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Effect of molecular interactions on retention and selectivity in reversed-phase liquid chromatography

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Abstract

The linear solvation energy relationships (LSERs) have been applied in the last years for description and prediction of retention and selectivity in reversed-phase liquid chromatography with good results. Widely different stationary phases have been compared and characterized by LSERs. In recent publications the influence of the type of the organic moderator and the composition of the mobile phase have also been described. However, the influence of the molecular properties of the solutes to be separated has never been discussed. According to the LSER model variation in retention factors (log *k*) with solute structure can be related to their potential for various intermolecular interactions. The retention factor is given as the sum of the terms of the LSER equation representing various types of molecular interactions. For this reason the influence of the structure and molecular properties of the solutes to be separated can also be investigated using the LSER equation. In this study we shall demonstrate how the specific molecular interactions influence chromatographic retention and selectivity. We intend to show that retention and selectivity depend on all participants of the system. In addition to the structure and properties of the stationary phase and the type and composition of the mobile phase the molecular properties of the solutes, characterized by the solvation parameters, will also influence the type and extent of the various molecular interactions governing retention and selectivity.

Keywords: Molecular interactions; Retention; Selectivity; Linear solvation energy relationships; Mobile phase composition; Stationary phases, LC

1. Introduction

Despite the great popularity of reversed-phase liquid chromatography (RPLC) the mechanisms of retention and selectivity at the molecular level are not well understood.

The classical thermodynamic expression relating the distribution coefficient with excess free energy can be employed to describe retention in thermodynamic terms. The basic thermodynamic process involved in chromatographic retention consists of (a) the creation of a solute-sized cavity in the stationary phase; (b) the transfer of the solute into that cavity from the mobile phase, and (c) the closing of the cavity in the mobile phase left behind by the solute.

The first detailed description of the retention process was proposed by Horváth and co-workers [1,2] called ''solvophobic theory'' based on the solvophobic theory of Sinanoglu developed to describe the binary association process of two solute molecules in a single solvent [3].

The solvophobic theory considers that the free energy change depends on the change of solute size cavities in the mobile phase only and treats the stationary phase as a passive entity that plays no role in the separation process other than providing a sorptive site for retention. The failure of the sol-

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vophobic theory to account for differences on the type and chain density of the bonded hydrocarbon phase arises from its reliance on an incorrect model of the relevant solution process. It supposes that retention can be modeled in terms of association of two solute molecules in a single solvent rather than on the transfer of a solute from one solvent to another [3].

The difficulty in providing a descriptive model of the interactions associated with solute retention lies in the complexity of the system. The bonded phase is a structurally complex surface presented by the highly porous silica support and the solvation layer. It has various contributions from the chemistry of the silica surface, the chemically bonded species and the solvent sorbed in the interphase region. All of these provide varying interactions with solutes of widely different polarity and geometry in chromatographic separations. The three-dimensional structure of the surface solvated layer acts as the stationary phase in terms of a temperature- and solvation-dependent change in conformation of the bonded hydrocarbon moieties.

A statistical mechanical lattice model was developed by Martire and Boehm [4] describing chain organization in the stationary phase as similar to a liquid crystalline material. Refinements to the lattice-interphase model were presented by Dill and coworkers [5–7] in terms of the surface anchored chains and their configurational entropy. According to these theories two driving forces dominate the retention process: (i) the difference in the chemistry of the contacts of the solute with its surrounding molecular neighbors in the stationary and mobile phase and (ii) the partial ordering of the grafted chains which leads to an entropic expulsion of solute relative to that which would be expected in simpler a amorphous oil phase/water partitioning process. The model of Dill [5] provides a more satisfactory treatment of (ii), but both approaches are similar in their accounting for (i), the solute contact interactions.

The thermodynamic properties of any physico–chemical system are, in fact, bulk properties of the system. Their magnitude represents the combination of a number of different effects that may take place on the molecular scale. Since the excess free enthalpy or excess free entropy cannot be allotted to any specific stationary or mobile phase effect, this renders the use of thermodynamic data useless for the prediction of retention or selectivity.

To avoid the ambiguity of bulk property data, attempts have been made to relate the molecular properties of the solute and phase system to retention and selectivity. The molecular interaction model suggests that distribution occurs between two phases as a result of the different molecular forces that exist between the solute molecule and the molecules of the two phases. Those solute molecules that experience stronger forces between them and the stationary phase will be retained longer than those molecules that experience stronger interactions with the mobile phase. This approach has been investigated both in normal- and reversed-phase chromatography to describe the effect of the type and composition of the mobile phase on retention [8–10].

The first approach to incorporate molecular interactions in the description of chromatographic retention and selectivity was the solubility parameter concept introduced originally by Hildebrand et al. [11] to cover regular solutions. The solubility parameter can be related to retention parameters by use of the activity coefficients. With some simplifying assumptions the retention factor can be described in terms of solubility factors derived for the solute, stationary phase and mobile phase [12]. The multicomponent solubility parameter model takes into account the different types of interactions, i.e., dispersion, dipole orientation, dipole induction and exchanges of electrons and protons between molecules. The total solubility parameter can be described as the combination of these different contributions [13,14]. Although the solubility parameter concept much contributed to the understanding of retention behavior and selectivity in high-performance liquid chromatography (HPLC), because of the simplifying assumptions and the uncertainty of the partial polarities and of their dependence on the operating conditions, it is regarded as describing chromatographic behavior only qualitatively, and fails to predict retention and selectivity in practical applications.

The molecular interaction models are recently called quantitative structure–retention relationships (QSRRs) derived by means of various statistical procedures [15–17]. There are theoretical approaches

aimed at the prediction of RPLC retention using the substituent/or fragmental contribution to retention parameters [18–20]. Several QSRR equations have been reported which contain molecular descriptors of obscure physico–chemical meaning difficult to assign to molecular properties. QSRRs that are not interpretable in physical terms are not very informative regarding the mechanism of retention.

Two main approaches to QSRRs are reported [21]. One method employs structural descriptors, provided solely by calculation chemistry as independent variables in QSRR equations. A large number of various structural descriptors have been tested describing RPLC retention [17,22–25]. A typical strategy is to generate a multitude of solute descriptor that are next regressed against retention data. Observing the statistical rules, one selects the minimum number of descriptors needed to produce an equation yielding the calculated retention data in satisfactory agreement with the observed values [26,27]. It is often difficult to assign any physical sense to some parameters. It is even more difficult to interpret QSRR equations consisting of terms produced by various transformations or combinations of such descriptors [18,21].

