ELSEVIER

Journal of Chromatography A, 799 (1998) 1–19

JOURNAL OF CHROMATOGRAPHY A

Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships II. The mobile phase

Lay Choo Tan[a,*], Peter W. Carr[b]

[a]*Novartis Pharmaceuticals Corporation, 556 Morris Avenue, Summit, NJ 07901, USA*

[b]*Department of Chemistry, Smith and Kolthoff Hall, University of Minnesota, 207 Pleasant Street S.E., Minneapolis, MN 55455, USA*

Received 13 February 1997; received in revised form 13 October 1997; accepted 15 October 1997

Abstract

The applicability of linear solvation energy relationships (LSERs) to reversed-phase liquid chromatography (RPLC) was studied by examining the retention of a wide variety of aliphatic and aromatic compounds over the range of 20–50% (v/v) acetonitrile, methanol and tetrahydrofuran. The role of cavity formation, dispersion interaction, polarity/polarizability, hydrogen bond acidity, and hydrogen bond basicity in determining the retention behavior as the mobile phase composition was changed has been investigated. The LSER coefficients were then examined in terms of the corresponding properties of the mobile phase (cohesive energy density, surface tension, the Abraham solvophobic parameter, polarity/polarizability, hydrogen bond basicity, and hydrogen bond acidity) and from these the influence of mobile phase and stationary phase on the retention behavior was explored. In order to chemically interpret the RPLC retention results we compared them to alkane–water and octanol–water partition coefficients. © 1998 Elsevier Science B.V.

Keywords: Linear solvation energy relationships; Mobile phase composition; Retention mechanism; Stationary phases, LC

1. Introduction

At a molecular level, solute retention in RPLC involves the following microscopic processes: (i) the creation of an appropriately shaped solute-sized cavity in the stationary phase, (ii) the transfer of a solute molecule from the mobile phase to the pre-formed cavity in the stationary phase, and (iii) the closing of a solute-sized cavity in the mobile phase [1]. Step (ii) corresponds to 'turning-off' the attractive interactions between the mobile phase and the solute and 'turning-on' the attractive interaction between the stationary phase and the solute. It follows that both the mobile phase and the stationary phase must be considered in studies of the retention mechanism in RPLC. However, many of the early theories of RPLC retention were greatly influenced by Horváth's solvophobic model [2] which focuses almost exclusively on step (iii). The solvophobic model argues that retention of a nonpolar solute is largely controlled by the free energy liberated by the formation of water–water contacts upon removal of the nonpolar solute [2].

Based on the use of linear solvation energy relationships (LSERs), we investigate the retention process as being due to the sum of the *differential* interactions of a solute with the mobile phase and with the stationary phase. These include the cavity

*Corresponding author

0021-9673/98/$19.00
PII S0021-9673(97)01054-6

formation, dipolarity/polarizability, hydrogen bond donor acidity, and hydrogen bond acceptor basicity processes. We examine how each of these interactions is affected as the mobile phase varies in composition. In this paper, we present studies done with the three most commonly used mobile phase modifiers: acetonitrile (ACN), methanol (MeOH) and tetrahydrofuran (THF), on a widely used type of alkyl bonded phase. In the previous paper in this series we used a fixed mobile phase and examined a variety of different bonded phases [3].

1.1. Mobile phase properties

We will discuss the mobile phase properties that correspond to each of the interactions; cavity formation, dipolarity/polarizability, hydrogen bond donor acidity, and hydrogen bond acceptor basicity processes. The most appropriate solvent property that controls cavity creation is not very obvious. Among the many macroscopic physical properties that have been investigated are the surface tension, the cohesive energy density, and Abraham's solvophobic scale. The surface tension (γ) of a liquid is a direct two-dimensional measure of the intermolecular forces [4]. It was used as the chief mobile phase parameter in Horváth's solvophobic theory [2]. The solvent cohesivity, or cohesive energy density is measured as the square of the Hildebrand solubility parameter (δ_H^2). The solvent solvophobic scale (S_p) of Abraham et al. is based on the Gibbs free energies of transfer of inert solutes from water to the solvent of interest [5]. The S_p values increase nonlinearly with volume fraction of organic (φ_O) of the solvent of interest. Since retention in RPLC is a composite factor and thus $\log k'$ does not vary linearly with φ_O, S_p must also be a composite solvent characteristic, represented by a combination of the dipolarity/polarizability and HBD acidity of the hydro-organic mixtures [6]. The correlation between S_p and surface tension (γ) of aqueous organic mixtures is poor and highly nonlinear.

The solvatochromic property that represents a solvent's ability to interact with a solute's dipolarity/polarizability is its Kamlet–Taft dipolarity/polarizability (π^*). Based on solvatochromic measurements, the π^* values of aqueous mixtures of methanol, acetonitrile and tetrahydrofuran increase monotonically with the volume fraction of water (φ_W) [7–9]. The relationship is non-linear and the measured π^* values deviate from those predicted by random mixing. The variation of π^* with organic composition is likely due to changes in the dielectric properties of the local medium, the so-called cybotactic region, experienced by the indicator [7].

A solvent's hydrogen bond donor (HBD) strength; denoted α, corresponds to its ability to share (donate) an active hydrogen atom with a hydrogen bond acceptor (base) solute. The α values of hydro–organic mixtures can be derived from $E_T(30)$ and $E_T(33)$, two solvatochromic solvent strength parameters based on betaine dyes [7,10–13]. For aqueous mixtures of acetonitrile and tetrahydrofuran, which are poor HBD acids, α values increase very rapidly as the first small amount of water is added to the neat solvent. Thereafter the α values approach a plateau, and subsequently rise slowly and monotonically to the HB acidity value of pure water. The trend in α values of aqueous methanol mixtures is more complicated. The α values first decrease with increasing water concentration, reaching a minimum and then increase to the α value of pure water [7]. The α values of hydro–organic mixtures can also be estimated using a neutral indicator. Using an uncharged Fe(II) complex as the α indicator, Park et al. [6] found that trends in the α values for aqueous mixtures of acetonitrile and tetrahydrofuran to be very similar to those obtained based on $E_T(30)$ or $E_T(33)$. However, with this indicator the trend for aqueous mixtures of methanol was different from those observed with the betaines. No minimum in α was observed, instead, the α values simply increased monotonically as φ_W was increased.

A solvent's hydrogen bond acceptor (HBA) strength; denoted β, corresponds to its ability to accept an active hydrogen atom from a hydrogen bond donor (acid) solute. β(OH) and β(NH) are two hydrogen bond basicity scales based on indicators with –OH and –NH donor groups, respectively [8,14–16]. Generally, for aqueous mixtures of acetonitrile and tetrahydrofuran, both β(OH) and β(NH) show a broad and flat maximum. According to Dallas, these maxima are due to specific solvation of the solvatochromic indicators by the organic solvent [14]. Aqueous mixtures of methanol do not exhibit maxima for either of these basicity scales.

The β values increase in a monotonic but nonlinear fashion with φ_W over the entire range of composition [14].

A general comment based on the above analysis is that hydro–organic mixtures are very complex. The chemical composition is microscopically inhomogeneous and various chemical properties of a hydro–organic mixture do not vary in a linear and simple relationship with chemical composition. Preferential solvation and microheterogeneity phenomena in hydro–organic mixtures have been widely reported [8,17–21]. Hydro–organic mixtures typically contain extended hydrogen bonding structures resulting from networks of linear and/or cyclic hydrogen bonded regions [22]. Self-association of solvent molecules, especially in water–alcohol mixtures, have a substantial influence on the bulk properties of the mixtures.

1.2. Bonded phase properties

In RPLC, these complex mobile phase mixtures are sorbed into the bonded phase [23–28] and significantly modify the chemical nature of the stationary phase. Enrichment is induced by the dispersive interactions of sorbed components with the bonded alkyl chains, as well as the dipolar and hydrogen bonding interactions with residual silanol groups [26,27]. The dispersive interactions increase as: THF>ACN>MeOH, which is the reversed order of the cohesive energy density values of these solvents. The HBD strength of the solvents decrease in the order: MeOH≫ACN≈THF. The HBA strengths of the solvents follow the order: MeOH> THF>ACN.

The following discussion focuses on the mobile phase composition range from 20 to 50% (v/v) organic, where our RPLC studies were carried out. Within this composition range, the volume fraction of organic modifier sorbed into bonded phases increase in the order: THF>ACN>MeOH [24–27]. The sorption of organic modifier *on* or *into* the bonded phase monotonically increases as the concentration of modifier in the mobile phase is increased [23,24,27]. Water, being a strong hydrogen bond donor and acceptor, is sorbed into the bonded phase by hydrogen bonding with the sorbed organic modifier and residual silanol groups [28,29]. At a particular modifier volume fraction, the amount of water sorbed is highest in systems using THF, followed by MeOH and is least with ACN. However, the sorption pattern of water as φ_O is varied is more complicated. In the range of 20–50% organic modifier, sorption of water into the bonded phase increases with organic composition in ACN and MeOH mobile phases. For THF mixtures, the sorption of water reaches a maximum at around 40% (v/v) THF and then decreases with further increases in the organic composition in the mobile phase. All sorption studies suggest that the formation of the stationary phase is a dynamic process under the control of the mobile phase composition [23,24,26,27,30]. The stationary phase should be viewed as a quaternary mixture of bonded organic moiety, sorbed water and organic modifier and residual silanol groups.

Despite their chemical similarity, alkyl bonded phases undergo greater and more complex sorption processes than does bulk liquid hexadecane when equilibrated with the same mobile phase [14]. Sorption onto the bonded phases is complicated by hydrogen bond interactions with residual silanol groups, the much lower density of bonded phases relative to a bulk liquid alkane, that is, the greater spacing between neighbors (nearly a factor of 2), and the inherent ordering of the bonded chains [1,31,32]. In addition, the distribution of the sorbed solvent molecules in the bonded phase is highly inhomogeneous. Several studies suggest that most of the sorbed molecules reside at the mobile phase-stationary phase interface, especially in highly polar mobile phases [28,31,32].

A growing body of spectroscopic and thermodynamic data suggests that solvated bonded phases have much higher dipolarity and hydrogen bonding ability than do bulk alkanes [14,33–39], or even bulk hexadecane saturated with organic aqueous mixtures [14]. The polarity of an octadecyl phase in contact with water is as high as that of bulk octanol [33]. Fluorescence studies show that the polarity of the bonded phase depends greatly on the type and concentration of organic modifier in the mobile phase [37]. Carr and Harris found that the effective polarity of a C_{18} phase decreases with increasing organic concentration in the mobile phase over the range of 0–50% methanol, but then increases with

the organic concentration over the range of 50–80% methanol [34,35]. On the other hand, the bonded phase polarity increases non-linearly with organic concentration over the range of 20–70% acetonitrile and 25–45% tetrahydrofuran. With fluorescence probes of higher polarity, Men and Marshall found that the bonded phase polarity remained virtually constant over the range of 50–80% methanol [36]. Based on an independent study using Kamlet–Taft π^* probes, Hu and Rutan [9] observed polarity trends in the bonded phase similar to those reported by Carr and Harris [34,35].

