-phase composition over this narrow range in mobile-phase composition. Since s is related to the difference in the π^* of the stationary and mobile phases (see eq 2), the negative sign of this coefficient indicates that the dipolarity/polarizability of the mobile-phase is higher than that of the stationary phase, as is

Table 5. Solvatochromic and Physical Properties of Bulk Solvents

	solvent property				
	δ_H^2 (MPa) ^a	n_D^b	π^* ^c	β^c	α^c
water	2294	1.333	1.17	0.47	1.17
methanol	876	1.327	0.61	0.66	0.93
benzene	353	1.501	0.59	0.10	0.00
pentane	206	1.358	-0.08	0.00	0.00
decane	249	1.411	0.10	0.00	0.00
perfluorobenzene	275	1.377			
perfluorooctane	<141 ^c	<1.30	-0.41	-0.08	0.00
HFIP ^d		1.371			

^a Cohesive energy density, from ref 22. ^b Refractive index from refs 21 and 22. ^c From ref 73. ^d 3-(Hepotafluoroisopropoxypropyl)trichlorosilane. ^e Value for perfluoroheptane.

expected since methanol and water are more polar than the stationary phase, even though it is solvated by mobile-phase components. Thus, for these phases an increase in the solute dipolarity/polarizability decreases retention slightly. For the aromatic and fluorinated stationary phases (C_6H_5 , $2[C_6H_5]$, $[CH_2]_2C_6F_{13}$, $[CH_2]_3OC_3F_7$, C_6F_5), the s coefficient is essentially zero for all the mobile-phase compositions. This, according to eq 2, means that the dipolar interactions between the solute and the stationary phase are of the same magnitude compared to dipolar interactions in the mobile phase and, then, they mutually cancel. In the absence of mobile-phase sorption, the dipolarity/polarizability of the phenyl-based phases should be significantly greater than that of the fluorocarbon phase. The alkyl phases should have an intermediate value. The essentially zero s coefficients on both the aromatic and fluorinated stationary phases indicate that mobile-phase sorption must play an important role in controlling dipolar interactions in the stationary phase.

The a Coefficient. The a coefficient is small (about 10–30% of the v coefficient) and is only slightly dependent on the type of stationary phase. This means that solute HBD acidity ($\Sigma\alpha_2^{H1}$) has only a small effect on retention. Except for the $C_{10}H_{21}$ phase, there is almost no variation in the a coefficient with mobile-phase composition. This suggests, according to eq 2, that β_s is strongly correlated with β_m . Since β_m decreases as the amount of methanol in the mobile phase is decreased, β_s must decrease by almost the same amount to keep the a coefficient almost constant. This reasoning is consistent with a decrease in the amount of sorbed methanol in the stationary phase as the percentage of methanol in the mobile phase is decreased, as observed solvatochromically by Ståhlberg.⁵⁶ The negative a values mean that the mobile phase is more basic than the stationary phase, as expected since both methanol and water are more basic than the aprotic bonded materials. Thus, an increase in HB acidity of the solute will decrease retention only slightly.

The b Coefficient. The b coefficients are all large and negative. Thus, solutes with stronger HB acceptor ability will be significantly less retained than non basic solutes. This indicates that the mobile phase is a much stronger HB donor than the stationary phase. As ϕ_{MeOH} is decreased, the b coefficient $[= B(\alpha_s - \alpha_m)]$ increases significantly (approximately 0.3 unit). On the other hand, α_m increases as ϕ_{MeOH} is decreased. Since b increases, α_s must remain

(56) Ståhlberg, J.; Almgren, M. *Anal. Chem.* **1985**, *57*, 817.

