ELSEVIER

Journal of Chromatography A, 818 (1998) 19–30

JOURNAL OF CHROMATOGRAPHY A

Characterization of various reversed-phase columns using the linear free energy relationship
II. Evaluation of selectivity

Ákos Sándi, László Szepesy*

Department of Chemical Technology, Technical University of Budapest, Budafoki u. 8, H-1521 Budapest, Hungary

Received 14 April 1998; received in revised form 16 June 1998; accepted 17 June 1998

Abstract

Using the database presented in Part I of this series [J. Chromatogr. A, 818 (1998) 1] selectivity factors for various solute pairs with known linear free energy relationship (LFER) solvation parameters have been studied. Selectivity factors of selected solute pairs have been used to characterize hydrophobic properties and different types of molecular interactions of widely different reversed-phase columns. For reversed-phase packings evaluated under the same mobile phase composition differences in chromatographic selectivity can be attributed to the structure and characteristics of the stationary phase. Significant correlations have been found between the regression coefficients of the LFER equation and the different types of selectivity, as hydrophobic selectivity and polar selectivities. It has been established that hydrophobic selectivity is not identical to the hydrophobic strength of the column and depends on the structure and polarity of the homologous series used for calculation. Polar selectivity for different types of compounds depends on the propensity of the stationary phase to enter into polar (mainly hydrogen-bond donor and hydrogen-bond acceptor) interactions with the compounds investigated.

Keywords: Linear free energy relationships; Selectivity; Column characterization; Stationary phases, LC

1. Introduction

In Part I of this series [1], we have compared and evaluated retention factors measured under isocratic conditions acetonitrile–water (30:70) on 15 reversed-phase high-performance liquid chromatography (RP-HPLC) columns with widely different characteristics (narrow-pore and wide-pore silica supports, various ligands, different bonding chemistry) using 34 solutes. Column characteristics were determined and compared by principal component analysis (PCA) as well as applying the linear free energy relationship (LFER) or solvation equation derived by Abraham et al. [2–5]. The coefficients or constants in the solvation equation for each column were determined by multiple linear regression. These coefficients are characteristic of the phase systems, i.e., of a given RP-HPLC column with a particular mobile phase composition. If a number of columns are studied with the same mobile phase composition, the coefficients will characterize the various RP-HPLC columns.

In the literature several hundreds of publications can be found on characterization and comparison of reversed-phase columns. Most of these studies compare retention factors of arbitrarily selected test

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00536-6

solutes using different mobile phase compositions. It is well recognized that solute selectivity is chromatographically more important than is absolute retention. In some cases it is required to vary separation selectivity by changing the stationary phase rather than the components of the mobile phase [6]. However, it is frequently reported that the same column type from different manufacturers or even different lots of packings from the same manufacturer often show selectivity differences suggesting variations in the properties of the silica support used or the bonding chemistry applied.

Relative retention or selectivity have often been calculated and used to compare hydrophobic properties of the columns [7–9]. Kimata et al. [10] studied the relative retentions of various types of solutes in order to characterize silanol activity and different types of molecular interaction. Claessens and co-workers [11,12] have also used selectivity factors in evaluation and comparison of RP columns. Solute shape may also play an important role in retention of non-polar compounds. Sander and Wise [13,14] developed a simple empirical test to assess column shape selectivity based on the relative retention of three properly chosen polycyclic aromatic hydrocarbons. More recently, Cruz et al. [15] have reported the characterization and classification of 30 C_{18} columns commercially available, based on selectivity factors suggested by Kimata et al. [10], using various chemometric methods (PCA, cluster analysis and radar plots).

In present study we investigated the relationship between LFER phase system coefficients and different selectivity terms. Correlation analysis was found useful in selecting solute pairs representing different types of molecular interactions. Columns were evaluated on the basis of selectivity differences displayed.

2. Theoretical

Chromatographic selectivity or selectivity factor (α) is an important experimental probe in studies of the solute retention process. It reflects the difference between two solutes in terms of Gibbs free energy of transfer from the mobile phase to the stationary phase

$$\alpha = \frac{k_j}{K_i} \quad \text{and} \quad \ln \alpha = \frac{-\Delta(\Delta G)}{RT} \tag{1}$$

where k_i and k_j are the retention factors for solutes i and j, ΔG is the Gibbs energy, R is the gas constant and T is the absolute temperature. If the same mobile phase composition is used when comparing different stationary phases, mobile phase contributions to the free energy of transfer are equivalent, cancelling each other in the selectivity ratio. In such a case, selectivity is indicative of differences in the stationary phases [16,17]. Generally it is not recognized that selectivity is a complex phenomenon comprising of different mechanisms. The extent and importance of the different mechanisms depend on the phase system investigated and operating conditions. Antle et al. [18] stated that there are two types of RP column selectivity, hydrophobic (or solvophobic) and chemical (or polar) selectivity. A third type of selectivity, shape or steric selectivity can also be exhibited by chemically bonded phases.

Hydrophobic or methylene selectivity is generally taken as the relative retention of the adjacent members of homologous series differing only in one CH_2 group. Methylene selectivity depends on the hydrophobic interaction between the stationary phase and the compounds investigated. It has been reported that methylene selectivity increases with chain length of the ligand [19–22]. Correlations between selectivity and bonded-phase carbon loading [23,24] as well as with bonded-group surface coverage [17,25,26] have also been confirmed. In addition to the characteristics of the stationary phase, the type of the organic modifier and the mobile phase composition will also influence selectivity [27,28].