The discrepancies between the patterns observed with different polarity probes is a clear sign of the heterogeneity of the stationary phase. There are some local environments which are more polar than others whose polarity varies differently over the same mobile phase compositions. Depending on their chemical characteristics, the probes reside in different bonded phase environments and give different polarity results. These differences in microenvironment were reported by Hu and Rutan in their studies of the α and β values of solvated bonded phase [9]. Measurements of π^*, α or β depend not only on the type and amount of sorbed modifier, but the amount of sorbed water, which in turn is controlled by the residual silanol groups [26,27].

1.3. Method

Based on the use of linear solvation energy relationships, we studied retention in RPLC using the following linear multivariable regression equation:

$$\log k' = \log k'_o + \mathbf{m}V_x + \mathbf{s}\pi_2^{*H} + \mathbf{a}\Sigma\alpha_2^H + \mathbf{b}\Sigma\beta_2^H \quad (1)$$

where V_x is a molecular volume calculated using McGowan's method [40,41], while π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are the solute's dipolarity/polarizability, hydrogen bond acidity and hydrogen bond basicity, respectively. The subscript 2 denotes a solute property. The parameters π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ were generally obtained from gas chromatographic measurements by Abraham et al. [42]. The intercept, $\log k'_o$, and the fitting coefficients **m**, **s**, **a** and **b** are characteristic of the pair of mobile and stationary phases and are obtained from multivariable simultaneous least-squares regressions [43]. Each coefficient reflects the *difference* in the same property of the mobile phase and the stationary phase:

$$\log k' = \log \mathbf{k}'_o + \mathbf{M}(\nu_s - \nu_m)V_x + \mathbf{S}(\pi_s^* - \pi_m^*)\pi_2^{*H} + \mathbf{A}(\beta_s - \beta_m)\Sigma\alpha_2^H + \mathbf{B}(\alpha_s - \alpha_m)\Sigma\beta_2^H \quad (2)$$

where the subscripts s and m denote the bulk stationary and mobile phase properties, respectively. The coefficients **M**, **S**, **A** and **B** are fitting parameters which ought to be independent of the solute and the chromatographic phases if the formalism were rigorously correct [44,45].

In preliminary studies, we tested the correlation with a variety of different solute parameters, such as the molar volume, the Bondi volume [46], the Leahy volume [47], with the π_2^*, α_2 and β_2 scales of Kamlet and co-workers [48–56], and Li and co-workers [45,57], and the monomer solute HB acidity and basicity scales of Abraham et al. [58,59]. Overall, the LSER regressions using the alternative parameters gave similar results, but with decidedly poorer fits. V_x, π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ were used for this work for three reasons. First, this parameter combination gave the best regression fit. *The fits are as good as those obtained in the gas chromatography experiments from which the parameters were derived* [42]. Second, these parameters have been successfully applied to the correlation of many other physical properties such as retention in various gas–liquid chromatographic stationary phases [42,60–65], toxicity of gases and vapors, water solubility of gaseous solutes, gas–liquid partition coefficients and octanol–water partition coefficients [66]. Third, unlike many of the original Kamlet–Taft solute parameters which were back-calculated from RPLC data or subjected to parameter estimation rules [48,49,56], π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ were derived from gas chromatographic measurements, *totally independent of the reversed-phase system which is being examined*. Thus, the regression result is chemically valid and non-biased.

Table 1 lists the 73 test solutes studied here along with their corresponding V_x, π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ values. The test solutes include both aliphatic and aromatic alcohols, aldehydes, amides, esters, ethers, ketones, nitriles, nitro and halogenated compounds, and alkylbenzenes, phenols and polyaromatic hydro-

Table 1
Test solutes and solute parameters[a]

Solute	V_x	π_2^{*H}	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$
1 Diethyl ether	0.7309	0.25	0	0.45
2 Acetonitrile	0.4042	0.90	0.07	0.32
3 2-Propanol	0.5900	0.36	0.33	0.56
4 Methanol	0.3082	0.44	0.43	0.47
5 1-Butanol	0.7309	0.42	0.37	0.48
6 Cyclohexanol	0.9041	0.54	0.32	0.57
7 Acetone	0.5470	0.70	0.04	0.49
8 2-Butanone	0.6879	0.70	0	0.51
9 Cyclopentanone	0.7202	0.86	0	0.52
10 2-Hexanone	0.9697	0.68	0	0.51
11 *n*-Propyl formate	0.7466	0.63	0	0.38
12 *n*-Butyl acetate	1.0284	0.60	0	0.45
13 Ethyl propionate	0.8875	0.58	0	0.45
14 Ethyl butyrate	1.0284	0.58	0	0.45
15 *n*-Propionitrile	0.5451	0.90	0.02	0.36
16 *n*-Nitropropane	0.7055	0.95	0	0.31
17 *n*-Valeronitrile	0.8269	0.90	0	0.36
18 Butyraldehyde	0.6879	0.65	0	0.45
19 2,2,2-Trifluoroethanol	0.5022	0.60	0.57	0.25
20 Methylene chloride	0.4943	0.57	0.10	0.05
21 Chloroform	0.6167	0.49	0.15	0.02
22 Dibromomethane	0.5995	0.67	0.10	0.10
23 *N*,*N*-Dimethylformamide	0.6468	1.31	0	0.74
24 *N*,*N*-Diethylformamide	0.9286	1.25	0	0.76
25 Dimethyl sulfoxide	0.6126	1.74	0	0.89
26 *N*,*N*-Dimethylacetamide	0.7877	1.33	0	0.78
27 *N*,*N*-Diethylacetamide	1.0695	1.30	0	0.78
28 Dioxane	0.6810	0.75	0	0.64
29 Benzene	0.7164	0.52	0	0.14
30 Toluene	0.8573	0.52	0	0.14
31 Benzaldehyde	0.8730	1.00	0	0.39
32 Acetophenone	1.0139	1.01	0	0.48
33 Propiophenone	1.1548	0.95	0	0.51
34 Benzonitrile	0.8711	1.11	0	0.33
35 *m*-Tolunitrile	1.0120	1.10	0	0.34
36 Nitrobenzene	0.8906	1.11	0	0.28
37 *m*-Nitrotoluene	1.0315	1.10	0	0.25
38 Anisole	0.9160	0.75	0	0.29
39 Methyl benzoate	1.0726	0.85	0	0.46
40 Ethyl benzoate	1.2135	0.85	0	0.46
41 Phenol	0.7751	0.89	0.60	0.30
42 *m*-Cresol	0.9160	0.88	0.57	0.34
43 Benzylalcohol	0.9160	0.87	0.33	0.56
44 2-Phenylethanol	1.0569	0.91	0.30	0.64
45 3-Phenylpropanol	1.1978	0.90	0.30	0.67
46 *N*-Benzylformamide	1.1137	1.80	0.40	0.63
47 Methyl phenyl sulfoxide	1.0795	1.58	0	0.92
48 Fluorobenzene	0.7341	0.57	0	0.10
49 Chlorobenzene	0.8388	0.65	0	0.07
50 Ethylbenzene	0.9982	0.51	0	0.15
51 *n*-Propylbenzene	1.1391	0.50	0	0.15
52 *p*-Xylene	0.9982	0.52	0	0.16
53 Mesitylene	1.1391	0.52	0	0.19
54 Bromobenzene	0.8914	0.73	0	0.09
55 Iodobenzene	0.9746	0.82	0	0.12
56 *n*-Butylbenzene	1.2800	0.51	0	0.15
57 *tert*-Butylbenzene	1.2800	0.49	0	0.16
58 Biphenyl	1.3242	0.99	0	0.22
59 Naphthalene	1.0854	0.92	0	0.20
60 Anthracene	1.4544	1.34	0	0.26
61 Benzophenone	1.4808	1.50	0	0.50
62 Benzyl cyanide	1.0120	1.15	0	0.45
63 Benzyl bromide	1.0323	0.98	0	0.20
64 *p*-Nitrobenzyl bromide	1.2065	1.50	0	0.40
65 *p*-Nitrobenzyl chloride	1.1539	1.34	0	0.40
66 *o*-Nitrotoluene	1.0315	1.11	0	0.27
67 *p*-Nitrotoluene	1.0315	1.11	0	0.28
68 *p*-Nitrophenol	0.9493	1.72	0.82	0.26
69 *p*-Cresol	0.9160	0.87	0.57	0.31
70 *o*-Cresol	0.9160	0.86	0.52	0.30
71 *p*-Ethylphenol	1.0569	0.90	0.55	0.36
72 *p*-Chlorophenol	0.8975	1.08	0.67	0.20
73 *p*-Chlorotoluene	0.9797	0.67	0	0.07
74 *p*-Bromotoluene	1.0323	0.74	0	0.09
75 *p*-Dichlorobenzene	0.9612	0.75	0	0.02

[a]Values of V_x were obtained from Refs. [40,41], while values of π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ were obtained from Ref. [42].

carbons. These solutes were judiciously chosen to span as wide a range as possible in the various solute characteristics, and cover both aliphatic and aromatic subsets. In addition, we attempted to obtain data at as high a volume fraction of water as possible. Most of the previous studies were carried out in mobile phases of high organic composition. However, we avoided studies at mobile phases with less than 10% (v/v) organic because such mobile phases may cause the collapse of bonded alkyl chains and limit pore accessibility that results in a different retention behavior [67,68]. Preliminary investigations reported in our previous paper [3] showed that the solute variables used here do not show strong covariance. The high degree of orthogonality among the solute parameters is desirable to produce reliable results in multiple regression analysis.

2. Experimental

The liquid chromatographic measurements were collected at four volume–volume ratios for each organic modifier (20, 30, 40 and 50% acetonitrile,

methanol and tetrahydrofuran). All the measurements were made at 25.0±0.1°C. The reported capacity factors were averages of at least triplicate determinations. The void-volume of the system was taken as the peak produced by ^2H_2O. All measurements were made with a Hewlett-Packard 1090 liquid chromatograph. Two detectors were used. The ultra-violet detector built into the HP 1090 was used at a wavelength of 254 nm to detect aromatic compounds; while an external refractive index detector (HP 1047A) was used to detect aliphatic compounds. Retention times were taken at the peak maximum reported by a Hewlett-Packard 9153 data system. HPLC-grade solvents were used for the preparation of the mobile phase: distilled water from Baker Analyzed (Phillipsburg, NJ, USA), acetonitrile and methanol from EM Science (Cherry Hill, NJ, USA), and tetrahydrofuran from Fisher Scientific (Fairlawn, NJ, USA). All test solutes were obtained commercially. Most samples were prepared in the mobile phase under study. For a few solutes which were not sufficiently soluble, the organic solvent used to modify mobile phase was added to dissolve the solute to a concentration that could be detected.

