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METHOD FOR CHARACTERIZATION OF SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

II. POSSIBILITIES FOR THE PREDICTION OF RETENTION DATA

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SUMMARY

The method suggested for characterization of selectivity, based on an adequate calibration homologous series, is used for predictions of relative retentions of various solutes. It allows the prediction of possible changes in the order of elution with changing composition of mobile phases (selectivity cross-overs) from the combination of constants characterizing lipophilic and polar contributions to selectivity. It also allows the characterization and prediction of selectivity in ternary mobile phases containing two different organic solvents and water. Using this method, it is possible to predict the retention on one column from the constants that characterize the lipophilicities and polarities of the functional groups of the solutes measured on another column (with bonded alkyl chains of the same length) with a probable relative error between 10 and 20%.

INTRODUCTION

In Part I of this series[1] the derivation of a new method for characterization of selectivity in reversed-phase liquid chromatography is presented and verified. Here, possibilities for the prediction of retention data are indicated and illustrated by means of practical examples.

THEORETICAL

Eqns. 4 and 5 in Part I[1] describe the separation of selectivity into two contributions, the lipophilic selectivity, α_L, and the polar selectivity, α_P

$$\alpha = \alpha_L \alpha_P \tag{1}$$

where

$$\log \alpha_P = -x\Delta q \tag{2}$$

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and

$$\log \alpha_L = a_1(1 - px)\Delta n_c \tag{3}$$

for systems where linear log k' *vs.* x relationships can be used, or

$$\log \alpha_L = [a_1(1 - px) + d_1x^2]\Delta n_c \tag{4}$$

for systems that require quadratic log k' *vs.* x equations. The concentration of the more efficient eluting component in the mobile phase, *i.e.*, the concentration of an organic solvent in binary aqueous–organic mobile phases, is x. The constants a_1, p (and, if necessary, d_1) depend on the nature of the organic solvent, but not on its concentration in the mobile phase. They are almost independent of the nature of the solute and are not very significantly influenced by the nature of the alkylsilica column-packing material provided the bonded alkyls have a constant length. These constants are determined from the k' values for the members of a calibration homologous series (*n*-alkylbenzenes). The constants Δn_c and Δq characterize a given compound in terms of its lipophilicity and of the polarity of its functional groups.

It is not necessary always to relate the selectivity to a single reference standard compound (such as toluene in Part I[1]), but the relative retention of two arbitrary compounds, the separation of which is of interest, can be described in the same way. It follows from eqns. 2–4 that the extrapolated selectivity in water and the quadratic term of the log α *vs.* x relationship are determined solely by the lipophilic contribution to selectivity, but the linear term of the log α *vs.* x relationship is comprised of both the lipophilic and polar contributions to selectivity.

For a better illustration of the two contributions to selectivity, we should recall that q_i is constant for the members of a given homologous series[2]. This means that $\Delta q = 0$ and, consequently, $\log \alpha_P = 0$ for pairs of homologues. The selectivity for the members of an homologous series is determined only by the lipophilic contribution, *i.e.*, $\alpha = \alpha_L$, in agreement with previous findings[2–6]. In other words, the lipophilic contribution to selectivity can be understood as the selectivity between compounds that differ only in the size of their alkyl substituents. On the other hand, compounds with equal lipophilic carbon equivalents, n_{ce}, have equal capacity factors in pure water (extrapolated), $\Delta n_c = 0$, and such pairs of compounds can be separated only on the basis of the polar contribution to selectivity. Such a separation would be possible only if $\Delta q \neq 0$, *i.e.*, if the two compounds possess different polar groups which are subject to different interactions with the polar components of the mobile phase.

The characterization of the contribution of each compound to retention and selectivity by means of two constants (n_{ce} and q_i) instead of a single index[7] not only permits a distinction between the lipophilic and polar contributions to retention (and selectivity), but also allows predictions of the changes in selectivity caused by changing the mobile phase composition and even possible changes in the order of elution (selectivity cross-overs), which is not possible if each compound is characterized only by a single index. This is illustrated schematically in Fig. 1, where the selectivity for a hypothetical pair of compounds i and j (described as $\log \alpha_{j,i}$) and various contributions to $\log \alpha_{j,i}$ are plotted *vs.* the concentration of an organic solvent in a binary mobile phase.

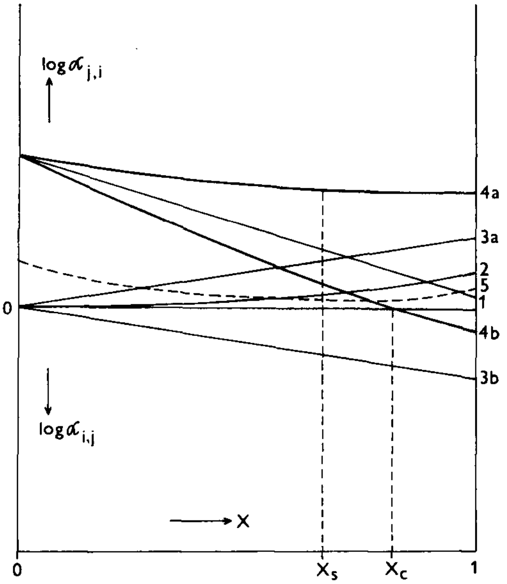

Fig. 1. Schematical plots of the selectivity, $\alpha_{j,i}$ and its components as a function of the concentration of organic solvent in the mobile phase, x (in % · 10^{-2}). Plots: 1, $a_1\Delta n_c(1 - px)$ *vs.* x; 2, $d_1x^2\Delta n_c$ *vs.* x; 3, $\log \alpha_P = - \Delta q \cdot x$ for $\Delta q < 0$ (a) and $\Delta q > 0$ (b); 4, $\log \alpha_{j,i}$ *vs.* x for $\Delta q < 0$ (a) and $\Delta q > 0$ (b); 5, a possible contribution of polar interactions in the stationary phase to selectivity. $x_c = x$ for $\alpha_{j,i} = 1$; for x_s see the text.

