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Normal-phase high-performance liquid chromatography of volatile compounds
Selectivity and mobile phase effects on polar bonded silica

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Abstract

Liquid chromatographic retention data of various volatile alcohols, phenols, ethers, aldehydes, ketones, esters, nitriles, isothiocyanates, and heterocyclic compounds were measured on diol-, cyano- and amino-bonded silica columns. The mobile phase was pentane–diethyl ether in varying compositions. The Snyder theory was applied to retention of polycyclic aromatic hydrocarbons and to selected other compounds. The role of diethyl ether in the retention process was examined. Restricted-access delocalization was observed on all three bonded phases, and was pronounced on diol and amino silica. Secondary solvent effects were important for most compounds, indicating differences in specific solvent–solute interactions between adsorbed and non-adsorbed mobile phase. Principal component analysis was performed on a large set of retention data. Esters and ethers showed clear affinity for diol silica, while alcohols and phenols were preferentially retained on amino silica.

1. Introduction

Reversed-phase high-performance liquid chromatography (HPLC) on octadecyl bonded silica continues to be the most popular technique in liquid chromatography. Nevertheless, polar bonded phases have gained in interest, both in the reversed-phase and in the normal-phase mode. Only three binding types have found wide application, dihydroxypropyl propyl ether (diol), aminopropyl, and cyanopropyl silica. In the normal-phase mode, these phases exhibit properties similar to bare silica, but they offer some important advantages, among which (1) no irreversible adsorption of very polar solutes, (2) faster column equilibration, and (3) less importance of water content of the mobile phase.

The Snyder model of adsorption chromatography [1] was originally designed for silica and alumina as adsorbents. It starts from the assumption that retention of a solute is governed by competitional adsorption on some active site on the adsorbent rather than by partition between two immiscible liquid phases. Several studies showed that this general assumption also holds

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true on diol- [2,3], cyano- [2,4–7] and amino- [5,6,8,9] bonded silica, while details of retention mechanisms are still controversial.

The present work was conducted in the context of a study of the possible advantages of normal-phase HPLC in prefractionation of aroma extracts of various origins. It aims to further elucidate retention characteristics and selectivity effects of these adsorbents for a wide range of volatile solutes, with consideration of the role of the mobile phase. Especially for diol bonded silica, there is still a deficiency of precise differentiation from the other two adsorbents. With respect to easy concentration of fractions in semi-preparative chromatography, only one mobile phase system, pentane–diethyl ether, was used in varying compositions throughout the study. Its volatility largely exceeds that of the solutes studied.

2. Experimental

2.1. Chemicals

Pentane and diethyl ether were of HPLC grade (Fisons, Loughborough, UK). Prior to use all solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA). The standards were from Sigma (St. Quentin Fallavier, France) or from our laboratory collection of volatile compounds.

2.2. Equipment

All experimental conditions were as previously published [10]. The columns, a LiChrospher 100 diol (5 μm), a Hypersil Cyanopropyl (5 μm), and a Hypersil Aminopropyl (5 μm), all 250 × 4.6 mm, were obtained from Shandon (Eragny, France). Dead time determination was performed using a Waters R 401 refractometer. The flow rate was set to 1.5 ml/min.

2.3. Procedures

Capacity factors were calculated from the mean of three injections. Correction for variations in the mobile phase composition via selectivity coefficients and for flow rate variations were performed as described previously [10].

3. Results and discussion

3.1. Dead time determination

Column dead time was measured by injection of 20 μl of a solution of 1% hexane in the mobile phase. It was found to depend on the mobile phase composition. Experimental data show a decrease in dead time, when varying the mobile phase composition from 100% pentane to 100% diethyl ether (Fig. 1). The maximum dead time shift is more pronounced on diol (9 s) than on amino (5 s) and cyano (2 s).

3.2. Solvent strength

The experimental data obtained in this study were interpreted using the Snyder displacement model [1]. For a non-localizing compound, retention is given by

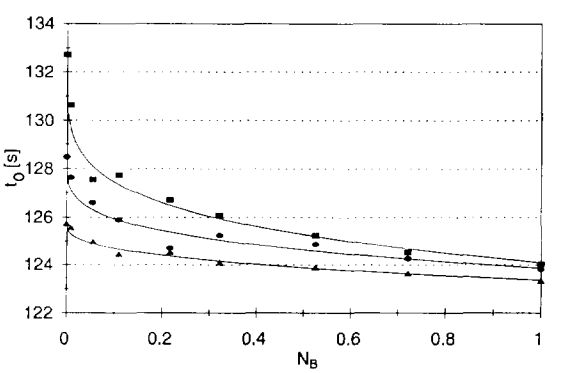

Fig. 1. Dead time on diol (■), cyano (▲) and amino (●) columns as a function of mobile phase diethyl ether content. Flow rate 1.5 ml/min. Dead time marker: hexane. Refractometric detection. Each point is a mean of 10 repetitions. Mean standard deviations are 0.15 for diol, 0.15 for cyano, and 0.20 for amino. Curves result from regression analysis with the model: $t_0 = a\,N_B^b + c$; N_B is molar fraction of diethyl ether.

$$\log (k'_{AB}/k'_{A}) = \alpha' A_S(\epsilon_A - \epsilon_{AB}) \quad (1)$$

where k'_A and k'_{AB} are the capacity factors for pure mobile phase A and for a mixture of A and B (A being a weaker eluent than B), α' is the adsorbent activity factor (arbitrarily defined as one for all adsorbents in this study), A_S is the molecular cross-sectional area of the solute, and ϵ_A and ϵ_{AB} are the solvent strength values of solvent A and the mixture of A and B, respectively. For pentane, ϵ_A is equal to zero.