Zorbax-C_8 (DuPont; particle size, 5 μm; pore size, 100 Å) was used throughout the study. Columns of different dimensions (5 cm×2.1 mm I.D., 5 cm×4.6 mm I.D., 7.5 cm×4.6 mm I.D. and 15 cm×4.6 mm I.D.) were packed from the same lot of packing material in order to accommodate the very wide range in k' values encountered with this highly variegated set of solutes and mobile phase compositions. Columns were packed with a pressurized upward-slurry technique with 2-propanol as the packing solvent at 4500 p.s.i. packing pressure. They were flushed with pure organic modifier and then brought to the analytical mobile phase composition via a gradient. A very shallow gradient was used to achieve the final mobile phase composition so as to ensure complete equilibration of the mobile and stationary phases. Typically, a column was flushed with 50 column volumes of mobile phase per each percentage change in composition from pure modifier to the analytical composition. Retention measurements were made at a flow-rate that generated a back pressure of no more than 80 bar. Due to the different sizes of columns used here the back pressure of each column was different.

3. Results and discussion

The regression results for $\log k'$ values as per Eq. (1) are given numerically in Table 2 and are shown graphically in Figs. 1–3 (the error bars indicate the 95% confidence intervals). Overall, the LSER equations for the modifiers ACN and MeOH give excellent goodness-of-fit statistics at all mobile phase compositions studied. The average residuals are in the range of 0.05–0.09 and the correlation coefficients are always better than 0.99. These results are as good as those obtained previously in our study of five different RP stationary phases at a single mobile phase composition [3], as well as in other studies of

Table 2
Coefficients of LSER equations for all compounds

	S_{po}	**m**	**s**	**a**	**b**	n^a	$\overline{sd}^a$	r^a
50% ACN	−0.28±0.03	1.47±0.03	−0.25±0.03	−0.41±0.04	−1.71±0.04	71	0.056	0.9946
40% ACN	−0.24±0.04	1.84±0.04	−0.27±0.03	−0.42±0.04	−2.09±0.04	71	0.062	0.9956
30% ACN	−0.27±0.04	2.35±0.04	−0.25±0.03	−0.47±0.04	−2.50±0.05	68	0.067	0.9961
20% ACN	−0.29±0.05	2.77±0.05	−0.23±0.04	−0.43±0.05	−2.68±0.05	57	0.073	0.9951
50% MeOH	−0.66±0.04	2.38±0.04	−0.47±0.03	−0.27±0.05	−1.77±0.05	71	0.073	0.9947
40% MeOH	−0.54±0.06	2.73±0.06	−0.54±0.05	−0.28±0.06	−1.93±0.07	64	0.095	0.9907
30% MeOH	−0.58±0.06	3.08±0.06	−0.50±0.04	−0.29±0.06	−1.98±0.07	55	0.086	0.9924
20% MeOH	−0.67±0.06	3.37±0.07	−0.43±0.05	−0.32±0.06	−1.94±0.08	48	0.089	0.9916
50% THF	−0.34±0.04	1.16±0.04	−0.19±0.04	−0.07±0.04	−1.50±0.05	63	0.065	0.9860
40% THF	−0.28±0.05	1.53±0.05	−0.17±0.05	0.04±0.06	−1.96±0.06	63	0.083	0.9864
30% THF	−0.25±0.05	2.13±0.05	−0.22±0.05	0.22±0.05	−2.64±0.06	61	0.080	0.9925
20% THF	−0.31±0.07	2.81±0.09	−0.23±0.08	0.32±0.08	−3.35±0.10	50	0.114	0.9877

[a] n is the number of test solutes, $\overline{sd}$ and r are the average residual and correlation coefficient of the fit, respectively.

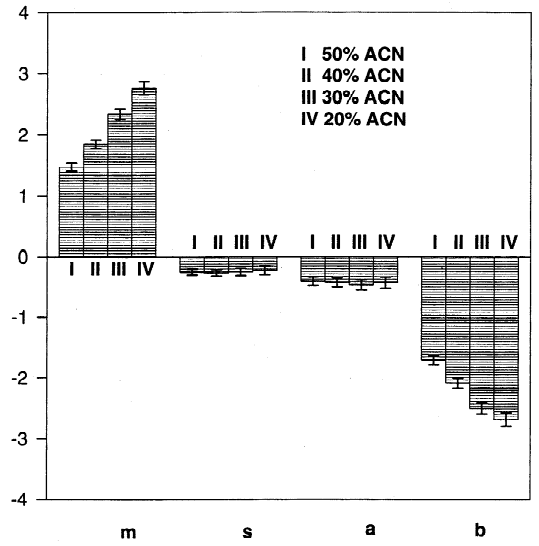

Fig. 1. Plots of LSER fitting coefficients (**m**, **s**, **a** and **b**) vs. volume fraction of water in mobile phase using acetonitrile as the organic modifier.

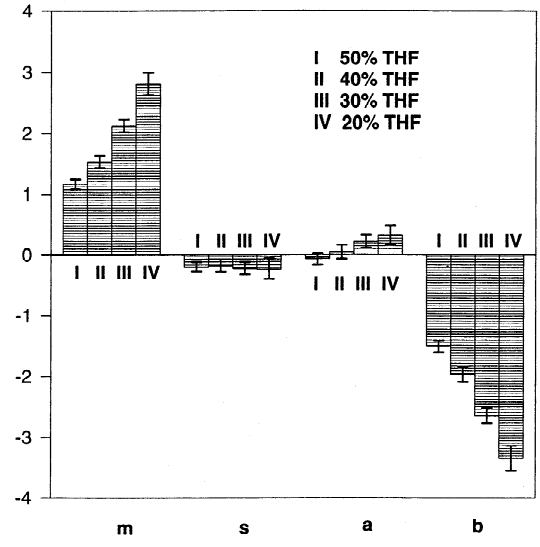

Fig. 3. Plots of LSER fitting coefficients (**m**, **s**, **a** and **b**) vs. volume fraction of water in mobile phase using tetrahydrofuran as the organic modifier.

bulk phase partitioning processes [49,69–73]. The goodness-of-fit for the LSER-RPLC equations for THF are slightly poorer in terms of their correlation coefficients. The average residual for the equations are still quite satisfactory except in the case of THF–water (20:80, v/v) ($\overline{sd} = 0.11$). Overall, the regression fits are quite satisfactory and support the efficacy of LSERs in modeling the retention behavior of RPLC using different organic modifiers. The results also suggest that the same set of solute solvatochromic parameters (π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$) can be used to model both gas-to-liquid and liquid-to-bonded phase transport processes [42].

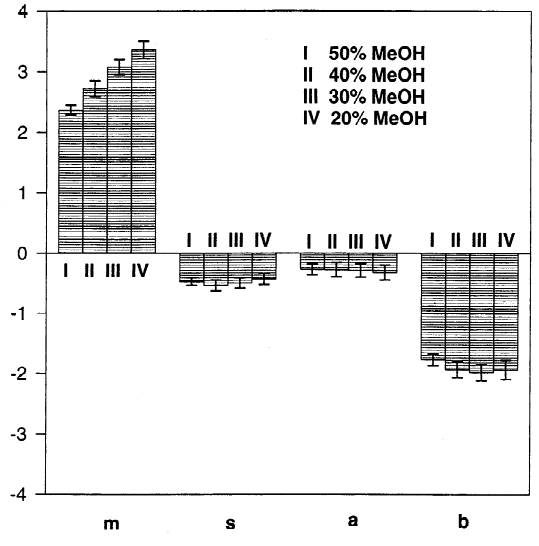

Fig. 2. Plots of LSER fitting coefficients (**m**, **s**, **a** and **b**) vs. volume fraction of water in mobile phase using methanol as the organic modifier.

To demonstrate the quality of the fits, the calculated log k' values are graphically compared with the experimental values. Fig. 4(a–c) shows plots of the experimental vs. calculated log k' values, with the aliphatic compounds contrasted with the aromatic compounds, at mobile phases, using ACN, MeOH, and THF as organic modifier, respectively. Notice that the data points fall close to the overall regression lines. These confirm the excellent fits shown by the low average residuals and high correlation coefficients (Table 2). Note that on average the aromatic solutes are more retained than the aliphatic solutes. However, both subsets fit equally well.

In general, the solute size (V_x) and HBA basicity ($\Sigma\beta_2^H$) are the most important solute descriptors governing retention, in accord with previous studies [43,44,67,74–79]. Overall, the magnitude of the coefficients **m** and **b** decrease significantly with the

