ELSEVIER

Journal of Chromatography A, 893 (2000) 215–234

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Characterization of reversed-phase columns using the linear free energy relationship
III. Effect of the organic modifier and the mobile phase composition

Ákos Sándi, Mária Nagy, László Szepesy*

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

Received 6 September 1999; received in revised form 30 June 2000; accepted 30 June 2000

Abstract

Retention factors determined for 31 solutes of widely different types on five columns of different chromatographic characteristics have been used to calculate the regression coefficients of the linear free energy relationship (LFER) equations. The mobile phases investigated consisted of acetonitrile–water and methanol–water, respectively, in a composition range of 20–70% (v/v) of organic modifiers. The regression coefficients of the LFER equations are characteristic of the given phase system (stationary phase, organic modifier and mobile phase composition) and represent the extent of the various molecular interactions contributing to the retention process. The effect of the characteristic of the stationary phase, the type of the organic modifier and the mobile phase composition is demonstrated and discussed. α selectivity factors have been determined for various pairs of compounds. Hydrophobic or methylene selectivity can be described by the variation of the v coefficient in Eq. (3) representing the difference in hydrophobicity between the stationary phase and the mobile phase. The polar or chemical selectivity of a phase system varies with the b coefficient in Eq. (3) representing the difference in acidity between the stationary phase and the mobile phase. Polar selectivity, i.e. the relative retention of polar solutes to that of a non-polar solute, e.g. toluene decreases with increasing polarity of the mobile phase. It depends also significantly on the polar characteristics of the columns. Specific selectivity, i.e. the relative retention of various polar solutes depends on the acidic or basic properties of the solutes to be separated and the chemical properties of the columns. The b regression coefficients can be used to describe the effect of mobile phase composition on the variation of specific selectivities. We have demonstrated that the LFER method provides a useful estimate of selectivity under different operating conditions by using the solvation parameters describing the different molecular interactions and the regression coefficients of the LFER equation characterizing the phase system.

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

1. Introduction

In previous publications we investigated the characterization of various RP-HPLC columns by the determination of the regression coefficients of the

*Corresponding author. Fax: +36-1-463-1913.
E-mail address: szepesy.ktt@chem.bme.hu (L. Szepesy).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00733-0

linear free energy relationship (LFER) describing the various types of molecular interactions in the retention process. In the first publication [1] we calculated the regression coefficients of the LFER equation by using the retention factors determined for a large number (34) of different types of solutes at fixed mobile phase compositions [acetonitrile–water (30:70%, v/v)]. The regression coefficients representing the different types of molecular interactions furnished information about the contribution of the individual interactions in the retention process characteristic of the stationary phases. Since the same mobile phase has been used with the different columns, the regression coefficients obtained can be used to characterize and compare the various columns investigated. We have demonstrated that the regression coefficients can be used to predict the retention factors by the LFER equation based on the known solvation parameters of the solutes.

In the second publication [2] 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, the 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 demonstrated that hydrophobic selectivity is not identical with the hydrophobic strength of the column and 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.

In a recent publication [3] we have investigated the evaluation and modulation of selectivity in RP-HPLC on five columns of different characteristics using acetonitrile–water and methanol–water mobile phases, respectively, in a composition range of 20–70% (v/v) of organic modifiers. Retention factors for 31 solutes of widely different types were determined and α selectivity factors were calculated for various types of solute pairs.

It was established that chromatographic selectivity is a very complex phenomenon, depending, on the one hand, on the phase system, i.e. characteristics of the stationary phase, type of the organic modifier and mobile phase composition, on the other hand, also on the type and structural characteristics of the compounds to be separated. We have demonstrated the influence of operating conditions on the different types of selectivities such as hydrophobic or methylene selectivity, polar or chemical selectivity and specific selectivity among compounds of different types.

In the present study we have investigated the effect of the type of the organic modifier and the composition of the mobile phase on the regression coefficients of the LFER equations. Because the characteristics of a phase system depend not only on the characteristics of the stationary phase but also on the type of the organic modifier and very strongly on the composition of the mobile phase, the variation of the regression coefficients provide information about the contribution and relative importance of the individual molecular interactions in the retention process under different mobile phase conditions.

In addition, we will discuss the effect of the type of the organic modifier and the mobile phase composition on the selectivity of separation and the prediction of selectivity by using the LFER approach.

2. Theoretical

In the last decade a number of studies have been published to correlate solute effects in various distribution processes based on the linear solvation energy relationships (LSER) or more generally expressed, on the linear free energy relationships (LFER) pioneered by Kamlet and Taft [4–9].

The LSER approach has been recently applied extensively to the study of gas chromatography (GC) [10–14] and HPLC [15–21] with generally good results. Based on this model a free energy related term in a phase transfer process can be separated into several molecular interaction terms. In HPLC the logarithmic retention factor, log k, can be correlated with various fundamental solute descriptor properties as shown in Eq. (1):

$$\log k = \log k_0 + m(\delta_s^2 - \delta_m^2)V_2 + s(\pi^*_s - \pi^*_m)\pi_2 + a(\beta_s - \beta_m)\alpha_2 + b(\alpha_s - \alpha_m)\beta_2 \quad (1)$$

where $\log k_0$ is an independent term; m, s, a and b are the regression coefficients; V_2 is the characteristic volume of the solute; δ is the Hildebrand solubility parameter; π is a measure of dipolarity/polarizability; β is hydrogen-bond acceptor (HBA) basicity and α is hydrogen-bond donor (HBD) acidity. The subscripts s, m and 2 denote the stationary phase, the mobile phase and the solute, respectively. In this model each solute property is multiplied by a term that represents the difference in the complementary property between the stationary phase and the mobile phase. The model postulates that retention results from the differential interactions of a solute in the two phases.

When a system with a fixed stationary phase and a fixed mobile phase composition is investigated, Eq. (1) becomes:

$$\log k = \log k_0 + mV_2 + s\pi^*_2 + a\alpha_2 + b\beta_2 \quad (2)$$

where each coefficient reflects the difference in a specific bulk property between the stationary phase and the mobile phase.

Several approaches have been put forward to measure, calculate or estimate the solute descriptors. The solute solvatochromic properties were derived from solvent solvatochromic measurements of the absorption bands for a series of indicator compounds [4–7]. Abraham and coworkers introduced new solute parameters derived from equilibrium measurements on the solutes themselves such as GC data, water–solvent partion coefficients and data relating to the molecular structure [22–25]. In this study we apply the LFER or solvation equation introduced by Abraham et al. to correlate chromatographic retention:

$$\log k = c + rR_2 + s\pi^*_2 + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + vV_x \quad (3)$$

where c is the intercept; R_2 is an excess molar refraction; π^*_2 is the solute dipolarity/polarizability; $\Sigma\alpha_2^H$ is the solute overall or effective HBD acidity; $\Sigma\beta_2^H$ is the solute overall or effective HBA basicity; V_x is the McGowan characteristic volume. The coefficients in Eq. (3) can be determined by multivariate regression analysis and characterize the phase system investigated. Value r is a measure of the propensity of the phase to interact with solute n- and π-electron pairs; s is a measure of the phase dipolarity/polarizability; a is a measure of the phase HB basicity; b is a measure of the phase HB acidity; v is a measure of phase hydrophobicity. If Eq. (3) is applied to the distribution between two phases, the coefficients will refer to differences between the phases concerned.

In recent years LFERs have been used to characterize and compare various RP stationary phases with good results [1,23,26–30]. The coefficients c, r, s, a, b and v in Eq. (3) are characteristic of the phase system, i.e. a particular RP-HPLC column with a fixed mobile phase composition. If different columns are studied with the same mobile phase composition, the coefficients obtained are characteristic to the individual columns, i.e. for the contribution of the stationary phases to the different molecular interactions.

It has been recognized, however, that the volume and composition of the stationary phases varies under changing mobile phase conditions. The stationary phase is actually a ternary combination of the bonded organic moiety, sorbed solvent molecules and residual silanols on the silica surface [31–34].

The overall stationary phase formation depends on the chain length of the bonded organic moiety and the residual silanol activity. The volume and composition of the sorbed solvent layer depend on the type of the organic modifier and the composition of the mobile phase [31–33]. In accordance with the above changes, the regression coefficients in Eq. (3) will vary by changing the mobile phase composition reflecting the changes in the individual molecular interactions. Until quite recently there were only a few studies in the literature examining the application of LFER relationships under varying mobile phase composition and for different types of columns [23,27,34].

In a recent study Tan and Carr [35] investigated the effect of the mobile phase on LSER coefficients. They used methanol, acetonitrile and tetrahydrofuran as the organic modifiers in a composition range of 20–50% (v/v) organic. All the measurements were carried out on Zorbax-C_8 columns of different length. Many of their conclusions are in accord with

our findings, but there are some points where our results are open to another interpretation.

During the preparation and submission of the manuscript some important studies have been published relating the effect of the mobile phase composition and the modification of the linear solvation energy relationship (LSER) to predict retention and optimize selectivity in RPLC. Wang et al. [36] developed a global linear solvation energy relationship by combining both the LSER model and the linear solvent strength theory (LSST) model into a single model. This approach assumes a linear relationship both in the LSST model (log k vs. mobile phase composition, ϕ) and between the LSER coefficients and ϕ. There are a number of studies in the literature demonstrating that the linearity of the log k–ϕ relationship is limited to a narrow range of mobile-phase compositions [37–41]. On the other hand, either the linearity between the LSER coefficients and mobile phase composition is also not true for a wide range of mobile phase compositions [23,27,34,35].

Zhao and Carr [42] used the combination of the LSST and LSER equations to define the ''effective'' selectivity of separation. They concluded that effective selectivity can be determined and different stationary phases can be compared only by using the ratios of the LSER coefficients and not the absolute values in accordance with the suggestion of Abraham et al. [27].

A very recent study by Reta et al. compares different types of bonded RPLC stationary phases by the LSERs [43]. The scope, and partly the conclusions, of that article are similar to our study and we shall compare our results with their results in the discussion section.

By using regression coefficients determined for a given phase system, the α selectivity factor for any two compounds (j and i) can be calculated:

$$\log \alpha = \log \frac{k_j}{k_i} = \log k_j - \log k_i$$
$$= r(R_{2j} - R_{2i}) + s(\pi^*_{2j} - \pi^*_{2i}) + a(\Sigma\alpha^H_{2j} - \Sigma\alpha^H_{2i}) + b(\Sigma\beta^H_{2j} - \Sigma\beta^H_{2i}) + v(V_{xj} - V_{xi}) \quad (4)$$

To characterize selectivity is a difficult task because chromatographic selectivity is a very complex phenomenon.