Line 1 shows the contribution of the term $a_1\Delta n_c(1 - px)$ to $\log \alpha_L$ and to selectivity (provided $\Delta n_c > 0$) and curve 2 illustrates the contribution of the quadratic term $d_1x^2\Delta n_c$ to $\log \alpha_L$ and to $\log \alpha$ (the quadratic term can often be neglected). Lines 3a and b characterize the polar contribution, $\log \alpha_P = -\Delta q \cdot x$. to selectivity for two different instances: a, $\Delta q < 0$ (line 3a); b, $\Delta q > 0$ (line 3b). Curves 4a and b show the selectivity ($\log \alpha_{j,i}$) as a function of x for these two different instances. Curve 4a shows the dependence of selectivity on the mobile phase composition for a pair of compounds with $\Delta n_c > 0$ and $\Delta q < 0$. Here, the selectivity is approximately constant or it decreases slightly with increasing x. (In the instance illustrated the sum of the polar contribution $\log \alpha_P$ and of the quadratic term just compensates the decrease in the term $-a_1\Delta n_c \cdot px$ for the mobile phase composition range where x increases over a certain limit, x_s, and the selectivity is practically independent of the mobile phase composition in this region.) In certain instances, the contribution of the quadratic term can be so significant that even minima on $\log \alpha_{j,i}$ *vs.* x curves can be observed. Other relationships between selectivity and mobile phase composition are obtained for a pair of compounds with both $\Delta n_c > 0$ and $\Delta q > 0$. One member of this pair contains not only a bulkier hydrocarbon part, but also a more polar functional group(s) than the other compound. The selectivity for such a pair decreases significantly with increasing content of the organic solvent in the mobile phase, and $\log \alpha_{j,i}$ can even acquire negative values (curve 4b in Fig. 1). A mobile phase with the concentration of an organic solvent x_c where $\log \alpha_{j,i} = 0$ and $\alpha_{j,i} = 1$ elutes the two compounds in a single peak without separation. In the mobile phase composition range where $x > x_c$, $\alpha_{j,i} < 1$, the order of elution is reversed and compound i is eluted later than compound j ($\alpha_{i,j} = (\alpha_{j,i})^{-1} > 1$). A reversal of the elution order (selectivity cross-over) is likely for the pairs of compounds with $\Delta n_c > 0$ and $\Delta q > 0$ and can be predicted on the basis of the present theory.

In the above description of the contributions to selectivity it is assumed that the mobile phase interactions (the hydrophobic effect and polar interactions in the mobile phase) are far more significant than the interactions of solutes with some polar adsorption centres on the surface of the stationary phase (such as the interactions with residual silanol groups[8,9]) or than possible interactions with the layer of organic solvent formed by preferential sorption of the organic solvent on to the surface of the bonded phase[10–13]. Antle *et al.*[14] have recently introduced the "effective phase ratio" to characterize relative differences in the contributions of various columns to retention. However, this quantity cannot characterize possible selective interactions of solutes in the stationary phase.

It is very difficult to describe such effects quantitatively, as they depend on the nature of the solutes (basic compounds would interact more strongly with silanol groups than neutral ones). It can only be suggested that interactions with the adsorbed solvent layer would be relatively more important for mobile phases with very low concentrations of an organic solvent, while the interactions with polar (silanol) groups on the surface of the bonded phase would be more significant for mobile phases with low concentrations of water. (Water obviously competes with solutes for interactions with silanol groups to a greater extent than does an organic solvent.) A possible contribution of the stationary phase interactions to selectivity is indicated schematically in Fig. 1 as the dashed line 5. In the present approach, the contribution of these interactions is neglected to a first approximation, which can lead to certain errors in the values of n_{ce}, q_i, Δn_c and Δq and in predicted retention volumes or selectivities. However, the results presented in Part I[1] indicate that, in spite of these potential errors, the prediction of retention and selectivity based on the present method can be useful in practice.

Recently, it has been shown that the retention (log k') in ternary mobile phases containing two organic solvents, X and Y, in concentrations x and y, can be calculated from the constants n_{ce} and q_i of the solutes determined in the binary mobile phases water–X and water–Y[7]. A similar equation for the selectivity in ternary mixtures (log α_T or log $\alpha_{j,i,T}$) can be derived, provided the interactions between the organic solvents, X and Y, do not influence the retention significantly:

$$\log \alpha_T = [a_1(1 - p_x x - p_y y) + (d_{1x} x^2 + d_{1y} y^2)]\,\Delta n_c - \Delta q_x \cdot x - \Delta q_y \cdot y \tag{5}$$

Eqn. 5 applies if approximately equal values of Δn_c (and a_1) are found in the binary mobile phases water–X and water–Y. If the differences between the Δn_c (and a_1) values, determined in the two binary mobile phases, are significant, as may often be the case, another equation for α_T should be used. This equation can be obtained after combination of the following equations from refs. 2 and 15:

$$a = \frac{x a_x + y a_y}{x + y}$$

$$\log k' = a - m_x x - m_y y + d_x x^2 + d_y y^2$$

$$m_x = a_x p_x + q_x,\ m_y = a_y p_y + q_y,\ a_x = a_{0x} + a_{1x} n_{ce\,x},$$
$$a_y = a_{0y} + a_{1y} n_{ce\,y},\ d_x = d_{0x} + d_{1x} n_{ce\,x},\ d_y = d_{0y} + d_{1y} n_{ce\,y}$$

then

$$\log \alpha_T = a_{1x} \Delta n_{cx}\left(\frac{1}{x+y} - p_x\right)x + a_{1y}\Delta n_{cy}\left(\frac{1}{x+y} - p_y\right)y - \Delta q_x \cdot x - \Delta q_y \cdot y + \left[d_{1x}\Delta n_{cx} \cdot x^2 + d_{1y}\Delta n_{cy} \cdot y^2\right] \tag{6}$$

The subscripts x and y denote the values in the binary mobile phases water–X and water–Y, respectively. The quadratic terms in eqns. 5 and 6 can be neglected for systems where d_{1x} and d_{1y} are close to zero.