The second approach is the application of the linear solvation energy relationships (LSERs) by using the solvation parameters determined experimentally to describe the various molecular properties of the solutes to be separated. We shall use this approach to study the effect of molecular interactions on retention and selectivity in RPLC.

2. Theoretical

The solvatochromic model was developed by Kamlet and co-workers to characterize solute–solvent interactions in different chemical processes [28–34]. The solvatochromic parameters derived by spectroscopic measurements serve to describe the different molecular interactions, i.e., cavity formation and dispersion interaction; dipolarity/polarizability; hydrogen-bond-acceptor strength (basicity); and hydrogen-bond-donor strength (acidity). The solvatochromic model and the derived LSER have been used extensively to study different distribution processes among them to describe gas chromatography (GC) [35–39] and HPLC [40–46] separations. The solute's solvatochromic properties were derived from solvent solvatochromic measurements of the absorption bands for a series of indicator compounds [28–32]. Difficulties arose because of the lack of solvatochromic parameters for less common solvents and because a huge number of solute parameters had to be estimated from a very small solvent database. Several approaches have been proposed for measurement, calculation or estimation of solute descriptors. For this reason there are fairly large differences between solvatochromic parameters published by different authors [34,44,47–49]. In addition, because the solvent parameters were derived from UV–visible shifts and other spectroscopic measurements, they are not thermodynamic parameters [50].

Abraham and co-workers have introduced new solute parameters called solvation parameters derived from equilibrium measurements on the solutes themselves such as GC data, water–solvent partition coefficients and data relating to the molecular structure [50,53]. These solute descriptors are thermodynamic Gibbs energy related quantities and they are the correct parameters to be used in the LSER equation to describe Gibbs energy related data such as chromatographic retention. The model postulates that retention results from the differential interactions of a solute with the two phases. Variation of retention factors with solute structure can be related to their tendencies to participate in different molecular interactions.

The methodology is in principle the same as that of the Kamlet–Taft system. In HPLC the logarithmic retention factor, log k, can be correlated with the various fundamental solute descriptor properties, and can be determined by multivariate linear regression of the solvation parameters characteristic of various molecular interactions. The main advantage of this model is that the phase transfer process can be separated into several terms characteristic of different molecular interactions.

The multivariate linear regression equation introduced by Abraham et al. to describe chromatographic retention reads:

$$\log k = c + rR_2 + s\pi_2^* + a\sum\alpha_2^{\mathrm{H}} + b\sum\beta_2^{\mathrm{H}} + vV_{\mathrm{x}} \quad (1)$$

where the solvation parameters denote specific solute

properties, for example R_2 is excess molar refraction; π_2^* is solute dipolarity/polarizability; $\Sigma\alpha_2^H$ is the overall or effective hydrogen-bond (HB)-donor acidity; $\Sigma\beta_2^H$ is the overall or effective HB-acceptor basicity; V_x is the McGowan characteristic volume; and c is the intercept. Solvation parameters for more than 3000 compounds can be found in the literature [50–53].

The coefficients in Eq. (1) can be determined by multivariate regression analysis and characterize the phase system investigated. The r is a measure of the propensity of the phase to interact with solute n- and π-electron pairs; s measures the phase dipolarity/polarizability; a is a measure of the phase HB-acceptor-basicity; b is a measure of the phase HB-donor-acidity; v is a measure of the phase hydrophobicity. If Eq. (1) is applied to the distribution between two phases as in HPLC, the coefficients will refer to differences in various properties between the phases concerned.

In addition to the retention factor, the selectivity of separation can also be determined. By using the regression coefficients obtained for a given phase system, the α selectivity factor for any two compounds (j and i) can be calculated:

$$\begin{aligned}\log \alpha &= \log k_j/k_i = \log k_j - \log k_i \\ &= r(R_{2j} - R_{2i}) + s(\pi_{2j}^* - \pi_{2i}^*) + a(\sum \alpha_{2j}^H \\ &\quad - \sum \alpha_{2i}^H) + b(\sum \beta_{2j}^H - \sum \beta_{2i}^H) + v(V_{xj} - V_{xi})\end{aligned} \tag{2}$$

In recent years several studies have been published to study HPLC retention and characterize different reversed-phase (RP) stationary phases by the LSER method [54–62] with good results.

Quite recently studies have been published also examining the application of the LSER relationships under varying mobile phase composition and for different types of columns [50,57,63–65]. Since the volume and composition of the sorbed solvent layer depend on the type of the organic modifier and the composition of the mobile phase, these properties will also influence the molecular interactions. The main advantage of the LSER approach relies in its ability to measure independently the contribution of individual molecular interactions to the retention process. The overall applicability of the LSER model to describe retention RPLC was proved in the above publications by regression statistics and the corresponding statistical descriptors (correlation coefficients, Fisher-test, standard deviation) showing very good fits.

In addition to the stationary phase and the mobile phase, the molecular properties of the solutes to be separated will also influence the type and extent of the various molecular interactions in the retention process. This factor has not been taken into account up to now in the studies and publications on the application of the LSER. The effect of the individual terms on the retention factor was investigated by a statistical method giving the % variance of log k accounted for by each term of the LSER equation [56,64]. Apart from the statistical validity of this approach because the covariances among the variables are not insignificant and cannot be neglected, the calculation was made for a large number of solutes with different solvation parameters—most of them having 0 proton donor property—and the % contributions obtained for the individual terms of the LSER equation reflect an average value for the solutes investigated.

In this publication we shall demonstrate that the retention and selectivity in chromatographic separation depend on the characteristics and molecular properties of all participants of the chromatographic system as:

(1) Characteristics of the stationary phase.

(2) Type of organic modifier and composition of the mobile phase.

(3) Molecular properties (solvation parameters) of the solutes to be separated.