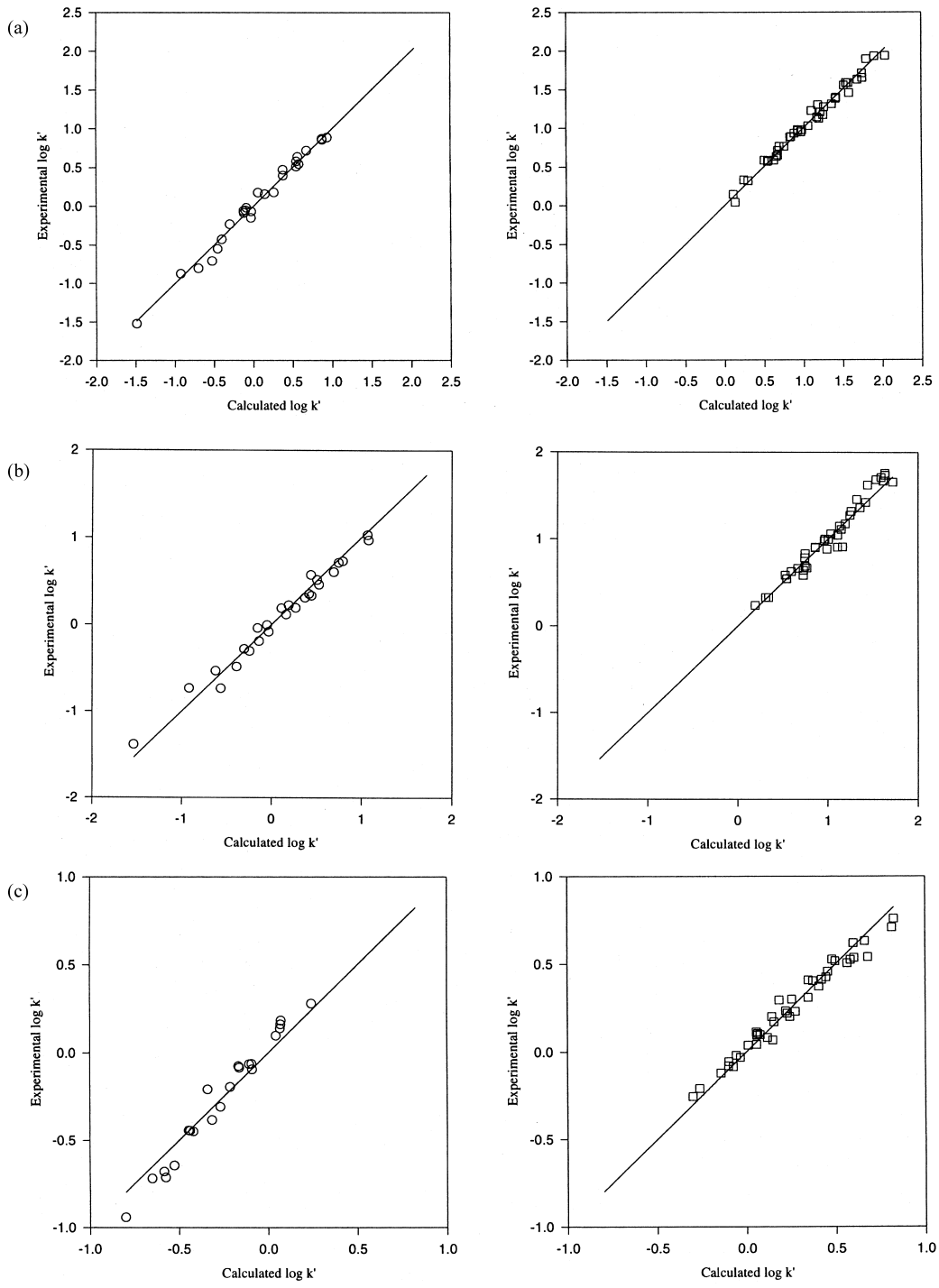

Fig. 4. (a) Plots of experimental vs. calculated log k' for aliphatic (○) and aromatic (□) obtained in: (a) acetonitrile–water (30:70, v/v); (b) in methanol–water (40:60, v/v); (c) in tetrahydrofuran–water (50:50, v/v).

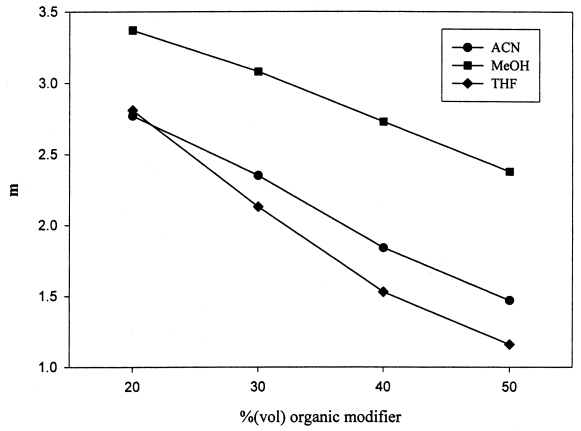

Fig. 5. Plots of **m** vs. % (v/v) organic modifier in the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

volume fraction of organic modifier in the mobile phase (see Fig. 5 Fig. 6), except for the **b** coefficient in MeOH mobile phase. The solute dipolarity/polarizability (π_2^{*H}) and HBD acidity ($\Sigma\alpha_2^H$) have almost no influence, and their fitting coefficients (**s** and **a**) are virtually the same at all mobile phase compositions. Because **s** and **a** are minor we can tolerate considerable error in π_2^{*H} and $\Sigma\alpha_2^H$ and still get rather good overall fits. Thus the small $\overline{sd}$ values shown in Table 2 are not good tests of whether the π_2^{*H} and $\Sigma\alpha_2^H$ values used here are accurate.

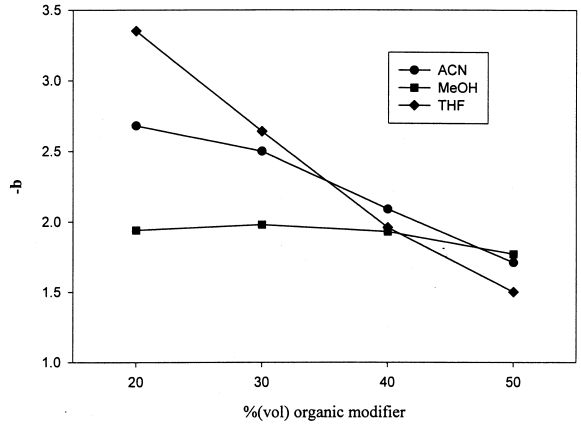

Fig. 6. Plots of $-$**b** vs. % (v/v) organic modifier in the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

3.1. The **m** coefficient

The **m** coefficients are large and positive in all cases (see Figs. 1–3), i.e. increasing the solute size leads to increased retention. Based on a great deal of recent work [3,80,81], much of which is still in progress, we now believe that the large, positive **m** value results from a complex set of opposing factors in both the aqueous mobile phase and the predominantly nonpolar stationary phase. In the process of retaining nonpolar moieties in RPLC, the contribution of the presumed highly unfavorable cavity formation process in water is actually smaller than thought compared to the net favorability of forming dispersive interactions in the stationary phase [80]. As a sign of this we note that the *net* free energy of transfer of a methylene group from the ideal gas phase to pure water and pure hexadecane at 25°C are $+159$ and -634 cal/mol, respectively [80] (1 cal = 4.184 J). The large negative (favorable) free energy of transfer of a methylene group from the gas phase to bulk hexadecane clearly shows that dispersive interactions between the solute and a hydrocarbon must play a major role in bonded phase liquid chromatography. It is also clear that the *net* free energy of transfer of a methylene group from pure water to hexadecane (-793 cal/mol) is due predominantly to the net favorable interactions in the alkane phase. It has also been shown [82] that the free energy of transfer of a methylene group from gas to *n*-pentane is virtually identical to that from gas to *n*-hexadecane. In consequence the solvent density has only a small effect. Currently we believe that bonded phase density only has small effect ($\ll 20\%$) on **m**. This needs to be tested in greater detail than we have in the preceding paper [3] in this series. In view of these observations we must discuss the $\mathbf{m}V_x$ term based on both the cavity effects and dispersion interactions. This is in great contrast to previous work by ourselves and others [2,39].

As discussed in our previous work [3], the aqueous–organic mobile phase is a highly cohesive medium, due primarily to the cohesive density of water. The water molecules form hydrogen bonding network structures, and thus the creation of any cavity within the mobile phase takes place at the cost of considerable free energy. The cohesive energy density of water is almost 10-fold greater than that of

Table 3
Solvatochromic properties of bulk solvents

Complementary solvent property	Cohesive energy density[a], δ^2 (cal/cm^3)	Refractive index[a], n	Dipolarity/polarizability[b], π^*	HB basicity[c], β	HB acidity[b], α
Octane	57	1.395	0.01[e]	0.00	0.00
Hexadecane	64[d]	1.432[d]	0.08[e]	0.00[f]	0.00[g]
Octadecane	66[d]	1.435[d]	0.10[e]	0.00	0.00
Water	554	1.333	1.17	0.47	1.17
Acetonitrile	147	1.342	0.75	0.40	0.19
Methanol	210	1.327	0.61	0.66	0.93
Tetrahydrofuran	98	1.405	0.60	0.55[g]	0.00

[a]Obtained from Ref. [83].
[b]Obtained from Ref. [7].
[c]Obtained from Ref. [84]; unless otherwise indicated.
[d]Estimated from values of lower alkanes.
[e]Obtained from Ref. [85].
[f]Obtained from Ref. [14].
[g] Obtained from Ref. [48].

pure octane (see Table 3). The organic modifiers (MeOH, ACN and THF) also have much higher cohesivities compared to an alkane. Moreover, any cohesivity contributed by the sorbed water and organic molecules to the stationary phase is reduced by the alkyl chains. Thus, the free energy of cavity formation in the mobile phase is much greater than that in the stationary phase. As the fraction of water is increased, the cohesive energy density of the mobile phase increases substantially. However, changes in the cohesivity of the bonded phase, which are largely controlled by the sorbed solvents, are minor. With ACN and MeOH as modifiers, sorption of both organic modifier and water into the bonded phase decreases as φ_W increases [27], and thus leads to even lower cohesivity for the bonded phase. Combining the effects in both the mobile phase and the stationary phase, the *differential* cohesivity between the two phases, and thus the *differential* cavity formation free energy, increases upon increasing φ_W. Therefore the **m** coefficient becomes increasingly positive as φ_W increases (see Fig. 5). In the THF–water system, sorption of organic modifier decreases with φ_W, but sorption of water reaches a maximum around 60% water [27]. Despite the slightly different sorption trend, the plot of the **m** coefficients in THF–water system vs. organic composition is no different from that in ACN–water and MeOH–water systems (see Fig. 5). This is attributed to the fact that cohesivity changes in the bonded phase are minor compared to those in the mobile phase.

The **m** coefficient is also influenced by the *differential* dispersive interactions of a solute with the stationary and the mobile phases. The stationary phase has a higher refractive index than the components of the mobile phase (see Table 3). The refractive index of bulk water (1.333) is especially low compared to that of bulk octane (1.395). This results in higher dispersive interactions between a solute and the stationary phase than with the mobile phase, and thus leads to large and positive **m** coefficients. As water, a species of low refractive index, is added to the mobile phase, the solute–mobile phase dispersive interaction decreases. For the ACN system, as φ_W increases, less water and organic solvent are sorbed into the bonded phase, and thus the *average* solute-bonded phase dispersive interactions increase accordingly. Combining the changes in both the mobile phase and the bonded phase, the *differential* dispersive increases as φ_W increases, and thus the **m** coefficient becomes increasingly positive. The same argument can be applied to the THF system. For the MeOH system, the sorption profiles of solvent and water are similar to that for ACN system, and thus the solute-bonded phase dispersive interaction increases with increasing φ_W. However, the refractive index of MeOH is slightly smaller than that of water, and thus the

solute–mobile phase dispersive interaction increases as φ_W increases. This *counter* effect certainly dampens the difference between solute-bonded phase and solute–mobile phase interaction. Notice that the increment in the **m** coefficient for MeOH system is smaller than those in the ACN and THF systems as φ_W increases (see Fig. 5). The fact that the **m** coefficient for the MeOH system still becomes increasingly positive as φ_W increased suggests two possibilities. First, the changes in the dispersive interactions with the bonded phase outweigh the changes in dispersive interaction with the mobile phase. Second, the influence of the cohesivity effect is greater than that of the dispersive interaction effect.