Thermodynamically, the α selectivity factor reflects the difference between the two solutes in terms of Gibbs free energy of transfer (ΔG) from the mobile phase to the stationary phase:

$$\alpha = \frac{k_j}{k_i} \quad \text{and} \quad \ln \alpha = -\frac{\Delta(\Delta G)}{RT} \quad (5)$$

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.

This overall characteristic, however, is composed of several different mechanisms, depending on the phase system, the operating conditions and the properties of the compounds to be separated.

Column selectivity can be classified according to the type of molecular interactions between the stationary phase and the solutes as hydrophobic, polar and steric selectivity [44,45].

Hydrophobic or methylene selectivity is defined as the relative retention of adjacent members of homologous series differing only in one CH_2 group. Hydrophobic selectivity depends on the strength of the hydrophobic interaction between the stationary phase and the compounds [46–48].

Polar or chemical selectivity comes about from polar interactions as hydrogen bonding, dipole and ionic interactions, complexation between the solute molecules and specific active sites on the surface of the stationary phase [44,45,49].

Steric or shape selectivity may be important in the separation of polycondensed multiring systems according to their molecular shape [50,51].

In the mobile phase both the modifier concentration (solvent strength selectivity) and the type of the organic modifier (modifier selectivity) will influence the selectivity of separation [52–54].

Recently, it has been shown that in addition to the stationary phase and the mobile phase temperature may also influence selectivity [55,56].

3. Experimental

Retention data were measured on a Merck-Hitachi LiChrograph consisting of a L-6200 programmable pump, a Rheodyne 7215 injector with a 10-μl loop and a L-4250 UV–Vis detector operating at 220 nm. Data acquisition was performed by the D-7000 HPLC System Manager Software.

In order to study the effect of column characteris-

tics on LFER coefficients we have selected five columns of different chromatographic characteristics from our set investigated, see Ref. [1]. The columns and their main characteristics, as provided by the manufacturers, are listed in Table 1. The first two columns are well covered quasi neutral C_{18} columns [1]. The third one is a nonendcapped C_8 column showing both acidic and basic character [1]. The SymmetryShield columns have basic character because of the carbamate groups embedded in the alkyl chain [1,57].

The solutes were of analytical grade and were purchased from different manufacturers. They were selected to cover a wide range of chemical properties. The list of the 31 solutes investigated and their corresponding solvation parameters pertaining to Eq. (3) are shown in Table 2 [22–26].

The retention time of test solutes were measured on all five columns in duplicate or triplicate using acetonitrile–water and methanol–water mobile phases, respectively, in a composition range of 20–70% (v/v) organic modifiers. Acetonitrile, methanol and water of chromatographic grade were obtained from Merck (Darmstadt, Germany). For the calculation of retention factors, column dead time was determined by injecting 0.05 m*M* sodium nitrate solution. The reproducibility of sequential measurements was excellent with an average deviation of 1% in *k* retention factors. In order to evaluate the different packing materials without modification of surface properties water was used without any additive for pH and ionic strength adjustment [58]. Sample mixtures were prepared using the actual mobile phase composition to approx. 1–2 mg/ml concentration, which corresponded to a load of 0.008–0.015 mg/g stationary phase. It was established that retention of even basic solutes is independent of the sample if the linear capacity range of around 0.1 mg of sample per g of stationary phase is not exceeded [59].

Log *k* values obtained with the two organic modifiers investigated in the 20–70% composition range were published as Tables 2–11 in Ref. [3]. In this publication we have used the above database to determine the regression coefficients of Eq. (3) by the multivariate linear regression technique using the Statistica 5.0 for Windows software (Stat Soft, USA). In all cases 31 solutes were used (Table 2) and no outliers were detected on the different columns and at various mobile phase compositions. Correlation coefficients (*R*) were in all cases over 0.97, the linear regression was significant at minimum 95% level. Uncertainties of *v* and *b* coefficients were generally within 10% and those for *r*, *a* and *s* were within 25%. These uncertainties of the regression coefficients do not influence the course of the regression coefficients with changing mobile phase composition.

4. Results and discussion

4.1. Effect of mobile phase composition on the regression coefficients

Several different methods have been used in the literature to study the effect of solute structure and mobile phase composition on the retention process in

Table 1
Characteristics of columns

Column	LiChrospher 100 RP-18e	Purospher RP-18e	LiChrospher 100 RP-8	Symmetry Shield RP-C_{18}	Symmetry Shield RP-C_8
Symbol	M-C_{18}e	M-PURe	M-C_8	SYM-C_{18}	SYM-C_8
Manufacturer	Merck (FRG)	Merck (FRG)	Merck (FRG)	Waters (USA)	Waters (USA)
Dimensions (mm×mm, I.D.)	125×4.0	125×4.0	125×4.0	150×3.9	150×3.9
Particle size (μm)	5.0	5.0	5.0	5.0	5.0
Pore size (nm)	10	12	10	10	10
Surface area (m^2/g)	350	350	350	340	340
Ligand type	C_{18}	C_{18}	C_8	C_{18}	C_8
Carbon loading (%C)	21.6	18.0	12.5	21.2	15.0
Endcapping	+	+	–	+	+
Chromatographic character	Neutral	Neutral	Acidic/basic	Basic (carbamate)	Basic (carbamate)

Table 2
Test solutes and solvation parameters

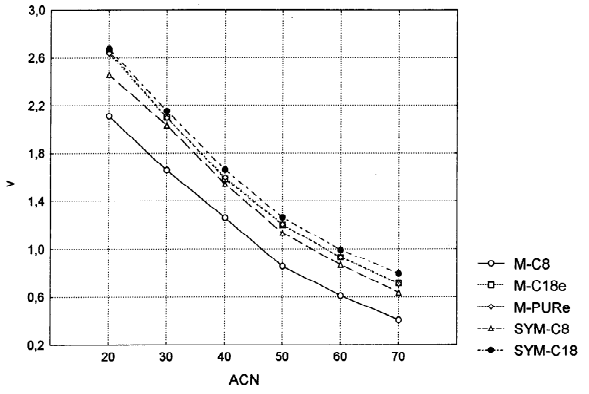
Compound	Symbol	R_2	π_2^*	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	V_x
Aniline	A	0.955	0.96	0.26	0.50	0.816
Methylbenzoate	MBO	0.733	0.85	0.00	0.46	1.073
Toluene	T	0.601	0.52	0.00	0.14	0.857
Ethylbenzene	EB	0.613	0.51	0.00	0.15	0.998
p-Cresol	PCR	0.820	0.87	0.57	0.32	0.916
2,6-Dimethylphenol	DP26	0.860	0.79	0.39	0.39	1.057
Ethylbenzoate	EBO	0.689	0.85	0.00	0.46	1.214
Chlorobenzene	CB	0.718	0.65	0.00	0.07	0.839
Bromobenzene	BRB	0.882	0.73	0.00	0.09	0.891
Caffeine	CAF	1.500	1.60	0.00	1.33	1.363
o-Toluidine	OT	0.966	0.92	0.23	0.59	0.957
Benzyl cyanide	BC	0.751	1.15	0.00	0.45	1.012
α-Naphtylamine	NA	1.670	1.26	0.20	0.57	1.185
o-Nitrotoluene	ONT	0.866	1.11	0.00	0.27	1.032
Hydroquinone	HQ	1.000	1.00	1.16	0.60	0.834
Phenol	P	0.805	0.89	0.60	0.30	0.775
o-Cresol	OCR	0.840	0.86	0.52	0.31	0.916
3,5-Dimethylphenol	DP35	0.820	0.84	0.57	0.36	1.057
β-Naphtol	BNA	1.520	1.08	0.61	0.40	1.144
Benzyl alcohol	BA	0.803	0.87	0.33	0.56	0.916
Acetophenone	AP	0.818	1.01	0.00	0.48	1.014
Dimethyl phtalate	PDM	0.780	1.41	0.00	0.88	1.429
p-Ethylphenol	PEP	0.800	0.90	0.50	0.30	1.057
α-Naphtol	ANA	1.520	1.05	0.60	0.37	1.144
Pyridine	PYR	0.631	0.84	0.00	0.52	0.675
Anisole	AN	0.708	0.75	0.00	0.29	0.916
N,N'-Dimethylaniline	DMA	0.957	0.84	0.00	0.42	1.098
Methylparaben	MP	0.900	1.37	0.69	0.45	1.131
Ethylparaben	EP	0.860	1.35	0.69	0.45	1.272
Propylparaben	PP	0.860	1.35	0.69	0.45	1.413
Butylparaben	BP	0.860	1.35	0.69	0.45	1.554

RP-HPLC. Contrary to the majority of chemometric methods, the linear free energy relationship (LFER) or solvation equation is based on a thermodynamically derived solvation parameter model. The unique advantage of the LFER approach relies in its ability to measure independently the contribution of individual molecular interactions to the retention process. This is achieved by constructing LFER regression equations in the general form of Eq. (3), using multivariate linear regression analysis. As it was discussed in Section 2, regression coefficients will characterize the difference in certain interactions between the stationary and mobile phase. If the composition of the mobile phase is varied, the characteristics of both the mobile phase and the stationary phase will vary resulting in changes in the regression coefficients. For this reason the regression coefficients should be determined for all mobile phase composition.

The regression coefficients of Eq. (3) determined for the five columns investigated are shown in Figs. 1–5. These figures indicate also the effect of the stationary phase and the differences among the columns investigated.

4.1.1. The v coefficient

In Fig. 1 *v* coefficients represent the difference in hydrophobicity between the stationary phase and the 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. As the alkyl-bonded stationary phase is far less cohesive than the water-rich mobile phase [26,31,32], a greater amount



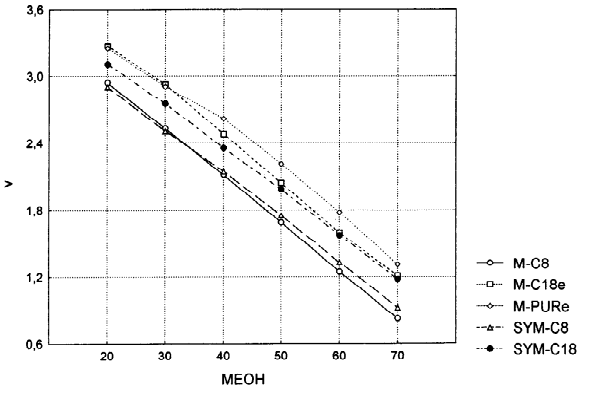
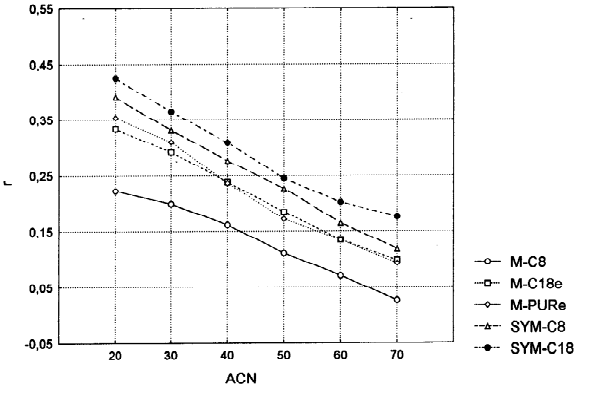


Fig. 1. Regression coefficients, v, of Eq. (3) as a function of percent of organic modifier (v/v).