3. Experimental

To study the effect of column characteristics on molecular interactions we have selected six columns of widely different properties from our set investigated, see Ref. [60]. The selected columns and their main characteristics as provided by the manufacturers, are listed in Table 1. The first column is a quasi-neutral, well-covered C_{18} column. The second one is a specialty column for polynuclear aromatic hydrocarbon (PAH) separation prepared by poly-

Table 1
Characteristics of the columns

Column	LiChrospher 100 RP-18e	LiChrospher PAH	SymmetryShield RP-C18	SymmetryShield RP-C8	LiChrosorb RP-select B	LiChrospher 100 RP-8
Symbol	M-C18e	M-PAH	SYM-C18	SYM-C8	M-RP-B	M-C8
Manufacturer	Merck (Darmstadt, Germany)	Merck	Waters (Milford, MA, USA)	Waters	Merck	Merck
Dimensions (mm×mm I.D.)	125×4.0	250×3.0	150×3.9	150×3.9	125×4.0	125×4.0
Particle size (μm)	5.0	5.0	5.0	5.0	5.0	5.0
Pore size (nm)	10	15	10	10	6	10
Surface area ($m^2\ g^{-1}$)	350	200	340	340	300	350
Ligand type	C18	C18	C18+carbamate	C8+carbamate	C8	C8
Bonded phase	Monomeric	Polymeric	Monomeric	Monomeric	Monomeric	Monomeric
% C	21.6	20.0	17.6	14.6	11.4	12.5
Endcapping	+	−	+	+	+	−

meric coverage. The third and fourth ones are ''polar'' SymmetryShield reversed-phases containing carbamate groups embedded into the alkyl ligands [66]. The fifth one is an acid-washed C_8 phase with high surface coverage (4.21 μmol m^{-2}), optimized for separation of basic solutes. Finally, the sixth one is a non-endcapped C_8 column showing both acidic and basic properties. Experimental conditions, list of the solutes and the corresponding solvation parameters are given in Ref. [54].

In order to study the effect of the type and composition of the mobile phase on molecular interactions we have selected three columns of different properties from our set investigated in Ref. [65], which figure as column Nos. 1, 4 and 6 in Table 1. Experimental conditions and the log k values measured are given in Refs. [55] and [65].

The effect of solute properties on the extent and proportion of the various molecular interactions was investigated by selection of eight compounds of different structure and molecular properties using principal component analysis (PCA) from the data set obtained for 15 columns [60]. The factor loadings of the selected solutes and their solvation parameters (molecular descriptors) are given in Table 2. in order to indicate the relative importance of the individual

Table 2
PCA loadings and solvation parameters of selected solutes

	Toluene (T)	Ethylbenzene (EB)	*p*-Cresol (PCR)	Methylparaben (MP)	Caffeine (CAF)	Pyridine (PYR)	Aniline (A)	Phenol (P)
PC1	**0.776**	**0.796**	0.592	0.541	0.269	0.378	0.577	0.507
PC2	0.471	0.442	**0.658**	**0.724**	0.284	0.245	**0.553**	**0.713**
PC3	0.359	0.355	0.408	0.402	**0.905**	**0.805**	**0.554**	0.431
R_2	0.601	0.613	0.820	0.900	1.500	0.631	0.955	0.805
π_2^*	0.52	0.51	0.87	1.37	1.60	0.84	0.96	0.89
$\Sigma\alpha_2^H$	0	0	0.57	0.69	0	0	0.26	0.60
$\Sigma\beta_2^H$	0.14	0.15	0.32	0.45	1.33	0.52	0.50	0.30
V_x	0.857	0.998	0.916	1.131	1.363	0.675	0.816	0.775

molecular properties on the retention process. The selection and molecular properties of the above solutes will be discussed in Section 4.3.

4. Results and discussion

4.1. Effect of the characteristics of the stationary phase

The characteristics of the stationary phase were determined from measurements on the selected columns using acetonitrile–water (30:70, v/v) mobile phase. The selection of this mobile phase was explained elsewhere [62]. The LSER regression equations were calculated using log k values as dependent variable and solvation parameters of the solutes as independent variable set.

The resulting regression coefficients and the corresponding statistical descriptors were also given [54]. The goodness-of-fit of the equations were very good ($R>0.98$), the validity of regression hypothesis were proved for all columns by the Fisher F-test. These statistical indicator values confirmed that the LSER model adequately described retention even for widely different stationary phases. The numerical range of the various coefficients were in good agreement with values reported for octadecyl and octyl columns [50,56,59,63,64].

As it was discussed in the Theoretical section, the regression coefficients account for differences in a particular interaction involved in the stationary phase and mobile phase. A positive sign in Eq. (1) would indicate that the respective molecular interaction is stronger in the stationary phase than in the mobile phase. This is the case for r and v coefficients, where r arises from n- and π-electron interactions, while v measures the combination of cavity formation and dispersive interactions. On the other side, coefficients with negative sign (s, a and b) correspond to molecular forces that act favorably in the mobile phase, and therefore decrease retention. Dipolar-type forces and HB-donor, HB-acceptor interactions fall into this category.

In order to evaluate stationary phase properties a term-by-term analysis of the regression coefficients will be provided. The corresponding statistical evaluation is given in Ref. [54]. In the 95% confidence interval the average error for the larger coefficients (v, b) was ±5–10%, for the smaller coefficients (r, a, s) ±10–15%.

In Fig. 1a the positive coefficients favoring the stationary phase are shown for all columns. The c intersect is near to zero, but should also be taken into account by applying Eq. (1).

The coefficient r in Eq. (1) refers to the difference between the solvated bonded stationary and mobile phase to interact with solute n- and π-electrons. The positive r obtained for all columns indicates that electron-involved interactions are slightly stronger in the stationary phase than in the mobile phase. The significantly larger r coefficients obtained for SymmetryShield columns suggest that solutes capable of donating n- and π-electrons are longer retained on these specialty columns.