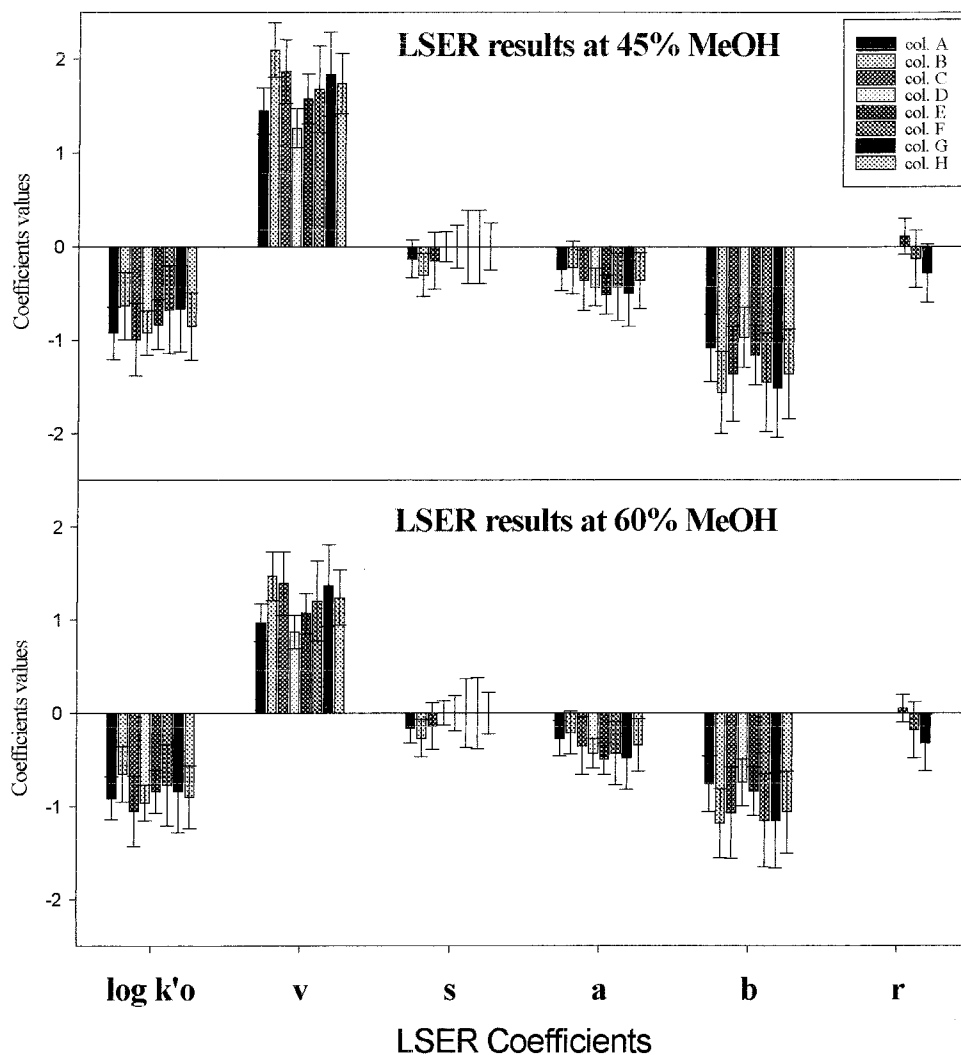


Figure 3. LSER coefficients from eq 1 for the different stationary phases at 45 and 60% methanol: (A) CH_3 , (B) C_5H_{11} , (C) $\text{C}_{10}\text{H}_{21}$, (D) C_6H_5 , (E) $2[\text{C}_6\text{H}_5]$, (F) $(\text{CH}_2)_3\text{OC}_3\text{F}_7$, (G) $(\text{CH}_2)_2\text{C}_6\text{F}_{13}$, and (H) C_6F_5 (see Figure 1 for chemical structures).

almost constant or diminish. It has been shown that changes in α_s are small compared to changes in α_m .³⁰ Again, this is consistent with a decrease in the amount of sorbed methanol in the stationary phase as ϕ_{MeOH} is decreased.⁵⁵

The C_6H_5 and CH_3 phases have the smallest b coefficients. This suggests that, for these two phases, α_s is closer to α_m than for the other stationary phases. This could be due to greater sorption of mobile-phase molecules or greater accessibility of residual silanols. Zhao and Carr³¹ observed that the b coefficient for aromatic phases is lower than for aliphatic phases and attributed this to the higher HB acceptor strength of aromatic rings compared to aliphatic moieties.³¹ Thus, sorption of mobile-phase components due to HB interactions is stronger on aromatic phases compared to alkyl phases. This is consistent with the statistically near-zero value of s for aromatic phases compared to the negative s for aliphatic phases (Table 3). The b coefficient for the CH_3 phase is lower than for the other alkyl phases. Again, this suggests that α_s is higher for this phase and that sorption of mobile-phase components is greater on it compared to longer chain alkyl phases.

The r Coefficient. The r coefficient is statistically zero except for the $2(\text{C}_6\text{H}_5)$, $(\text{CH}_2)_2\text{C}_6\text{F}_{13}$, and $(\text{CH}_2)_3\text{OC}_3\text{F}_7$ phases. In these cases, adding R_2 to the regression improved both the correlation

coefficient and the average standard deviation, whereas for the other stationary phases it does not significantly change these statistics. However, the relative standard deviations of the r coefficients are high (20–50%). For the $2(\text{C}_6\text{H}_5)$ phase, the r coefficient is positive and tends to zero as ϕ_{MeOH} is increased. This can be attributed to the higher polarizability of the stationary phase compared to the mobile phase. A positive r coefficient implies that the solute undergoes stronger π – π interactions with the stationary phase than with the mobile phase. This is consistent with the higher polarizability of aromatic rings compared to aliphatic chains. Table 5 shows that benzene has a much higher refractive index than any of the other liquids, which means that it is much more polarizable. As ϕ_{MeOH} is increased, the magnitude of r decreases. Since the refractive index of methanol is slightly lower than that of water, r_m decreases slightly as ϕ_{MeOH} is increased. However, r_s must decrease faster than r_m so that the r coefficient decreases as observed. This can be explained by the preferential sorption of methanol onto the stationary phase and desorption of water as ϕ_{MeOH} is increased. For the two most highly fluorinated phases ($(\text{CH}_2)_3\text{OC}_3\text{F}_7$ and $(\text{CH}_2)_2\text{C}_6\text{F}_{13}$), the r coefficient is negative. This is due to the lower polarizability of the stationary phase compared to the mobile phase. The r coefficient for these two

columns is almost independent of ϕ_{MeOH} . Again, this indicates that r_s decreases at the same rate as does r_m , which can occur upon sorption of methanol by the stationary phase.

One of the most interesting findings of this study is that the LSER model correctly leads to the conclusion that phenyl phases are more polarizable than aliphatic phases and that fluorinated phases are less polarizable.

The ν Coefficient. As observed previously,^{8,30} the ν coefficient becomes increasingly more positive as ϕ_{MeOH} is decreased. This observation is consistent with the higher cohesivity of water ($\delta_{\text{H}}^2 = 2294 \text{ MPa}$) as compared to methanol ($\delta_{\text{H}}^2 = 876 \text{ MPa}$), which affects the dependence of retention on solute volume (V_2). As the fraction of water, φ_{w} , is increased, the cohesive energy density of the mobile phase increases substantially. Changes in the cohesivity of the bonded phase, which are largely controlled by the sorbed solvents, are minor because the strong HB interaction between methanol and water prevent these solvents from strongly interacting with the alkyl chains.⁵⁷