Chemical or polar selectivity comes about from strong interactions as hydrogen-bonding, dipole and ionic interactions, complexation between the solute molecules and specific active sites, such as silanol groups or trace metal contaminants on the silica surface [17,18,29]. These effects are relatively unimportant for non-polar solutes. In addition to the polarity of the stationary phase polar selectivity depends also on the type of organic modifier and the mobile phase composition.

In the literature polar selectivity, generally related to silanol activity of a stationary phase is characterized by the relative retention of arbitrarily selected

polar solutes [7–9,12]. Silanol activity is usually regarded as an undesirable characteristic of the stationary phase causing severe peak tailing for basic solutes. However, in general, polar selectivity of a stationary phase can contribute to the resolution of solutes with similar structure and physical characteristics. In order to characterize polar selectivity of the various phases we have investigated relative retention of a large number of polar solutes to that of toluene as a non-polar solute.

Steric or shape selectivity may play an important role in the separation of non-polar compounds, particularly isomers. Isomers with rigid, well defined structures such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), steroids and carotenoids can often be separated in RP-HPLC on the basis of molecular shape [13,14,30,31]. Shape selectivity depends first of all on the bonding chemistry i.e., the structure of the bonded phase favoring polymeric phases. But it depends also on pore size, alkyl phase length, bonding density, temperature and to a lesser degree on the mobile phase composition. Sander and Wise's test method (SRM 869) provides a sensitive measure of polymeric or monomeric character of the phases [32,33].

In contrast to the empirical testing procedure using arbitrarily chosen solutes, the LFER concept furnishes a theoretically sound basis by using physicochemical parameters to correlate retention and identify molecular interactions. The master equation of the LFER model is defined [2–5] as

$$\log k = c + rR_2 + s\pi_2^* + a\sum\alpha_2^{\mathrm{H}} + b\sum\beta_2^{\mathrm{H}} + vV_{\mathrm{x}} \quad (2)$$

where the solvation parameters that denote specific solute properties are: solute excess molar refractivity (R_2), dipolarity/ploarizability (π_2^*), HBD acidity ($\Sigma\alpha_2^{\mathrm{H}}$), HBA basicity ($\Sigma\beta_2^{\mathrm{H}}$) and McGowan's molecular volume (V_{x}). Regression coefficients of Eq. (2) (c, r, s, a, b, v) can be obtained by multivariate linear regression technique and are characteristic of the phase system investigated. In Part I [1] regression coefficients pertaining to 15 phase systems [RP columns and acetonitrile–water (30:70) mobile phase] were determined and evaluated. By using the LFER solvation equation for any given column with known regression coefficients, the selectivity factor of any two compounds (j and i) can be calculated as

$$\begin{aligned}\log\alpha = \log\frac{k_{\mathrm{j}}}{k_{\mathrm{i}}} &= \log k_{\mathrm{j}} - \log k_{\mathrm{i}} \\ &= r(R_{2,\mathrm{j}} - R_{2,\mathrm{i}}) + s(\pi^*_{2,\mathrm{j}} - \pi^*_{2,\mathrm{i}}) \\ &+ a\left(\sum\alpha^{\mathrm{H}}_{2,\mathrm{j}} - \sum\alpha^{\mathrm{H}}_{2,\mathrm{i}}\right) + b\left(\sum\beta^{\mathrm{H}}_{2,\mathrm{j}} - \sum\beta^{\mathrm{H}}_{2,\mathrm{i}}\right) \\ &+ v(V_{\mathrm{x,j}} - V_{\mathrm{x,i}}) \quad (3)\end{aligned}$$

For different stationary phases evaluated under the same mobile phase conditions differences in chromatographic selectivity can be attributed to the structure and composition of the stationary phase [16,34].

3. Experimental

Experimental conditions, characteristics of columns investigated, list of the solutes and the corresponding solvation parameters were given in Part I of this series [1]. The same database has been used to calculate and evaluate selectivity factors (relative retentions) for comparison and characterization of packing materials investigated. Linear regression and correlation analysis were performed with Statistica 5.0 for Windows software (StatSoft, USA).

4. Results and discussion

4.1. Hydrophobic or methylene selectivity

Among the various selectivity terms, hydrophobic or methylene selectivity has been the most extensively studied property of RP phase systems. According to the definition (Eq. (1)), its logarithm is proportional to the Gibbs free energy of transfer per methylene group from the mobile phase to the stationary phase. It is usually calculated by dividing retention factors of neighboring members in a homologous series (such as of ethylbenzene and toluene) or, more accurately, from the slope of regression line in the log k vs. n_{CH2} (number of methylene units) plot.

Based on the retention data collected in our previous paper [1] we were able to calculate methylene selectivity for compounds of different polarity: (1) $\alpha_{\mathrm{EB/T}}$, relative retention of ethylbenzene to

toluene, (2) $\alpha_{EBO/MBO}$, relative retention of ethylbenzoate to methylbenzoate and (3) methylene selectivity of paraben homologues from the slope of the ln k–n_{CH2} plot for the four *p*-hydroxybenzoates.