EXPERIMENTAL

The instrumentation, solutes, mobile phases and the method for evaluating the experimental data are described in Part I[1]. In addition to the columns used there, the following columns were also employed: Silasorb C_8, 7.5 μm, 300 × 3.8 mm I.D. (packed in this laboratory); LiChrosorb RP C_8, 7 μm, 250 × 4.0 mm I.D. (commercial stainless-steel column); Silasorb C_{18}, 10 μm, 300 × 4.2 mm I.D. (packed in this laboratory); LiChrosorb RP C_{18}/Si 100, 10 μm, 300 × 4.2 mm I.D. (packed in this laboratory); LiChrosorb RP C_{18}/Si 60, 10 μm, 300 × 3.8 mm I.D. (packed in this laboratory); Hypersil C_{18}, 5 μm, 150 × 4.2 mm I.D. (packed in this laboratory); μBondapak C_{18}, 300 × 3.9 mm I.D. (commercial stainless-steel column); Separon Six C_{18}, 5 μm, 150 × 3.9 mm I.D. (commercial cartridge glass column); Silasorb C_{18}, 5 μm, 300 × 4.2 mm I.D. (packed in this laboratory).

RESULTS AND DISCUSSION

Order of elution and selectivity cross-overs

To verify the possibilities of predicting selectivity for various compounds, including predictions of the order of elution and possible selectivity cross-overs, the lipophilic and polar contributions to selectivity ($\log \alpha_L$ and $\log \alpha_P$) and the resulting selectivity, $\log \alpha$ (related to toluene as the standard compound), were calculated for several pairs of compounds on the Silasorb C_8 column with methanol–water and acetonitrile–water as mobile phases. These data are listed in Table I, together with calculated (c) and experimental (e) selectivities, $\alpha_{j,i}$, characterizing the relative retention of the pairs of compounds of interest.

For the pair benzophenone/chlorobenzene (the first example in Table I), both Δn_c(benzophenone/chlorobenzene) > 0 and Δq(benzophenone/chlorobenzene) > 0, because benzophenone contains a more polar functional group (carbonyl) and a bulkier hydrocarbon part (two benzene rings) than chlorobenzene with one chloro substituent and a single benzene ring. According to the present theory, a selectivity

TABLE I

EXAMPLES OF THE INFLUENCE OF THE LIPOPHILIC (α_L) AND POLAR (α_P) CONTRIBUTIONS TO SELECTIVITY (α) ON THE ORDER OF ELUTION

Column: Silasorb C_8. Mobile phases: methanol–water (A) and acetonitrile–water (B). α_L, α_P and α are related to toluene as the standard compound; $\alpha_{j,i}$ denote the selectivities between the individual sample compounds. c = calculated, e = experimental.

Compound	*Mobile phase*		*x [%(v/v) of the organic solvent · 10^{-2}]*							
			0.4	*0.5*	*0.55*	*0.6*	*0.7*	*0.8*	*0.9*	*1*
Benzophenone (1)	A	log α_L	0.198	0.168	0.154	0.139	0.110	0.080	0.051	0.022
(Δn_c = 0.63,		log α_P	−0.056	−0.070	−0.077	−0.084	−0.098	−0.112	−0.126	−0.140
Δq = 0.14)		log α	0.142	0.098	0.077	0.055	0.012	−0.032	−0.075	−0.118
Chlorobenzene (2)		log α_L	0.079	0.067	0.061	0.055	0.044	0.032	0.020	0.008
(Δn_c = 0.25,		log α_P	−0.024	−0.030	−0.033	−0.036	−0.042	−0.048	−0.054	−0.060
Δq = 0.06)		log α	0.055	0.037	0.028	0.019	0.002	−0.016	−0.034	−0.052
		$\alpha_{2,1}$ (c)	0.82	0.87	0.89	0.92	0.98	1.04	1.10	1.17
		$\alpha_{2,1}$ (e)				0.90	1.01	1.06	1.07	
Benzophenone (1)	B	log α_L	0.150	0.130	0.120	0.110	0.090	0.071	0.051	0.031
(Δn_c = 0.78,		log α_P	−0.064	−0.080	−0.088	−0.096	−0.112	−0.128	−0.144	−0.160
Δq = 0.16)		log α	0.086	0.050	0.032	0.014	−0.022	−0.057	−0.093	−0.129
Chlorobenzene (2)		log α_L	0.084	0.073	0.068	0.062	0.051	0.040	0.029	0.018
(Δn_c = 0.44,		log α_P	−0.040	−0.050	−0.055	−0.060	−0.070	−0.080	−0.090	−0.100
Δq = 0.10)		log α	0.044	0.023	0.013	0.002	−0.019	−0.040	−0.061	−0.082
		$\alpha_{2,1}$ (c)	0.91	0.94	0.96	0.97	1.01	1.04	1.08	1.11
		$\alpha_{2,1}$ (e)		0.93	0.97	0.99	1.02	1.06		
n-Propyl phenyl ether (3)	A	log α_L	0.176	0.150	0.137	0.124	0.098	0.071	0.045	0.019
(Δn_c = 0.56,		log α_P	−0.036	−0.045	−0.050	−0.054	−0.063	−0.072	−0.081	−0.090
Δq = 0.09)		log α	0.140	0.105	0.087	0.070	0.035	−0.001	−0.036	−0.071
Bromobenzene (4)		log α_L	0.173	0.147	0.134	0.122	0.096	0.070	0.044	0.019
(Δn_c = 0.55,		log α_P	−0.024	−0.030	−0.033	−0.036	−0.042	−0.048	−0.054	−0.060
Δq = 0.06)		log α	0.149	0.117	0.101	0.086	0.054	0.022	−0.010	−0.041
		$\alpha_{4,3}$ (c)	0.98	0.97	0.97	0.96	0.96	0.95	0.94	0.93
		$\alpha_{4,3}$ (e)				0.97	0.95	0.95	0.94	
n-Propyl phenyl ether (3)	B	log α_L	0.140	0.122	0.112	0.103	0.084	0.066	0.048	0.029
(Δn_c = 0.73,		log α_P	−0.032	−0.040	−0.044	−0.048	−0.056	−0.064	−0.072	−0.080
Δq = 0.08)		log α	0.108	0.082	0.068	0.055	0.028	0.020	−0.024	−0.051