The ν coefficient is closely related to the "hydrophobic selectivity" of the stationary phase since it explains changes in retention in a series of solutes with constant π_2^{H} , $\Sigma\alpha_2^{\text{H}}$, and $\Sigma\beta_2^{\text{H}}$ values,³⁰ e.g., a homologous series. We see from Table 3 and Figure 3 that the retention order for a nonpolar, non-HB-interacting solute according to the size of the ν coefficient should be as follows: $\text{C}_5\text{H}_{11} > \text{C}_{10}\text{H}_{21} \approx (\text{CH}_2)_2\text{C}_6\text{F}_{13} > (\text{CH}_2)_3\text{OC}_3\text{F}_7 \approx \text{C}_6\text{F}_5 > 2(\text{C}_6\text{H}_5) > \text{CH}_3 > \text{C}_6\text{H}_5$. This order is quite complicated, but we believe it can be rationalized by considering the physical properties (see Table 5) of the liquids analogous to the bonded phases. On the basis of eq 3 we see that, at a fixed mobile-phase composition, an increase of the stationary-phase dispersive interactions (the λ_s term) should increase the ν term (and retention) while an increase of the stationary-phase cohesive energy (the σ_s term) decreases the ν coefficient (and retention).

In general, perfluoro compounds have lower cohesive energy densities than do the corresponding hydrogenated compounds.⁵⁷ On this basis alone, ν and the retention of nonpolar solutes should be larger on fluorocarbon phases than for hydrocarbon phases. However, this is opposite to what is observed. To rationalize these results, we must also consider the role of dispersive interactions. Hydrocarbon phases are significantly more dispersive than highly fluorinated phases (the λ_s term is higher for alkanes due to their higher refractive index). We conclude that the higher ν coefficient of alkyl phases as compared to highly fluorinated phases can be explained by weaker dispersive interactions of the solute with the fluorocarbon phase. This is consistent both with the free energy of transfer for a methylene group from gas phase to bulk hexadecane⁵⁸ as compared to bulk perfluorohexane (-685 ± 1.5 vs $-444 \pm 3.2 \text{ cal/mol}$, respectively) and with earlier studies wherein a decyl phase proved to have a larger methylene selectivity than on analogous fluorocarbon phase.^{15,16} We note that the methyl phase has a smaller ν coefficient than do the fluorocarbon phases. This could be due to the much smaller polarizability of this phase compared to the longer alkyl-bonded phases or to a difference in retention mechanism on the very short "chain" methyl phase.⁵⁸

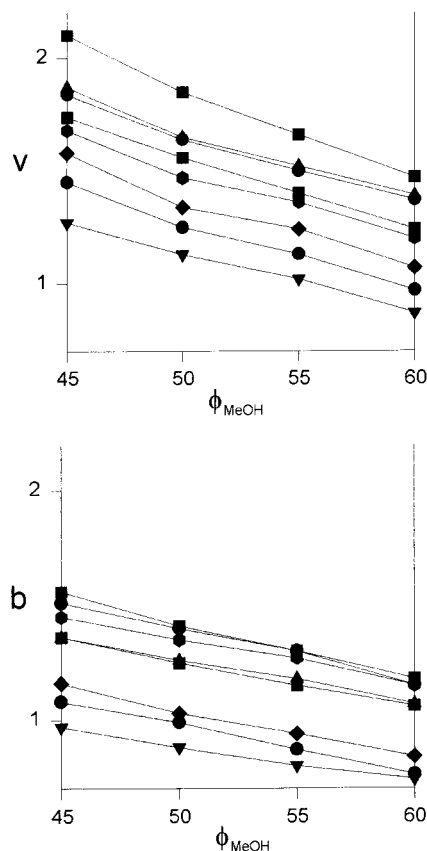


Figure 4. Variation of the b and ν coefficients with mobile-phase composition.

The aromatic phases have the smallest ν coefficient of any phase examined. These results are in agreement with Zhao and Carr's work.³¹ According to the refractive indexes in Table 5, the aromatic phases should have the largest ν values since they are much more polarizable. Before going further, we point out that the free energy of transfer of a methylene group from gas phase to bulk benzene and toluene are virtually identical to the free energy of transfer into bulk hexadecane.⁵⁹ On this basis alone we would expect the ν coefficient of the aromatic phases to be similar to the aliphatic phases but it is clearly much smaller (see Table 3). There are several possible explanations for the small ν coefficient of the aromatic phases. The one we currently favor³¹ is much stronger sorption of mobile phase into the aromatic bonded phase compared to sorption into aliphatic bonded phases. We point out that at room temperature benzene and methanol are far more miscible than are decane and methanol. Significant sorption of methanol makes the stationary phase environment more like the mobile-phase environment and thus reduces all LSER coefficients.

Correlation of the b and ν Terms. We see from Figure 4 that for all stationary phases the b and ν terms change linearly with mobile-phase composition. In Figure 5 we show that the b coefficient is linearly related to the ν coefficient for all stationary phases; i.e., the b coefficient changes linearly with ν as the mobile-phase composition is changed. Such results have previously been obtained by Abraham for a series of aliphatic C_{18} phases.⁹ Here, we extend the observation to aromatic and short-chain alkyl

(57) Yonker, C. R.; Zwier, T. A.; Burke, M. F. *J. Chromatogr.* **1982**, *241*, 269.

(58) Tan, L. C.; Carr, P. W. *J. Chromatogr., A* **1997**, *775*, 1.

(59) Eikens, D. I. Ph.D. Thesis, University of Minnesota, Minneapolis, 1993.