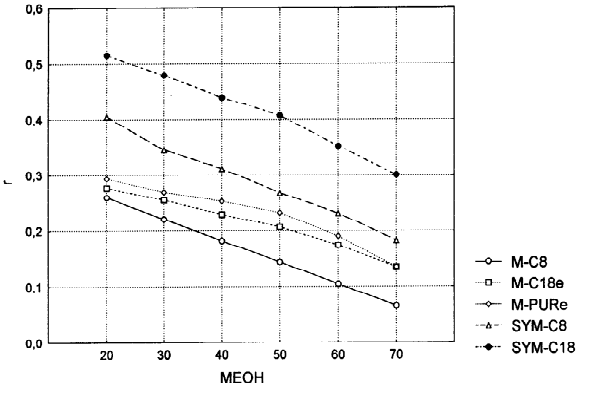


Fig. 2. Regression coefficients, r, of Eq. (3) as a function of percent of organic modifier (v/v).

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 dispersion interactions between the alkyl bonded phase and the solute than between the aqueous mobile phase and the solute. For this reason the coefficient v is positive and increases considerably by increasing water ($\delta^2_W = 549$) content of the mobile phase. At the other composition end the v coefficient should become small following from the cohesive energy densities of the organic modifiers ($\delta^2_{ACN} = 138$, $\delta^2_{MEOH} = 205$). Actually, the v coefficient reflects the strength of retention in the given phase system.

The more polar the mobile phase is the higher the v coefficient is. For this reason and in accordance with the higher polarity of methanol, the retention factor and correspondingly the v coefficient is lower for acetonitrile than for methanol, for a given mobile phase composition.

As it is expected, columns with longer alkyl chain, i.e. higher retentive capacity are characterized by greater (positive) v coefficients [1]. In Fig. 1 the C_{18} columns show greater v coefficients than the C_8 columns.

In Tan and Carr's interpretation the large positive v (in their publication m) and its increase with increasing water content is the combined results of the increase of the differential cohesivity between the two phases and the increase of dispersive interactions between a solute and the stationary phase [35]. We agree with this explanation with two remarks. First, the refractive index, used in their paper is not an unambigous characteristic of dispersion interactions. Second, the cohesive energy density (δ^2) of the

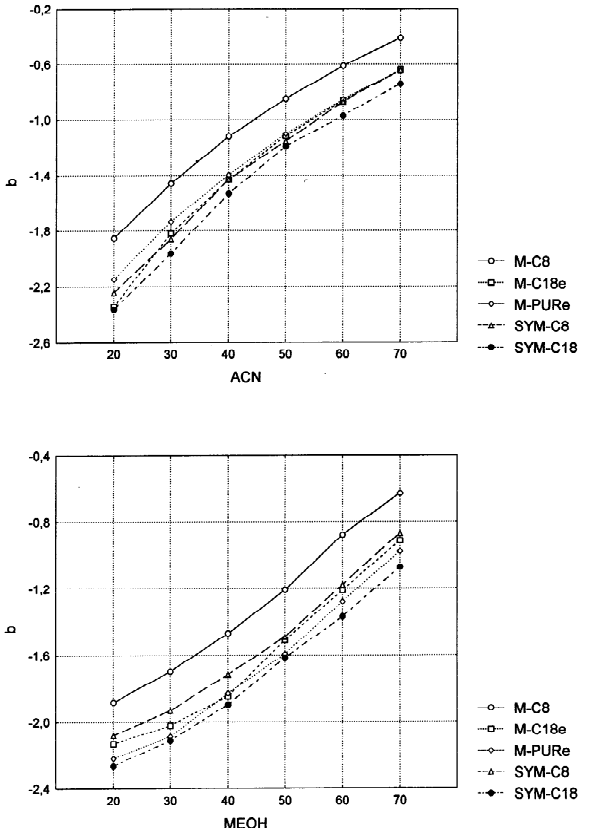


Fig. 3. Regression coefficients, *b*, of Eq. (3) as a function of percent of organic modifier (v/v).

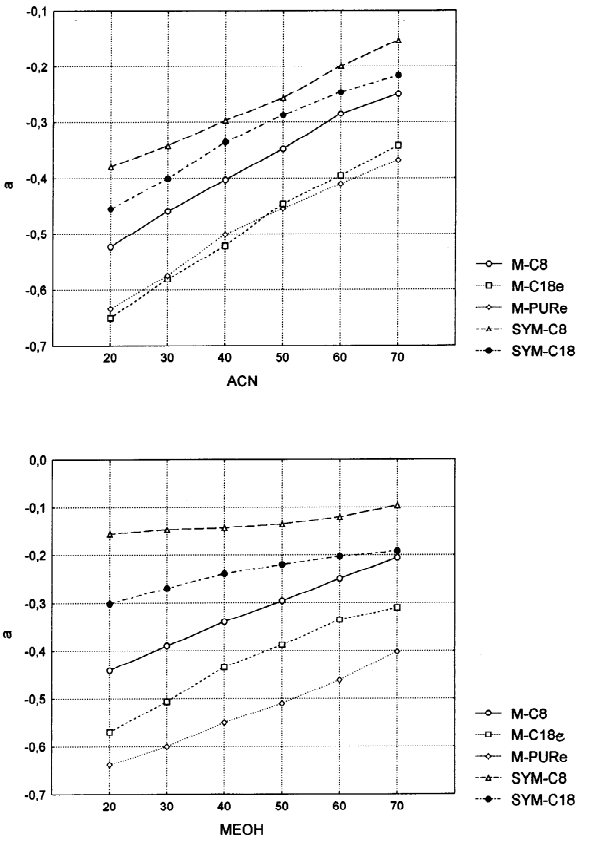


Fig. 4. Regression coefficients, *a*, of Eq. (3) as a function of percent of organic modifier (v/v).

mobile phase – which characterizes its polarity – is the dominating parameter influencing retention and the magnitude of the v coefficient.

As regards the dependence of the v coefficient on mobile phase composition, Wang et al. [36] found a linear correlation in the composition range of 20–50% organic modifier (Fig. 3). Our results in Fig. 1 show also a quasi linear correlation in the above composition range but it can be seen that in a wider composition range the dependence is not linear. Nevertheless, the data can be well described by a linear correlation as it is shown in Table 3.

4.1.2. The r coefficient

The coefficient r in Fig. 2 refers to the difference between the solvated bonded and mobile phase to interact with solute n- and π-electrons. The positive r obtained for all columns indicated that electron involved interactions are slightly stronger in the stationary phase than in the mobile phase. This can be interpreted as that the stationary phase is more polarizable than the mobile phase [23].

The r coefficient is also increasing by increasing water content of the mobile phase but the values of the r coefficients are much lower than the v coefficients in the whole composition range. The type of the organic modifier seems to have small influence on the r coefficient. Considerable differences can be observed, however, among the different columns. The large r coefficients obtained for SymmetryShield columns suggest that these specialty phases can enter into enchanced electron involved interactions due to their special surface chemistry, namely the π-electron-rich carbamate groups embedded in the ligand.

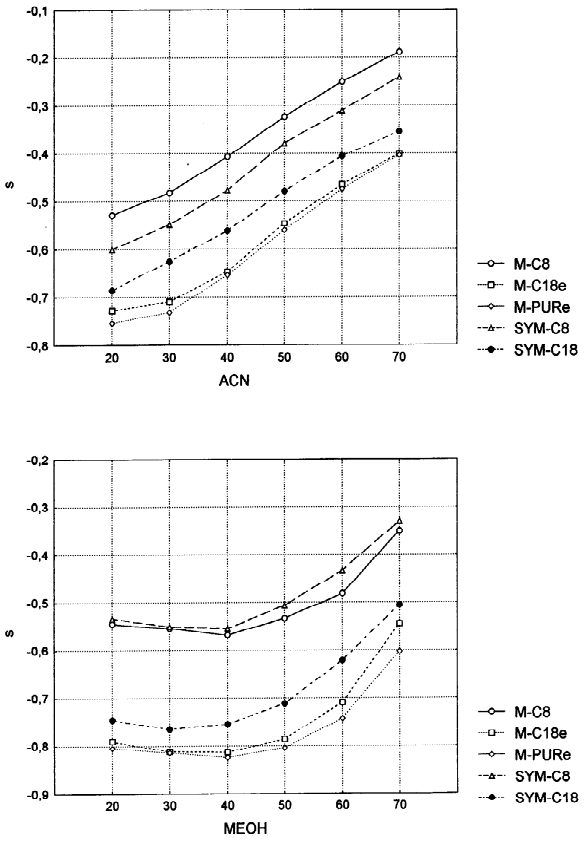


Fig. 5. Regression coefficients, *s*, of Eq. (3) as a function of percent of organic modifier (v/v).

4.1.3. The *b* coefficient

The coefficient *b* in Fig. 3 reflects the difference in hydrogen-bond donor (HBD) acidity between the stationary and mobile phases. The aqueous mobile phase has strong HBD acidity [26] which increases with increasing water content. Reversed-phase packing 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 ($\alpha_1 = 1.17$) of the mobile phase. As the solvation parameter, α_1, representing solvent HBD acidity is higher for methanol ($\alpha_1 = 0.93$) than for acetonitrile ($\alpha_1 = 0.19$), at the same composition the mobile phase is more acidic in the case of methanol than with acetonitrile resulting in an increase in the difference between the stationary and mobile phases, i.e. an increase of the absolute values of the *b* coefficients [23].

A similar interpretation has been given in the paper of Tan and Carr [35] for ACN and THF but not for MeOH. They have found that the *b* coefficient for the MeOH–water system remained reasonably constant over the range of the mobile phase composition investigated. They reason that the 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.