The solvent strengths of pure solvent A and B are related to that of their mixture by

$$\epsilon_{AB} = \epsilon_A + \left[\log \left(N_B\ 10^{\alpha' n_B(\epsilon_B - \epsilon_A)} + 1 - N_B\right)\right] / \alpha' n_B \quad (2)$$

where N_B is the mole fraction of solvent B in the mixture, and n_B is the molecular cross-sectional area of solvent B (in multiples of 8.5 Å^2, equal to 4.5 for diethyl ether). When the capacity factor of a solute for pure mobile phase A and for a mixture of A and B is known along with its molecular cross-sectional area, substitution of ϵ_{AB} in Eq. 2 by Eq. 1 and subsequential solution for ϵ_B allows calculation of the solvent strength of pure solvent B in this mixture.

Retention of phenanthrene and triphenylene was measured on all three adsorbents with seven mobile phase compositions (ranging from 100% pentane to 50% diethyl ether). Capacity factors and calculated ϵ_B are given in Table 1. As can be seen, ϵ_B increases with decreasing molar fraction

Table 1
Retention of aromatic hydrocarbons and experimental mobile phase strengths

Modifier (%)	log k'		ϵ_{AB}	ϵ_B	θ_B	ϵ''	ϵ'	Determination coefficient[c]
	Phenanthrene[a]	Triphenylene[b]						
Diol								
0	0.078	0.486				0.101	0.291	0.996
0.8	− 0.042	0.320	0.012 ± 0.001	0.265 ± 0.006	0.123			
5	− 0.182	0.131	0.025 ± 0.001	0.180 ± 0.005	0.273			
10	− 0.268	0.029	0.033 ± 0.001	0.150 ± 0.004	0.368			
20	− 0.345	− 0.080	0.041 ± 0.002	0.119 ± 0.004	0.487			
50	− 0.639	− 0.331	0.064 ± 0.002	0.099 ± 0.003	0.755			
Cyano								
0	− 0.386	− 0.096				0.061	0.094	0.934
0.8	− 0.401	− 0.112	0.0013 ± 0.0001	0.091 ± 0.003	0.022			
5	− 0.485	− 0.202	0.0086 ± 0.0005	0.095 ± 0.004	0.135			
10	− 0.538	− 0.252	0.0129 ± 0.0011	0.080 ± 0.005	0.221			
20	− 0.608	− 0.350	0.0198 ± 0.0007	0.069 ± 0.002	0.362			
50	− 0.814	− 0.587	0.0382 ± 0.0012	0.063 ± 0.002	0.681			
Amino								
0	0.309	0.818				0.145	0.423	0.998
0.8	− 0.106	0.278	0.040 ± 0.001	0.392 ± 0.004	0.342			
5	− 0.380	− 0.053	0.065 ± 0.001	0.280 ± 0.002	0.516			
10	− 0.456	− 0.143	0.072 ± 0.001	0.232 ± 0.002	0.576			
20	− 0.567	− 0.276	0.082 ± 0.001	0.190 ± 0.001	0.664			
50	− 0.770	− 0.522	0.100 ± 0.001	0.145 ± 0.001	0.832			

[a] $A_S = 10.84$.
[b] $A_S = 13.26$.
[c] Non-linear regression model: $\%_{Ic} = 1 - \int_0^x e^{-25(0.5-x^4)^2} dx / \int_0^1 e^{-25(0.5-x^4)^2} dx$.

of diethyl ether. The pure solvent strength of diethyl ether can furthermore be related to its fractional surface coverage on the adsorbent θ_B by

$$\theta_B = N_B 10^{\alpha' n_B(\epsilon_B - \epsilon_A)} / \left[1 - N_B + N_B 10^{\alpha' n_B(\epsilon_B - \epsilon_A)}\right] \quad (3)$$

The plot of ϵ_B against θ_B (Fig. 2) permits us to visualize the capability of solvent B to localize on the adsorbent surface. For solvent–adsorbate combinations where the solvent does not localize, a horizontal graph is obtained (ϵ_B is independent of concentration). If the solvent molecule is able to undergo strong interactions with active sites on the adsorbent, the graph will be non-linear. In order to quantitatively describe the relationship of ϵ_B and θ_B in these cases, Snyder [11,12] introduced a localization function $\%_{lc}$ as follows:

$$\epsilon_B = \epsilon'' + \%_{lc}(\epsilon' - \epsilon'') \quad (4)$$

At low solvent B concentration, ϵ_B will be independent of the surface coverage (plateau on the left side of the graphs, $\epsilon_B = \epsilon'$), but with increasing concentration the active sites will become saturated with solvent B and solvent strength decreases until a state where all active sites are occupied by solvent B molecules and solvent strength becomes again independent of surface coverage, now reflecting the solvent strength of delocalized B ($\epsilon_B = \epsilon''$). This effect is commonly referred to as restricted-access delocalization.