Bromobenzene (4)		log α_L	0.109	0.095	0.088	0.080	0.066	0.052	0.037	0.023
(Δn_c = 0.57,		log α_P	−0.028	−0.035	−0.039	−0.042	−0.049	−0.056	−0.063	−0.070
Δq = 0.07)		log α	0.081	0.060	0.049	0.038	0.017	−0.004	−0.026	−0.047
		$\alpha_{4,3}$ (c)	1.06	1.05	1.04	1.04	1.03	1.01	1.00	0.99
		$\alpha_{4,3}$ (e)		1.05	1.03	1.04	1.04	0.97		
Linuron (5)	A	log α_L	0.185	0.158	0.144	0.130	0.103	0.075	0.048	0.020
(Δn_c = 0.59,		log α_P	−0.088	−0.110	−0.121	−0.132	−0.154	−0.176	−0.198	−0.220
Δq = 0.22)		log α	0.097	0.048	0.231	−0.002	−0.051	−0.101	−0.150	−0.200
Chlorbromuron (6)		log α_L	0.295	0.252	0.230	0.208	0.164	0.120	0.076	0.032
(Δn_c = 0.94,		log α_P	−0.100	−0.125	−0.138	−0.150	−0.175	−0.200	−0.225	−0.250
Δq = 0.25)		log α	0.195	0.127	0.092	0.058	−0.011	−0.080	−0.149	−0.218
n-Butyl-N-phenylcarbamate (7)		log α_L	0.292	0.249	0.227	0.205	0.162	0.118	0.075	0.032
(Δn_c = 0.93,		log α_P	−0.144	−0.180	−0.198	−0.216	−0.252	−0.288	−0.324	−0.360
Δq = 0.36)		log α	0.148	0.069	0.029	−0.011	−0.090	−0.170	−0.249	−0.328
		$\alpha_{6,5}$ (c)	1.25	1.20	1.17	1.14	1.10	1.05	1.00	0.96
		$\alpha_{6,5}$ (e)				1.09	1.11	1.14	0.92	
		$\alpha_{7,6}$ (c)	0.90	0.88	0.86	0.85	0.83	0.81	0.80	0.78
		$\alpha_{7,6}$ (e)				0.84	0.88	0.76	0.83	
		$\alpha_{7,5}$ (c)	1.12	1.05	1.01	0.98	0.92	0.85	0.80	0.74
		$\alpha_{7,5}$ (e)				0.92	0.97	0.86	0.77	
Linuron (5)	B	log α_L	0.075	0.065	0.060	0.055	0.045	0.035	0.025	0.016
(Δn_c = 0.39,		log α_P	−0.076	−0.095	−0.104	−0.114	−0.133	−0.152	−0.171	−0.190
Δq = 0.19)		log α	−0.001	−0.030	−0.044	−0.059	−0.088	−0.117	−0.146	−0.174
Chlorbromuron (6)		log α_L	0.084	0.073	0.068	0.062	0.051	0.040	0.029	0.018
(Δn_c = 0.44,		log α_P	−0.064	−0.080	−0.088	−0.096	−0.112	−0.128	−0.144	−0.160
Δq = 0.16)		log α	0.020	−0.007	−0.020	−0.034	−0.061	−0.088	−0.115	−0.142
n-Butyl-N-phenylcarbamate (7)		log α_L	0.152	0.132	0.122	0.112	0.092	0.072	0.052	0.032
(Δn_c = 0.79,		log α_P	−0.088	−0.110	−0.121	−0.132	−0.154	−0.176	−0.198	−0.220
Δq = 0.22)		log α	0.064	0.022	0.001	−0.020	−0.062	−0.104	−0.146	−0.188
		$\alpha_{6,5}$ (c)	1.05	1.06	1.06	1.06	1.06	1.07	1.07	1.08
		$\alpha_{6,5}$ (e)		1.07	1.06	1.07	1.06	1.07		
		$\alpha_{7,6}$ (c)	1.10	1.07	1.05	1.03	1.00	0.96	0.93	0.90
		$\alpha_{7,6}$ (e)		1.03	1.08	1.01	1.02	0.97		
		$\alpha_{7,5}$ (c)	1.16	1.13	1.11	1.09	1.06	1.03	1.00	0.97
		$\alpha_{7,5}$ (e)		1.10	1.14	1.08	1.08	1.04		