3.2. *m* vs. mobile phase's δ^2_{mix}, S_p and γ

The **m** coefficient is closely related to the slope of plots of $\log k'$ vs. n_{CH2} for a homolog series. This results because, in a homolog series, π_2^{*H}, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are essentially constant and solute size (V_x) varies linearly with n_{CH2}. Thus **m** is closely related to the 'hydrophobic selectivity'. If the LSER methodology were completely rigorous, then the **m** coefficient should represent that part of the free energy of retention due solely to cavity formation and dispersive (London) interactions. Dielectric effects (dipole–dipole, dipole-induced dipole) and hydrogen bond acid–base interactions should have been completely removed. In consequence plots of **m** vs. the *correct* complementary mobile phase property should then all fall on the same line or curve for all mobile phase modifiers as the mobile phase composition is varied. Such plots are considered below for a series of common mobile phase descriptors of cohesiveness and solvophobicity of hydroorganic mixtures.

The bulk properties of hydroorganic mixtures are shown in Table 4. Fig. 7 shows plots of the **m** coefficient vs. the cohesivity of hydro–organic mixtures (δ^2_{mix}), computed via $\delta^2_{mix} = \varphi_W\delta^2_W + (1-\varphi_W)\delta^2_O$ where δ^2_W and δ^2_O are the cohesive energy densities for water and the organic modifier, respectively. The plots for all three modifiers are rather linear. This suggests that the cavity formation energy in the mobile phase contributes greatly to *establishing the variation* of **m** coefficient as φ_W varies. In addition, the slope of the plots are quite similar, suggesting that δ^2_{mix} is a good descriptor of the **m** coefficient. The plots for ACN and THF virtually overlap, but the plot for MeOH is consistently much higher. The fact that the plots do not all overlap suggests either that (1) the bonded phase in the MeOH system has consistently much *lower* cohesivity than those in the ACN and THF systems, or (2) the random mixing model used in calculating δ^2_{mix} fails in quantifying δ^2_{mix} of MeOH–water mixtures, or (3) δ^2_{mix} does not capture the dispersive interaction in the MeOH–water system the same way as it does in the THF–water and ACN–water mixtures (**m** is also controlled by dispersive interaction in both phases). Argument (1) is less probable in view of the fact that the amount of solvents sorbed into the bonded phase in the MeOH system does not vary greatly from those in the ACN and THF systems. Arguments (2) and (3) are more probably because water and MeOH form hydrogen bonds and cause microheterogeneity [8,17–21], which in turn affects the cavity formation energy.

The surface tension (γ) of a liquid is a direct two-dimensional measure of interfacial intermolecular forces [4]. It is the chief solvent parameter in Horváth's solvophobic theory [2]. In accord with LSER theory, the **m** coefficient is plotted against measured values of $\gamma^{3/2}$ of the mobile phases (see Fig. 8). The 3/2 power was chosen to make the corresponding dimension comparable to volume units, which are the basis for the **m** coefficient. These plots (Fig. 8) are grossly nonlinear and non-superimposing. The **m** coefficients were plotted vs. γ, in accord with the solvophobic theory [2], but none of the plots are superimposable nor are they linear (results are not shown). This clearly shows that the surface tension is not a good complementary parameter to solute size as measured by its volume (V_x). We must point out that there is a very good linear relationship between any measure of solute area and V_x for the set of solutes studied here. We conclude that the mobile phase surface tension is not a good universal indicator of the effect of changes in mobile phase composition on change in retention. We infer that there must be significant contribution to variation in **m** and the nonpolar selectivity with mobile phase not captured in the surface tension descriptor. Because the LSER deconvolves all dipo-

Table 4
Bulk properties of hydro–organic mixtures

Mobile phase	CED[a] (cal/cm^3)	γ[b] (dyne/cm)	S_p[c]	π^*[d]	β(OH)[e]	β(NH)[f]	β(Kry)[g]	$\alpha(E_T(30))$[h]	$\alpha(E_T(33))$[i]	α(Fe)[j]
Water	554	71.66	1	1.17	0.404	0.144	0.19	1.09	1.01	1.24
ACN	147	28.49	0.3408	0.75	0.404	0.339	0.37	0.33	0.35	0.32
MeOH	210	22.35	0.3273	0.61	0.814	0.628	0.62	1.09	1.06	1.02
THF	98	26.88	0.1281	0.60	0.572	0.551	0.54	0.00	−0.23	0.04
ACN–water										
(50:50)	350.5	33.10	0.5463	0.95	0.683	0.381	0.402	0.89	0.858	0.90
(40:60)	391.2	34.20	0.6262	0.99	0.655	0.358	0.382	0.90	0.880	0.95
(30:70)	431.9	37.10	0.7212	1.06	(0.616)[k]	(0.322)[k]	0.354	0.90	0.883	1.01
(20:80)	472.6	42.50	0.8392	1.12	0.568	0.274	0.308	0.94	0.903	1.08
MeOH–water										
(50:50)	382.0	35.40	0.6920	1.03	0.456	0.399	0.434	0.86	0.906	1.07
(40:60)	416.4	38.70	0.7824	1.08	0.449	0.342	0.380	0.88	0.923	1.10
(30:70)	450.8	43.30	0.8416	1.11	(0.434)[k]	(0.278)[k]	0.320	0.92	0.942	1.15
(20:80)	485.2	49.10	0.9016	1.15	0.409	0.209	0.260	0.93	0.957	1.17
THF–water										
(50:50)	326.0	30.40	0.3477	0.89	0.750	0.564	0.526	0.58	0.641	0.83
(40:60)	371.6	31.70	0.4296	0.95	0.670	0.524	0.542	0.60	0.675	0.86
(30:70)	417.2	33.70	0.5659	1.05	(0.600)[k]	(0.460)[k]	0.528	0.62	0.705	0.90
(20:80)	462.8	38.10	0.7586	1.14	0.543	0.368	0.466	0.72	0.772	0.98

[a]CED, cohesive energy density; values for mixtures (δ^2_{mix}) are computed through $\delta^2_{mix}=\varphi_W\delta^2_W+(1-\varphi_W)\delta^2_O$ where δ^2_W and δ^2_O are the CED for water and the organic modifier, respectively.
[b]Surface tension [86].
[c]Solvophobic parameter [5], values calculated based on experimental data taken from Ref. [87].
[d]Dipolarity/polarizability [7].
[e]Hydrogen bond basicity based on the OH indicator [14].
[f]Hydrogen bond basicity based on the NH indicator [14].
[g]Hydrogen bond basicity based on the NH indicator [15,16].
[h]Hydrogen bond acidity based on indicator $E_T(30)$ [7].
[i]Hydrogen bond acidity based on indicator $E_T(33)$ [13].
[j]Hydrogen bond acidity based on the Fe complex [6].
[k]Estimated by interpolation.

lar and hydrogen bonding effects on retention, the above analysis and plots (Figs. 7 and 8) constitute a much more complete test of models of mobile phase cohesivity than merely plotting $\log k'$ against the corresponding solvent parameters (δ^2_{mix}, $\gamma^{3/2}$). Finally, Fig. 8 can be made more linear by plotting against γ to a power higher than 3/2 but such is physically nonsensical and, in any case, will not remove the lack of superimposability among the three modifiers.

Fig. 9 shows plots of **m** vs. Abraham's solvophobic parameter, S_p [5]. S_p values of the hydro–organic mixtures were computed based on the free energy of transfer of a homolog series from bulk water into these mobile phase mixtures [87]. Thus, S_p represents the sum of the cavity formation energy and dispersive interaction in water as opposed to in the hydro–organic mixtures. All three plots of **m** are approximately linear with S_p. The linear correlation confirms the role of the $\mathbf{m}V_x$ term in probing the changes in the retention due to the nonpolar and size-dependent parameter as separated from the other terms ($\mathbf{s}\pi_2^{*H}$, $\mathbf{a}\Sigma\alpha_2^H$ and $\mathbf{b}\Sigma\beta_2^H$) which respond to changes in the polar parts of the solutes. Similar to the $\mathbf{m}$–δ^2_{mix} plots, the slope of the $\mathbf{m}$-S_p plots for different modifier systems are quite similar. This suggests that S_p is a good complementary solvent parameter to V_x. However, the plots for the three systems are not statistically the same although they are closely grouped. This suggests that the stationary

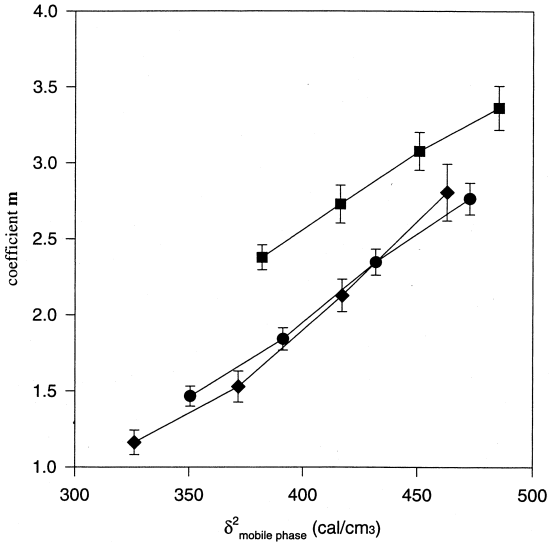

Fig. 7. Plots of coefficient **m** vs. cohesive energy density of the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

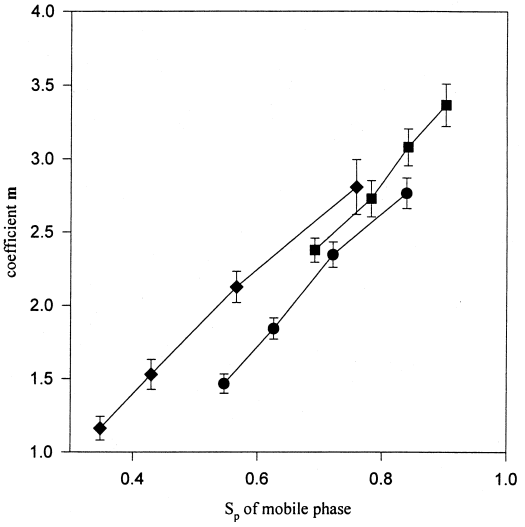

Fig. 9. Plots of coefficient **m** vs. S_p of the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

phase has at least some influence in establishing the dependence of **m** on φ_W. This suggestion is supported by our prior work wherein we showed that the **m** coefficients for C_{18} and C_8 phases (on the same silica) are significantly different [3], as well as other work in which a clear cut monotonous trend in the slope of log k' vs. solute homolog number could be seen with the chain length of the bonded phase [88].