First we examined ''classical'' hydrophobic selectivity as measured by $\alpha_{EB/T}$. This quantity is sometimes erroneously used in the literature, as an alternative to characterize RP hydrophobicity. However, as it was pointed out by Sentell and Dorsey [17,35], methylene selectivity does not depend significantly on bonding density of monomeric C_{18} phases. They arrived at this conclusion by examining monomeric octadecyl phases with surface coverage between 1.74 and 4.07 $\mu mol/m^2$. Moreover, Engelhardt and Jungheim [36] showed that methylene selectivity increases with carbon content of the stationary phase only up to a certain level (approx. 12% C) then it becomes nearly independent of the carbon load. In contrast, both of the above physical characteristics of stationary phases (bonding density and carbon content) is indeed positively correlated with the retention of non-polar compounds, thus, with the so-called hydrophobic strength.

A very similar picture was obtained for our 15 RP column set differing considerably in hydrophobic retentive power. Since data on surface coverage was not available in all instances we plotted methylene selectivity against the retention factor of a hydrophobic test solute, ethylbenzene (k_{EB}). Fig. 1 indicates that the large differences observed in hydrophobic retention is not obviously accompanied by the same selectivity variations. For instance, the two wide-pore (WP) C_{18} columns (A-C_{18} and S-C_{18}) have comparable methylene selectivity to narrow-pore (NP) C_{18} packings, although ethylbenzene was much less retained on the WP columns. Among the NP-C_{18} materials as well, hydrophobic retention varied considerably, while differences in methylene selectivity did not exceed 0.1 α units (between 1.99–2.08). Highly covered, end-capped stationary phases resulted in highest methylene selectivity values (M-PURe, M-C_{18}e). The lower hydrophobic retentivity of M-PUR and SYM-C_{18} columns is a consequence of polar groups inserted into their C_{18} alkyl chains. The NP-octyl columns investigated were quite similar to each other concerning retentive strength and hydrophobic selectivity, in spite of their different surface chemistry. (M-C_8 is a non-end-capped octyl phase, M-RP-B is type B silica obtained by acidic treatment, while SymmetryShield C_8 contains a carbamate shielding group inserted into the octyl ligand). The retention of test solute ethylbenzene on these phases reached values near that of obtained for the least hydrophobic C_{18} columns (M-PUR and SYM-C_{18}), but on methylene selectivity scale C_8 columns differed significantly from C_{18} ones ($\alpha_{average}=1.91$ for NP-C_8 and $\alpha_{average}=2.05$ for NP-C_{18} columns).

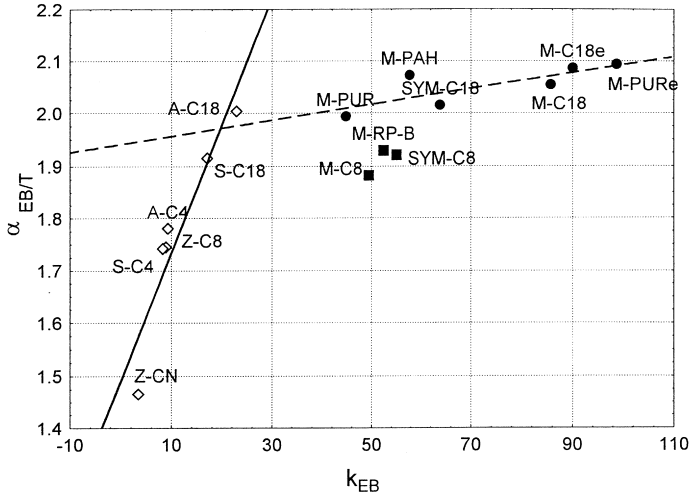

Fig. 1. Relationship between absolute retention and hydrophobic selectivity of different RP columns. (◇) WP columns; (●) NP-C_{18} columns; (■) NP-C_8 columns. Linear regression line for WP columns (—); for NP-C_{18} columns (– – –).

Concerning wide-pore materials, large differences in methylene selectivity were observed while k_{EB} varied between 3.4–22.9. This variation may primarily be due to the widely diverse ligand type of the WP columns studied. It is interesting to note however, that the relationship between methylene selectivity and hydrophobic retention is largely different on the packings of diverse pore size. To assess this, we constructed regression lines for different subsets of columns, i.e., for NP-C_{18}, NP-C_8 and WP columns, respectively (Fig. 1). The results of regression analysis is summarized in Table 1 (note that M-PAH column was not considered in the NP-C_{18} subgroup on account of its completely different polymeric bonding chemistry). Significant linear correlations between $\alpha_{EB/T}$ and k_{EB} were obtained for subsets NP-C_{18} and WP, although the extent of selectivity dependence on hydrophobic retention greatly varied. (For the subset NP-C_8 columns, linear regression was not significant at 95% level, therefore the regression line was not indicated in Fig. 1). It can be seen, that methylene selectivity on the weakly covered WP packings was much sensitively dependent on the hydrophobic properties of the stationary phase, than on the high-carbon containing NP-C_{18} phases (see slopes of 0.024 and 0.002). Previous studies confirmed [36,37], that for phases of low surface coverage, methylene selectivity significantly increases with increasing ligand length. According to Tan and Carr [37], adsorption is likely to play a significant role on such reversed-phase columns and as a consequence, hydrophobic selectivity is more strongly influenced by local environment, i.e., chain length and surface coverage. This is supported by the steep regression line obtained here for WP columns. However, if the interphase region reaches a certain density and thickness so that solutes can fully be embedded into, methylene selectivity will not increase considerably further. The almost constant, and on hydrophobic retention just slightly dependent methylene selectivity observed for longer-chain and/or densely bonded stationary phases (here for WP-C_{18} and the all NP columns) is most likely a consequence of the dominating partition-like retention behavior.