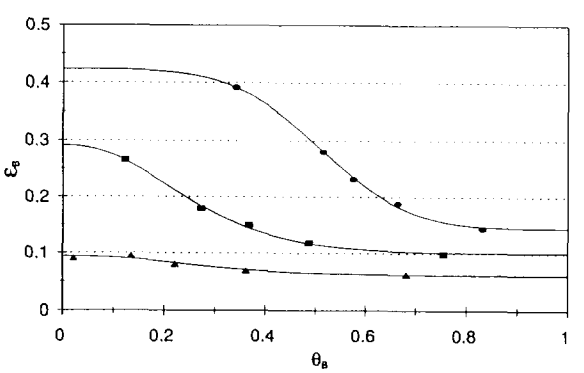

Fig. 2. Solvent strength of pure diethyl ether ϵ_{AB} as a function of its fractional surface coverage θ_B. Symbols of columns as in Fig. 1.

The empirical term of function $\%_{lc}$ proposed by Snyder for silica cannot be sufficiently adapted to the bonded phases of this study, because an additional minimum occurs on the right half of the graph when varying the parameters in this function so that it approaches the experimental points. Therefore, another function, the integral of a slightly modified Gaussian curve, was used for the regression analysis generating the curves in Fig. 2 (see legend of Table 1).

Snyder and Schunk [8] assumed that no restricted-access delocalization should occur on polar bonded phases, since the flexibility of the functional group, attached to the silica by a propyl chain, precludes formation of strong interactions. The authors found this theory confirmed by the experimental data they obtained on an amino column with hexane–tetrahydrofurane mixtures as a mobile phase. However, ϵ_B values calculated in the above described way (Eq. 2) from their experimental ϵ_{AB} data show significant decrease when increasing the mobile phase modifier content. In a subsequent publication [13], Snyder argued that this variation of ϵ_B does not signify the occurrence of restricted access delocalization. This hypothesis was based on the observation that the shapes of their graphs of ϵ_B against θ_B differ markedly from the equivalent graph on bare silica, and that ϵ_B varied even when non-localizing solvents like chlorinated hydrocarbons were used as modifiers.

Cooper and Smith [2,7] studied retention on diol, cyano and amino columns with hexane–methyl *tert.*-butyl ether as a mobile phase and found that ϵ_B varies with the ether content only in the case of the cyano column. They attributed this phenomenon to remaining accessible silanols rather than to interactions with the cyano group, although this column was the only endcapped column in their experiments. From retention data of an aromatic nitro compound with localization effects on all three columns, however, the authors concluded that restricted-access delocalization may also occur on diol and amino, but

that this phenomenon does not affect the retention of the large aromatic hydrocarbons that served for ϵ_B calculation, due to a lesser extent of accessibility of the silanols.

Our data show localization of diethyl ether on active sites of all three columns. While the cyano column exhibits only a slight non-linearity, this effect is pronounced on the diol and amino columns. The sigmoidal shapes of the graphs of ϵ_B against θ_B (Fig. 2) resemble the relationship observed on silica [12]. Total obstruction of the active sites for large molecules by the aliphatic groups bonded to the silica could not be observed. We believe furthermore that the functional groups on the organic moiety are involved in localization. Two observations support this hypothesis.

First, Fig. 2 reveals that the position of the point of inflection depends on the bounding type. It was found at $\theta_B = 0.25$ (diol), 0.27 (cyano) et 0.51 (amino). On bare silica, independently of the solvents used, this transition point is found at $\theta_B = 0.75$ [12]. This last value means that, at saturation of the active sites by the modifier, 75% of the adsorbent surface are occupied by localized modifier molecules. 25% of the surface remain unoccupied due to steric hindrance of the modifier molecules among each other. An analogous conclusion suggests that on diol and cyano, a quarter of the surface can be occupied by localized solvent molecules, whereas on amino, one half is accessible. It is not clear why, at comparable bonding densities (ca. 4 μmol/m^2) and thus comparable silanol density of all three adsorbents, silanol accessibility is double on amino compared to diol and cyano.

Second, we found that the value of the delocalization function $f(Q_k^\circ)$, defined by

$$f(Q_k^\circ) = 1 - \epsilon''/\epsilon' \quad (5)$$

also depends on the type of adsorbent. This function indicates to which amount the standard adsorption energy Q_k° of a group k (here–O–in diethyl ether) is lowered as a result of its delocalization. On diol, cyano and amino, this function takes the values 0.64, 0.35 and 0.66, respectively. When supposing only residual silanols as sites of localization, $f(Q_k^\circ)$ should be independent of the adsorbent type. For cyano, this is clearly not the case. Its $f(Q_k^\circ)$ value is similar to that of bare silica (0.45).

We conclude that localization of ether molecules on residual silanols occurs on all three stationary phases, and that, on diol and amino, it also takes place on the functional groups of the organic moieties. The cyano phase appears, in terms of the analysis of primary solvent effects, equivalent to partially deactivated silica.