Table 3
Linear correlation of *v* and $-b$ coefficents with mobile phase composition

	ACN (20–70%)			MEOH (20–70%)		
	R^2	SD	*F*	R^2	SD	*F*
Column (*v*)						
M-C_8	0.9798	0.1033	194.03	0.9998	0.0125	20 047.51
M-C_{18}e	0.9699	0.1431	129.03	0.9986	0.0321	2996.80
M-PURe	0.9726	0.1358	142.18	0.9942	0.0620	681.69
SYM-C_8	0.9811	0.1075	208.07	0.9995	0.0175	8867.85
SYM-C_{18}	0.9701	0.1390	129.85	0.9994	0.0192	7121.64
Column ($-b$)						
M-C_8	0.9850	0.0740	261.86	0.9921	0.0477	504.77
M-C_{18}e	0.9749	0.1120	155.39	0.9723	0.0894	140.52
M-PURe	0.9858	0.0753	277.23	0.9868	0.0612	299.83
SYM-C_8	0.9871	0.0769	304.97	0.9834	0.0661	237.47
SYM-C_{18}	0.9868	0.0954	204.08	0.9893	0.0525	369.32
$-b$ vs. *v*	0.9906	0.0562	2935.88	0.9439	0.1113	471.67

In contrast, we have found that the *b* coefficient varies similarly in the MeOH system as in the ACN system (Fig. 3). Comparing the MeOH and ACN systems by using the α_1 solvation parameters given above and the sorption data of the solvents on the stationary phase published by Yonker et al. [31,32] it turned out that the change in the acidity of the MeOH mobile phase system is really smaller (φ_w = 0.3: $\alpha_1 = 1.00$; $\varphi_w = 0.8$: $\alpha_1 = 1.11$; $\Delta\alpha_1 = 0.11$) with increasing water concentration than for the ACN mobile phase system ($\varphi_w = 0.3$: $\alpha_1 = 0.85$; φ_w 0.8: $\alpha_1 = 1.02$; $\Delta\alpha_1 = 0.17$), but the difference in acidity between the mobile and stationary phases increases in both systems with increasing water concentration (MeOH: $\varphi_w = 0.3$: $\Delta\alpha_{m/s} = 0.02$; $\varphi_w = 0.8$: $\Delta\alpha_{m/s} = 0.06$; ACN: $\varphi_w = 0.3$: $\Delta\alpha_{m/s} = 0.07$; $\varphi_w = 0.8$: $\Delta\alpha_{m/s} = 0.78$) resulting in an increase of the *b* coefficient. This interpretation, however, is a rough approximation, because it is based on the presumable composition of the sorbed mobile phase components on the stationary phase and does not take into account the role of the silanol groups on the surface influencing significantly the acidity of the stationary phase.

For the columns investigated the two endcapped C_{18} columns and the SYM columns seem to have similar acidities resulting in similar *b* coefficients over the whole composition range. For the nonendcapped M-C_8 column, however, the absolute values of the *b* coefficients are considerably lower, indicating enhanced acidity of this column due to the availability of acidic silanol sites on the surface.

Wang et al. [36] found a linear correlation between *b* and mobile phase composition in the range of 20–50% organic modifier (Fig. 3). As it can be seen in Fig. 3 the correlation is not linear over a wider composition range but it can be really well approximated by linear correlation, as shown in Table 3.

4.1.4. The a coefficient

The *a* coefficients shown in Fig. 4 reflect the difference in hydrogen-bond acceptor (HBA) basicity of the stationary and mobile phases. The solvation parameter, β_1, representing HBA basicity of the solvents are as follows: β_1 water = 0.43, β_1 methanol = 0.62, β_1 acetonitrile = 0.37 [23]. 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. As the difference in stationary and mobile phase basicities is less pronounced [26], the coefficients obtained are remarkably smaller than that for HBD acidity. Tan and Carr [35] have found that the *a* coefficient is virtually independent of φ_w, i.e. the concentration of water in the mobile phase. Our results show a small but definite increase of the *a* coefficient with increasing water concentration (Fig. 4). If we compare the differences in basicity between the mobile phase and stationary phase the above results seem to be well supported. The basicity is represented by the β_1 solvation parameter given above, the approximate compositions of the stationary phase were taken again from the study of Yonker et al. [31,32]. The difference in basicity in the MeOH system with increasing water content between mobile phase and stationary phase is also increasing ($\varphi_w = 0.3$: $\Delta\beta_{m/s} = 0.02$; $\varphi_w = 0.8$: $\Delta\beta_{m/s} = 0.07$) somewhat less than in the ACN system ($\varphi_w = 0.3$: $\Delta\beta_{m/s} = 0.01$; $\varphi_w = 0.8$: $\Delta\beta_{m/s} = 0.09$) resulting in a small increase of the *a* coefficient also in the MeOH system. The amount and composition of the sorbed mobile phase on the stationary phase depends both on the type of the organic modifier and the stationary phase [31,32]. For this reason there are some differences among the *a* coefficients obtained for acetonitrile and methanol but the range of the values is practically the same. There are, however, significant differences among the various columns. The columns of lowest HBA basicity were M-PURe and M-C_{18}e, i.e. the well covered, endcapped C_{18} columns. The nonendcapped M-C_8 column has shown somewhat higher basicity due to the acidic/basic character of the accessible surface. The SymmetryShield columns exhibited stronger HBA activity than the other packing as a consequence of the pronounced basic character of the carbamate functionality built into the ligands.

4.1.5. The s coefficient

Difference between stationary and mobile phase dipolarity/polarizability is measured through the *s* coefficients, as shown in Fig. 5. The solvent property complementary to the solute's π^*_2 parameter is the solvent's dipolarity/polarizability, π^*_1. The mobile phase is highly dipolar because its components,

water ($\pi^*_1 = 1.09$), acetonitrile ($\pi^*_1 = 0.75$) and methanol ($\pi^*_1 = 0.60$) are strongly dipolar substances [23]. Although the bonded alkyl chains are almost incapable of dipole interactions, the sorbed modifier and water molecules may substantially increase its π^* value. In addition, accessible silanol groups on the packings and special compounds built into the ligands can contribute to the polarity of the stationary phase. However, the difference in polarity between the bonded phase and mobile phase is small and generally slightly increases by increasing the water content of the mobile phase. Tan and Carr [35] found that the *s* coefficient is virtually independent of φ_w in all three systems (MeOH, ACN and THF) investigated. Our results show a definite increase of the *s* coefficient with increasing water concentration in the ACN system and between 30–50% water concentration in the MeOH system (Fig. 5). In the range of $\varphi_w = 0.5$–0.8, the range investigated by Tan and Carr, we also found a virtual constancy of the *s* coefficient. Considering again the π^*_1 solvation parameters of the solvents and the approximate composition of the stationary phase [31,32] the difference in dipolarity between the mobile phase and stationary phase increases with increasing water concentration. However, in the MeOH system this difference is significantly lower ($\varphi_w = 0.3$: $\Delta\pi^*_{m/s} = 0.06$; $\varphi_w = 0.8$: $\Delta\pi^*_{m/s} = 0.15$) than in the ACN system ($\varphi_w = 0.3$: $\Delta\pi^*_{m/s} = 0.02$; $\varphi_w = 0.8$: $\Delta\pi^*_{m/s} = 0.27$). The difference in the course of the plots between acetonitrile and methanol modifier can be explained by the different amount and composition of the sorbed mobile phase for the two modifiers [31,32].

The characteristics of the columns have significant influence on the polar interactions. Here again M-PURe and M-C_{18}e columns displayed the largest negative *s* values indicating a diminished ability to interact with the solute's polarizable functional groups. The SYM-C_{18} column shows somewhat higher polarity presumably due to the carbamate groups in the ligands. The most dipolar/polarizable columns were the C_8 phases, which may be interpreted by the more accessible surface, the availability of free silanols and in case of SYM-C_8 also by the carbamate groups in the ligands.

Since among the polar interactions influencing retention in RP-HPLC the most important are the differences in HBD acidity (*b*) and HBA basicity (*a*) between the stationary phase and mobile phase, we have plotted the ratio of a/b of the two coefficients obtained for the columns investigated.

In Fig. 6 the variation of the a/b ratio is shown as a function of the mobile phase composition. As expected, this ratio will decrease with increasing water content indicating a decrease in the role of the polar interactions with the stationary phase in highly aqueous mobile phases. The effect of the organic modifier corresponds to that discussed in connection with the *b* and *a* coefficients.

There is, however, a marked difference between the ''normal'' columns and the SymmetryShield columns. As a consequence of the enhanced basic character of these columns (low *a* coefficients) the a/b ratio is significantly lower for the SYM columns

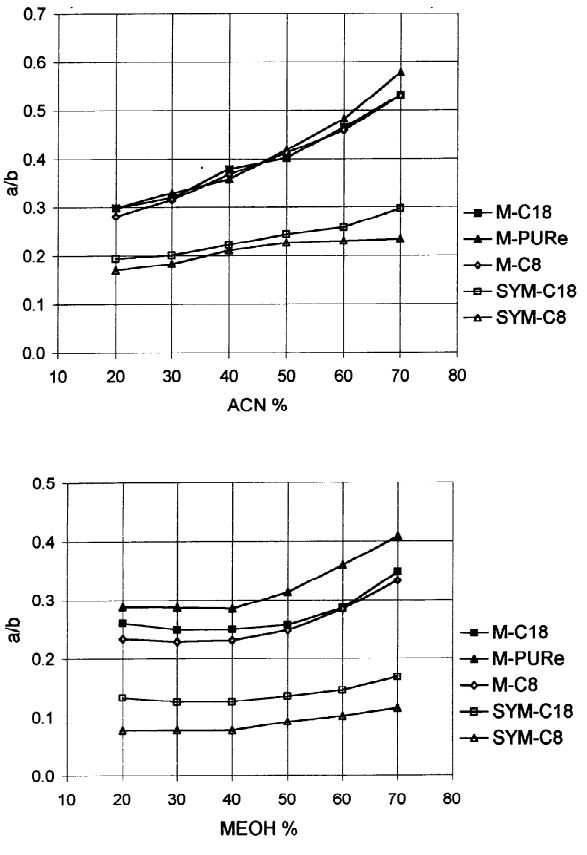


Fig. 6. Ratio of a/b regression coefficients as a function of percent of organic modifier (v/v).

and the effect of the mobile phase composition is less pronounced than for the other columns.

In order to compare the effect of mobile phase composition on the regression coefficients of the LFER relationship in Fig. 7 all the five coefficients are plotted as a function of mobile phase composition for the M-C_{18}e column. In publications presenting LFER analysis for RP-HPLC processes it was established that the most important retention-governing solute parameters are solute size (V_x) and hydrogen bond basicity ($\Sigma\beta_2^H$) [1,15–18,21–23], this is reflected also in the values of the v and b coefficients. For fixed mobile phase composition percent contribution of the individual solute terms to the log k value was also calculated. Depending on the type of solutes investigated Tan et al. [26] found that the vV_x term accounts for about 40–70% and the $b\Sigma\beta^H$ term accounts for about 30–45% of the variance in log k values [Eq. (3)]. They concluded that the above two terms account for more than 90% of the variance in log k values and the other terms are not needed to adequately define log k. However, apart from the statistical problems in these calculations because of the strong covariances among the solute parameters, Fig. 7 clearly shows that the extent and proportion of the individual terms depend significantly on mobile phase composition.