Methylene selectivity can also be calculated by using other homologous series than aromatic hydrocarbons. Nevertheless, if parent molecular skeleton contains polar functionalities as well, the obtained selectivity factors will be affected by secondary interactions. To show this, methylene selectivity was calculated using retention factors of ethylbenzoate (EBO) and methyl benzoate (MBO). We also constructed a log k vs. n_{CH2} plot for the four paraben homologues available, where the slope of the linear equation corresponds to an average methylene selectivity (log α, from which α is computed) for this compound group. Similarities among different methylene selectivity values was evaluated by regression analysis, where the correlation coefficient shows to what extent these quantities are proportional to each other. In Fig. 2, the relationship between ''classical'' methylene selectivity and $\alpha_{EBO/MBO}$ or $\alpha_{Paraben}$ are displayed. (For clarity reasons, only C_{18} and C_8 columns were indicated.) In general, a good correlation exists between the different methylene selectivity terms calculated, but selectivity of the less polar benzoate esters is more similar to $\alpha_{EB/T}$ ($R=0.99$) than that of the parabens ($R=0.96$). Interestingly, for columns containing special basic functionality built in the alkyl ligand environment (carbamate for SYM columns and amino-group for M-PUR) the paraben methylene selectivity was higher than $\alpha_{EBO/MBO}$ or even $\alpha_{EB/T}$. We attribute this effect to the basic (HB-acceptor) character of these stationary phases, by which the phenolic OH containing, strong HB-donor parabens are better separated. The smallest deviations between the three different types of methylene selectivities investigated were found on columns M-PURe and

Table 1
Regression equations and statistics between hydrophobic selectivity ($\alpha_{EB/T}$) and hydrophobic retention (k_{EB})

Subset of columns	Intercept	Slope	R	S.D.	F	n
NP-C_{18}	1.90	0.002	0.97	0.011	52	5
NP-C_8	1.54	0.007	0.80	0.021	1.7	3
WP	1.49	0.024	0.93	0.074	27	6

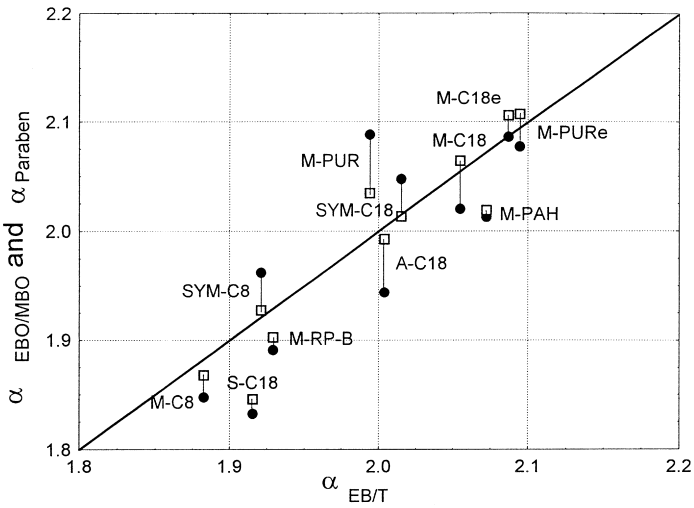

Fig. 2. Comparison of different methylene selectivities: (□) $\alpha_{EBO/MBO}$; (●) $\alpha_{Paraben}$. Straight line with a slope of unity indicates theoretical values.

M-C_{18}e. The end-capping procedure applied here seemingly yields a more homogenous stationary phase, where polarity differences of parent molecules do not alter remarkably the separation process.

Individual molecular interactions involved in reversed-phase chromatography can be uncovered and examined separately by applying the LFER concept of Abraham [2]. The v coefficient in Eq. (2) measures differences between the dispersive interactions and energies required to form solute-size cavity in the stationary and mobile phase. For homologous series, one methylene group increment in the molecule does not substantially alter other molecular properties than size, resulting in a constant change only in V_x McGowan volume. This is especially true for higher members in any of the homologous series. Therefore, the v coefficient obtained by multivariate regression should be strongly correlated with the logarithm of methylene (hydrophobic) selectivity. For computing selectivity, solute pair of propylparaben/ethylparaben (PP/EP) were selected since they were first in the paraben series differing only in V_x solute parameter. In Fig. 3, log $\alpha_{PP/EP}$ of different columns was regressed against the v coefficients (LFER regression coefficients were taken from Ref. [1]). Details of regression analysis is given in Table 2. The regression line comprising all the columns confirmed a statistically significant linear relationship between the measured hydrophobic selectivity and v. But again, if regression was split according to different type of columns (wide-pore, narrow-pore C_{18} and C_8), the WP materials showed the strongest correlation with the v coefficient. Selectivity dependence on the v coefficient was less significant for the most hydrophobic NP-C_{18} phases, presumably due to small differences in methylene selectivity. Since measured log k values are inevitably affected by experimental error, and moreover, multivariate regression coefficients (here the v coefficient) are always loaded with some degree of uncertainty (see confidence intervals [1]), it seems reasonable to assume that the LFER model cannot precisely distinguish small differences in selectivity of *very* similar RP packings. However, a general trend of increasing selectivity with the increasing ease of cavity formation and stronger dispersive interactions was confirmed by the v coefficient of LFER equations. Similar trends in regression equations have been obtained by using other methylene selectivity terms as $\alpha_{EBO/MBO}$ or $\alpha_{EB/T}$.