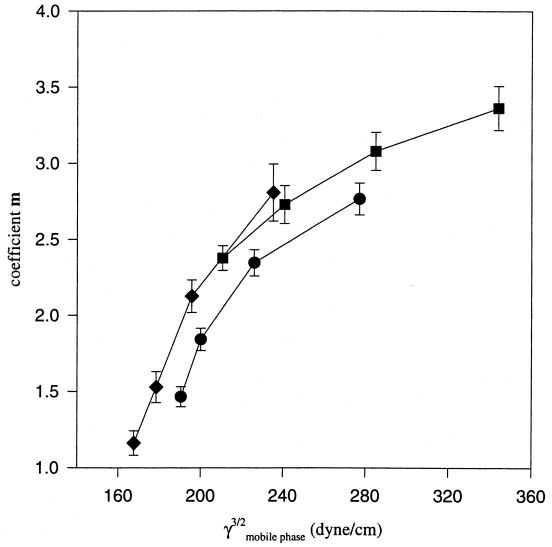

Fig. 8. Plots of coefficient **m** vs. $\gamma^{3/2}$ of the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

*3.3. The **b** coefficient*

The **b** coefficients are all large and negative (see Table 2) thus, all other parameters being held constant, solutes with greater HB acceptor ability are significantly less retained. The large and negative **b** coefficient indicates that the mobile phase is a much stronger hydrogen bond acid than the stationary phase.

For the ACN–water and THF–water systems, the size of the **b** coefficient increases with φ_W (see Fig. 6). Water ($\alpha_{H2O} = 1.17$) is a much stronger HBD acid than either ACN ($\alpha_{ACN} = 0.19$) or THF ($\alpha_{THF} = 0.00$). Thus, adding water to the mobile phase causes a significant increase in the HBD acidity of the mobile phase. On the other hand, as φ_W increases within the range $0.5 \leq \varphi_W \leq 0.8$, sorption of water and organic modifiers into the bonded phase decreases [26,27] and this should lead to a decrease in the HBD acidity of the bonded phase. As a result, there is a significant increase in $\Delta(\alpha_s - \alpha_m)$, and hence a significant

increase in the size of the **b** coefficient (see Eq. (2)). The increase is especially significant in the THF–water system because THF is not an HBD acid. In contrast, the **b** coefficient for the MeOH–water system remains reasonably constant over the range of $0.5 \leq \varphi_W \leq 0.8$ (see Fig. 6). This is expected since the difference in HBD acidity between water and MeOH ($\alpha_{MeOH} = 0.93$) is much smaller than with ACN or THF. Thus, addition of water to the mobile phase does not cause significant changes in the HBD acidity of the mobile phase or the stationary phase in the MeOH system. This result also indicates why ACN- and THF-modified mobile phases afford different chromatographic selectivities compared to the MeOH modified mobile phase.

Fig. 10 shows the **b** coefficients vs. the HBD acidity of the mobile phase (α_m). The α_m values are *average* values of bulk phase α scales based on the indicators $E_T(30)$ [7], $E_T(33)$ [13] and the Fe(II) complex [6] (Preliminary studies (not shown) of plots of the **b** coefficients against each individual scale do not clearly indicate any differences among the indicators). Fig. 10 shows that plots for the ACN, MeOH and THF systems are located far from one another and have different slopes. The slope for THF is the largest followed by ACN and then MeOH. The magnitude of the slope correlates with the differential HBD acidity between water and the modifier: $\Delta\alpha_{water-THF} > \Delta\alpha_{water-ACN} > \Delta\alpha_{water-MeOH}$. These results show that although α_s is altered by changes in φ_W, the alterations are minor compared to those in α_m. In turn, the *variation* in the **b** coefficient with changes in the mobile phase composition is largely controlled by α_m.

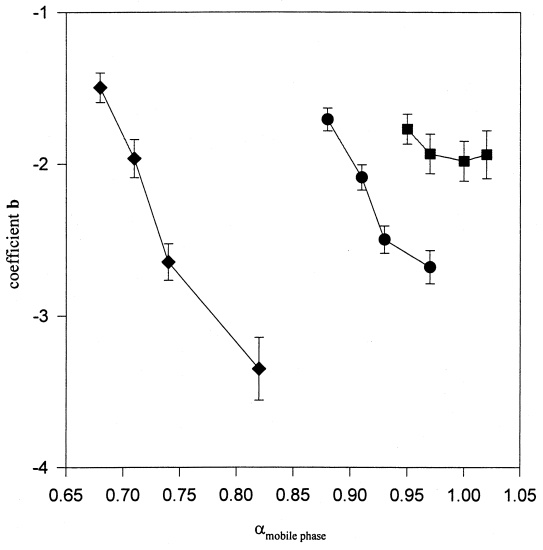

Fig. 10. Plots of coefficient **b** vs. hydrogen bond donor acidity (α) of the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

3.4. The s coefficient

The **s** coefficient is small and negative in all cases (see Figs. 1–3), thus a solute's retention decreases only slightly as its dipolarity is increased. Because the **s** coefficient is proportional to the *difference* in dipolarity/polarizability between the mobile phase and the stationary phase, $(\pi_s^* - \pi_m^*)$, these results strongly suggest that over the range of $0.5 \leq \varphi_W \leq 0.8$ the stationary phase is only slightly less dipolar/polarizable than is the mobile phase. Thus, this result agrees with literature spectroscopic and thermodynamic studies that clearly indicate the high polarity of the solvated bonded phase [14,32–39]. This agreement further suggests that the typically rather polar solvatochromic indicators and the highly variegated solutes used here experience the same stationary phase environment.

Figs. 1–3 show that the **s** coefficient is virtually independent of φ_W, in all three systems. The π^* of the mobile phase should increase as water, a substance of much higher π^* than the organic component, is added [7–9]. The constancy of the **s** coefficient suggest that π^* of the stationary phase (π_s^*) increase accordingly as water is added to the mobile phase. However, the studies of Yonker et al. [27] showed that, in this range, sorption of both water and organic modifiers decreases as φ_W increases. This suggests that the π^* of the bonded phase does not depend only on its chemical composition. We propose the following: as φ_W increases, the bonded chains adopt the collapsed conformation [68]. Instead of sorbing into the denser and nonpolar collapsed phase, the sorbed solvent molecules accumulate in the relatively polar interface region [28,31,32]. As a result, a stationary phase region of high π^* is formed.

Overall, the **s** coefficient in the MeOH system is consistently larger than that observed for the ACN

system. This in turn is larger than that seen in the THF system. This implies that π_s^* increases in the order of modifier used: THF>ACN>MeOH. This result is consistent with Carr and Harris's findings regarding the surface polarity which follows the same order of solvent strength [34].

Fig. 11 shows a plot of the **s** coefficient vs. the dipolarity/polarizability of the mobile phase mixtures, π_m^*, as measured by Cheong and Carr [7]. Three important points are noted. First, the **s** coefficients for all three systems do not show significant variation with π_s^*. This indicates that changes in π_m^* by themselves have only a minor influence in establishing the variation in the **s** coefficients. *This in turn suggests that the stationary phase is playing a significant role*. Second, the curves for ACN and THF virtually overlap, which indicates that the π^* value of the bonded phase in ACN and THF systems overlap in this φ_W range. This result agrees with literature work which shows that the polarity of bonded phase solvated with ACN–water and THF–water mixtures are very similar [35]. Third, at any given π_m^*, the **s** coefficient for MeOH is more negative than the others, which suggests that the stationary phase in equilibrium with MeOH–water mixtures is less dipolar/polarizable. Again, this suggests that the stationary phase is important in establishing the **s** coefficient, for otherwise the **s** coefficients should fall on the same curve vs. π_m^* for all modifier systems.

3.5. The *a* coefficient

For all RPLC systems studied here and elsewhere, the **a** coefficient is small and negative, and virtually independent of φ_W. The **a** coefficients for THF system appear to increase with φ_W, but the variations are not outside the relatively large standard deviations. As the $\Sigma\alpha_2^H$ of a solute increases, the affinity of the solute towards the mobile phase relative to its affinity for the stationary phase increases slightly, thereby leading to lower retention. *This result suggests that the bonded phase is only slightly less basic than is the mobile phase in the composition ranges studied*. The HBA basicity of water is roughly equal to those of the organic modifiers (see Table 3). Thus, an increase in the water content does not significantly alter the HBA basicity of the chromatographic phases, and hence has little effect on the corresponding **a** coefficient. This effect is further dampened by the small values of the **a** coefficient.

Fig. 12 shows the **a** coefficient as a function of

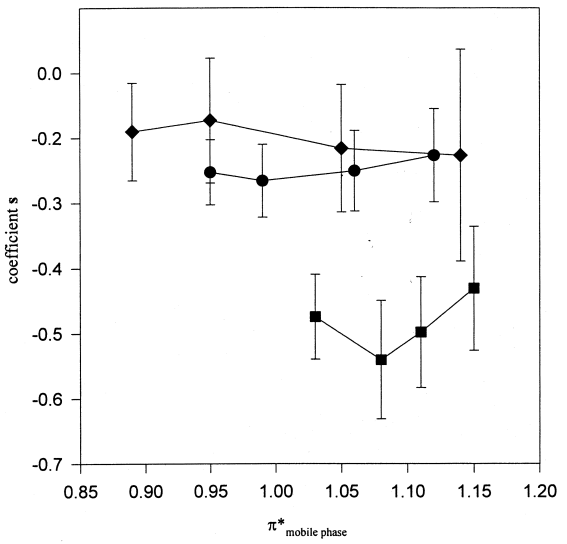

Fig. 11. Plots of coefficient **s** vs. dipolarity/polarizability (π^*) of the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

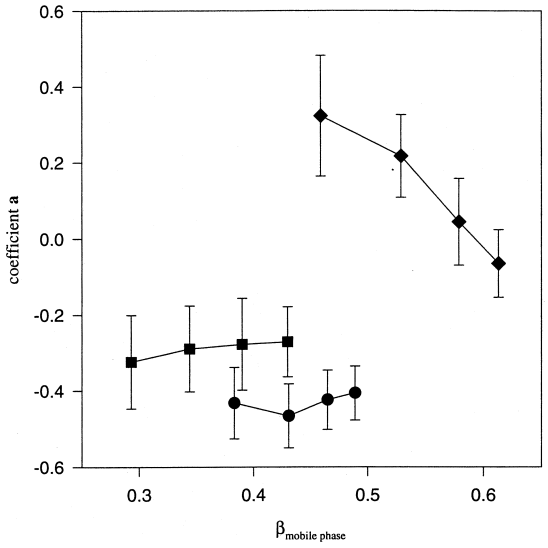

Fig. 12. Plots of coefficient **a** vs. hydrogen bond acceptor basicity (β) of the mobile phase for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

the HBA basicity of the mobile phase, β_m. The β_m values are *average* values of three HB basicity scales determined using Kamlet–Taft solvatochromic indicators [14–16] (Preliminary results do not clearly show if there are differences between these scales.) The **a**–β_m plots for ACN, MeOH and THF systems are clearly different. The **a** coefficient for the ACN and MeOH systems remains constant over the HB basicity range explored. This suggests that $\Delta(\beta_s - \beta_m)$ remains constant despite changes in β_m. This might be explained by assuming that the HB basicity of the stationary phase (β_s) is dependent on β_m. The plot for the THF system is quite different; the **a** coefficient decreases with increasing β_m, i.e. $\Delta(\beta_s - \beta_m)$ becomes smaller as β_m increases. This result suggests that β_s varies with changes in the mobile phase, but to a lesser degree than does β_m. Overall, the study shows that β_s depends greatly on the type of organic modifier and the composition of the mobile phase [23,24,26,27,30].