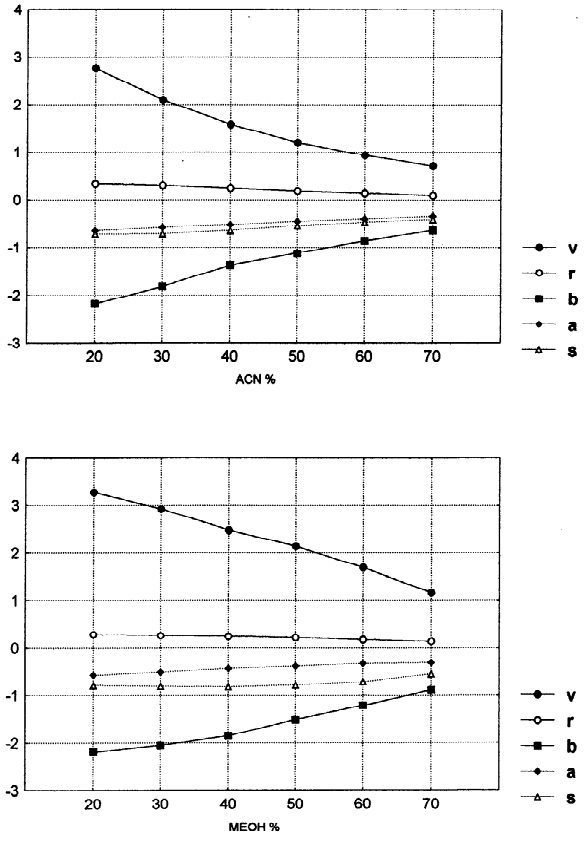


Fig. 7. Regression coefficients of Eq. (3) obtained for M-C_{18}e column as a function of percent of organic modifier (v/v).

4.1.6. Correlation of the b and v coefficients

First it was suggested by Abraham et al. [27] that instead of the LSER coefficients the ratios of b/v, a/v, r/v and s/v should be used to characterize C_{18} stationary phases over a wide composition range of mobile phase. Quite recently Reta et al. [43] have demonstrated that the b coefficient is linearly related to the v coefficient for a number of different stationary phases; i.e. the b coefficient changes linearly with v as the mobile phase composition is changed. They investigated this correlation in a fairly narrow composition range: 45–60% methanol in the mobile phase. Our results in a much wider composition range and also for acetonitrile modifier support their findings as it is shown in Fig. 8.

Next we have investigated how the regression coefficients of the LFER equation representing different types of molecular interactions will influence the different types of selectivities with changing mobile phase composition. A further objective was to study the role of the stationary phase in establishing different selectivities.

4.2. Effect of mobile phase composition on the selectivity factors

4.2.1. Hydrophobic or methylene selectivity

As we have discussed before, the v coefficient of Eq. (3) represents the difference in hydrophobicity between the stationary phase and the mobile phase which depends on the difference in cohesivity of the two phases and the magnitude of dispersive interactions between the solute and the stationary and mobile phase, respectively. We have seen that the more polar the mobile phase is the higher the v coefficient is.

In Fig. 9a the variation of the α selectivity factor of ethylbenzene (EB) to toluene (T) representing hydrophobic or methylene selectivity is shown as a function of the v coefficients obtained under various

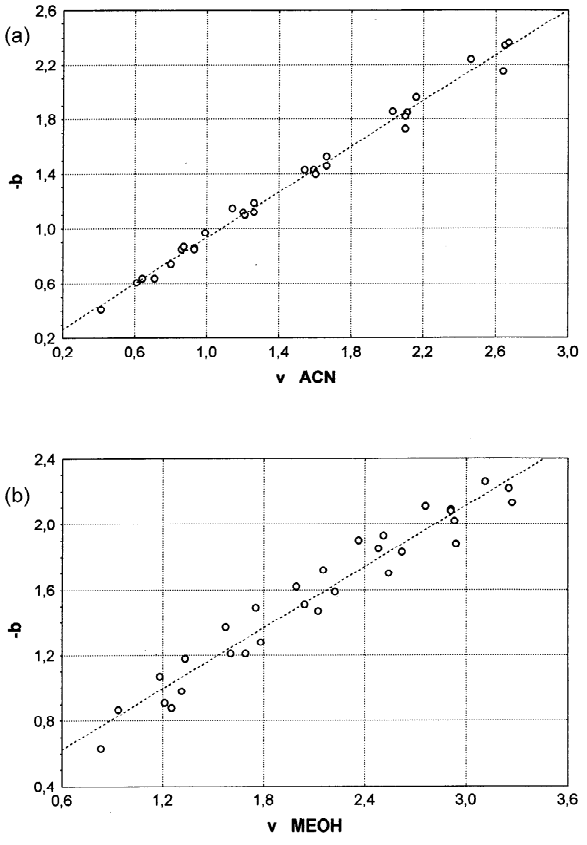


Fig. 8. Correlation of b vs. v regression coefficients for the columns investigated (a) ACN 20–70%; (b) MEOH 20–70%.

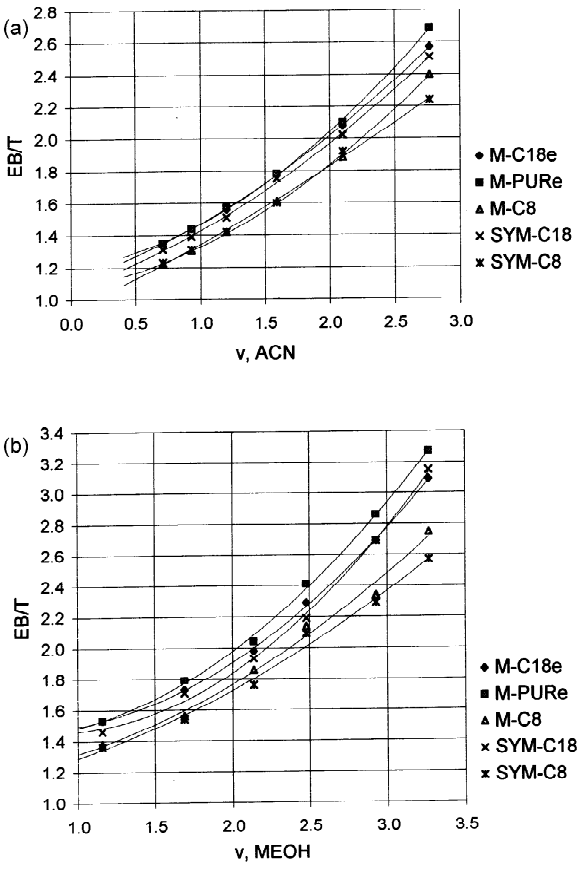


Fig. 9. The α selectivity factor of ethylbenzene to toluene (EB/T) as a function of v coefficient on different columns: (a) ACN 20–70%; (b) MEOH 20–70%.

mobile phase compositions with an ACN modifier for the five columns investigated. In Fig. 9b the same plots are shown, obtained with MeOH as the organic modifier. These plots are quite similar to those obtained by plotting $\alpha_{EB/T}$ as a function of the organic concentration in the mobile phase [3] taking into account the fact that v increases with increasing water concentration. 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. It is interesting to note that at fixed v values the selectivity factors are practically the same in both systems. It follows that the v coefficient is a good indication of hydrophobic or methylene selectivity in a given phase system.

It has been established that methylene selectivity depends also on the hydrophobic strength of the column. For that reason C_8 columns provide lower methylene selectivity than C_{18} columns [3]. This can also be observed in Fig. 9. C_8 columns have lower v coefficients and consequently lower methylene selectivity than C_{18} columns.

The r coefficient in Eq. (3) refers to the difference between the solvated bonded phase and mobile phase to interact with solute n- and π-electrons. The r coefficient is also positive and increases with increasing water content similarly to the v coefficient. However, the values of the r coefficients are much lower (0.05–0.5) than the v coefficients (1.2–3.2) and do not influence methylene selectivity.

4.2.2. Polar or chemical selectivity

Polar 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 [3]. The higher the α is, the stronger the polar solute is retained compared to toluene.

Polar selectivity depends on the regression coefficients characterizing the polar interactions between the stationary phase and the mobile phase. In Eq. (3) the polar interactions are characterized by the regression coefficients *b*, *a* and *s*.

From among the above coefficients, the *b* coefficient representing the difference in acidity between the stationary phase and the mobile phase exerts the highest influence on the retention process [1,15,16,26,43]. The *b* coefficient is always negative and its absolute value increases considerably by increasing the water content of the mobile phase [26,27].

In Fig. 10a the variation of the α selectivity factors for two basic (caffeine, aniline) and two acidic (methylparaben, *p*-cresol) compounds, i.e. the relative retentions of the polar solutes to that of toluene, are shown as a function of *b* coefficients obtained under various mobile phase compositions with an ACN modifier for the M-C_{18}e column. In Fig. 10b the same plots are shown obtained with MeOH as the organic modifier. In Figs. 11–13 the same plots are shown for M-8 (Fig. 11), SYM–C_{18} (Fig. 12) and SYM-C_8 (Fig. 13) columns.

In general, it can be concluded that the variation

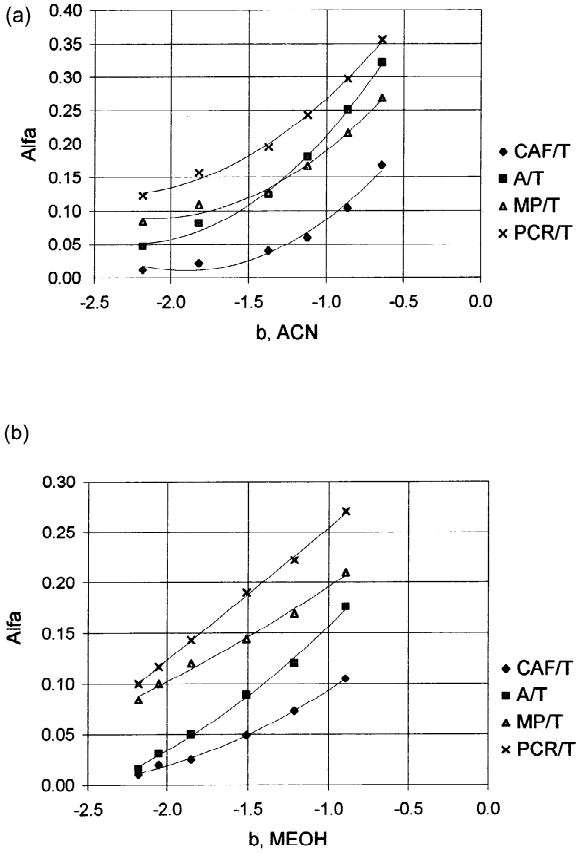


Fig. 10. Polar selectivity factors, relative retentions of polar solutes (CAF, A, MP, PCR) to that of toluene (T) as a function of *b* coefficient on M-C_{18}e column: (a) ACN 20–70%; (b) MEOH 20–70%.