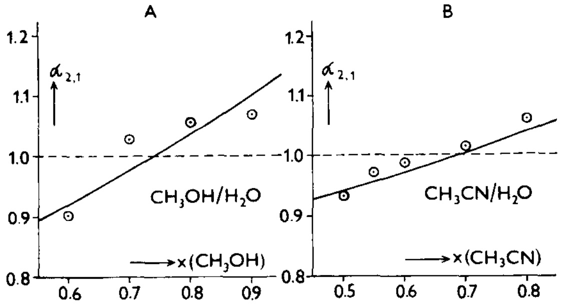

Fig. 2. Example of the change in elution order with changing concentration of the organic solvent in the mobile phase, x. Column: Silasorb C_8, 10 μm. Compounds: 1 = benzophenone (Δn_c = 0.63, Δq = 0.14 in methanol–water; Δn_c = 0.78, Δq = 0.16 in acetonitrile–water); 2 = chlorobenzene (Δn_c = 0.25, Δq = 0.06 in methanol–water; Δn_c = 0.44, Δq = 0.10 in acetonitrile–water). $\alpha_{2,1}$ = Relative retention, lines represent predicted values, points the experimental values. (A) mobile phase, methanol–water; (B) mobile phase, acetonitrile–water [x in % (v/v) $\cdot$ 10^{-2}].

cross-over with changing mobile phase composition is likely for such a pair of compounds and could be calculated by using eqn. 4 from Part I[1] and the values of Δn_c and Δq. It was found experimentally for this pair of compounds in methanol–water as the mobile phase at approximately 70% methanol and in acetonitrile–water as the mobile phase at approximately 65% acetonitrile (Table I and Fig. 2).

In the second example in Table I, the pair of compounds *n*-propyl phenyl ether and bromobenzene, having Δn_c (ether/bromobenzene) $\approx$ 0 and Δq(ether/bromobenzene) > 0 in methanol–water as mobile phase show approximately equal lipophilicities. Their separation, if possible, would be based only on the polar contribution to selectivity and, consequently, the selectivity of separation would improve with increasing concentration of the organic solvent in the mobile phase. As the difference Δq is relatively small, the selectivity $\alpha_{j,i}$ is relatively close to zero over the whole range of mobile phase compositions and the separation would be difficult. In acetonitrile–water as mobile phase, Δn_c(ether/bromobenzene) > 0 and Δq(ether/bromobenzene) $\approx$ 0. Here, the selectivity cross-over occurs (predicted for 90% acetonitrile and found experimentally at 80% acetonitrile). The selectivity, α, is also close to 1 for the mobile phases containing at least 40% acetonitrile and would improve at lower concentrations of acetonitrile, but at the cost of analysis time.

The last example in Table I considers the selectivity for two phenylurea herbicides, linuron and chlorbromuron, and *n*-butyl-N-phenylcarbamate with potential herbicidal properties. With methanol–water as mobile phase, Δn_c(chlorbromuron/linuron) > 0 and Δq(chlorbromuron/linuron) > 0. Consequently, the reversal of the elution order occurs at $\approx$90% methanol. Δn_c(phenylcarbamate/chlorbromuron) $\approx$ 0 and Δq(phenylcarbamate/chlorbromuron) > 0, which means that *n*-butyl-N-phenylcarbamate is eluted prior to chlorbromuron over the whole range of the mobile phase composition; Δn_c(phenylcarbamate/linuron) > 0 and Δq(phenylcarbamate/linuron) > 0, and the reversal of the elution order occurs at approximately 55% methanol. Consequently, the elution order linuron, *n*-butyl-N-phenylcarbamate, chlorbromuron is predicted and observed for mobile phases with less than 55% methanol, *n*-butyl phenylcarbamate, linuron, chlorbromuron for mobile phases containing 55–90% methanol and *n*-butyl phenylcarbamate, chlorbromuron, linuron for mobile phases containing more than 90% methanol. In acetonitrile–water as mobile phase, Δn_c(chlorbromuron/linuron) > 0 and Δq(chlorbromuron/linuron) < 0, which means that the elution order linuron, chlorbromuron does not change with changing concentration of acetonitrile in the mobile phase and that the selectivity is almost independent of the acetonitrile/water ratio of the mobile phases. For the pairs *n*-butyl-N-phenylcarbamate/chlorbromuron and *n*-butyl-N-phenylcarbamate/linuron $\Delta n_c > 0$ and $\Delta q > 0$, and the reversal of the elution order occurs at approximately 70% acetonitrile for the first pair and at approximately 90% acetonitrile for the second pair of compounds. Consequently, the three compounds are eluted in the order linuron, chlorbromuron, *n*-butyl-N-phenylcarbamate in the mobile phases containing less than 70% acetonitrile, in the order linuron, *n*-butyl-N-phenylcarbamate, chlorbromuron by mobile phases containing 70–90% acetonitrile and in the order *n*-butyl-N-phenylcarbamate, linuron, chlorbromuron by mobile phases with more than 90% acetonitrile.

Selectivity in ternary mobile phases: homologous series

The selectivity of separation of the individual homologues is given by the lipophilic contribution only, because q_i is constant, $\Delta q = 0$ and $\log \alpha_P = 0$ for the members of an homologous series. Consequently, $\log \alpha = \log \alpha_L$. The selectivity in homologous series allows the investigation of various influences on the lipophilic contribution.

To investigate the influence of the composition of ternary mobile phases acetonitrile–methanol–water on α_L, the lipophilic selectivities were calculated for several different compositions, using two different methods.

(a) Assuming a linear dependence of log k' on the concentration of the organic solvent both in the binary mobile phases methanol–water and acetonitrile–water, the constants a_1 and p, determined from the experimental data for *n*-alkylbenzenes in binary mobile phases of several different compositions (see Table III in Part I[1]) were employed for calculations of α_L on a Silasorb C_{18} column, using a simplified linear form of eqn. 6 ($\Delta q_x = \Delta q_y = 0$)

$$\log \alpha_{L,T} = a_{1x}\Delta n_{cx}\left(\frac{1}{x+y} - p_x\right)x + a_{1y}\Delta n_{cy}\left(\frac{1}{x+y} - p_y\right)y \qquad (7)$$

where $\Delta n_{cx} = \Delta n_{cy} = 1$ for the neighbouring pairs of homologues; in methanol–water, $a_{1x} = 0.536$, $p_x = 0.871$; in acetonitrile–water, $a_{1y} = 0.277$, $p_y = 0.599$.