We note that the above studies of the π^*, α, and β of the bulk phases are complicated by the preferential solvation of the indicators used. Although Marcus and co-workers reported that the Kamlet–Taft π^*, α, and β indicators are able to sense the actual bulk solvent environment in hydro-organic mixtures without significantly perturbing the environment [8,17] there are others who report differently [14,89]. Many studies have effectively shown that the Kamlet–Taft indicators are preferentially solvated by one of the mixture components through dielectric enrichment [90–93], hydrogen bonding [93,94], or hydrophobic interactions [92,95]. In other words, the solvatochromic parameters actually reflect the 'local' properties, not the 'bulk' properties of a solvent.

Despite the complications, the above analysis strongly suggests that the chemical properties of the stationary phase depend greatly on the type of organic modifier and the organic concentration. The interactions between the stationary phases and solutes are greatly affected by the mobile phase composition. The fact that the LSER fitting coefficients do not vary in proportion to the bulk properties of mobile phase mixtures indicates that the changes in retention upon changes in the mobile phase composition result from alteration in the solute–mobile phase processes, and mobile phase-induced perturbation in the solute–stationary phase interactions. As the mobile phase is varied, the stationary phase is significantly changed and thus is the chemical environment experienced by the solute when it is in the stationary phase.

3.6. Comparison with hexadecane–water and octanol–water bulk phase transfer system

In order to more completely understand these very complex chemical systems we now compare the LSERs obtained in the RPLC system with a bulk phase transfer system to demonstrate that, although the bonded phase per se has the same alkane-like composition as hexadecane, its chemical properties are very different. K_{C16-w} is the hexadecane–water partition coefficient:

$$K_{C16-w} = [A]_{C16}/[A]K_{water} \quad (3)$$

where [A] is the equilibrium concentration of solute A in the two bulk phases, hexadecane and water:

$$A_{C16} \rightleftharpoons A_{water} \quad (4)$$

$\log K_{C16-w}$ is a free energy term which is well related to the solute parameters through the following LSER, which was obtained based on a very similar solute set [96],

$$\log K_{C16-w} = -0.05 + 4.85V_x/100 - 1.10\pi_2^{*H} - 3.13\Sigma\alpha_2^H - 5.78\Sigma\beta_2^H$$
$$n = 76,\ \overline{sd} = 0.245,\ r = 0.9899 \quad (5)$$

It is evident that the **a** and the **s** coefficients in Eq. (5) are much larger than in any RPLC system. Recent LSER studies on experimental $\log k'$ obtained in water, which is strongly polar and basic, shows similar results as we observed here, where **m** and **b** are relatively very large, and **s** and **a** are minor [67]. All available results indicate that a bonded phase is much more basic and polar than is bulk hexadecane. The alkyl bonded phase and hexadecane are similar in terms of their alkane composition. The great difference in the **a** and **s** coefficients observed between these two phases strongly supported the findings that significant amounts of water and organic modifier are extracted into the bonded phase [14].

In contrast, K_{oct-w}, the octanol–water partition

coefficient, which can be represented through the following LSER [96],

$$\log K_{\text{oct-w}} = -0.13 + 4.11V_x/100 - 0.44\pi_2^{*\text{H}} - 0.27\Sigma\alpha_2^{\text{H}} - 4.18\Sigma\beta_2^{\text{H}}$$

$$n = 76,\ \overline{sd} = 0.146,\ r = 0.9923 \quad (6)$$

is chemically more similar to the RPLC retention system. The relative values of the **m**, **s**, **a** and **b** coefficients of $\log K_{\text{oct-w}}$ are much closer to those of RPLC system, than $\log K_{\text{C16-w}}$. This is clearly due to the high hydrogen bond donor acidity of octanol compared to hexadecane.

We cannot altogether state that the surface silanol groups have no effect on establishing the **b**, **s**, and **a** coefficients of the LSER. The silanol groups may influence the sorption of mobile phase components. However, in the previous paper in this series we studied five rather different bonded phase at fixed mobile phase composition and observed only minor differences in the LSER coefficients compared to those seen here [3]. In addition, recent studies on a horizontally polymerized phase, with a very large fraction of all the surface groups being occupied, shows small changes in the LSER coefficients despite a big change in accessibility to silanol groups [97]. Results of another investigation [98], in which triethylamine was added to the mobile phase, show little change in the solvatochromic coefficient, further indicating that the silanol groups do not play a primary role in determining the coefficients. It is our view that sorption of mobile phase components is principally responsible for establishing the solvatochromic coefficients.

*3.7. −**b** vs. **m***

Fig. 13 shows a plot of $-\mathbf{b}$ vs. **m**. Apparently each modifier establishes its own relationship between $-\mathbf{b}$ and **m**. The plot for THF is the most linear of the three. The plot for ACN is slightly curved, while the plot for MeOH is greatly curved. If a line is force fitted to these points, the slopes for THF, ACN and MeOH are 1.12, 0.75 and 0.17, respectively. These values are related to the difference between the HB acidity of bulk water and the corresponding organic, $\Delta(\alpha_{\text{water}} - \alpha_{\text{organic}})$. The $\Delta(\alpha_{\text{water}} - \alpha_{\text{organic}})$ values for THF, ACN, and MeOH

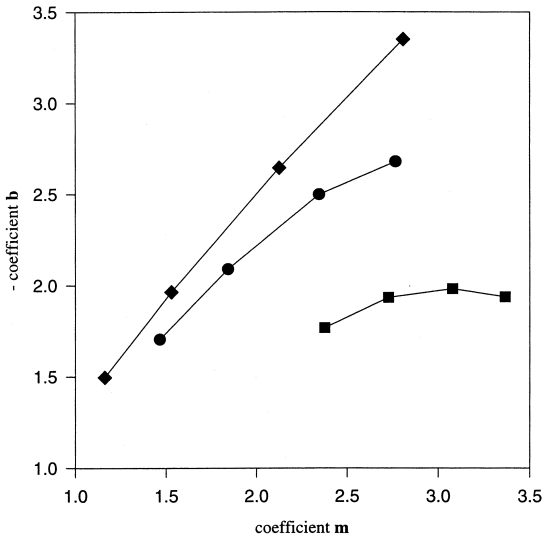

Fig. 13. Plots of the $-\mathbf{b}$ vs. **m** for RPLC using acetonitrile (●), methanol (■) and tetrahydrofuran (♦) as organic modifier.

are 1.17, 0.98 and 0.24, respectively. The value of the slope denotes the importance of the $\mathbf{b}\Sigma\beta_2^{\text{H}}$ term *relative* to the $\mathbf{m}V_x$ term as the mobile phase composition is varied. Thus, the data in Fig. 13 suggest that the $\mathbf{b}\Sigma\beta_2^{\text{H}}$ term becomes increasing important in establishing the $\log k'$ as more water is added to the mobile phase. The effect is greatest for the THF–water system, followed by the ACN–water system. For the MeOH–water system, the relative contribution of the $\mathbf{b}\Sigma\beta_2^{\text{H}}$ term remains approximately constant upon variation in the mobile phase composition.

4. Conclusions

Linear solvation energy relationships and the related gas chromatograhically derived solvatochromic parameters successfully represent solute interactions with the mobile phase and stationary phase as the mobile phase composition is varied. This was shown to be so for a wide variety of aliphatic, aromatic, polar and nonpolar solutes. Solute size (V_x) and hydrogen bond acceptor basicity ($\Sigma\beta_2^{\text{H}}$) are the main factors governing the retention in aqueous mobile phases using acetonitrile, methanol and tetrahydrofuran as modifiers. Over the range of 50–80%

(v/v) water, these two solute terms become increasingly more important as the volume fraction of water in the mobile phase increases. Solute dipolarity/polarizability (π_2^{*H}) and HBD acidity ($\Sigma\alpha_2^H$) are of minor significance in establishing retention in RPLC, and their contributions are virtually constant over the whole mobile phase composition range explored. The present work clearly indicates that the chemical properties of the stationary phase greatly depend on the mobile phase composition. Due to sorbed solvents, the bonded phase is much more polar and basic than is bulk hexadecane. Thus, the octanol–water partition coefficient (K_{oct-w}) provides a better model of retention in RPLC than does the hexadecane–water partition coefficient (K_{C16-w}). In general, the cohesive energy density and solvophobic parameter of the mobile phase linearly correlate with the LSER **m** coefficient, while surface tension, the chief solvent parameter in Horváth's solvophobic theory, shows concave plots. HBD acidity of the mobile phase is a major factor in controlling the variations in the **b** coefficient in the THF system, less so in the ACN system and least in the MeOH system. Dipolarity/polarizability and HBD basicity of the mobile phase do not have a really significant effect on controlling the variation in **s** and **a**, respectively. Our results strongly suggest that the stationary phase plays an important role in governing the retention mechanism in RPLC. Thus, a better understanding and quantitation of the various chemical properties of the bonded phase can help in predicting retention in RPLC.

Acknowledgements

This work was supported by a grant from the National Science Foundation.