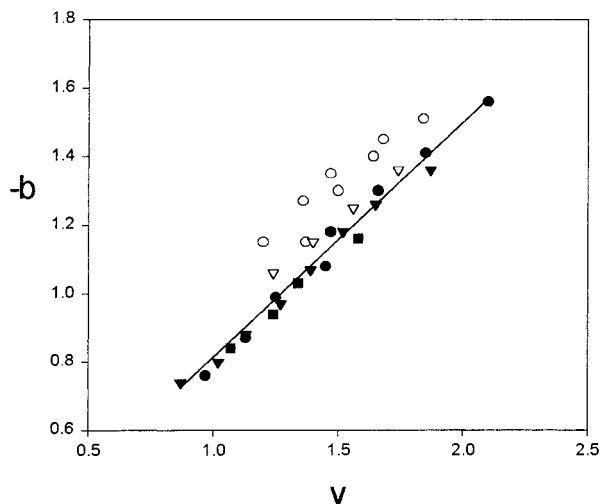


Figure 5. Plot of b vs v coefficient for the different stationary phases studied as mobile-phase composition is changed.

phases. The alkyl and phenyl phases are described by the same straight line. However, the fluorinated phases, mainly the C_6F_{13} and $(CH_2)_3OC_3F_7$ phases, fall on a different but parallel line. Considering the well-known relationship between $\log K'$ and mobile-phase composition, ϕ ,⁶⁰

$$\log K' = \log K'_w - S\phi \quad (4)$$

b and v coefficients should vary linearly with changes in mobile-phase composition in order for eq 4 to be satisfied unless for a sufficient short mobile-phase composition range. This suggestion is the basis for a recent paper by Wang and Carr.⁶¹ However, there is nothing in eq 4 that suggests or requires covariance in b and v as the stationary phase is varied.

Plots of b vs v coefficient for the different stationary phases at a fixed mobile phase are shown in Figure 6. Again we can see that alkyl and phenyl phases fall on the same straight line but the fluorinated phases do not. This behavior was first observed over a decade ago by Sadek et al.⁶² for different ODS phases in equiulotropic mixtures of acetonitrile, methanol, or tetrahydrofuran with water. Interestingly, as in this work, they observed a linear relationship between b and v when they did not consider fluorocarbon phases together.⁶² If we do not consider the fluoralkyl phases, the slopes and intercepts of v vs b plots are very similar when we change the stationary phase at a fixed mobile-phase composition or when we change the mobile-phase composition for a fixed stationary phase. This is shown in eq 4. Here, n includes

$$b = (0.71 \pm 0.03)v - (0.06 \pm 0.05) \quad (4)$$

$$r = 0.9974 \quad SD = 0.019 \quad n = 20$$

the different nonfluorinated phases and the different acetonitrile–water mobile-phase compositions. Abraham et al.⁹ have recently shown that although the regression coefficients r , s , v , a , and b vary widely between different C_{18} columns and with different

mobile phases, all coefficients are linearly related over the solvent composition range from 30 to 90% acetonitrile and from 30 to 90% methanol. For example, the slope they obtained between b and v for different C_{18} phases with different acetonitrile eluents is 0.92 and the intercept is zero. This results are in very good agreement with eq 4 considering the different chemical nature of the stationary phases included in the regression. This is a very important result since if b and v are linearly related and the relationship is independent of the mobile-phase composition and type of stationary phase we can describe retention in RPLC with only a single parameter, at least for the alkyl and phenyl phases.

Hence these and previous results from Cheong and Carr⁶³ together with the findings of Rosés and Bosch⁶⁴ that a single solvent parameter is sufficient to predict retention over a fairly wide range of solvent compositions are in agreement with the results presented in this work. Recently, Wang and Carr in a detailed study of a single octyl-bonded phase, carried out a so-called “global LSER”.⁶¹ They showed that a single LSER regression can be carried out to describe retention on a given stationary phase by assuming that all LSER coefficients are a linear function of ϕ_{org} . The results were tested in methanol–water, acetonitrile–water, and tetrahydrofuran–water mixtures.

II. PCA Studies. In previous work, Tan et al.³⁰ showed, using principal component factor analysis (PCA) results for 87 solutes on 5 different C_{18} phases in 50% acetonitrile–water, that 99.8% of the variance could be explained with a single solute-dependent factor (variable). We applied PCA methods to our rather different database as follows: we subtracted $\log K'$ for benzene as a reference compound from the other $\log K'$ values on each of the eight stationary phases. In this way, we remove the effect of the phase ratio. This also obviates the need for mean centering the data. The results obtained (see Table 6), in a fixed mobile-phase composition, show that nearly 99.8% of the variance can be explained by three factors when we consider all three types of stationary phases (aliphatic, aromatic, and fluorinated phases), by two factors when we omit the fluorinated phases, and by only one factor when we consider only the aliphatic phases. In each case, the average standard deviation considering three, two, or one factors for each set of stationary phases is less than 0.03. If we consider that the a and s coefficients vary only slightly among the different stationary phases and that there is almost no variation with mobile-phase composition, then intuitively we might assert that a single PCA factor should suffice to explain a very large fraction of the variance and it is possible that the main contributor to that factor is the b/v ratio.^{9,31} The r coefficient is different from zero for only three stationary phases and it changed slightly with mobile-phase composition. Thus, we hypothesize that these three PCA factors could be related to dominance by the aliphatic, aromatic, or perfluorinated nature of the bonded phase.

The fact that the average standard deviation in fitting the various phases is 0.03–0.07 points out that the LSER model is not fully adequate and we reach the same conclusion from the systematic patterns of residuals discussed above (see Figure 2).

In deciding on the completeness and accuracy of a model, it is obviously very important to have a good measure of the experimental imprecision of the data used to test the model or

(60) Valkó, K.; Snyder, L. R.; Glajch, J. L. *J. Chromatogr., A* **1993**, 656, 501.

(61) Wang, A.; Carr, P. W. *J. Chromatogr., A*, in press.

(62) Sadek, P. C.; Carr, P. W.; Doherty, R. M.; Kamlet, M. J.; Taft, R. W.; Abraham, M. H. *Anal. Chem.* **1985**, 57, 2971.