In this context it is interesting to look at the observed dead time shifts when increasing the modifier concentration (Fig. 1). At a bonded phase load of 1.4 mmol/g on diol, and 0.8 mmol/g on cyano and amino, and at an apparent density of 0.6 g/cm^3 for these bonded silicas, solvation volumes are 16 μl/mmol on diol, 7.5 μl/mmol on cyano, and 15 μl/mmol on amino. This reflects quite well the order of importance of localization.

3.3. Solute retention

When the solute to be injected is at least moderately polar, it specifically interacts with the adsorbent, and competes with solvent molecules for active sites on the adsorbent. This effect, referred to as site-competition delocalization [14], leads to a weakening of the theoretical adsorption energy of the solute (i.e., the adsorption energy that would be observed without any interacting solvent molecule), and thus, because ϵ is by definition independent of solute nature, to an apparent increase in A_S values of Eq. 1. On the other hand, one condition of validity of Eq. 1 (interactions of solutes with solvent molecules in the mobile phase and in the stationary phase essentially cancel) may no longer be true. Deviations can be described quantitatively by the introduction of a secondary solvent term [1]. The variation of this secondary solvent term is responsible for changes in selectivity in adsorption chromatography. Eq. 1 becomes

$$\log(k'_{AB}/k'_A) = \alpha'(A_S + \Delta A_S)(\epsilon_A - \epsilon_{AB}) + (\Delta_{AB} - \Delta_A) \quad (6)$$

where Δ_A and Δ_{AB} are the secondary solvent terms for solvent A and the mixture of A and B, respectively. Since pentane can be considered not to induce such effects, Δ_A is assumed equal to zero. With $\alpha' = 1$ and $\epsilon_A = 0$ Eq. 6 can be simplified to

$$\log (k'_{AB}/k'_A) = -(A_S + \Delta A_S)\epsilon_{AB} + \Delta_{AB} \quad (7)$$

It can be seen from Eq. 7 that, if the secondary solvent term remains constant, a plot of $\log (k'_{AB}/k'_A)$ against ϵ_{AB} should be linear, with a slope of $-(A_S + \Delta A_S)$ and an intercept of Δ_{AB}.

Table 2 presents capacity factors of some moderately to highly polar solutes as a function of mobile phase composition (ϵ_{AB}). Figs. 3 to 6 show graphs of log k' versus mobile phase strength for the same solutes. Aldehydes and ketones were not injected on the amino column, since formation of Schiff's bases may occur. Obvious non-linearity can be observed in some cases, especially on the diol and cyano column. This effect is not correlated with solute polarity, since strongly retained 1-octene-3-ol (A16) and anisaldehyde (ALD6) show linear behaviour, and moderately retained esters do not. We believe Δ_{AB} to be responsible for the non-linearity of the graphs. For $\epsilon = 0$, $\Delta_{AB} = \Delta_A = 0$, and thus, for solutes with Δ_B different from zero, Δ_{AB} is dependent on diethyl ether concentration. This leads to non-linearity of the log k' versus mobile phase strength plot. However, since solute concentration is infinitely lower than diethyl ether concentration even at low modifier percentages, rapid stabilization of Δ_{AB} to a maximum value of Δ_B with increasing diethyl ether concentration should be expected.

We attribute this discrepancy with the experimental behaviour to the heterogeneity of the surface of polar bonded silicas. At low modifier concentrations, ether molecules occupy preferentially the residual silanols, due to the higher resulting adsorption energies compared to the functional groups of the organic moieties. At higher concentrations, the mobile phase will also progressively occupy the bonded moieties. The solvent–solute interactions in the adsorbed mobile phase depend on properties of solvent, solute and stationary phase. Since the nature of the latter varies with increasing modifier concentration, secondary solvent effects, which are the difference between solvent–solute interactions in the adsorbed and the non-adsorbed mobile phase, should vary as well.

We intended to model changes in secondary solvent effects throughout the entire concentration range from 0% to 50% diethyl ether. Therefore, all capacity factors measured, including those at 0% modifier, were considered in our mathematical treatment in Table 2. The regression analysis is based on the approximation that the ratio Δ_{AB}/Δ_B is constant at a given diethyl ether concentration, and independent of the stationary phase. The substitution of the empirical relationship

$$\Delta_{AB} = \Delta_B \log (1 + N_B^{0.25}) / \log 2 \quad (8)$$

for Δ_{AB} in Eq. 7 yields, via regression, $(A_S + \Delta A_S)$ and Δ_B. Theoretical A_S were calculated from van der Waals surfaces [15] of the parts of the molecules which are totally adsorbed, plus increments for the partially adsorbed alkyl chains [1]. Due to the experimental error inherent in small k', precision of the values seems rather low. Calculation of the ratio of experimental $(A_S + \Delta A_S)$ and theoretical A_S lets us estimate the extent of site-competition delocalization. As expected, this effect occurs for most of the solute–stationary phase combinations of Table 2. On diol, it seems in general to be less pronounced than on cyano and amino. The regression also makes Δ_B directly accessible, and the relative importance of solvent–solute interactions can be discussed. The observed secondary solvent terms significantly different from zero have all negative values, which means that diethyl ether further weakens retention by solute–solvent interaction.