(b) Assuming a linear dependence of log k' on the concentration of methanol in methanol–water as the binary mobile phases and a quadratic dependence on the concentration of acetonitrile–water as the binary mobile phases (with a_y in acetonitrile–water equal to a_x in methanol–water as mobile phases), the constants a_1 and p_x for methanol–water as mobile phase are determined as in the first method, but the constants m_y and d_y from the linear regression of the plots

$$\frac{\log k' - a_x}{y} = -m_y + d_y y \qquad (8)$$

where y is the concentration of acetonitrile in acetonitrile–water as mobile phase.

Linear regressions of the following plots were then used to determine the constants p_x, p_y, a_1 and d_{1y}

$$m_y = q_y + p_y a_x, \quad m_x = q_x + p_x a_x \tag{9a,b}$$

$$a_x = a_0 + a_1 n_c \tag{10}$$

and

$$d_y = d_{0y} + d_{1y} n_c \tag{11}$$

The constants a_1, p_x, p_y and d_{1y} were employed for calculations of log $\alpha_{L,T}$ in ternary mobile phases according to the adapted eqn. 5 (assuming Δq_x, Δq_y and $d_{1x} = 0$):

$$\log \alpha_{L,T} = [a_1(1 - p_x x - p_y y) + d_{1y} y^2]\Delta n_c \tag{12}$$

Here again, $\Delta n_c = 1$ for the neighbouring members of an homologous series; in methanol–water, $a_1 = 0.536$, $p_x = 0.871$, $d_{1x} = 0$; in acetonitrile–water, $p_y = 1.580$, $d_{1y} = 0.446$.

The values of α_L for pairs of neighbouring homologues, calculated by means of the two methods, are compared in Table II with the experimental values, determined for the homologous series of *n*-alkylbenzenes, 3,5-dinitrobenzoates of aliphatic alcohols and *p*-bromophenacyl esters of aliphatic *n*-carboxylic acids on a Silasorb C_{18} column in several acetonitrile–methanol–water ternary mobile phases of different compositions. Method a, where linear regression of log k' *vs.* x or y plots and different values of a_{1x} and a_{1y} are used, yields selectivities that are in a good agreement with the experimental values and are better than those by method b. The lipophilic selectivities for various homologous series in ternary mobile phases can be schematically plotted in diagrams with three axes, x, y and log α_L, as the "lipophilic selectivity

TABLE II

CALCULATED (c) AND EXPERIMENTAL (e) LIPOPHILIC SELECTIVITIES, α_L, IN TERNARY MOBILE PHASES METHANOL–ACETONITRILE–WATER

The experimental values are the selectivities between the neighbouring members of homologous series: (I) *n*-alkylbenzenes; (II) 3,5-dinitrobenzoates of aliphatic *n*-alcohols and (III) *p*-bromophenacyl esters of aliphatic *n*-carboxylic acids. Column: Silasorb C_{18}. a, Values calculated from eqn. 7 (linear log k' *vs.* x plots); b, values calculated from eqn. 12 (quadratic log k' *vs.* x plots).

Mobile phase composition (v/v/v)	*α_L (c)*		*α_L (e)*		
	a	*b*	*I*	*II*	*III*
70: 0:30	1.60	1.62	1.63	1.61	1.58
55:15:30	1.57	1.45	1.61	1.58	1.52
40:30:30	1.53	1.37	1.58	1.54	1.49
30:40:30	1.51	1.34	1.55	1.52	1.43
15:55:30	1.48	1.36	1.51	1.48	1.46
0:70:30	1.45	1.45	1.45	1.43	1.42

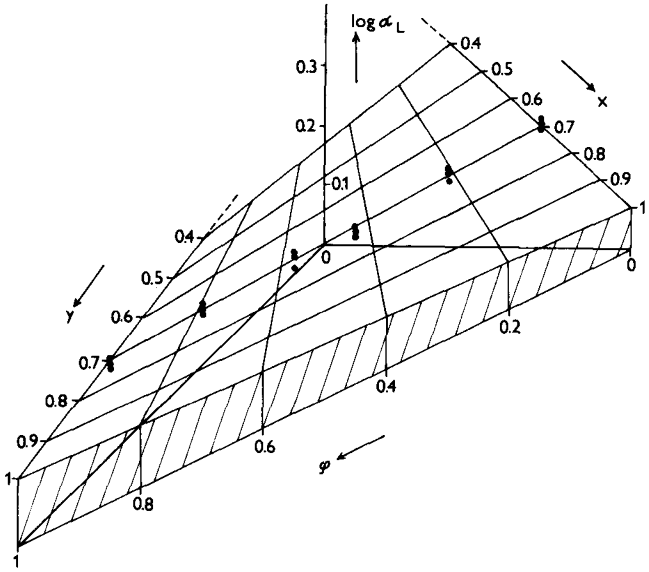

Fig. 3. Lipophilic selectivity surface (selectivity in homologous series), log α_L, as a function of the concentration of methanol x, and acetonitrile y, in ternary mobile phases of methanol–acetonitrile–water. Column: Silasorb C_{18}, 10 μm; φ is the proportion (v/v) of acetonitrile to methanol for ternary mobile phases with a constant sum of the concentrations of acetonitrile and methanol (70%); x and y are in % (v/v) · 10^{-2}. The points represent the experimental selectivities (Table II) for the homologous series of *n*-alkylbenzenes, 3,5-dinitrobenzoates of aliphatic *n*-alcohols and *p*-bromophenacyl esters of aliphatic carboxylic acids.

surface". Fig. 3 shows such a diagram for the Silasorb C_{18} column and acetonitrile–methanol–water as mobile phase. The lines on the surface were calculated using eqn. 7 and the points represent the experimental values of log α_L for the three homologous series as a function of the proportion, φ, of the binary mobile phase 70% acetonitrile–water to the mobile phase 70% methanol–water in ternary mobile phases containing a constant percentage of water (30%). Such diagrams may be useful for predicting the selectivity for separations of the members of homologous series.