The coefficient v represents the difference in

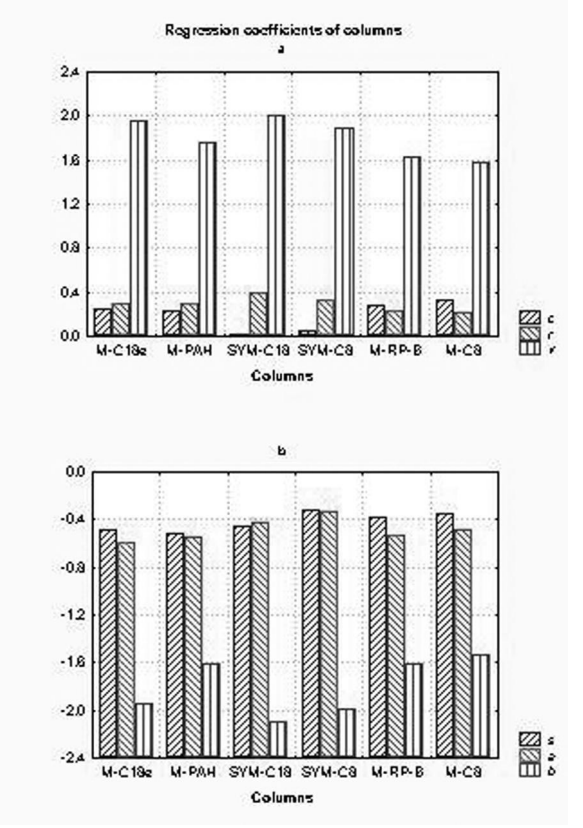

Fig. 1. Regression coefficients of the columns investigated. (a) Positive coefficients: c, r, v. (b) Negative coefficients: s, a, b.

hydrophobicity (hydrophobic strength) between the stationary and mobile phase, which depends on the difference in cohesivity of the two phases and the extent of dispersive interactions between the solute and the bonded and mobile phase, respectively. It is expected that columns of longer alkyl chain or more excessively covered surface can be identified by their greater v coefficients. In fact, as can be seen in Fig. 1a the two C_{18} columns with the highest retentive capacity are characterized by greater v coefficients than the C_8 columns. The M-PAH column presumably due to its smaller surface area and the polymeric coverage shows lower v coefficient and consequently lower hydrophobic strength than the other C_{18} columns. The v coefficient and the corresponding term in Eq. (1) is the dominating parameter to define chromatographic retention.

In Fig. 1b the negative coefficients of Eq. (1), s, a and b are shown which act favorably in the mobile phase and therefore decrease solute retention. The height of the bars indicate the actual value of the coefficients concerned. The less negative values indicate the less difference in the actual property between the stationary and mobile phase.

Difference between stationary and mobile phase dipolarity–polarizability is measured through the s coefficient. The mobile phase is highly dipolar because its components water and acetonitrile are strongly dipolar substances. Although the bonded alkyl chains are almost incapable of dipole interactions, the sorbed mobile phase components may substantially increase its dipolarity. In addition accessible silanol groups on the packings and polar compounds built into the ligands can contribute to the polarity of the stationary phase. The less negative s coefficients mean increased polarity of the stationary phase, i.e., less difference between the polarity of the stationary and mobile phase. In Fig. 1b it can be seen that the C_8 columns are more dipolar than the C_{18} columns due to their lower coverage and more accessibility of surface silanol groups. However, the s values and the differences among the columns are relatively small and have no decisive influence on retention.

The coefficient a reflects the difference in hydrogen-bond acceptor (HBA) basicity of the stationary and mobile phase. The mobile phase components have some HBA basicity indicated by their β_1 solvation parameters (water $\beta_1 = 0.43$; acetonitrile $\beta_1 = 0.37$). Since the mobile phase will sorb on the packing both the mobile phase and the stationary phase will have some basicity resulting in small negative a coefficients. Here again, the less negative coefficients mean increased HBA basicity of the stationary phase. SymmetryShield columns exhibited stronger HBA activity than other columns as a consequence of the pronounced basic character of the carbamate functionality built into the ligands. From among the other columns the C_8 columns show somewhat higher basicity than the C_{18} columns.

The coefficient b reflects the difference in hydrogen-bond donor (HBD) acidity between the stationary and mobile phase. The aqueous mobile phase has strong HBD acidity shown by the solvation parameters α_1 (water $\alpha_1 = 1.17$; acetonitrile $\alpha_1 = 0.19$). The reversed-phase packing materials exhibit considerably smaller HBD acidity originating mostly from water molecules sorbed in the interphase region and accessible silanol sites. Thus, large negative b values are obtained for the less acidic stationary phases, whereas smaller negative coefficients reveal increasing column acidity. The least acidic columns were the SymmetryShield phases due to the embedded carbamate groups followed by the well covered endcapped M-C18e column. The highest HBD acidity was shown by the non-endcapped M-C8 column followed closely by the M-RP-B and M-PAH columns. On the M-PAH column the different bonding chemistry seems to play a decisive role in affecting bonded phase HBD acidity. Among the polar forces the b coefficient and the corresponding term in Eq. (1) is the dominating parameter to influence chromatographic retention.

The separation power of a column can be characterized by the selectivity of separation. The overall selectivity comprises the combined effects of several different mechanisms because of the different types of molecular interactions. The contribution and extent of these interactions depend on all participants of the chromatographic system. Now we intend to demonstrate the contribution of the stationary phase to the molecular interactions involved in the separation process.

In order to take into account different types of interactions we have defined and used different selectivities in the comparison of stationary phases

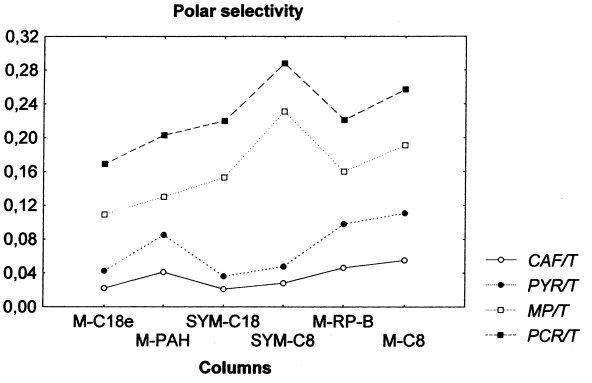

Fig. 2. Polar selectivity of columns for selected pairs of solutes.

[49,54,65]. The various selectivity factors determined by using Eq. (2) are shown in Figs. 2 and 3.

Polar or chemical selectivity comes about from polar interactions as hydrogen bonding (HB), dipole or ionic interactions. The magnitude of the overall or composite polar interactions can be characterized by the relative retention of polar solutes to that of a nonpolar solute, e.g., toluene [54,55]. The higher the α, the stronger is the polar solute retained compared to toluene. In Fig. 2 the polar selectivity factors obtained for two basic (CAF, PYR) and two acidic (MP, PCR) compounds are presented for the columns investigated. For the basic solutes higher polar (HBD) activity is shown for the most acidic (M-PAH, M-RP-B, M-C8) columns (see Fig. 1b). For the acidic solutes the relatively more basic SymmetryShield columns and less covered the C_8 columns exhibit stronger HBA activity.