4.2. Polar selectivity

More striking differences in relative retention can be observed when one attempts to separate polar molecules rather than members of homologous

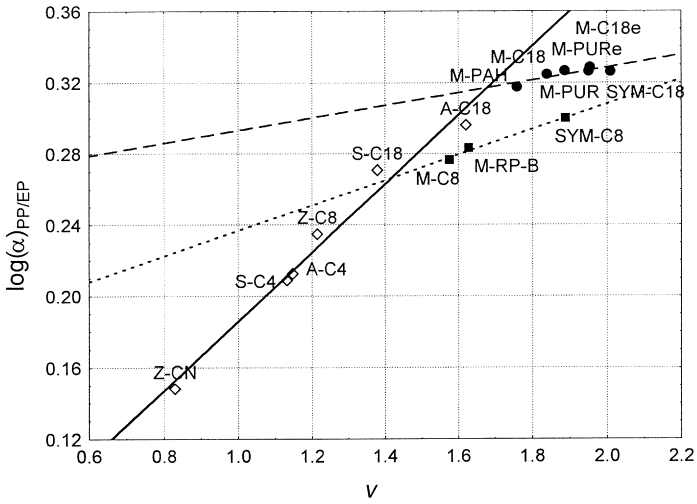

Fig. 3. Relationship between LFER system coefficient v and log $\alpha_{PP/EP}$, (◇) WP columns; (●) NP-C_{18} columns; (■) NP-C_8 columns. Linear regression line for WP columns (—); for NP-C_{18} columns (– – –); for NP-C_8 columns (- - -).

series. These deviations, such as reversal of the elution order, or unexpectedly large gaps between peaks cannot be explained on the basis of ligand-length, surface coverage or bonding density of the stationary phase. Secondary interactions [hydrogen-bonding (HB), n–π electron interactions, dipole or ionic forces) with specific binding sites of the packing material contribute more severely to the retention of polar solutes.

In this study, we defined polar or chemical selectivity as the relative retention of test solutes compared to toluene. If we assume that retention in RP-HPLC can roughly be broken down in two parts, hydrophobic and polar retention, differences that come about the non-specific, hydrophobicity variations of stationary phases are cancelled out by this calculation. Thus, polar selectivity computed in this way accounts for specific polar properties of the phases, and is better indicator value than ''pure'' retention factor which is always affected by column hydrophobicity. Furthermore, it is worth examining how selectivity terms can be correlated with LFER coefficients, that are the thermodynamically based descriptors of the particular phase system.

The result of correlation analysis between polar selectivities and LFER system constants is summarized in Table 3. It is obviously seen, that some of the selectivities are well correlated ($R>0.9$) with LFER system constants, and thus could serve as an initial estimate of secondary interactions involved in retention.

The b regression coefficient in Eq. (2) – which measures stationary phase HBD acidity – can be linearly related to the polar selectivity of some basic solutes, especially caffeine. Previous studies also suggested that caffeine was a sensitive indicator of hydrogen-bond involved retention characteristic [10]. Similarly, polar selectivities of specific HBD acidic

Table 2
Regression equations and statistics between log $\alpha_{PP/EP}$ (logarithm of methylene selectivity) and the v system coefficients of LFER equations (Eq. (2))

Subset of columns	Intercept	Slope	R	S.D.	F	n
NP-C_{18}	0.25	0.035	0.82	0.002	52	5
NP-C_8	0.16	0.071	0.99	0.002	64	3
WP	−0.01	0.193	0.99	0.009	164	6
All columns	0.04	0.146	0.97	0.013	231	15

Table 3
Correlation coefficients between LFER system constants and polar selectivities