Selectivity in ternary mobile phases: non-homologous compounds

As eqn. 7, based on linear log k' *vs.* x plots, yielded better predicted values of selectivities for homologous series in ternary mobile phases than the other method of calculation tested, the selectivities (α_T, related to toluene as the standard compound) of several non-homologous compounds in various ternary mobile phases, acetonitrile–methanol–water, were calculated from eqn. 6 without the quadratic terms ($d_{1x} = d_{1y} = 0$):

$$\log \alpha_T = a_{1x}\Delta n_{cx}\left(\frac{1}{x+y} - p_x\right)x + a_{1y}\Delta n_{cy}\left(\frac{1}{x+y} - p_y\right)y - \Delta q_x \cdot x - \Delta q_y \cdot y \qquad (13)$$

TABLE III

EXPERIMENTAL (e) AND CALCULATED (c) SELECTIVITIES, α, AND CAPACITY FACTORS, k', IN TERNARY PHASES OF METHANOL–ACETONITRILE–WATER

Column: Silasorb C_{18}. α are related to toluene as the standard (eqn. 13).

Compound	*Mobile phase composition (v/v/v)*					
	70:0:30	*55:15:30*	*40:30:30*	*30:40:30*	*15:55:30*	*0:70:30*
Benzene						
α (c)	0.616	0.630	0.643	0.653	0.667	0.681
k' (c)	1.25	1.04	0.98	0.94	0.91	0.73
k' (e)	1.24	1.04	0.93	0.94	0.91	0.74
Anisole						
α (c)	0.560	0.569	0.579	0.585	0.594	0.604
k' (c)	1.14	0.94	0.88	0.84	0.81	0.65
k' (e)	1.14	0.96	0.88	0.85	0.84	0.65
p-Dichlorobenzene						
α (c)	1.587	1.570	1.552	1.541	1.524	1.507
k' (c)	3.22	2.59	2.38	2.22	2.09	1.62
k' (e)	3.28	2.76	2.55	2.33	2.15	1.63
m-Chlorotoluene						
α (c)	1.674	1.634	1.594	1.568	1.530	1.493
k' (c)	3.40	2.70	2.44	2.26	2.10	1.60
k' (e)	3.46	2.80	2.48	2.31	2.15	1.60
Acetophenone						
α (c)	0.293	0.305	0.318	0.327	0.341	0.356
k' (c)	0.59	0.50	0.49	0.47	0.47	0.38
k' (e)	0.59	0.52	0.53	0.50	0.51	0.39
Benzonitrile						
α (c)	0.254	0.275	0.298	0.314	0.340	0.369
k' (c)	0.52	0.45	0.46	0.45	0.47	0.40
k' (e)	0.51	0.46	0.48	0.48	0.51	0.40
Nitrobenzene						
α (c)	0.428	0.436	0.443	0.448	0.455	0.462
k' (c)	0.87	0.72	0.68	0.65	0.62	0.50
k' (e)	0.87	0.72	0.71	0.68	0.66	0.51
m-Bromonitrobenzene						
α (c)	0.948	0.924	0.901	0.886	0.864	0.843
k' (c)	1.92	1.53	1.38	1.28	1.18	0.91
k' (e)	1.92	1.57	1.43	1.34	1.22	0.92
Toluene						
k'_T (e)	2.03	1.65	1.53	1.44	1.37	1.075

It is easy to calculate the capacity factors of solutes from the selectivities, α_T, calculated from eqn. 13 and from the experimental capacity factor of toluene, $k'_{T(e)}$, in various mobile phases:

$$k' = \alpha_T k'_{T(e)} \tag{14}$$

The k' values calculated from eqns. 13 and 14 are compared with the experimental capacity factors in Table III for the Silasorb C_{18} column. The constants of eqn. 13 necessary for calculations were taken from Table III in Part I[1]. The agreement between the calculated and experimental capacity factors is satisfactory for the ternary mobile phases tested.

Prediction of retention on other columns

The agreement between the relative retentions, measured on different columns for the same solutes and mobile phases, is usually better than the agreement between the absolute retentions. Therefore, the selectivity related to toluene as the standard was used as the basis in attempts to predict retention on one column using the data obtained for another column. To predict the retention on a certain column, a, from the selectivity, α_T, determined with another column, the retention of toluene, k'_T, on column a should be determined experimentally, and then the retention is calculated from eqn. 14. Table IV lists the experimental and calculated capacity factors of six compounds on nine different C_{18} and C_8 columns in 70% methanol–water as the mobile phase. The values of α_T were calculated by using eqns. 1–3, provided linear log k' *vs.* x relationships apply. The values of a_1, p, Δn_c and Δq, determined with one Silasorb C_{18} column (for all the other octadecylsilica columns) and with one Silasorb C_8 column (for all the other octylsilica columns) were used. An attempt to use the data from the Silasorb C_{18} column also for octylsilica columns and the data from the Silasorb C_8 column for octadecylsilica columns resulted in calculated k' values that were significantly different from the experimental values. When the selectivity, α_T, was transferred to other columns with the same lengths of bonded alkyl chains (Table IV), the relative deviations of the predicted k' from the experimental values were lower than 20%, the mean deviation being between 7 and 10%. The failure of the attempt to transfer the α_T values to the columns with different lengths of bonded alkyl chains can be attributed to differences in slight curvatures of log k' *vs.* x plots over the whole mobile phase composition ranges for column packing materials with different lengths of bonded alkyl chains. Consequently, differences are observed in the constants a and m of the regression straight lines and in the values of n_{ce} and q_i for the same solute and binary mobile phases, but for different lengths of the bonded alkyls. For the same reason, differences in a, m, n_{ce} and q_i can be found for a given column and mobile phase components, but for different ranges of the mobile phase composition.