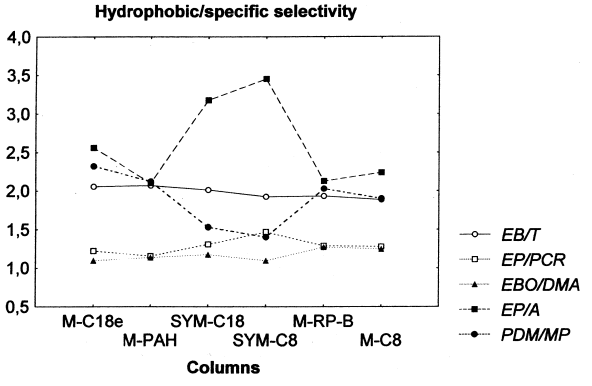

Fig. 3. Hydrophobic and specific selectivity of columns for selected pairs of solutes.

Hydrophobic or methylene selectivity is defined as the relative retention of the adjacent members of homologous series differing only in one CH_2 group. Methylene selectivity depends on the extent of hydrophobic interaction between the stationary phase and the compounds investigated. Methylene selectivity increases with the hydrophobic strength of the column [67–70]. In Fig. 3 the hydrophobic selectivity of the columns defined as the relative retention of ethylbenzene (EB) and toluene (T) are shown. In accordance with the magnitude of the v coefficients the C_8 columns exhibit lower hydrophobic selectivity than the C_{18} columns [54,55].

The relative retention of various polar solutes can be defined as ''*specific selectivity*'' because it depends considerably on the acidic or basic properties of the solutes to be separated. In addition, it depends also on the acidic or basic properties of the columns investigated. In Fig. 3 the relative retention of one acidic–acidic: ethylparaben/*p*-cresol (EP/PCR), one basic–basic: ethylbenzoate/dimethylaniline (EBO/DMA), one acidic–basic: ethylparaben/aniline (EP/A) and one basic–acidic: dimethyl phtalate/methylparaben (DMP/MP) pairs of solutes are presented for all columns For the acidic–acidic solute pair (EP/PCR) the SymmetryShield columns and the less covered C_8 columns show somewhat higher selectivity. For the basic–basic solute pair (EBO/DMA) also the C_8 columns show higher selectivity, but the SymmetryShield columns show similar selectivities than the C_{18} columns. For the acidic–basic solute pair (EP/A) the SymmetryShield columns produced much higher selectivity factors than the other columns because of the preferential retention of acidic solutes. In the contrary, for the basic–acidic solute pair (PDM/MP) these columns furnished considerably lower selectivity factors showing again the preferential retention of acidic compounds.

4.2. Effect of the type and composition of the mobile phase

In RPLC the most widely used organic modifiers in the mobile phase are the acetonitrile (ACN) and methanol (MeOH). In this section we wish to demonstrate the effect of the above organic modifiers and the composition of the mobile phase on the

molecular interactions governing retention and selectivity in chromatographic separation.

As was discussed in Section 2, regression coefficients will characterize the difference in certain interactions between the stationary and mobile phase. When the composition of the mobile phase is varied, the characteristics of both the mobile phase and stationary phase will vary resulting in changes in the regression coefficients. For this reason the regression coefficients should be determined for all mobile phase compositions. In Fig. 4 the change of the regression coefficients with varying ACN% is shown measured on the M-C18e column. Fig. 4a represents the positive, retention increasing coefficients (*c*, *r*, *v*), Fig. 4b the negative, retention decreasing coefficients (*s*, *a*, *b*).

As regards the positive coefficients *r* exhibit small increase with increasing water content of the mobile phase while the *v* coefficient representing the difference in hydrophobicity between the stationary phase and the mobile phase increases considerably by increasing water content. As the alkyl bonded phase is far less cohesive than the water rich mobile phase [56,71,72], a greater amount of free energy is required to create solute size cavity in the mobile phase compared to that in the stationary phase. This leads to stronger and increasing dispersion interactions between the alkyl bonded phase and the solute than between the aqueous mobile phase and the solute with increasing water content. The dependence of the *v* coefficient on mobile phase composition can be approximated by linear correlation [65,73].

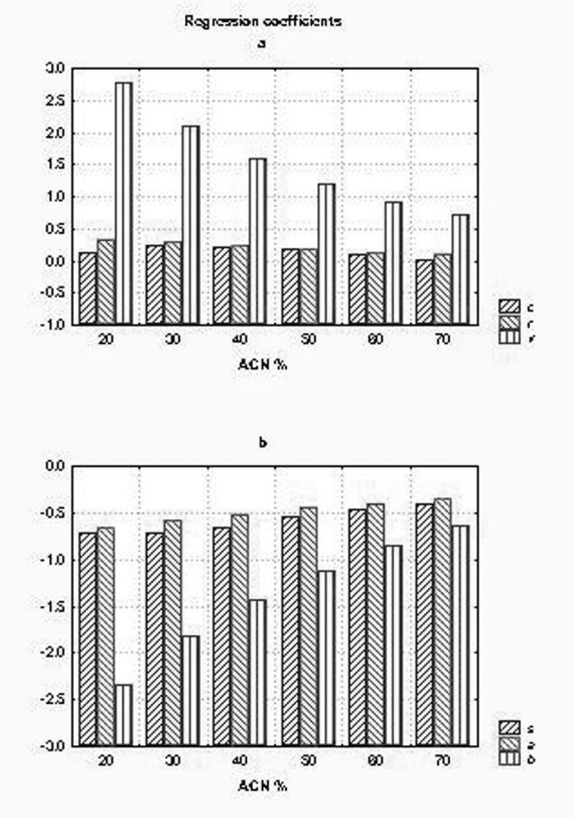

Fig. 4. Change of the regression coefficients with mobile phase composition with acetonitrile (ACN) modifier. (a) Positive coefficients: *c*, *r*, *v*. (b) Negative coefficients: *s*, *a*, *b*.