The weakly polar isothiocyanates (butyl isothiocyanate, I1, and 2-phenylethyl isothiocyanate, I2) show Δ_B values near zero, as does the moderately polar aliphatic ketone (2-undecanone, K2). On diol and cyano, esters [ethyl acetate, ES4, and ethyl (E)-2-butenoate, ES13] exhibit high secondary solvent terms,

Table 2
Retention data of moderately to highly polar compounds

Modifier (%)	ϵ_{AB}	log k'									
		I1[a]	I2	ES4	ES13	K2	L2	A16	ALD6	HE8	P1
A_S[b]		[c]	[c]	4.6	6.8	6.0	8.1	7.3	8.6	7.4	6.8
Diol											
0	0	−0.27	0.21	0.34	0.35	0.37	–	–	–	–	–
0.8	0.0118	−0.37	0.07	−0.07	−0.02	0.03	0.99	1.02	0.69	–	–
5	0.0253	−0.55	−0.07	−0.30	−0.28	−0.27	0.63	0.66	0.42	0.90	0.98
20	0.0408	−0.70	−0.27	−0.60	−0.62	−0.64	0.25	0.18	0.13	0.40	0.39
50	0.0639	−0.82	−0.62	−0.82	−0.80	−0.85	−0.16	−0.28	−0.19	−0.05	−0.08
$A_S + \Delta A_S$		6.5	15.0	5.8	6.7	21.4	10.8	13.8	9.0		
$(A_S + \Delta A_S)/A_S$				1.3	1.0	3.6	1.3	1.9	1.1		
Δ_B		−0.17	0.16	−0.90	−0.84	0.00	−1.17	−1.20	−0.82		
Determination coefficient[d]		0.982	0.998	0.998	0.993	0.941	1.000	0.998	1.000		
Degrees of freedom		3	3	3	3	3	2	2	2		
Cyano											
0	0	−0.46	−0.16	−0.32	−0.30	−0.41	0.94	0.04	0.38	0.69	1.17
0.8	0.0013	−0.47	−0.18	−0.46	−0.43	−0.44	0.79	−0.04	0.26	0.60	0.83
5	0.0086	−0.55	−0.29	−0.66	−0.62	−0.62	0.53	−0.30	0.09	0.24	0.21
20	0.0198	−0.72	−0.43	−0.82	−0.82	−0.77	0.27	−0.68	−0.10	−0.17	−0.34
50	0.0382	−0.92	−0.74	−0.96	−0.85	−1.10	−0.09	−1.22	−0.39	−0.62	−0.80
$A_S + \Delta A_S$		12.2	14.9	6.6	14.6	18.3	17.6	29.8	13.5	27.4	25.6
$(A_S + \Delta A_S)/A_S$				1.4	2.1	2.1	2.2	4.1	1.6	3.7	3.8
Δ_B		0.00	0.00	−0.46	−0.32	0.00	−0.41	−0.15	−0.29	−0.34	−1.19
Determination coefficient[d]		0.996	0.997	0.987	0.998	0.991	0.997	0.999	1.000	0.990	0.984
Degrees of freedom		3	3	3	2	3	3	3	3	3	3
Amino											
0	0	−0.17	0.34	0.40	0.42		–	–		–	–
0.8	0.0395	−0.47	0.02	−0.12	−0.09		–	–		–	–
5	0.0646	−0.64	−0.16	−0.51	−0.51		1.17	0.64		1.04	–
20	0.0816	−0.85	−0.41	−0.82	−0.82		0.26	0.11		0.49	0.82
50	0.1003	−1.00	−0.90	−1.00	−1.00		−0.18	−0.37		0.00	0.33
$A_S + \Delta A_S$		7.6	10.3	13.2	14.4						
$(A_S + \Delta A_S)/A_S$				1.9	1.1						
Δ_B		−0.05	0.00	−0.11	0.00						
Determination coefficient[d]		0.991	0.902	0.995	0.994						
Degrees of freedom		3	3	3	2						

[a] Explanation of compound codes: see Table 3.
[b] From molecular dimensions.
[c] A_S for these compounds not available.
[d] Regression model: $\log k_{AB} = \log k_A - (A_S + \Delta A_S)\epsilon_{AB} + \Delta_B \log(1 + N_B^{0.25}) / \log 2$.

while on amino, although most strongly retained, they do not. The aliphatic alcohol (1-octen-3-ol, A16) shows a high Δ_B on diol, but not on cyano. On amino, although no regression could be performed, values suggest a secondary solvent term even higher than on diol.

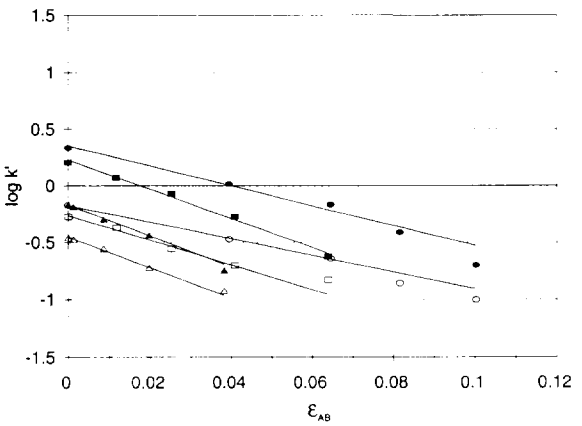

Fig. 3. Logarithm of capacity factors for butyl isothiocyanate (I1, open symbols) and 2-phenylethyl isothiocyanate (I2, black symbols) as a function of solvent strength ϵ_{AB}. Column symbols: rectangles are on diol, triangles on cyano, and circles on amino.