CONCLUSIONS

The present method for the characterization of selectivity in reversed-phase liquid chromatography makes it possible to predict relative or absolute retention of various solutes and possible selectivity cross-overs and to explain the observed selec-

TABLE IV

EXPERIMENTAL (k'_{ex}) AND CALCULATED (k'_c) CAPACITY FACTORS OF VARIOUS COMPOUNDS ON DIFFERENT C_{18} AND C_8 COLUMNS WITH 70% METHANOL–WATER AS THE MOBILE PHASE

Toluene was used as the standard compound (k' given in parentheses). $k'_c = \alpha_T k'$ (toluene). The α_T values were calculated from eqns. 1–3 (linear log k' *vs.* x dependence), using the values Δn_c and Δq for Silasorb C_{18} ($a_1 = 0.536$, $p = 0.871$) and Silasorb C_8 ($a_1 = 1.542$, $p = 0.932$) columns.

1Column	*Parameter*	*Anisole*	*Nitro-benzene*	*Benzo-nitrile*	*Aceto-phenone*	*n-Propyl 3,5-di-nitro-benzoate*	*n-Hexyl*	*p-Bromophenacyl*	
								Butyrate	*Capronate*
Silasorb C_{18}	α_T	0.560	0.428	0.254	0.293	1.171	5.007	1.069	2.626
Silasorb C_8	α_T	0.653	0.519	0.384	0.415	–	–	–	–
Silasorb C_{18}	Δn_c	−1.149	−1.288	−1.849	−1.942	1.392	4.378	1.243	3.192
	Δq	0.016	0.141	0.298	0.182	0.318	0.309	0.330	0.355
Silasorb C_8	Δn_c	−0.700	−0.87	−1.46	−1.47	–	–	–	–
	Δq	0.09	0.19	0.23	0.18	–	–	–	–
Silasorb C_8	k'_c	0.72	0.57	0.42	0.46	–	–	–	–
(1.101)	k'_{ex}	0.69	0.59	0.41	0.46	–	–	–	–
LiChrosorb C_8	k'_c	0.86	0.68	0.50	0.55	–	–	–	–
(1.315)	k'_{ex}	0.78	0.66	0.42	0.47	–	–	–	–
Silasorb C_{18} (5 μm)	k'_c	1.46	1.11	0.66	0.76	3.05	13.03	2.78	6.83
(2.602)	k'_{ex}	1.34	0.91	0.54	0.65	2.81	14.05	2.54	6.84
LiChrosorb SI 100/C_{18}	k'_c	1.43	1.09	0.65	0.75	2.98	12.75	2.72	6.69
(2.546)	k'_{ex}	1.38	0.98	0.58	0.69	2.91	13.42	2.55	6.59
Lichrosorb SI 60/C_{18}	k'_c	1.43	1.09	0.65	0.75	2.99	12.77	2.73	6.70
(2.550)	k'_{ex}	1.36	0.95	0.54	0.64	2.79	13.13	2.51	6.59
Hypersil C_{18}	k'_c	1.16	0.89	0.53	0.61	2.43	10.37	2.21	5.44
(2.071)	k'_{ex}	1.09	0.75	0.45	0.54	2.61	10.90	2.25	6.00
μBondapak C_{18}	k'_c	0.75	0.57	0.34	0.39	1.57	6.69	1.43	3.51
(1.337)	k'_{ex}	0.75	0.57	0.35	0.41	1.46	5.80	1.28	2.95
Separon Six C_{18}	k'_c	1.51	1.16	0.69	0.79	3.17	13.54	2.89	7.10
(2.705)	k'_{ex}	1.47	1.02	0.62	0.81	2.82	12.46	2.66	6.60
Silasorb C_{18} (10 μm)	k'_c	1.18	0.90	0.53	0.62	2.46	10.52	2.25	5.52
(2.101)	k'_{ex}	1.16	0.83	0.48	0.60	2.41	10.63	2.10	5.18

tivities on the basis of a different rôle of the lipophilic and polar contributions to the selectivity for various pairs of compounds. Relative and absolute retentions can be also predicted for ternary mobile phases containing two organic solvents, X and Y, from the data measured in the binary mobile phases water–solvent X and water–solvent Y. Linear plots of log *k'* *vs.* the concentrations of organic solvents in the binary mobile phases can provide sufficiently accurate predictions of relative and absolute retentions in ternary mobile phases. In the experiments with ternary mobile phases, the sum of the concentrations of the two organic solvents in the mobile phase was held constant, and it was not attempted to adjust a constant solvent strength as in many other investigations of the retention in ternary mobile phases[16–20], because it is virtually impossible to adjust exactly equal solvent strengths for more than a single compound. In the mobile phases where only the ratio of the concentrations of acetonitrile and methanol changed but their sum remained constant, no selectivity maxima or minima were observed, but rather a continuous change (decrease or increase) from methanol-rich to acetonitrile-rich mobile phases.

It is possible to use the present method to transfer the selectivity from one column to another column with bonded alkyls of equal lengths and to calculate the capacity factors on this column with the aid of experimental *k'* values for a standard compound (toluene), but larger deviations of the experimental values from the predicted *k'* (up to 20%) in comparison to the predictions for a single column should be expected.

Correlations between the structure of solutes and the constants Δn_c and Δq, reported in Part I[1], seem promising for a reduction in the number of experiments necessary for the determination of the constants for predictions by calculation, but this is still open to further investigations.

The present conclusions and those in Part I[1] are valid only for mobile phases containing more than 40–50% of an organic solvent; the possibilities of using this method for predicting relative and absolute retentions in mobile phases containing less than 40% of an organic solvent are currently being studied.

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