Log α	b	a	s	r
DMA/T	0.42	0.52	0.50	−0.35
ANA/T	0.33	0.86	0.70	−0.14
ONT/T	0.84	0.72	0.72	−0.77
BNA/T	0.44	**0.90**	0.73	−0.24
CAF/T	**0.99**	0.78	0.76	−0.92
PYR/T	**0.97**	0.71	0.77	−0.94
HQ/T	**0.93**	**0.91**	0.80	−0.80
DP26/T	0.72	**0.95**	0.80	−0.55
EBO/T	0.83	0.72	0.83	−0.80
PP/T	0.54	**0.91**	0.84	−0.41
PCR/T	0.78	**0.97**	0.85	−0.62
NA/T	**0.93**	0.75	0.85	−0.61
DP35/T	0.78	**0.97**	0.85	−0.63
A/T	**0.95**	0.87	0.85	−0.86
PDM/T	**0.92**	0.88	0.86	−0.83
BA/T	**0.93**	0.89	0.86	−0.83
NB/T	0.83	0.89	0.86	−0.74
BC/T	0.89	0.88	0.86	−0.81
PEP/T	0.72	**0.95**	0.87	−0.58
OT/T	**0.93**	0.87	0.87	−0.84
OCR/T	0.83	**0.97**	0.87	−0.69
AP/T	**0.92**	0.87	0.88	−0.83
P/T	0.85	**0.96**	0.88	−0.72
PNA/T	0.78	**0.96**	0.88	−0.64
MBO/T	0.89	0.86	0.89	−0.81
MP/T	0.81	**0.97**	0.89	−0.68
EP/T	0.72	**0.96**	**0.90**	−0.59
AN/T	0.81	**0.91**	**0.92**	−0.72
PNP/T	0.57	0.84	**0.96**	−0.55

Correlations higher than 0.9 in bold. Symbols of test solutes are given in Ref. [1].

solutes (primarily phenolic compounds) were found in strong correlation with stationary phase HBA basicity (*a* coefficient). Correlations with phase system dipolarity/polarizability (*s* coefficient) and with its tendency to interact through n–π electrons (*r* coefficients) could not be reliably evaluated since only a few test components have shown significant correlation with these properties. Nevertheless, these type of interactions reportedly play inferior role in the RP retention process [38].

The fairly good correlation coefficients found above suggest that certain polar selectivities can alternatively be used to characterize HB donor and acceptor properties of phase systems. Polar selectivities of solutes that were most strongly correlated with LFER system coefficient *b* and *a*, and therefore were good estimators of hydrogen-bond involved interactions, are shown in Fig. 4a,b. Higher selectivity values on both diagram correspond to higher hydrogen-bonding-activity (HBD acidity on Fig. 4a and HBA basicity on Fig. 4b) of phases relative to their hydrophobic retentive strength. On the contrary, smaller polar selectivities were observed for phases of excessively covered or specially treated surfaces. This means, that these phases can differentiate better between polar and non-polar solutes since H-bond involved secondary interactions play only inferior role during the retention process. Relative HBD acidity of two NP-C_8 columns (M-C_8 and M-RP-B) are greater than NP-C_{18} materials, which is obviously due to the greater accessibility of the silica surface (Fig. 4a). For the same reason, almost all WP columns were found to be more acidic than the NP ones. Lack of HBD acidic sites were confirmed for the specialty SymmetryShield columns and for M-PURe. On the relative HBA basicity scale (Fig. 4b) SYM columns and M-C_8 were scored high among the NP materials, which indicates larger amount of surface moieties with HBA basic properties on these columns. Among WP columns, Z-CN, Z-C_8 and A-C_4 had above average HBA basicity. In general, non-endcapped, non-specialty Merck columns (M-C_{18}, M-C_8) and also WP materials exhibited increased affinity toward basic and acidic solutes. This is an evident sign that accessible silica surface is capable of displaying both HB-donor and HB-acceptor sites, as well.

Sample constituents can only be successfully separated in a given phase system (mobile and stationary phase) when their selectivity differs from unity. We examined the possibility to use the LFER model for selectivity prediction based on 561 compound pairs generated from test solutes. Comparison of measured versus calculated selectivities on an arbitrarily selected column, M-C_{18}e showed good agreement between calculated and experimental data. Fig. 5 indicates that the model can describe selectivity factor in a wide numerical range. Similar results can be obtained for all the columns investigated, with an average deviation between predicted and observed selectivity factors of 0.097 for NP columns