From among the negative coefficients (Fig. 4b) the *s* coefficient representing the difference in dipolarity/polarizability between the stationary phase and the *a* coefficient reflecting the difference in HBA basicity between the stationary and mobile phase exhibit small decrease with increasing water content, indicating increased differences in both properties between the two phases. There are much larger changes in the *b* coefficient, reflecting the difference in HBD acidity between the stationary and mobile phases. The aqueous mobile phase has strong HBD acidity [50,56,60] which increases with increasing water content. The bonded phase materials exhibit considerably smaller HBD acidity originating from water molecules sorbed in the interphase region and accessible silanol sites. For this reason the *b* coefficients are always negative and the absolute values considerably increase by increasing water content of the mobile phase.

In Fig. 5 the variation of the regression coefficients with mobile phase composition is shown for MEOH modifier. The values of *r* coefficients show small increase with increasing water content. The magnitude of the *v* coefficient is considerably higher at all compositions than in the ACN system but its variation is quite similar. As it was discussed before the *v* coefficient depends on the polarity of the mobile phase. In accordance with the higher polarity of methanol, the *v* coefficient and correspondingly the retention factor is higher for methanol than for acetonitrile, for a given mobile phase composition.

The negative coefficients obtained in MEOH system (Fig. 5b) show similar values and similar

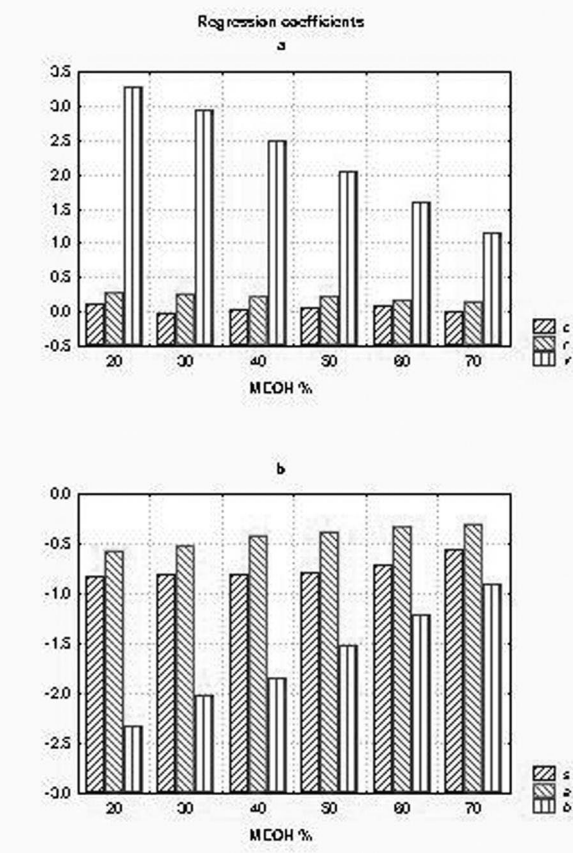

Fig. 5. Change of the regression coefficients with mobile phase composition with methanol (MEOH) modifier. (a) Positive coefficients: *c*, *r*, *v*. (b) Negative coefficients: *s*, *a*, *b*.

changes as in the ACN system. Coefficients *s* and *a* exhibit small decrease with increasing water content as explained before. The *b* coefficients show somewhat higher differences in HBD acidity between the stationary and mobile phases as a consequence of the higher acidity of methanol (ACN $\alpha_1 = 0.19$; MeOH $\alpha_1 = 0.93$).

In both mobile phase system the ratio and consequently the relative importance of the various coefficients changes considerably with mobile phase composition influencing the extent and relative contribution of the different molecular interactions to chromatographic retention.

Next we investigate the effect of the type and composition of the mobile phase on the selectivity of separation by determining the various selectivity

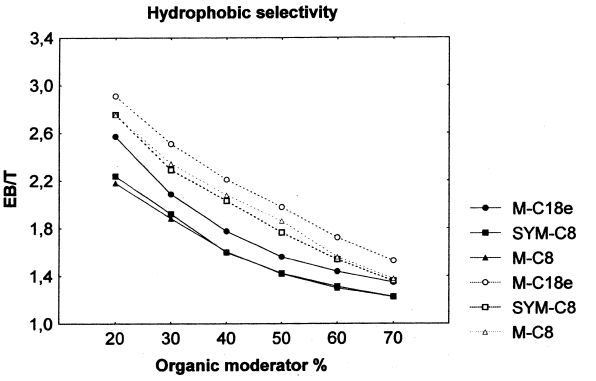

Fig. 6. Effect of mobile phase composition on hydrophobic selectivity. ——— ACN modifier; --------- MEOH modifier.

factors representing different molecular interactions as discussed in the previous section. In Fig. 6 the effect of the mobile phase composition is shown on the *hydrophobic/methylene selectivity* demonstrated on three widely different columns in both mobile phase system. It has been established that methylene selectivity depends on the hydrophobic strength of the column and the difference in hydrophobicity between the stationary phase and the mobile phase. For this reason methylene selectivity increases with increasing water content as a consequence of the increasing difference between the hydrophobicity of the two phases represented by the increasing *v* coefficient as discussed before. In accordance with the higher polarity of MEOH than ACN, higher *v* and consequently higher $\alpha_{EB/T}$ values are obtained with the MeOH modifier. As regards the hydrophobic strength of the column, C_{18} columns provide higher methylene selectivity than C_8 columns [54].

Polar or chemical selectivity characterize the effect of the various polar interactions on the retention process. To describe the magnitude of the overall polar interactions we have used the relative retention of polar solutes to that of a nonpolar solute, e.g., toluene [54,55]. The higher the relative retention the stronger is the polar solute retained compared to toluene. In Fig. 7 the relative retentions of two basic solutes (CAF, PYR) and two acidic solutes (MP, PCR) are shown as a function of mobile phase composition for both organic modifier. Polar selectivity, i.e., the relative retention of polar solutes to that of toluene decreases with increasing water

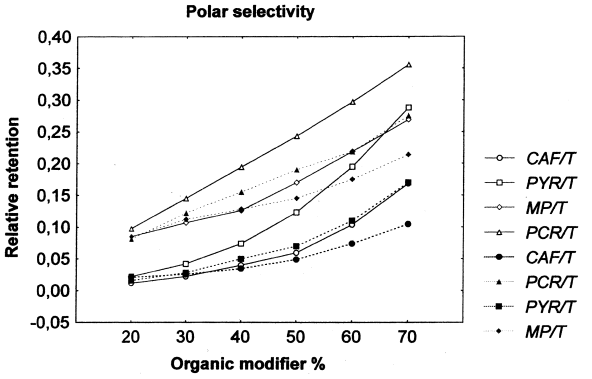

Fig. 7. Effect of mobile phase composition on polar selectivity. ——— ACN modifier; --------- MEOH modifier.

content because by increasing the polarity of the mobile phase, the retention of polar solutes decreases more rapidly than that of the nonpolar (toluene) solute. Since the polar selectivity depends to great extent on the polarity of the mobile phase, acetonitrile as the less polar organic modifier provides higher polar selectivities than methanol.