(63) Cheong, W. J.; Carr, P. W. *Anal. Chem.* **1988**, 60, 820.

(64) Rosés, M.; Bosch, E. *Anal. Chim. Acta* **1993**, 274, 147.

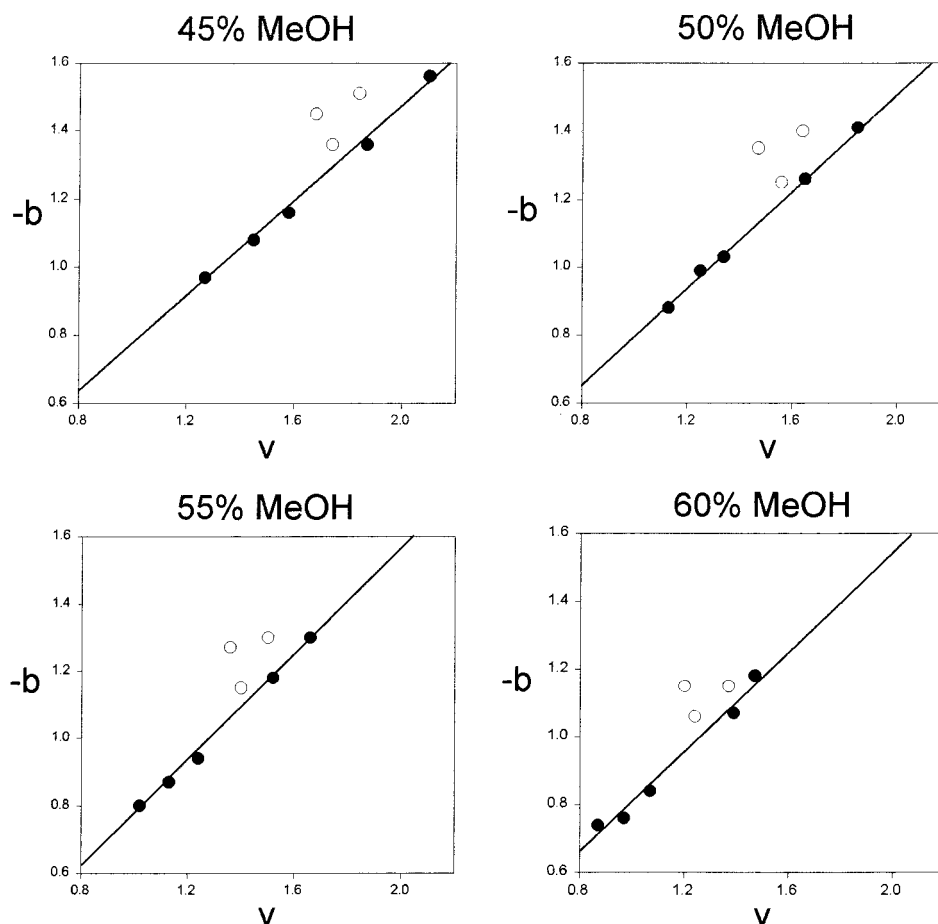


Figure 6. Plot of b vs v coefficient at a fixed mobile-phase composition as stationary phase is changed.

Table 6. PCA Result at the Different Studied Mobile-Phase Compositions

no. of PCA factors	stat phases considered	mobile-phase comp							
		45% MeOH		50% MeOH		55% MeOH		60% MeOH	
		% total variance	SD ^a	% total variance	SD ^a	% total variance	SD ^a	% total variance	SD ^a
3	all stat phases	99.86	0.020	99.84	0.019	99.78	0.02	99.75	0.019
2	aliphatic and aromatic	99.80	0.026	99.84	0.016	99.93	0.015	99.81	0.021
1	aliphatic phases	99.70	0.03	99.81	0.021	99.77	0.022	99.70	0.025

^a Average standard deviation of the fit.

differentiate between competing models. On the basis of the above results, it is evident that the PCA approach is more accurate than the LSER model since for all of the data sets and subsets examined in Table 6, the average standard deviations obtained by PCA were substantially smaller than those observed by LSER (Table 3). Our decision to stop at three principal components was somewhat arbitrary. In view of Table 6, it is clear that the experimental precision is better than 0.03.

We attempt to define the experimental precision of the overall fit in the following way. First, we note that the fits of $\log K$ vs $\log K$ on a given column using two different mobile phases are much better, in general, than fits of $\log K$ vs $\log K$ for two different stationary phases in a fixed mobile phase. Second, the quality of the fits deteriorate as the difference between mobile-phase compositions increases. This is due to real changes in relative retention in the chemically different mobile phases. Clearly, if the

same mobile phase and the same column were used, the fit in the absence of experimental imprecision should be perfect. Thus, we obtained an estimate of long-term precision by extrapolating the average residuals of the fits of $\log K$ vs $\log K$ to zero difference in mobile-phase composition. We found that the largest average standard deviation was only 0.013. The mean for all eight stationary phases was 0.005, and the median was 0.006. This is much smaller than what is observed with the PCA method and very much less than observed with the LSER method. We must conclude that the LSER approach is deficient to some extent and that more than three PCA factors are needed to fully explain variation among the different types of phases.