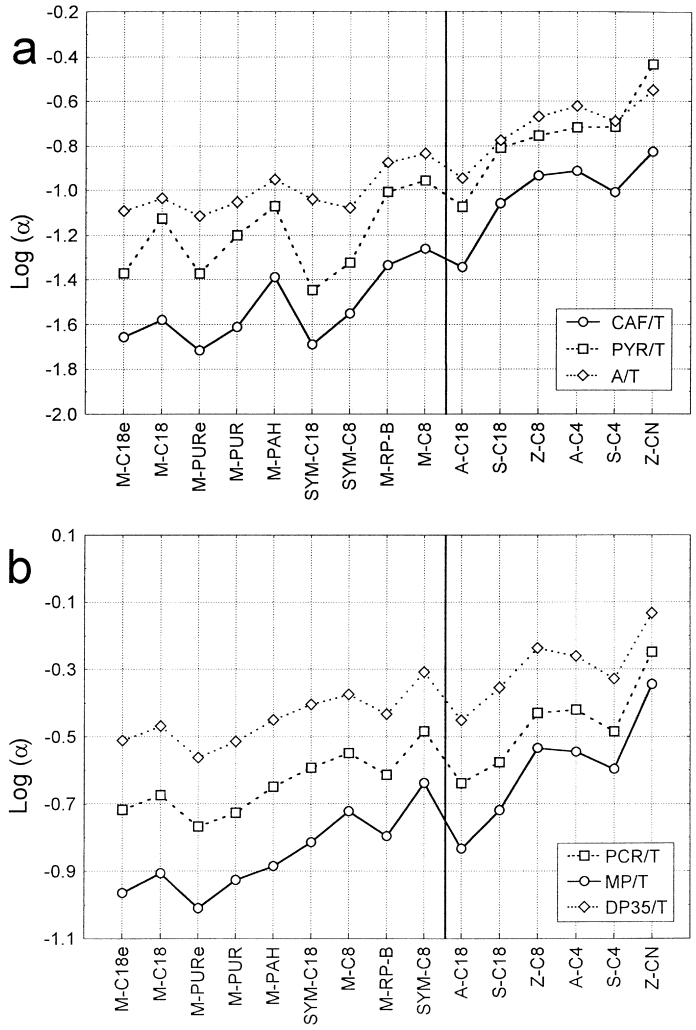

Fig. 4. Polar selectivity of (a) HB-acceptor solutes, (b) HB-donor solutes. Vertical line separates NP and WP columns.

and of 0.084 for WP columns, in log α, respectively. The accuracy of selectivity prediction using the LFER equations may not be enough for some solute pairs, especially in the low α range (1.0–1.5) e.g., OCR/AP, PDM/BC, AN/PEP. Nevertheless, the LFER model in most cases can be used to estimate which of the columns available will furnish the highest selectivity for the separation of the components of a sample to be investigated. Improving the accuracy of solvation parameter determination and using a larger set of test compounds when calculating system coefficients will enhance the overall predictive power of LFER equations.

5. Conclusions

The LFER model of Abraham et al. can be used to evaluate the presence and extent of various selectivities in a given phase system.

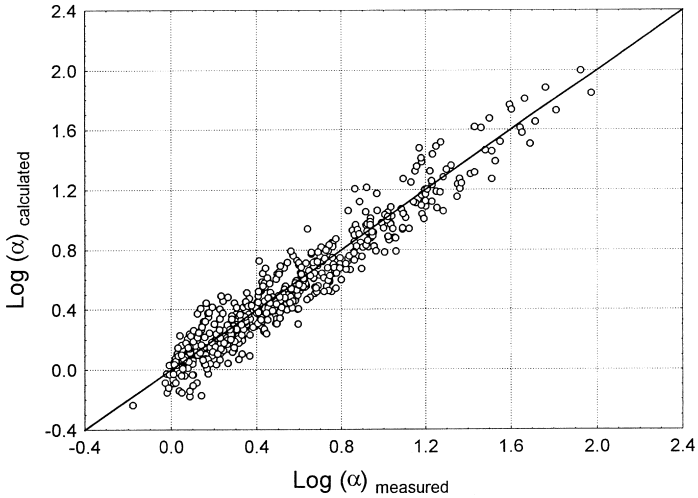

Fig. 5. Logarithm of selectivity factors on an M-C_{18}e column, measured vs. calculated values.

Hydrophobic or methylene selectivity was well correlated with the *v* coefficient of LFER indicating that dispersion interactions are the main forces in determining hydrophobic selectivity. It has been established that methylene selectivity strongly depends on the type of stationary phase, i.e., alkyl-chain length, bonding density and pore size. Highly covered, endcapped stationary phases (M-PURe, M-C_{18}e) furnished the highest methylene selectivity while C_8 columns had lower selectivities than C_{18} columns. Wide-pore stationary phases have shown a much wider range of methylene selectivity depending on the length of the ligand and the correspondingly varying secondary interactions. While WP-C_{18} columns had comparable methylene selectivity to NP-C_{18} columns, WP materials with shorter ligands exhibited much less selectivities. In accordance with the LFER system coefficients it can be concluded that highest methylene selectivities can be obtained for the most hydrophobic stationary phases and it decreases with increasing influence of secondary interactions.

Our results have shown that methylene selectivity is not identical with hydrophobicity or hydrophobic strength of the stationary phase. In addition, methylene selectivity depends also on the structure and polarity of the homologous series investigated.

Polar or chemical selectivity has been defined as the relative retention of various types of polar solutes to toluene. Good correlations have been found between specific polar solutes and LFER system coefficients. Correlation between the *b* coefficient – representing HBD acidity of the stationary phase – and log α values were the highest for strongly basic solutes (caffeine, pyridine and aniline). For basic solutes, polar selectivity was smaller on stationary phases of less acidic character. Similarly, correlations between the *a* coefficient – representing HBA basicity of the phase – and log α values were the most significant for strongly acidic solutes (*p*-cresol, methylparaben, 3,5-dimethylphenol). In general, when acidic solutes were concerned, polar selectivity was smaller on stationary phases with diminished basic character. The effect of dipolarity/polarizability (*s* coefficient) and molar refraction (*r* coefficient) cannot be unambiguously explained on the basis of the retention data of solutes investigated, but they exert less significant effect on the retention process.

The log α values calculated using the LFER model can be used to predict selectivity factors for the separation of any compound pairs with known solvation parameters. The accuracy and reliability of the LFER approach to predict retention and selectivity for various solutes could be enhanced by improv-

ing the precision of the determination of solvation parameters and also by extending the number and chemical type of the test solutes investigated.