The relative retention of various polar solutes can be defined as *specific selectivity*. It depends considerably on the acidic or basic properties of the solutes to be separated. Specific selectivity comes about as a composite of the various polar interactions in the given phase system. Specific selectivity depends on the acidic or basic properties of the columns as was discussed in Section 4.1. In addition, it depends also on the type of the organic modifier and the composition of the mobile phase. In Fig. 8

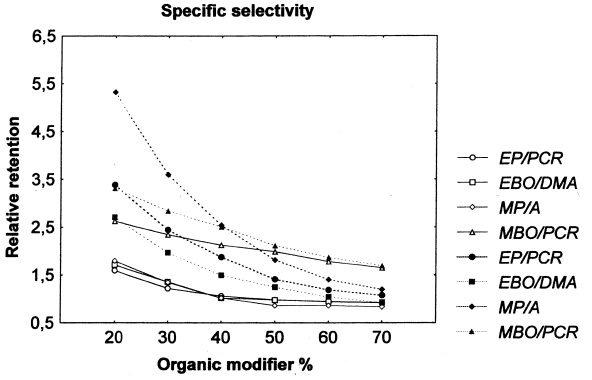

Fig. 8. Effect of mobile phase composition on specific selectivity. ——— ACN modifier; --------- MEOH modifier.

the relative retentions of solute pairs with different molecular properties are shown for both mobile phase system measured on the M-C18e column. These are as follows, ethylparaben/*p*-cresol (EP/PCR: acidic–acidic); ethylbenzoate/dimethyl aniline (EBO/DMA: basic–basic); methylparaben/aniline (MP/A: acidic–basic) and methylbenzoate/*p*-cresol (MBO/PCR: basic–acidic). For all solute pairs and all columns investigated it can be generally established that the specific selectivity increases with increasing water content, i.e., with increasing polarity of the mobile phase. For this reason the more polar methanol provides higher specific selectivities than acetonitrile.

4.3. Effect of the molecular properties of the solutes to be separated

The third participants of the phase system are the solutes to be separated with their different structures and molecular properties. Up to now this factor has not been taken into account in the description of chromatographic separation and retention mechanism. The retention process can be visualized that the stationary phase and the mobile phase compete for the retention of the solutes. The larger is the difference in the molecular forces acting in the stationary phase and mobile phase, respectively, the larger will be the extent of retention, i.e., the k retention factor. Quite obviously the role and extent of these forces depend considerably on the molecular properties and structures of the solutes to be separated.

In order to study the influence of the molecular properties of the solutes from among the solutes investigated eight solutes have been selected having different molecular properties. In an earlier study we applied PCA to characterize a column set comprising of 15 different RPLC columns [60]. We used the retention factors of 33 solutes measured on the 15 columns as input data. The principal component (PC) extraction was followed by VARIMAX rotation to yield an easier interpretable PC structure. The first three extracted PCs represented about 94% of the original variance in the retention data matrix. From the table containing the factor loadings [60] we have selected eight compounds of different types to illustrate the effect of solute structure on the re-

tention process. The factor loadings of the selected compounds and their solvation parameters (molecular descriptors) are given in Table 2. The factor loadings shown in the table can be regarded as correlation coefficients between the retention of the test solutes and the respective PC. A general characteristic of most factor analytical techniques is that each solute contributes with smaller or larger extent to each factor as a consequence of the possibility of different types of molecular interactions. Thus, with PCA individual interactions governing retention process on a RPLC column cannot be unravelled. However, on the basis of PCA solutes can be selected for which particular molecular interactions will have similar effects on chromatographic retention.

In our case PC1 extracted about 41% of original variance and was the most important factor to influence retention. Strongly retained test solutes with pronounced hydrophobic character displayed the highest loadings on PC1. From this group we have selected T and EB for further investigation.

PC2 with about 34% variance was found of commeasurable importance to PC1. As more than the half of 33 test solutes were carrying phenolic OH groups, the relatively high importance of these acidic solutes is not surprising. We have selected two from among the most acidic solutes (MP, PCR) for investigation.

PC3 accounted for smaller portion about 20% of variance and includes basic compounds, like CAF and PYR with considerable weight.

We have selected two other compounds, A and P, which show both basic and acidic properties.

In Table 2 the solvation parameters characterize the relative importance of molecular properties. As we have seen, in the LSER model (Eq. (1)) the products of the solvation parameters and the regression coefficients characterizing the phase system will furnish the contribution of the individual terms to the log k values. For this reason the molecular properties of the solutes should influence the retention process.

In the next figures the relative contribution of the individual terms of the LSER equation to the retention factor (log k) will be shown. For the sake of comparison of course the absolute values of the individual terms are presented.

In Fig. 9 the contribution of the individual terms

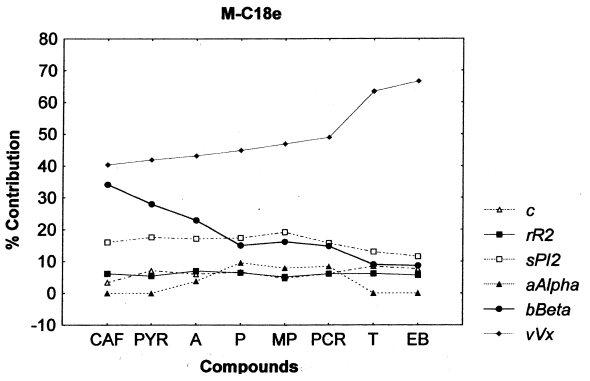

Fig. 9. Contribution of the individual terms of the LSER equation to retention of solutes of different molecular properties on M-C18e column.

of Eq. (1) to the retention process is presented on the M-C18e column and for the eight compounds selected. It can be seen that for all compounds the vV_x term representing hydrophobic interactions dominates the retention process. However, while for the nonpolar T and EB this contribution is over 60%, by increasing polarity of the solutes this contribution decreases to 40%.