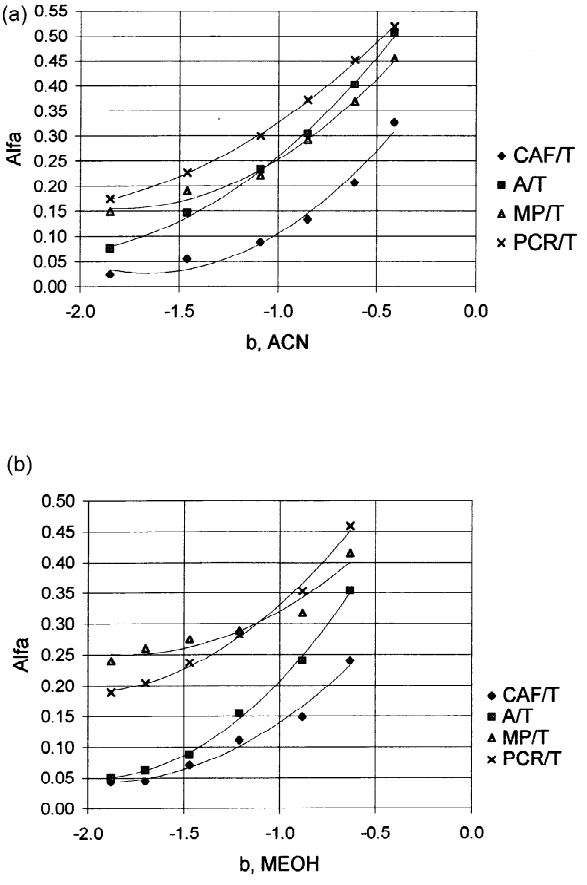


Fig. 11. Polar selectivity factors, relative retentions of polar solutes (CAF, A, MP, PCR) to that of toluene (T) as a function of *b* coefficient on M-C_8 column: (a) ACN 20–70%; (b) MEOH 20–70%.

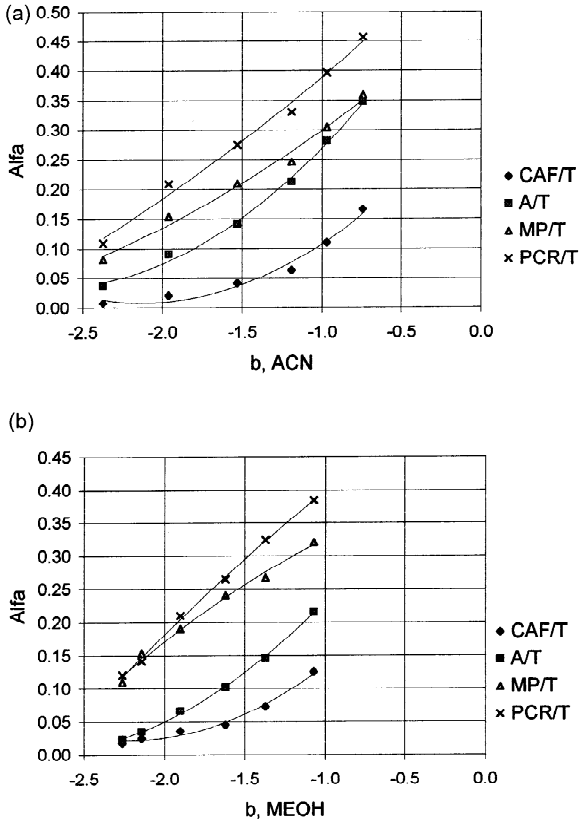


Fig. 12. Polar selectivity factors, relative retentions of polar solutes (CAF, A, MP, PCR) to that of toluene (T) as a function of *b* coefficient on SYM-C_{18} column: (a) ACN 20–70%; (b) MEOH 20–70%.

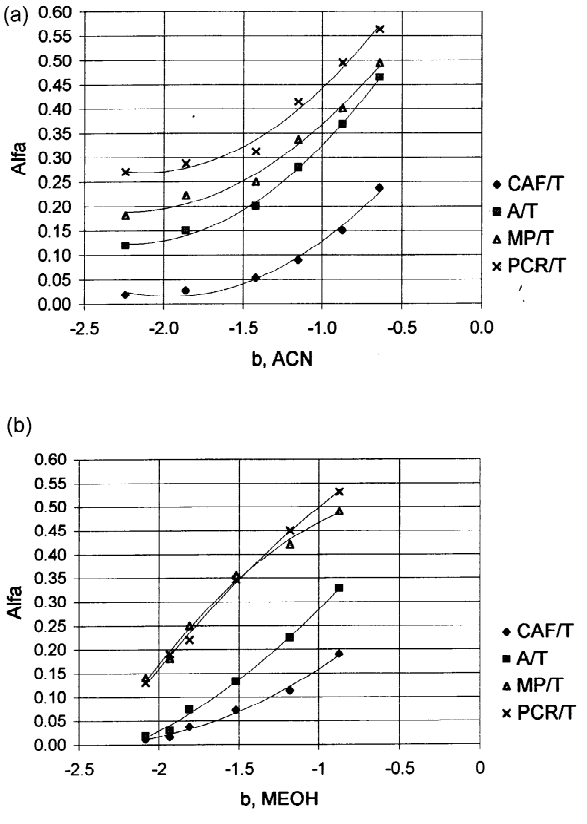


Fig. 13. Polar selectivity factors, relative retentions of polar solutes (CAF, A, MP, PCR) to that of toluene (T) as a function of *b* coefficient on SYM-C_8 column: (a) ACN 20–70%; (b) MEOH 20–70%.

of the polar selectivity factor can be well described by the *b* coefficient. Polar selectivity, i.e. the relative retention of polar solutes to that of toluene decreases with increasing water 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.

However, the properties of the solutes and the characteristics of the stationary phase will exert a significant influence on the polar selectivity obtained for different compounds.

Since the M-PURe column is quite similar to the M-C_{18}e column, the selectivity factors and their dependence on the *b* coefficient are quite similar for both columns.

In Fig. 11 the polar selectivity factors are plotted for the non-endcapped C_8 column. In accordance with the higher polarity/silanol activity of this column the selectivity factors are higher in both mobile phase systems than for the C_{18} columns.

In Figs. 12 and 13 the polar selectivity factors for the two basic type SymmetryShield columns are plotted. Here the selectivity factors for the acidic type compounds (MP, PCR) are significantly higher than on the neutral C_{18} columns because of the preferential retention of acidic solutes, while for the basic compound (CAF) there is little change in the α values. For aniline (A) the selectivity factors are higher than for the C_{18} columns because of the

basic/acidic character of aniline (see Table 2). Comparing the two SYM columns it can be seen that the C_8 column provides higher polar selectivities for all compounds than the C_{18} column.

4.2.3. Specific selectivity

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. In addition, it depends also on the acidic or basic properties of the columns investigated. Specific selectivity comes about as a composite of the various polar interactions in the given phase system. These various polar interactions provide different specific selectivities in the relative retention of acidic, basic, acidic to basic and basic to acidic compounds. All of these specific selectivities depend also on the type of the organic modifier and the composition of the mobile phase.

Since the most important regression coefficient describing polar interactions is the *b* coefficient [24,43] we have plotted the α selectivity factors determined for different types of compounds as a function of the *b* coefficient. In Fig. 14 the specific selectivity factors of different solutes are plotted for the M-C_{18}e column as a function of the *b* coefficient. The pairs of solutes selected were: ethylparaben–*p*-cresol (EP–PCR; acidic/acidic); methylbenzoate–benzylcyanid (MBO–BC; basic/basic); 2,6-dimethylphenol–*o*-toluidine (DP26–OT; acidic/basic) and dimethylphtalate–methylparaben (PDM–MP; basic/acidic).

In Figs. 15–17 the same plots are shown for M-C_8, SYM-C_{18} and SYM-C_8 columns. For all solute pairs and all columns investigated it can be

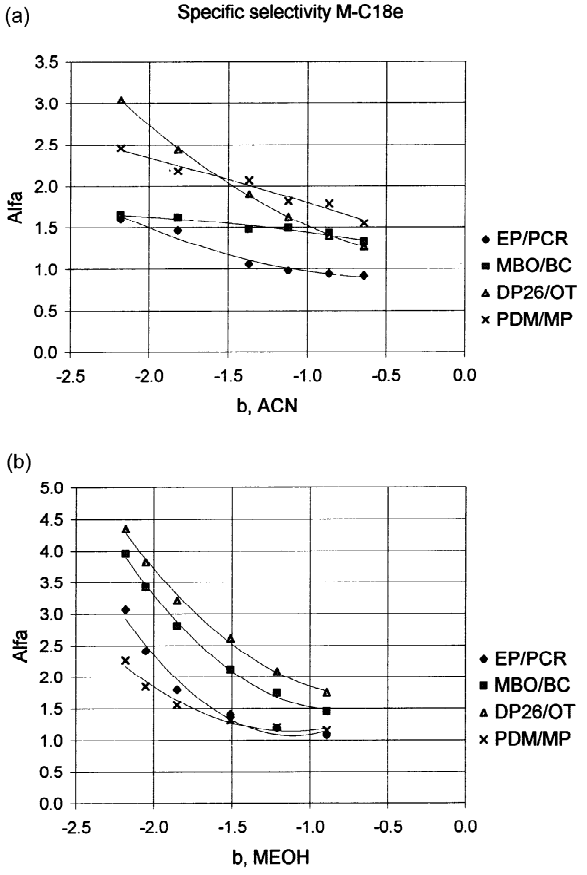


Fig. 14. Specific selectivity factors of selected solutes on a M-C_{18}e column: (a) ACN 20–70%; (b) MEOH 20–70%.