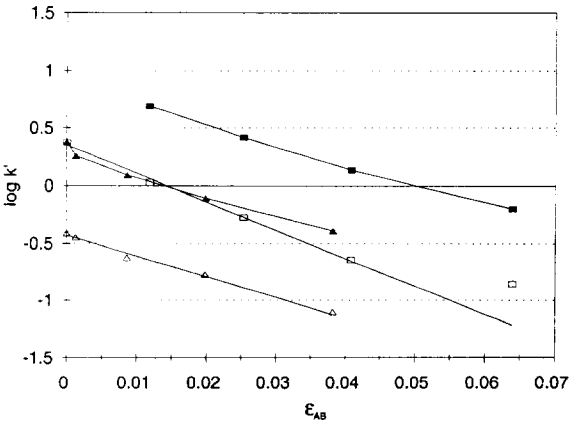

Fig. 5. Logarithm of capacity factors for 2-undecanone (K2, open symbols) and anisaldehyde (ALD6, black symbols) as a function of solvent strength ϵ_{AB}. Symbols of columns as in Fig. 3.

These few examples illustrate that (1) only solutes capable of forming hydrogen bonds exhibit significant secondary solvent terms; (2) the characteristics of the stationary phase are largely responsible for the importance of secondary solvent effects. In fact, while hydroxyl groups on diol can form hydrogen bonds with alcohols (acidic compounds) as well as with esters (basic compounds), no such interaction occurs between amino groups and esters. This means that, although interactions between diethyl ether and esters certainly exist in the non-adsorbed mobile phase regardless of adsorbent type, they are, due

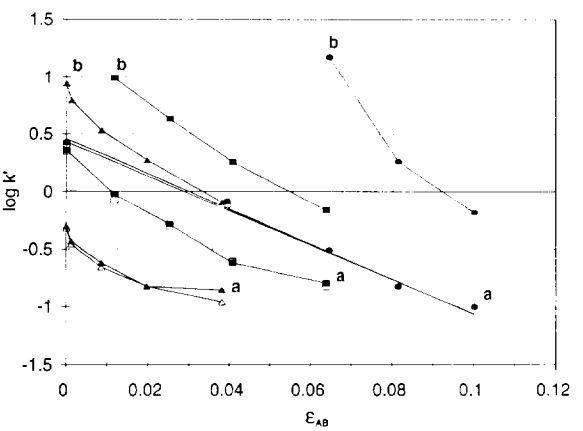

Fig. 4. Logarithm of capacity factors for ethyl acetate (ES1, open symbols), ethyl (E)-2-butenoate (ES2, a) and γ-decalactone (L2, b) as a function of solvent strength ϵ_{AB}. Symbols of columns as in Fig. 3.

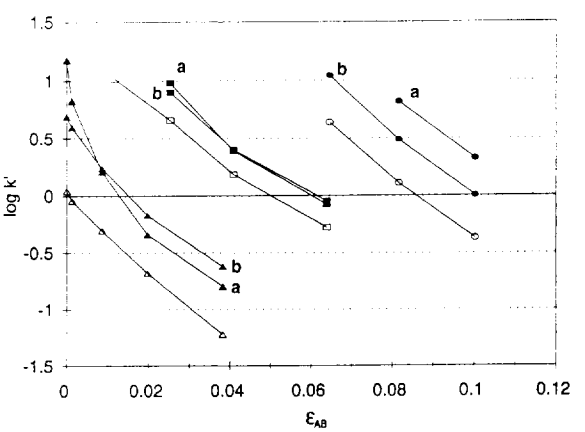

Fig. 6. Logarithm of capacity factors for 1-octene-3-ol (A16, open symbols), phenol (P1, a) and indole (HE8, b) as a function of solvent strength ϵ_{AB}. Symbols of columns as in Fig. 3.

to the low interaction of esters with amino groups on the silica, cancelled by equivalent interactions in the adsorbed mobile phase.

3.4. Column selectivity

About 100 volatile compounds of a wide variety of chemical classes were injected with pentane–diethyl ether mixtures on all three columns. The diol data were taken from a previous publication [10]. The mobile phase composition used for each compound was as previously defined for the diol column. Table 3 summarizes the capacity factors obtained.

The great number of values makes it difficult to interpret this table directly. We therefore believed it was useful to apply principal component analysis (PCA) to the data. This statistical tool allows to define an n-dimensional space (n being the variables, here the three adsorbent types) containing the total variation of the subjects (the solutes). Within this space, $n-1$ planes are defined so that the projection of the subjects and the vectors of the variables onto the first plane visualizes the maximum of variation, the projection onto the second plane the maximum of the remaining variation, and so on. Several types of information are now accessible:
- the greater the angles between the vectors of the variables, the greater their differences;
- the farther the subjects from the center of the plot, the farther their characteristics from the totality of the subjects;
- the nearer two subjects, the closer their relationship;
- the longer the orthogonal projection of a subject onto a vector, the stronger its correlation with the corresponding variable.