III. Selectivity Studies. If we choose nonpolar, non-HB solutes and compare their selectivity factors on alkyl and fluorinated phases, we can see from Table 7 that the selectivities are solute size dependent. Small solutes such as fluorobenzene and

Table 7. Selectivity Factors, α , for Two Pairs of Solutes Differing in Their Relative Size

	stationary phase			
	C ₁₀ H ₂₁	C ₅ H ₁₁	(CH ₂) ₂ C ₆ F ₁₃	(CH ₂) ₃ OC ₃ F ₇
fluorobenzene/ benzene	1.16	1.18	1.37	1.28
anthracene/ biphenyl	1.88	1.64	1.27	1.57

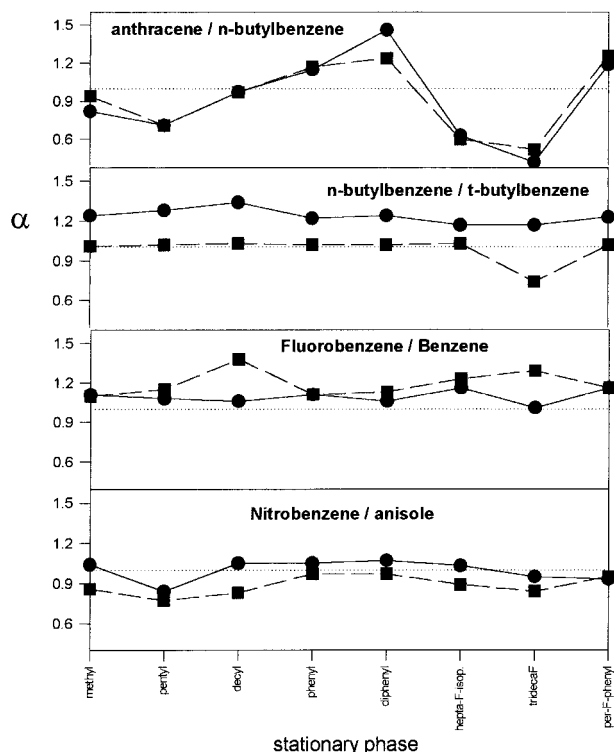


Figure 7. Selectivities of the different stationary phases for different carefully chosen solute pairs: (■) predicted values; (●) experimental values.

benzene show better selectivities on fluorocarbon phases than on hydrocarbonaceous phases, but larger solutes such as anthracene and biphenyl show better selectivities on hydrocarbonaceous phases. This behavior was observed before¹⁵ and was attributed to the differential solubility of small and big solutes in these types of phases. Larger solutes are more soluble in hydrocarbon phases because dispersive interactions are stronger. For small solutes, for which dispersive interactions are smaller, the effect of cohesive interactions predominates. The solubility parameters, δ_H , of small molecules are closer to those of the fluorocarbon phases than hydrocarbon phases. Billiet et al. also found that a (CH₂)₃C₃F₇ phase had a greater selectivity compared to C₁₈ bonded phase for esters, ketones, and fluoro-substituted solutes.⁶⁵

To evaluate the model given in eq 1 as a predictive tool, we have compared the chromatographic selectivity factors, α , for various pairs of solutes in 60% methanol with the predicted ones. The α values are shown in Figure 7. The first plot shows α values for anthracene relative to *n*-butylbenzene. This is an approximate measurement of the "aromatic selectivity" of the different columns.

(65) Billiet, H. A. H.; Schoenmakers, P. J.; de Galan, L. *J. Chromatogr.* **1981**, *218*, 443.

As expected, anthracene is much more strongly retained on the phenyl phase relative to *n*-butylbenzene, due to its higher aromatic character. In contrast, *n*-butylbenzene is more strongly retained than is anthracene on the fluoroalkyl and alkyl phases. For the 2(C₆H₅) phase we find that inclusion of the rR_2 term produces better predictions of chromatographic selectivity, but we must also note that adding this term improves both the regression coefficient and the average residual. The same is true for the (CH₂)₂C₆F₁₃ and (CH₂)₃OC₃F₇ phases except for k' (fluorobenzene)/ k' (benzene) in which the prediction without R_2 is better. The agreement between the predicted and experimental values is quite good, except for the 2(C₆H₅) phase. Thus, the LSER model is able to account for the degree of "aromatic selectivity" observed in practice.

"Shape selectivity" was evaluated using the retention of *n*-butylbenzene relative to *tert*-butylbenzene. The parameters for these two compounds are almost identical. The LSER method predicts that selectivity for this pair of solutes should be close to 1 as is shown in the second part of Figure 7. The prediction for the selectivity of the (CH₂)₂C₆F₁₃ phase is opposite to the observed retention sequence. This is due to the term rR_2 since the magnitude of r coefficient is important and the R_2 parameter for *tert*-butylbenzene is higher than for *n*-butylbenzene. As a consequence, dispersive interactions are predicted to be higher for *tert*-butylbenzene compared to *n*-butylbenzene. However, the linear isomer is considerably more retained on all of these stationary phases. This elution order is predicted by the solvophobic theory⁶⁶ since the molecular area of *n*-butylbenzene is higher than that of *tert*-butylbenzene. We conclude that a simple size parameter (*i.e.*, the solute molecular volume) is incapable of modeling this effect. We can see that the decyl column produces the highest selectivities and the fluorinated (CH₂)₂C₆F₁₃ and (CH₂)₃OC₃F₇ phases produce the lowest selectivities, which is in agreement with previous results since both solutes are quite small.^{15,16} The absence of any molecular shape term and the reliance of the LSER exclusively on molecular volume and not molecular surface area is a very real limitation of the approach. It is very well-known, both theoretically⁶⁷ and experimentally, that shape and molecular area^{68–70} are more important in establishing solubility in water and octanol–water partition coefficient than is the molecular volume.

The selectivity of fluorobenzene relative to benzene was also evaluated, and the results are shown in the third plot of Figure 7. The highest selectivities are obtained in the (CH₂)₃OC₃F₇ and C₆F₅ phases, and the lowest value is obtained in the (CH₂)₂C₆F₁₃ phase. The predicted values are quite good except for the C₁₀H₂₁ and (CH₂)₂C₆F₁₃ phases.