6. Symbols

a, b, c, r, s, v	Regression coefficients of Eq. (2)
F	Fischer's F-test on the significance of the regression
HB	Hydrogen bond
HBA	Hydrogen-bond acceptor
HBD	Hydrogen-bond donor
k	Retention factor
LFER	Linear free energy relationship
n	Number of variables used in regression
NP	Narrow-pore
R	Correlation coefficient (Pearson R)
S.D.	Standard deviation
WP	Wide-pore
α	Selectivity factor
$\Sigma\alpha_2^H$	LFER solvation parameter for hydrogen-bond donor acidity
$\Sigma\beta_2^H$	LFER solvation parameter for hydrogen-bond acceptor basicity
π_2^*	LFER solvation parameter for dipolarity/polarizability
R_2	LFER solvation parameter for excess molar refractivity
V_x	LFER solvation parameter for McGowan molecular volume

Acknowledgements

This work was supported by grants OTKA 014997 and OTKA F023120. We gratefully acknowledge the donation of the LiChrospher 100 RP-18e and the Purospher RP-18e columns Merck Kft. (Budapest, Hungary) and the donation of the SymmetryShield C_8 and C_{18} columns to Dr. U.D. Neue (Waters, Milford, MA, USA). Special thanks are due to Professor Michael H. Abraham (University College London) for helpful discussion and suggestions.

References

[1] Á. Sándi, L. Szepesy, J. Chromatogr. A 818 (1998) 1.
[2] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[3] M.M.H. Abraham, Pure Appl. Chem. 65 (1993) 2503.
[4] M.H. Abraham, J. Phys. Org. Chem. 7 (1994) 672.
[5] M.H. Abraham, J. Andonian-Haftvan, G.S. Whiting, A. Leo, J. Chem. Soc. Perkin Trans., II (1994) 1777.
[6] J.J. Kirkland, B.E. Boyes, J.J. DeStefano, Int. Chromatogr. Lab. 20 (1994) 2.
[7] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[8] S.J. Schmitz, H. Zwanziger, H. Engelhardt, J. Chromatogr. 544 (1991) 381.
[9] H. Engelhardt, M. Arangio, GIT Spez. Chromatogr. 16 (1990) 54.
[10] K. Kimata, K. Iwaguchi, S. Oniski, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[11] H.A. Claessens, J.W. de Haan, L.J.M. van de Ven, P.C. de Bruyn, C.A. Cramers, J. Chromatogr. 436 (1988) 345.
[12] H.A. Claessens, E.A. Vermeer, C.A. Cramers, LC·GC Int. 6 (1993) 692.
[13] L.C. Sander, S.A. Wise, CRC Crit. Rev. Anal. Chem. 18 (1987) 299.
[14] L.C. Sander, S.A. Wise, LC·GC 8 (1990) 378.
[15] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151.
[16] C.H. Lochmüller, M.L. Hunnicnut, J.F. Mullaney, J. Phys. Chem. 89 (1985) 5770.
[17] K.B. Sentell, J.G. Dorsey, J. Chromatogr. 461 (1989) 193.
[18] P.E. Antle, A.P. Goldberg, L.R. Snyder, J. Chromatogr. 321 (1985) 1.
[19] A.M. Krstulović, H. Colin, A. Tchapla, G. Guiochon, Chromatographia 17 (1983) 228.
[20] A. Tchapla, H. Colin, G. Guiochon, Anal. Chem. 56 (1984) 621.
[21] N. Tanaka, K. Sakagami, M. Araki, J. Chromatogr. 199 (1980) 327.
[22] N. Tanaka, K. Kimata, K. Hosoya, H. Miyanashi, T. Araki, J. Chromatogr. A 656 (1993) 265.
[23] B. Shaikh, J.E. Tomaszewski, Chromatographia 17 (1983) 675.
[24] A.L. Colsmjo, M.W. Ericsson, J. Liq. Chromatogr. 9 (1986) 2825.
[25] N. Tanaka, H. Godell, B.L. Karger, J. Chromatogr. 158 (1978) 233.
[26] H. Engelhardt, G. Ahr, Chromatographia 14 (1981) 227.
[27] C.R. Yonker, T.A. Zwier, M.F. Burke, J. Chromatogr. 241 (1982) 257.
[28] C.R. Yonker, T.A. Zwier, M.F. Burke, J. Chromatogr. 241 (1982) 269.
[29] P. Jandera, J. Chromatogr. 352 (1986) 91.
[30] L.C. Sander, S.A. Wise, J. Chromatogr. A 656 (1993) 335.
[31] L.C. Sander, S.A. Wise, in: R.M. Smith (Ed.), Retention and Selectivity in Liquid Chromatography, Elsevier, Amsterdam, 1995, p. 337.

[32] L.C. Sander, J. Chromatogr. Sci. 26 (1988) 380.
[33] L.C. Sander, S.A. Wise, J. High Resolut. Chromatogr., Chromatogr. Commun. 11 (1988) 383.
[34] K.B. Sentell, N.I. Ryan, A.N. Henderson, Anal. Chim. Acta 307 (1995) 203.
[35] K.B. Sentell, J.G. Dorsey, Anal. Chem. 61 (1989) 930.
[36] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[37] L.C. Tan, P.W. Carr, J Chromatogr. A 775 (1997) 1.
[38] L.C. Tan, P.W. Carr, M.H. Abraham, J. Chromatogr. A 752 (1996) 1.