References

[1] J.G. Dorsey, K.A. Dill, Chem. Rev. 89 (1989) 331.
[2] Cs. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129.
[3] L.C. Tan, P.W. Carr, M.H. Abraham, J. Chromatogr. A 752 (1996) 1.
[4] K. Shinoda, Principles of Solution and Solubility, translated in collaboration with P. Becker, Mercel Dekker, New York, 1978.
[5] M.H. Abraham, P.L. Grelier, R.A. McGill, J. Chem. Soc. Perkin Trans. II (1988) 339.
[6] J.H. Park, M.D. Jang, D.S. Kim, P.W. Carr, J. Chromatogr. 513 (1990) 107.
[7] W.J. Cheong, P.W. Carr, Anal. Chem. 60 (1988) 820.
[8] Y. Marcus, Y. Migron, J. Phys. Chem. 95 (1991) 400.
[9] H. Lu, S.C. Rutan, Anal. Chem. 68 (1996) 1387.
[10] J.G. Dorsey, B.P. Johnson, J. Liq. Chromatgr. 10 (1987) 2695.
[11] B.P. Johnson, M.G. Khaledi, J.G. Dorsey, Anal. Chem. 58 (1986) 2354.
[12] B.P. Johnson, M.G. Khaledi, J.G. Dorsey, J. Chromatogr. 384 (1987) 221.
[13] J.H. Park, A.J. Dallas, P. Chau, P.W. Carr, J. Chromatogr. A 677 (1994) 1.
[14] A.J. Dallas, Ph.D. Thesis, University of Minnesota, 1994.
[15] T.M. Krygowski, P.K. Wrona, U. Zielkowska, C. Reichardt, Tetrahedron 41 (1985) 4519.
[16] T.M. Krygowski, C. Reichardt, P.K. Wrona, C. Wyszomirska, U. Zielkowska, J. Chem. Res. (1983) 116.
[17] Y. Migron, Y. Marcus, J. Chem. Soc., Faraday Trans. 87 (1991) 1339.
[18] Y. Marcus, J. Chem. Soc., Faraday Trans. I 85 (1989) 381.
[19] Y. Marcus, J. Chem. Soc., Faraday Trans. I 86 (1990) 2215.
[20] E. Matteoli, L. Lepori, J. Chem. Phys. 80 (1984) 2856.
[21] M.J. Blandamar, N.J. Blundell, J. Burgess, H.J. Cowles, I.M. Horn, J. Chem. Soc., Faraday Trans. I 86 (1990) 277.
[22] S. Backlund, H. Hoiland, I. Vikholm, J. Sol. Chem. 13 (1984) 749.
[23] E.H. Slaats, W. Markovski, J. Fekete, H. Poppe, J. Chromatogr. 207 (1981) 299.
[24] R.M. McCormick, B.L. Karger, Anal. Chem. 52 (1980) 2249.
[25] R.M. McCormick, B.L. Karger, Anal. Chem. 199 (1980) 259.
[26] C.R. Yonker, T.A. Zwier, M.F. Burke, J. Chromatogr. 241 (1982) 257.
[27] C.R. Yonker, T.A. Zwier, M.F. Burke, J. Chromatogr. 241 (1982) 269.
[28] R.P.W. Scott, P. Kucera, J. Chromatogr. 142 (1977) 213.
[29] C.S. Koch, F. Köster, G.H. Findenegg, J. Chromatogr. 406 (1987) 257.
[30] A. Tilly-Melin, Y. Askemark, K.G. Wahlund, G. Schill, Anal. Chem. 51 (1979) 976.
[31] D.E. Martire, R.E. Boehm, J. Phys. Chem. 87 (1983) 1045.
[32] M.R. Böhmer, L.K. Koopal, R. Tijssen, J. Phys. Chem. 95 (1991) 6285.
[33] J. Stahlberg, M. Almgren, Anal. Chem. 57 (1985) 817.
[34] J.W. Carr, J.M. Harris, Anal. Chem. 58 (1986) 626.
[35] J.W. Carr, J.M. Harris, Anal. Chem. 59 (1987) 2546.
[36] Y.-D. Men, D.B. Marshall, Anal. Chem. 62 (1990) 2606.
[37] C.H. Lochmuller, D.B. Marshall, P.R. Wilder, Anal. Chim. Acta 130 (1981) 31.
[38] J.L. Jones, S.C. Rutan, Anal. Chem. 63 (1991) 1318.

[39] W.J. Cheong, P.W. Carr, J. Chromatogr. 499 (1990) 373.
[40] J.C. McGowan, J. Chem. Technol. Biotechnol. 34A (1984) 38.
[41] M.H. Abraham, J.C. McGowan, Chromatographia 23 (1987) 243.
[42] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, J. Chromatogr. 587 (1991) 213.
[43] P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W. Melander, Cs. Horváth, Anal. Chem. 58 (1986) 2674.
[44] M.J. Kamlet, M.H. Abraham, P.W. Carr, R.M. Doherty, R.W. Taft, J. Chem. Soc., Perkin Trans. II (1988) 2087.
[45] J. Li, Y. Zhang, H. Ouyang, P.W. Carr, J. Am. Chem. Soc. 114 (1992) 9813.
[46] A. Bondi, J. Phys. Chem. 68 (1964) 441.
[47] D.E. Leahy, J. Pharm. Sci. 75 (1986) 629.
[48] M.J. Kamlet, J.M. Abboud, M.H. Abraham, R.W. Taft, J. Org. Chem. 48 (1983) 2877.
[49] M.J. Kamlet, R.M. Doherty, M.H. Abraham, Y. Marcus, R.W. Taft, J. Phys. Chem. 92 (1988) 5244.
[50] R.W. Taft, M.J. Kamlet, J. Am. Chem. Soc. 98 (1976) 2886.
[51] M.J. Kamlet, R.W. Taft, J. Am. Chem. Soc. 98 (1976) 377.
[52] M.J. Kamlet, J.M. Abboud, R.W. Taft, J. Am. Chem. Soc. 99 (1977) 6027.
[53] R.W. Taft, J.M. Abboud, M.J. Kamlet, M.H. Abraham, J. Sol. Chem. 14 (1985) 153.
[54] J.M. Abboud, K. Sraidi, G. Guiheneuf, A. Negro, M.J. Kamlet, R.W. Taft, J. Org. Chem. 50 (1985) 2870.
[55] B. Frange, J.M. Abboud, C. Benamou, L. Bellon, J. Org. Chem. 47 (1982) 4553.
[56] M.J. Kamlet, R.M. Doherty, P.W. Carr, D. Mackay, M.H. Abraham, R.W. Taft, Environ. Sci. Technol. 22 (1988) 503.
[57] J. Li, Y. Zhang, A. Dallas, P.W. Carr, J. Chromatogr. 550 (1991) 101.
[58] M.H. Abraham, P.L. Grellier, D.V. Prior, P.P. Duce, J.J. Morris, P.J. Taylor, J. Chem. Soc., Perkin Trans. II (1989) 699.
[59] M.H. Abraham, P.L. Grellier, D.V. Prior, J.J. Morris, P.J. Taylor, J. Chem. Soc., Perkin Trans. II (1990) 521.
[60] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, J. Chromatogr. 587 (1991) 229.
[61] M.H. Abraham, G.S. Whiting, J. Andonian-Haftvan, J.W. Steed, J.W. Grate, J. Chromatogr. 588 (1991) 361.
[62] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, P. Sakellariou, Polymer 33 (1992) 2162.
[63] M.H. Abraham, I. Hamerton, J.B. Rose, J.W. Grate, J. Chem. Soc., Perkin Trans. II (1991) 1417.
[64] M.H. Abraham, G.S. Whiting, J. Am. Oil Chem. Soc. 69 (1992) 1236.
[65] M.H. Abraham, J. Andonian-Haftvan, J.P. Osei-Owusu, P. Sakellariou, J.S. Urieta, M.C. López, R. Fuchs, J. Chem. Soc., Perkin Trans. II (1993) 299.
[66] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[67] M.-M. Hsieh, J.G. Dorsey, J. Chromatogr. 631 (1993) 63.
[68] R.P.W. Scott, C.F. Simpson, J. Chromatogr. 197 (1980) 11.
[69] M.J. Kamlet, R.M. Doherty, M.H. Abraham, P.W. Carr, R.F. Doherty, R.W. Taft, J. Phys. Chem. 91 (1987) 1996.
[70] M.J. Kamlet, D.J. Abraham, R.M. Doherty, R.W. Taft, M.H. Abraham, J. Pharm. Sci. 75 (1986) 350.
[71] M.H. Abraham, P.L. Grellier, R.A. McGill, R.M. Doherty, M.J. Kamlet, T.N. Hall, R.W. Taft, P.W. Carr, W.J. Koros, Polymer 28 (1987) 1363.
[72] M.H. Abraham, G.J. Buist, P.L. Grellier, R.A. McGill, R.M. Doherty, M.J. Kamlet, R.W. Taft, S.G. Maroldo, J. Chromatogr. 409 (1987) 15.
[73] M.J. Kamlet, R.M. Doherty, V. Fiserova-Bergerova, P.W. Carr, M.H. Abraham, R.W. Taft, J. Pharm. Sci. 76 (1987) 14.
[74] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, M.H. Abraham, Anal. Chem. 57 (1985) 2978.
[75] C. Altomare, S. Cellamare, A. Carotti, M. Ferappi, Quant. Struct.-Act. Relatsh. 12 (1993) 261.
[76] J.H. Park, M.D. Jang, S.T. Kim, Bull. Korean Chem. Soc. 11 (1990) 297.
[77] J.H. Park, P.W. Carr, M.H. Abraham, R.W. Taft, R.M. Doherty, M.J. Kamlet, Chromatographia 25 (1988) 373.
[78] D.E. Leahy, P.W. Carr, R.S. Pearlman, R.W. Taft, M.J. Kamlet, Chromatographia 21 (1986) 473.
[79] J.H. Park, J.J. Chae, T.H. Nah, M.D. Jang, J. Chromatogr. A 664 (1994) 149.
[80] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, J. Chromatogr. A 656 (1993) 113.
[81] M.H. Abraham, G.S. Whiting, R. Fuchs, E.J. Chambers, J. Chem. Soc., Perkin Trans. II (1990) 291.
[82] D.I. Eikens, Ph.D. Thesis, University of Minnesota, 1993.
[83] J.A. Riddick, W.B. Bunger, T.K. Sakano, Organic Solvents, 4th ed., Wiley-Interscience, New York, 1986.
[84] Y. Marcus, M.J. Kamlet, R.W. Taft, J. Phys. Chem. 94 (1988) 3613.
[85] J.E. Brady, P.W. Carr, J. Phys. Chem. 89 (1985) 1813.
[86] W.J. Cheong, P.W. Carr, J. Liq. Chromatogr. 10 (1987) 561.
[87] J. Li, Ph.D. Thesis, University of Minnesota, 1992.
[88] A. Tchapla, H. Colin, G. Guiochon, Anal. Chem. 56 (1984) 621.
[89] J.H. Park, A.J. Dallas, P. Chau, P.W. Carr, J. Phys. Org. Chem 7 (1994) 757–769.
[90] K.S. Nitsche, P. Suppan, Chimia 36 (1982) 346.
[91] C. Lerf, P. Suppan, J. Chem. Soc., Faraday Trans. 88 (1992) 963.
[92] J.G. Dawber, J. Ward, R.A. Williams, J. Chem. Soc., Faraday Trans. I 84 (1988) 713.
[93] W.P. Zurawsky, S.F. Scarlata, J. Phys. Chem. 96 (1992) 6012.
[94] P. Chatterjee, S. Bagchi, J. Chem. Soc., Faraday Trans. 86 (1990) 1785.
[95] J.G. Dawber, J. Chem. Soc., Faraday Trans. 86 (1990) 287.
[96] L.C. Tan, Ph.D. Thesis, University of Minnesota, 1994.
[97] L. Li, M.S. Thesis, University of Minnesota, 1995.
[98] J.H. Park, Unpublished work, 1996.