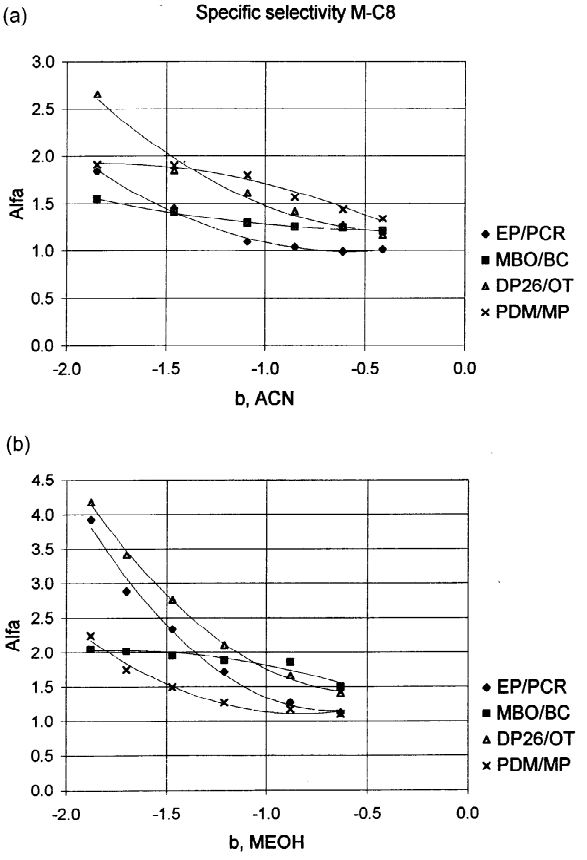


Fig. 15. Specific selectivity factors of selected solutes on a M-C_8 column: (a) ACN 20–70%; (b) MEOH 20–70%.

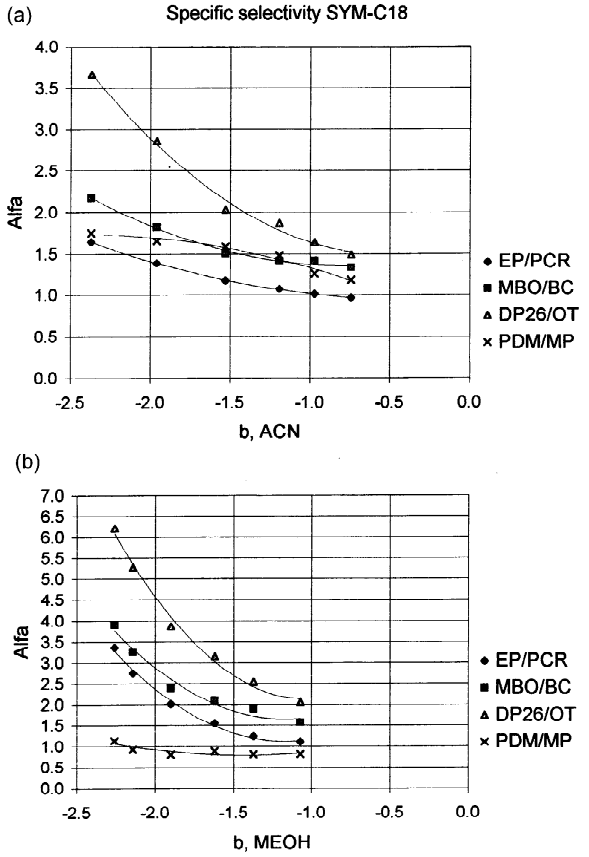


Fig. 16. Specific selectivity factors of selected solutes on a SYM-C_{18} column: (a) ACN 20–70%; (b) MEOH 20–70%.

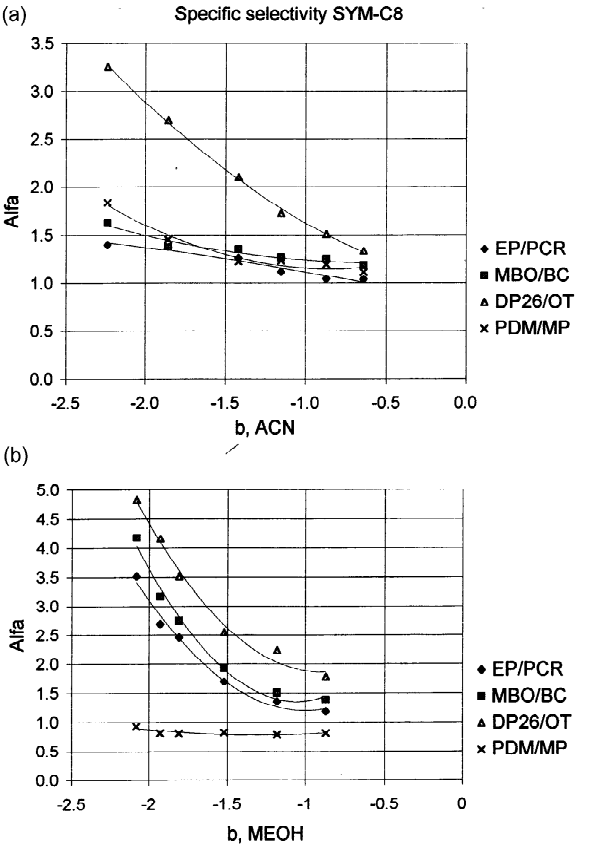


Fig. 17. Specific selectivity factors of selected solutes on a SYM-C_8 column: (a) ACN 20–70%; (b) MEOH 20–70%.

generally established that the specific selectivity increases with an increasing absolute value of *b* coefficient, i.e. with increasing polarity of the mobile phase. For this reason the more polar methanol provides higher specific selectivities than acetonitrile.

The characteristics of the columns, however, will influence differently the specific selectivities of different types of compounds. Both the selectivity factors and the course of the values are quite similar for the two neutral C_{18} columns. In Fig. 15 the plots are somewhat different for the nonendcapped C_8 column. But if you compare the α values obtained at the same *b* coefficient, the differences are quite small.

In Figs. 16 and 17 the α specific selectivity factors are plotted for the basic SymmetryShield columns. Here the differences among the specific selectivity factors obtained for different types of compounds are significantly higher. The relative retention of DP26–OT (acidic/basic) is significantly higher, while the relative retention of PDM–MP (basic/acidic) is significantly lower because of the preferential retention of the acidic solutes. The relative retentions of EP–PCR (acidic/acidic) and MBO–BC (basic/basic) at a given *b* coefficient do not vary significantly in comparison with the neutral C_{18} columns. It can be established that the *b* coefficient determined under various mobile phase compositions can be used to estimate and compare specific selectivities for different types of polar compounds.

4.3. Prediction of selectivity by LFER method

Since the selectivity of separation depends not only on the chemical properties of the solvent but

also significantly on the properties of the solutes and the stationary phase, neither of the solvent selectivity triangle (SST) approaches [60–63] can provide a useful estimate of selectivity.

Selectivity of separation can be estimated more reliably by the LFER method using the solvation parameters describing the different molecular interactions and the regression coefficients of the LFER equation characterizing the phase system.

In Fig. 18 the measured and calculated α selectivity factors are shown for the M-C_{18}e column at fixed mobile phase composition [ACN–water (30:70%, v/v)]. The figure indicates that the LFER model can describe selectivity factors over a wide numerical range. In principle, it is possible to predict selectivity factors for any pair of solutes in a given phase system using the LFER coefficients without chromatographic experiments.

In Fig. 19a the measured and calculated α selectivity factors are plotted, obtained over a wide composition range (20–70% ACN) with an ACN modifier. In Fig. 19b the same selectivity factors are plotted obtained with a methanol modifier (20–70%) . The average error of the calculation is in the range of 5–20%.

By all means, it can be concluded that the LFER method [Eq. (4)] provides a useful prediction of selectivities under different operating conditions. The overall predictive power of the LFER method could be enchanced by improving the accuracy of the solvation parameters and using a larger set of greatly different test compounds for the determination of system regression coefficients.

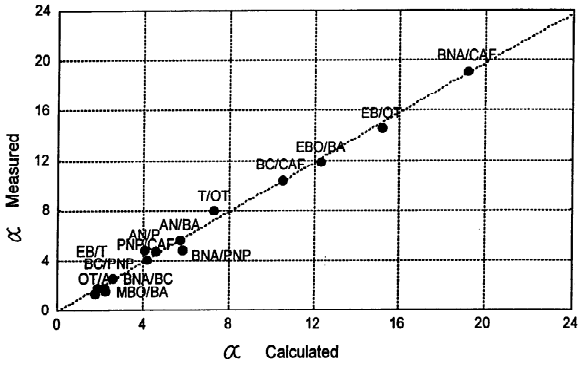


Fig. 18. Measured and calculated α selectivity factors on a M-C_{18}e column. Mobile phase: ACN–water (30:70%, v/v).

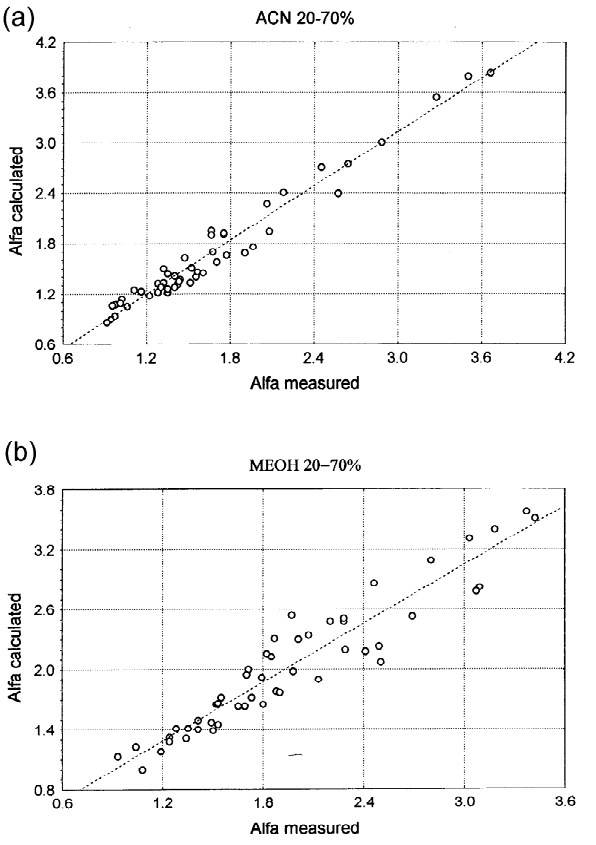


Fig. 19. Measured and calculated selectivity factors for various solutes on a M-C_{18}e column: EB–T, EP–PCR, DP26–OCR, PEP–OCR, EBO–DMA, OT–A, EP–A, MBO–MP, NA–PCR; mobile phase: (a) 20–70% acetonitrile, (b) 20–70% methanol.

5. Conclusion

The regression coefficients of the LFER equation [Eq. (3)] determined over a wide composition range (20–70%, v/v, organic modifier) for acetonitrile and methanol revealed the extent and relative importance of the individual molecular interactions with varying mobile phase conditions.