The selectivity factors for nitrobenzene relative to anisole were calculated (last curve of Figure 7) since the parameters for these compounds are very similar, except for the π_2^H parameter (1.11 for nitrobenzene and 0.52 for anisole) and a small difference in polarizabilities as indicated by the R_2 parameter. The predictions for this pair of compounds are not good and inversion in the

(66) Horváth, Cs.; Melander, W. R.; Molnár, I. *J. Chromatogr.* **1976**, *125*, 129.

(67) Vailaya, A.; Horváth, Cs. *J. Phys. Chem. B* **1997**, *101*, 5875.

(68) Harris, S. M. J.; Higuchi, T.; Rytting, J. H. *J. Phys. Chem.* **1973**, *77*, 22, 2694.

(69) Valvani, S. C.; S. H. Yalkowsky; Amidon, G. L. *J. Phys. Chem.* **1976**, *80*, 8, 829.

(70) Hermann, R. B. *J. Phys. Chem.* **1972**, *76*, 2754.

Table 8. Analysis of κ - κ Plots^a

phases	CH ₃			C ₅ H ₁₁			C ₁₀ H ₂₁			C ₆ H ₅		
	slope	SD	<i>r</i>	slope	SD	<i>r</i>	slope	SD	<i>r</i>	slope	SD	<i>r</i>
CH ₃				1.42	0.03	0.996	1.35	0.02	0.997	1.02	0.05	0.988
C ₅ H ₁₁	0.70	0.03	0.996				1.06	0.05	0.995	1.45	0.10	0.976
C ₁₀ H ₂₁	0.74	0.02	0.997	0.94	0.05	0.995				1.41	0.05	0.989
C ₆ H ₅	0.97	0.05	0.988	0.67	0.10	0.976	0.71	0.05	0.989			
2C ₆ H ₅	0.86	0.08	0.979	0.81	0.10	0.969	0.87	0.07	0.984	0.81	0.03	0.996
C ₆ F ₅	0.80	0.04	0.993	0.86	0.06	0.989	0.92	0.04	0.996	0.78	0.06	0.990
OC ₃ F ₇	0.81	0.05	0.986	0.88	0.09	0.983	0.89	0.08	0.98	0.76	0.08	0.970
C ₆ F ₁₃	0.76	0.08	0.973	0.93	0.12	0.968	1.00	0.12	0.963	0.71	0.10	0.951

phases	2C ₆ H ₅			C ₆ F ₅			(CH ₂) ₂ OC ₃ F ₇			(CH ₂) ₂ C ₆ F ₁₃		
	slope	SD	<i>r</i>	slope	SD	<i>r</i>	slope	SD	<i>r</i>	slope	SD	<i>r</i>
CH ₃	1.16	0.08	0.979	1.25	0.04	0.993	1.23	0.05	0.986	1.31	0.08	0.973
C ₅ H ₁₁	1.23	0.10	0.969	1.16	0.06	0.989	1.13	0.09	0.983	1.07	0.12	0.968
C ₁₀ H ₂₁	1.14	0.07	0.984	1.08	0.04	0.996	1.12	0.08	0.98	1.00	0.12	0.963
C ₆ H ₅	1.23	0.03	0.996	1.28	0.06	0.99	1.31	0.08	0.97	1.41	0.10	0.951
2C ₆ H ₅				1.06	0.06	0.987	1.07	0.12	0.954	1.16	0.15	0.929
C ₆ F ₅	0.94	0.06	0.987				1.00	0.08	0.979	1.08	0.12	0.957
OC ₃ F ₇	0.93	0.12	0.954	0.996	0.08	0.979				1.06	0.06	0.987
C ₆ F ₁₃	0.86	0.15	0.929	0.925	0.12	0.957	0.94	0.06	0.987			

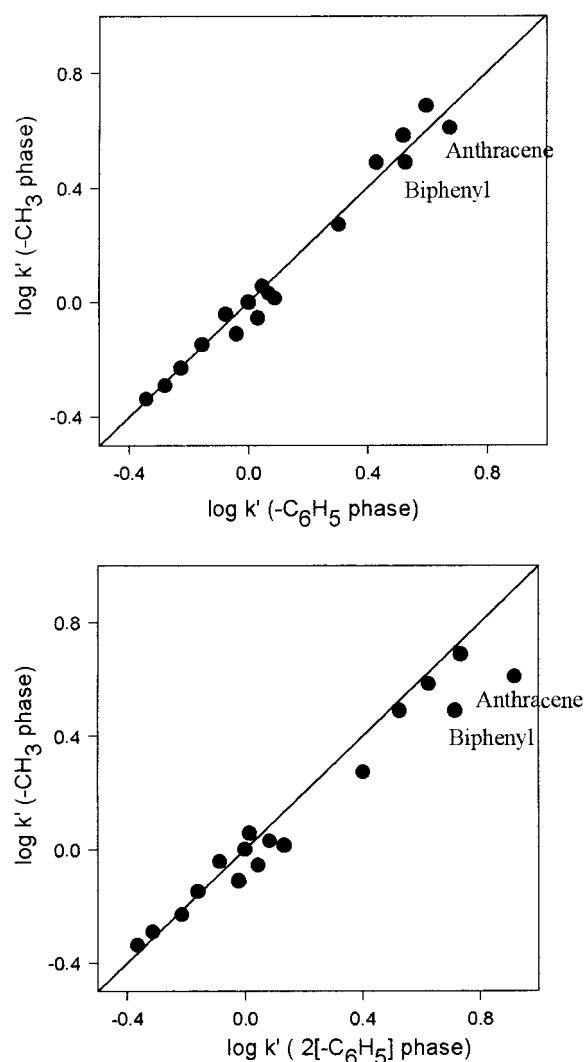
^a Based on retention data in 55% MeOH–water.

retention orders are frequently predicted. The agreement between predicted and observed selectivity is good only for the C₆F₅ phase. Silanol interactions and differences in bonded phase coverage and, as a consequence, in the number of silanol groups plus the inability of the model in dissecting dispersion, dipolar induction, and cavity effects are the reasons for the failure in predicting the selectivities for these compounds.