The two axes spanning the first plane represent the principal factors responsible for the variations. Its is however rarely possible to attribute to them a physical meaning. Thus, PCA cannot be used for prediction of solutes not included in the original data matrix. Some researchers have already applied this tool to the analysis of chromatographic data [16–19].

PCA was performed on the log (k'/mean k') values, mean k' referring to the mean capacity factor of one solute on all three columns. Fig. 7 shows the plot corresponding to the two principal axes, representing together 97.5% of the variation. All three columns are well distinguished from another and occupy different sectors of the plot. This illustrates important differences in the interaction of solutes with the diol, cyano and amino silica and hence differences in selectivity. Contrary to the discussion of secondary solvent effects in the preceding section, this analysis shows differences in absolute retention, and does not differentiate between the effects responsible for it (ΔA_S or Δ_B). As expected, essentially acidic compounds (alcohols, phenols) are, due to hydrogen bonding with the adsorbent, clearly correlated with the amino column. Predominantly dipolar compounds (nitriles, isothiocyanates), and, more surprisingly, also lactones, are correlated with the cyano column. Ethers and esters are, in their great majority, correlated with the diol column. This indicates a strong interaction of this phase with ether bridges (sp^3 hybridized oxygen). On the other hand, a negative correlation of these compounds with the cyano column can be observed. Ethyl lactate (ES23) behaves like an alcohol because it contains a hydroxyl group.

These results are in general agreement with those obtained in earlier studies on diol, cyano and amino columns. However, it must be pointed out that meaningful comparisons can only be made for specific column–mobile phase combinations, not for columns as a whole, since the same column can exhibit very different characteristics with two different mobile phases. Salotto et al. [3] used group retention values to characterize selectivity differences. They found, for dichloromethane as mobile phase modifier, no significant difference between the amino and the diol column, while the cyano column was described as retaining preferentially dipolar solutes. Smith and Cooper [2] used nitrobenzenes, phenol and aniline as test solutes. They presented their results as selectivity matrices, which describe column characteristics as a function of the selectivity of the mobile phase modifier. In

Table 3
Capacity factors on diol, cyano and amino columns

Code	Compound	Mobile phase[a]	Diol	Cyano	Amino
Saturated alcohols					
A1	Ethanol	4	3.40	0.39	
A2	1-pentanol	4	2.82	0.35	1.86
A3	1-Dodecanol	4	2.41	0.30	1.88
A4	2-Pentanol	4	2.39	0.28	1.81
A5	2-Methyl-1-butanol	4	2.20	0.29	1.84
A6	3-Methyl-1-butanol	4	2.75	0.34	1.87
A7	2-methyl-2-butanol	4	2.39	0.25	1.71
A8	3-Methyl-2-butanol	4	1.99	0.24	1.60
A9	Menthol	4	1.85	0.24	1.45
A10	Fenchyl alcohol	3	4.18	0.14	2.79
Unsaturated alcohols					
A11	2-Buten-1-ol	4	3.16	0.43	3.21
A12	(*E*)-2-Octen-1-ol	4	2.72	0.35	2.50
A13	(*Z*)-3-Octen-1-ol	4	2.68	0.34	2.38
A14	(*Z*)-6-Nonen-1-ol	4	3.22	0.37	2.60
A15	1-Penten-3-ol	3	7.19	0.57	4.86
A16	1-Octen-3-ol	2	9.53	0.91	
A17	α-Terpineol	4	2.38	0.28	1.70
A18	4-Terpineol	3	4.36	0.33	2.27
A19	Carveol	4	2.25	0.32	1.97
A20	α-Bisabolol	3	6.84	0.45	3.11
A21	Geraniol	4	3.70	0.45	3.11
A22	Nerol	4	3.17	0.40	2.60
Aromatic alcohols					
A23	Benzyl alcohol	4	3.88	0.59	4.75
A24	Cinnamyl alcohol	4	5.42	0.79	6.11
A25	Veratryl alcohol	5	5.31	0.68	6.65
A26	Furfuryl alcohol	4	4.15	0.70	5.69
Phenols					
P1	Phenol	4	2.01	0.50	6.59
P2	4-Vinylphenol	4	1.58	0.56	3.33
P3	2-Methoxyphenol	3	4.39	0.76	1.84
P4	2,6-Dimethoxyphenol	5	3.33	0.57	7.64
Ethers and epoxides					
ET1	Diethylether	1	1.09		0.60
ET2	Vitispirane	1	1.15	0.05	0.34
ET3	Methoxybenzene	1	0.74	0.15	0.26
ET4	1,4-Dimethoxybenzene	1	3.83	0.33	1.24
ET5	α-Caryophyllene oxide	2	3.54		1.31
Thiols and sulfides					
S1	Butanethiol	1		0.06	0.05
S2	Dimethyldisulfide	1		0.11	0.15
S3	Dimethyltrisulfide	1		0.11	0.15

Table 3 (*continued*)