The second most important term $b\beta$ is the product of the b coefficient representing the difference in the HBD acidity between the stationary and mobile phase and the $\Sigma\beta_2^H$ solvation parameter describing the HBA basicity of the solutes. The contribution of this term is over 30% for the most polar solutes and decreasing by decreasing polarity of the solutes investigated. For the nonpolar T and EB its contribution is only about 10%. The third term is in order of succession the product of the s coefficient representing the difference in the dipolarity/polarizability of the two phases and the π^* solvation parameter describing the polarity of the solutes. Its contribution for polar solutes is nearly 20%, decreasing to 10–12% for the nonpolar T and EB.

The product of the a coefficient ($a\alpha$) representing the difference in the HBA basicity between the two phases and the $\Sigma\alpha_2^H$ solvation parameter describing the HBD acidity of the solutes is naturally zero for those solutes where $\Sigma\alpha_2^H = 0$, i.e., can act only as acceptors in the hydrogen bond. For the solutes with acidic properties (P, MP, PCR) its contribution is about 8–10%.

The rR_2 term representing interactions with n- and π-electrons is practically the same for the various solutes and presents about 5–6% contribution to the retention process.

The c intersect represents 2–5% of the contributions.

In Fig. 10 the % contributions of the individual terms are shown on the SYM-C8 column containing carbamate groups. Also on this special column the hydrophobic interaction (vV_x) dominates, reaching 70% contributions for the nonpolar T and EB and decreasing to 40% by increasing polarity of the solutes. The second most important term is also on this column the $b\beta$ product showing somewhat higher contribution for the polar solutes (38→18%) than on the M-C18e column and the same about 10% contribution for the nonpolar T and EB.

The contribution of dipolarity/polarizability ($s\pi^2$) is somewhat lower on this column in accordance with the smaller absolute value of the s coefficient indicating smaller difference in dipolarity/polarizability between the stationary and mobile phase. This contribution is 13–18% for the polar solutes and decreases to 10% for the nonpolar solutes.

The effect of the $a\alpha$ term is somewhat lower for the relevant solutes ($\Sigma\alpha_2^H > 0$) with about 6–7% contribution. The electron interactions (rR^2) are also somewhat higher than on the M-C18e column with 8–9% contribution in accordance with the higher r coefficients measured on this column. The contribution of the c intersect is near to zero.

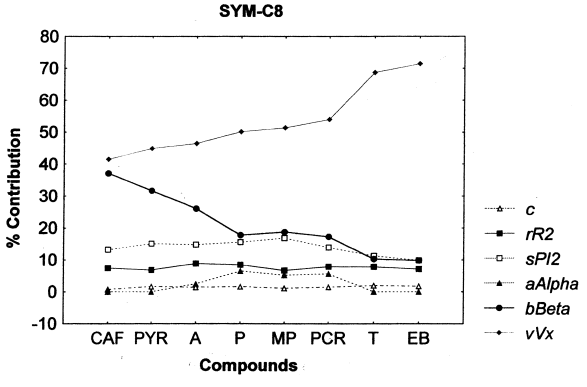

Fig. 10. Contribution of the individual terms of the LSER equation to retention of solutes of different molecular properties on SYM-C8 column.

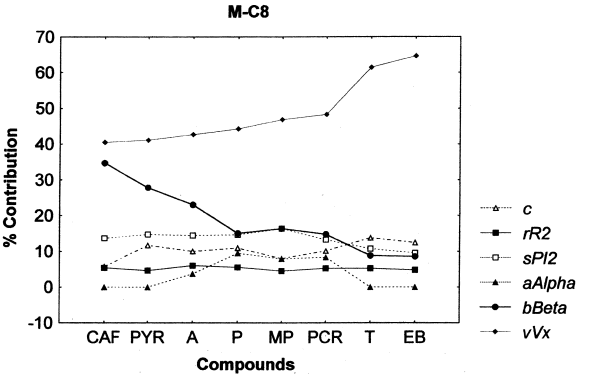

Fig. 11. Contribution of the individual terms of the LSER equation to retention of solutes of different molecular properties on M-C8 column.

In Fig. 11 a very similar picture of % contributions can be seen for the M-C8 column in spite of the different quality of this non-endcapped column. The contributions of the dominating hydrophobic interaction term (vV_x) and the HBA solvation parameter containing term ($b\beta$) are nearly the same and change similarly with changing polarity and hydrophobicity of the solutes investigated.

The contribution of dipolarity/polarizability is similar to that of the SYM-C8 column in accordance with their very similar s coefficients, showing 13–17% contribution for the polar solutes and about 10% for the nonpolar ones.

The contribution of the $a\alpha$ term for the relevant solutes is somewhat higher than on the SYM-C8 column in accordance with its somewhat lower (more negative) a coefficient showing 8–9% contribution.

The electron involved interactions are somewhat lower showing 4–6% contribution in accordance with the lowest r coefficient measured in this column.

5. Conclusions

The main advantage of the LSER model to describe chromatographic retention is that the change of Gibbs free energy can be described as the sum of the individual molecular interactions and furnishes

some insight into the mechanism of the retention process.

It has been demonstrated that the type and relative importance of the individual molecular interactions depend on all participants of the chromatographic system, i.e., the characteristics of the stationary phase, the type and composition of the mobile phase and the molecular properties of the solutes to be separated.

The characteristics of six widely different columns were evaluated and compared by using the regression coefficients of the LSER equation. In addition to differences in retention factors, the various selectivity terms as hydrophobic selectivity, polar selectivity and specific selectivity characterizing the separation of different types of solutes were also evaluated and compared.

The effect of the type and composition of the mobile phase were investigated by using acetonitrile and methanol as organic modifiers in a wide composition range (20–70% organic modifier). Changes in the mobile phase will influence significantly the values and proportion of the regression coefficients representing different types of interactions between the stationary and mobile phase. The various selectivity factors change also considerably with changing the type and composition of the mobile phase.

The effect of the molecular properties of the solutes to be separated was demonstrated by selecting eight solutes of different properties and comparing the % contribution of the individual terms of the LSER equations describing the different types of molecular interactions.

Acknowledgements

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