IV. Energetics of Retention (κ - κ Plots). Plots of $\log K$ vs $\log K$ for two phases as the solute is varied (so-called κ - κ plots)⁷¹ can be used to compare the energetics of retention. A linear correlation with unit slope indicates a *homoeenergetic* retention process.⁷¹ This means that the two phases have identical intrinsic thermodynamic behavior. Good linear correlation with a slope other than unity suggests that the physicochemical bases for retention on the two phases are similar. This is termed *homeoenergetic* retention. If correlation is poor, this means that the two columns are *heteroenergetic* and have different retention mechanisms. We will consider here that a standard error lower than 0.08 is very good and smaller than 0.08 is relatively poor. Taking this into consideration, from Table 8, we observe some differences in the retention behavior.

As expected, all alkyl phases show similar retention behavior. However, while the C₅H₁₁ and C₁₀H₂₁ phases show homoeenergetic behavior, the CH₃ phase shows slopes quite different from 1, so the retention process in this phase compared with the other alkyl phases is similar but not identical.^{59,72} As we observed before, the main differences between the CH₃ phase and the other alkyl phases are the *b* and *v* coefficients.

The two aromatic nonfluorinated phases show similar retention behavior. We see from Figure 8 for the CH₃ phase that the highly aromatic solutes anthracene and biphenyl are more retained in

Figure 8. κ - κ plots. Comparison between the retention behavior of aromatic phases and CH₃ phase.

- (71) Melander, W.; Stoveken, J.; Horváth, Cs. *J. Chromatogr.* **1980**, *199*, 35.
 (72) Park, J. H.; Lee, Y. K.; Weon, Y. C.; Tan, L. C.; Li, J. W.; Li, L.; Evans, J. F.; Carr, P., W. *J. Chromatogr. A* **1997**, *767*, 1.
 (73) Marcus, Y. *Chem. Soc. Rev.* **1993**, 409.

the aromatic phases. The magnitude of the deviation is bigger for the more highly aromatic 2(C₆H₅) phase. This preferential sorption of the highly aromatic solutes into these aromatic phases is presumably due to π - π interactions.

The fluorinated nonaromatic phases, C₆F₁₃ and OC₃F₇, show homoenergetic retention behavior. These phases have similar b/v ratios. On the other hand, the regression coefficients and standard deviations with the other phases are poor. This means that the fundamental molecular interactions involved in the retention mechanism are different. However, the C₆F₅ phase is more similar to the aromatic phases than to the fluorinated phases. Actually, the correlation coefficients between the C₆F₅ phase and the aromatic phases are strong and with the fluoroalkyl phases they are less strong. Surprisingly, the correlation is still good between this aromatic fluorinated phase and the alkyl phases and the slope is close to 1 with the C₁₀H₂₁ phase. These conclusions are in agreement with the LSER results (b vs v plots), since the C₆F₅ phase is seen to be more similar to the alkyl and phenyl phases than to the other fluorinated phases.

CONCLUSIONS

Once again LSERs are shown to be a powerful approach to the characterization of the chemical interactions present in different RPLC stationary phases. The statistical measures of the goodness of fit are very good for all stationary-/mobile-phase systems studied. The most important retention factors governing solute-dependent parameters are the solute size and hydrogen bond acceptor basicity. These two factors change significantly with mobile-phase composition whereas the other factors (hydrogen bond basicity and polarity/polarizability) are almost independent of the mobile-phase volume fraction. The b coefficient changes linearly with v upon change in both mobile-phase composition and stationary phase, except for the fluoroalkyl phases, which show a different behavior. This is an important finding since it could reduce the number of factors needed to explain retention data, at least in alkyl and aromatic stationary phases.

However, selectivity predictions based on the LSER are quite good for some pairs of solutes in some stationary phases but very bad for some others. Predictions based on differences in solute shapes are very bad. This means that *the V_2 solute parameter does not completely account for both cohesive and dispersive interactions.*

This is an important limitation of the LSER approach. Comparisons of average standard deviations between PCA and LSER studies indicate that the PCA approach is more accurate. However, three PCA factors are still not enough to explain all data in the three classes of stationary phases.

The higher v coefficient for pentyl and decyl phases as compared to the highly fluorinated phases can be explained by weaker dispersive interactions of the solute with the fluorinated phases. On the other hand, the small v coefficient for aromatic phases as compared to the other phases can be explained by the higher sorption of the protic solvents on the HBA rings of the aromatic phases.

The LSER model was able to account for the different polarizabilities of the stationary phases. Fluorinated and aromatic stationary phases show a small dependence on solute polarizability (R_2). This is attributed to the very different polarizabilities of the mobile-phase and stationary-phase components. The highly polarizable diphenyl phase showed a positive r coefficient whereas fluorinated phases (CH₂)₂C₆F₁₃ and (CH₂)₃OC₃F₇ appeared to be less polarizable than the mobile phase since the r coefficient is negative. This is due to the inductive effect of the fluorine atoms. The near-zero value for the r coefficient of the alkyl phases shows that stationary- and mobile-phase polarizabilities are similar. However, the s coefficient for these phases is negative, meaning that the dipolarity/polarizability is lower than that of the mobile phase, as expected, despite the fact that these phases are solvated by the polar components of the mobile phase. On the other hand, aromatic phases C₆H₅ and C₆F₅ show no dependence on solute R_2 or π_2^H . This indicates that polarity/polarizability effects between the stationary and mobile phases cancel out.

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