In accordance with the literature, the main characteristics influencing retention are the difference in hydrophobicity between the stationary phase and mobile phase (coefficient v) as well as the difference in hydrogen bond donor (HBD) acidity between the stationary and mobile phase (coefficient b). However, the proportion of the above coefficients to the other coefficients of Eq. (3) (a, s, r) considerably

decreases by increasing organic modifier concentration, indicating changes in the relative contribution of the individual molecular interactions.

The influence of the organic modifiers on the individual regression coefficients (and the various molecular interactions) can be largely explained by the physical characteristics (Hildebrand solubility factor, solvatochromic parameters) of the mobile phase compositions.

In contrast to several studies [23,26,27] it was established that the type and characteristics of the columns exert a significant influence on the mechanism of the retention process reflected also in the regression coefficients of the LFER equation.

It has been established that the regression coefficients of the LFER equation determined over a wide range of mobile phase compositions with acetonitrile and methanol as the organic modifiers can be applied to estimate the selectivity for the separation of different types of sample compounds. The effect of the type of the organic modifier and the composition of the mobile phase on the different types of selectivities can be described and evaluated by using the regression coefficients representing the various molecular interactions.

Hydrophobic or methylene selectivity depends on the difference in hydrophobicity of the stationary phase and the mobile phase and can be well described by the v coefficient of the LFER equation in the whole composition range investigated. These plots show also the influence of the type of the organic modifier as well as of the characteristics of the column on hydrophobic selectivity.

The variation of *polar or chemical selectivity* – relative retention of polar solutes to that of a nonpolar solute, e.g. toluene – with mobile phase composition can be described and evaluated by using the b coefficient of Eq. (3) representing the difference in acidity between the stationary phase and the mobile phase. This type of interaction has been established as the most important polar interaction influencing chromatographic retention [1,15,16, 26,43]. Polar selectivity decreases with increasing polarity of the mobile phase. For this reason acetonitrile provides higher polar selectivity than methanol. The effect of column type on polar selectivity has also been demonstrated.

Relative retention of various polar solutes defined as *specific selectivity* can also be well described by using the b coefficient determined under different mobile phase compositions. Specific selectivity increases with increasing polarity of the mobile phase, for this reason methanol generally provides higher specific selectivity than acetonitrile. The characteristics of the column will influence differently the specific selectivity of different types of compounds.

By using Eq. (4) the α selectivity factors can be predicted for any pairs of compounds with known solvation parameters using the regression coefficients determined for the given phase system.

6. Nomenclature

a, b, c, r, s, v	Regression coefficients of Eq. (3)
HBA	Hydrogen-bond acceptor
HBD	Hydrogen-bond donor
k	Retention factor
LFER	Linear free energy relationship
R_2	LFER solvation parameter for excess molar refractivity
V_x	LFER solvation parameter for McGowan molecular volume
α	Selectivity factor
$\Sigma\alpha_2^H$	LFER solvation parameter for HBD acidity
$\Sigma\beta_2^H$	LFER solvation parameter for HBA basicity
π^*_2	LFER solvation parameter for dipolarity/polarizability
δ^2	Hildebrand solubility parameter

Acknowledgements

This work was supported by grants OTKA 025041 and OTKA F 023120. We gratefully acknowledge the donation of the LiChrospher 100 RP-18e and the Purospher RP-18e columns to the Merck Kft. (Budapest, Hungary) and the donation of the SymmetryShield C_8 and C_{18} columns to Dr. U.D. Neue (Waters Co., Milford, MA, USA). Special thanks are due to Mrs. Katalin Fazekas for technical assistance.

References

[1] Á. Sándi, L. Szepesy, J. Chromatogr. A 818 (1998) 1.
[2] Á. Sándi, L. Szepesy, J. Chromatogr. A 818 (1998) 19.

[3] Á. Sándi, L. Szepesy, J. Chromatogr. A 845 (1999) 113.
[4] M.J. Kamlet, R.W. Taft, J. Am. Chem. Soc. 98 (1976) 377.
[5] M.J. Kamlet, J.L.M. Abboud, R.W. Taft, Prog. Phys. Org. Chem. 19 (1977) 6027.
[6] M.J. Kamlet, J.L.M. Abboud, R.W. Taft, Prog. Phys. Org. Chem. 13 (1981) 485.
[7] M.J. Kamlet, J.L.M. Abboud, M.H. Abraham, R.W. Taft, J. Org. Chem. 48 (1983) 2877.
[8] P.C. Sadck, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, M.H. Abraham, Anal. Chem. 57 (1885) 2971.
[9] M.J. Kamlet, R.M. Doherty, J.L.M. Abboud, M.H. Abraham, R.W. Taft, CHEMTECH 16 (1986) 566.
[10] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, J. Chromatogr. 587 (1991) 229.
[11] M.H. Abraham, G.S. Whiting, R.M. Doherty, W.J. Shuely, J. Chromatogr. 518 (1990) 329.
[12] T.O. Kollie, C.F. Poole, Chromatographia 33 (1992) 551.
[13] M.H. Abraham, D.P. Walsch, J. Chromatogr. 627 (1992) 294.
[14] C.F. Poole, T.O. Kollie, Anal. Chim. Acta 282 (1993) 1.
[15] P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W. Melander, Cs. Horváth, Anal. Chem. 58 (1986) 2674.
[16] M.J. Kamlet, M.H. Abraham, P.W. Carr, R.M. Doherty, R.W. Taft, J. Chem. Soc. Perkin Trans. II (1998) 2087.
[17] J.H. Park, P.W. Carr, M.H. Abraham, R.W. Taft, R.M. Doherty, M.J. Kamlet, Chromatographia 25 (1988) 373.
[18] M.J. Kamlet, R.M. Doherty, M.H. Abraham, T. Marcus, R.W. Taft, J. Phys. Chem. 92 (1988) 5244.
[19] J.H. Park, P.W. Carr, J. Chromatogr. 465 (1989) 123.
[20] W.J. Cheong, P.W. Carr, Anal. Chem. 61 (1989) 1524.
[21] M. Rosés, E. Bosch, Anal. Chim. Acta 274 (1993) 147.
[22] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[23] M.H. Abraham, M. Rosés, J. Phys. Org. Chem. 7 (1994) 672.
[24] M.H. Abraham, Pur. Appl. Chem. 65 (1993) 2503.
[25] M.H. Abraham, J. Andonian-Haftvan, G.S. Whiting, A. Leo, J. Chem. Soc. Perkin Trans. II (1994) 1777.
[26] L.C. Tan, P.W. Carr, M.H. Abraham, J. Chromatogr. A 752 (1996) 1.
[27] M.H. Abraham, M. Rosés, C.F. Poole, S.K. Poole, J. Phys. Org. Chem. 10 (1997) 358.
[28] P.T. Jackson, M.R. Schure, T.W. Weber, P.W. Carr, Anal. Chem. 69 (1997) 358.
[29] A. Nasal, P. Haber, R. Kaliszan, E. Forgács, T. Cserháti, M.H. Abraham, Chromatographia 43 (1996) 484.
[30] J.H. Park, J.J. Chae, T.H. Nah, M.D. Jang, J. Chromatogr. A 664 (1994) 149.
[31] C.R. Yonker, T.A. Zwier, M.F. Burke, J. Chromatogr. 241 (1982) 257.
[32] C.R. Yonker, T.A. Zwier, M.F. Burke, J. Chromatogr. 241 (1982) 269.
[33] T.C. Schunk, M.F. Burke, J. Chromatogr. A 656 (1993) 289.
[34] N. Tanaka, H. Kimata, K. Hosoya, M. Miyanashi, T. Araki, J. Chromatogr. A 656 (1993) 265.
[35] L.C. Tan, P.W. Carr, J. Chromatogr. A. 799 (1998) 1.
[36] A. Wang, L.C. Tan, P.W. Carr, J. Chromatogr. A. 848 (1999) 21.
[37] P. Jandera, H. Colin, G. Guiochon, Anal. Chem. 54 (1982) 435.
[38] P. Jandera, J. Chromatogr. 314 (1984) 13.
[39] B.P. Johnson, M.G. Khaledi, J.G. Dorsey, Anal. Chem. 58 (1986) 2354.
[40] B.P. Johnson, M.G. Khaledi, J.G. Dorsey, J. Chromatogr. 384 (1987) 221.
[41] M.R.P. Breuer, H.A. Claessens, C.A. Cramers, Chromatographia 38 (1994) 137.
[42] J. Zhao, P.W. Carr, Anal. Chem. 71 (1999) 2623.
[43] M. Reta, P.W. Carr, P.C. Sadek, S.C. Rutan, Anal. Chem. 71 (1999) 3484.
[44] P.E. Antle, A.P. Goldberg, L.R. Snyder, J. Chromatogr. 321 (1985) 1.
[45] K.B. Sentell, J.G. Dorsey, J. Chromatogr. 461 (1989) 193.
[46] B. Shaikh, J.E. Tomaszewski, Chromatographia 17 (1983) 675.
[47] A. Tchapla, H. Colin, G. Guiochon, Anal. Chem. 56 (1984) 621.
[48] N. Tanaka, H. Kimata, K. Hosoya, M. Miyanashi, T. Araki, J. Chromatogr. A 656 (1993) 265.
[49] P. Jandera, J. Chromatogr. 352 (1986) 91.
[50] L.C. Sander, S.A. Wise, CRC Crit. Rev. Anal. Chem. 18 (1987) 299.
[51] L.C. Sander, S.A. Wise, LC·GC 8 (1990) 378.
[52] R.K. Gilpin, M. Jaroniec, S. Lin, Chromatographia 30 (1990) 393.
[53] D.V. Mc Calley, J. Chromatogr. A 708 (1995) 185.
[54] D.V. McCalley, J. Chromatogr. A 738 (1996) 169.
[55] K.B. Sentell, N.I. Ryan, A.N. Henderson, Anal. Chim. Acta 307 (1995) 203.
[56] P.L. Zhu, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 756 (1996) 41.
[57] U.D. Neue, Anal. Chem. 67 (1995) 3809.
[58] H. Engelhardt, H. Lôw, W. Götzinger, J. Chromatogr. 544 (1991) 371.
[59] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[60] L.R. Snyder, J. Chromatogr. 92 (1974) 223.
[61] L.R. Snyder, J. Chromatogr. Sci. 16 (1978) 223.
[62] S.C. Rutan, P.W. Carr, W.J. Cheong, J.H. Park, L.R. Snyder, J. Chromatogr. 463 (1989) 21.
[63] L.R. Snyder, P.W. Carr, S.C. Rutan, J. Chromatogr. A 656 (1993) 537.