Code	Compound	Mobile phase[a]	Diol	Cyano	Amino
Saturated aldehydes					
ALD1	Formaldehyde	4	1.59	0.37	
ALD2	Nonanal	1	1.65	0.24	
Unsaturated aldehydes					
ALD3	Geranial	2	5.49	1.04	
ALD4	Citronellal	1	2.61	0.38	
Aromatic aldehydes					
ALD5	4-Hydroxybenzaldehyde	4	9.33	1.78	
ALD6	Anisaldehyde	3	4.67	1.06	
ALD7	Vanillin	4	7.04	1.66	
ALD8	Coniferaldehyde	5	3.32	1.18	
ALD9	Sinapaldehyde	5	6.72	2.08	
Saturated ketones					
K1	Acetone	3	4.68	1.11	
K2	2-Undecanone	2	2.37	0.36	
K3	5-Undecanone	1	3.17	0.23	
K4	Camphor	2	3.43	0.52	
K5	Menthone	1	2.42	0.36	
Unsaturated ketones					
K6	Artemisia ketone	1	2.20	0.19	
K7	Carvone	2	4.43	0.61	
K8	Dihydrocarvone	2	2.61	0.50	
K9	Verbenone	3	6.76	1.16	
K10	Damascenone	2	2.99	0.47	
Aromatic ketones					
K11	Acetophenone	2	3.73	0.65	
K12	Indonone	3	5.16	0.97	
K13	Benzyl methyl ketone	2	6.32	0.94	
K14	Acetovanillone	4	7.74	1.54	
Miscellaneous ketones					
K15	Acetoine	4	5.00	0.75	
K16	Diacetyl	2	1.69	0.86	
K17	Sotolon	2	3.61	0.59	
Saturated esters					
ES1	Methyl acetate	1	5.40	0.32	1.72
ES2	Methyl tetradecanoate	1	2.31	0.15	0.85
ES3	Ethyl formiate	1	2.47	0.28	
ES4	Ethyl acetate	1	5.42	0.27	1.52
ES5	Pentyl pentanoate	1	1.98	0.15	0.77
ES6	Isopentyl pentanoate	1	1.89	0.16	0.80
ES7	Pentyl isopentanoate	1	1.86	0.15	0.80
ES8	Pentyl 2-methylbutyrate	1	1.75	0.14	0.76
ES9	Isopentyl 2-methylbutyrate	1	1.68	0.14	0.73
ES10	Isopentyl isopentanoate	1	1.72	0.14	0.77
ES11	Bornyl acetate	1	1.87	0.25	1.38
ES12	Fenchyl acetate	1	2.02	0.18	0.82

(Continued on p. 52)

Table 3 (*continued*)

Code	Compound	Mobile phase[a]	Diol	Cyano	Amino
Unsaturated esters					
ES13	Ethyl (*E*)-2-butenoate	1	4.71	0.28	1.60
ES14	Ethyl (*E*)-4-decenoate	1	2.71	0.18	1.03
ES15	Ethyl (*Z*)-4-decenoate	1	2.55	0.17	0.94
ES16	Geranyl acetate	1	5.07	0.27	1.64
ES17	Terpinyl acetate	1	3.49	0.21	1.16
Aromatic esters					
ES18	Benzyl acetate	1	6.90	0.48	2.36
ES19	2-Phenylethyl acetate	2	3.01	0.46	0.62
ES20	Methyl benzoate	1	3.22	0.34	1.24
ES21	Ethyl benzoate	1	2.96	0.32	1.28
ES22	Methyl cinnamate	2	3.39	0.56	1.51
Miscellaneous esters					
ES23	Ethyl lactate	4	2.74	0.34	3.87
ES24	Diethyl malonate	3	2.87	0.66	1.04
ES25	Tributyrine	3	5.10	0.94	1.23
ES26	Tristearine	3	9.09	0.40	0.43
Lactones					
L1	γ-Butyrolactone	2	6.47	1.28	3.20
L2	γ-Decalactone	2	6.43	1.29	3.19
L3	δ-Decalactone	2	6.39	1.27	3.14
Nitriles					
N1	Allyl cyanide	2	2.86	0.79	2.88
N2	Benzyl cyanide	3	3.56	1.52	2.20
Isothiocyanates					
I1	Butyl isothiocyanate	1	0.54	0.23	0.41
I2	2-Phenylethyl isothiocyanate	1	1.73	0.69	1.31
Heterocyclics					
HE1	Menthofuran	1	0.21	0.08	0.05
HE2	Thiophene	1	0.17	0.09	
HE3	Furfural	3	4.34	1.00	
HE4	5-Hydroxymethylfurfural	5	4.63	0.87	
HE5	Pyridine	5	3.50	2.50	0.84
HE6	2-Acetylpyridine	3	4.19	0.44	
HE7	Pyrrole	3	3.90	0.92	1.00
HE8	Indole	4	2.20	0.68	3.08
HE9	2-Acetylpyrrole	4	4.81	0.70	3.52
HE10	Pyrazine	5	2.28	0.34	0.56
HE11	Ethylpyrazine	4	2.48	0.22	0.60
HE12	2-Isobutyl-3-methoxypyrazine	3	1.78	0.07	0.35
HE13	Thiazole	4	2.38	0.48	0.83
HE14	4-Methyl-5-vinylthiazole	4	1.77	0.22	0.54
HE15	2-Acetylthiazole	3	3.11	0.46	1.07

[a] 1 = 100% pentane, 2 = 0.8%, 3 = 5%, 4 = 20%, 5 = 50% diethyl ether.

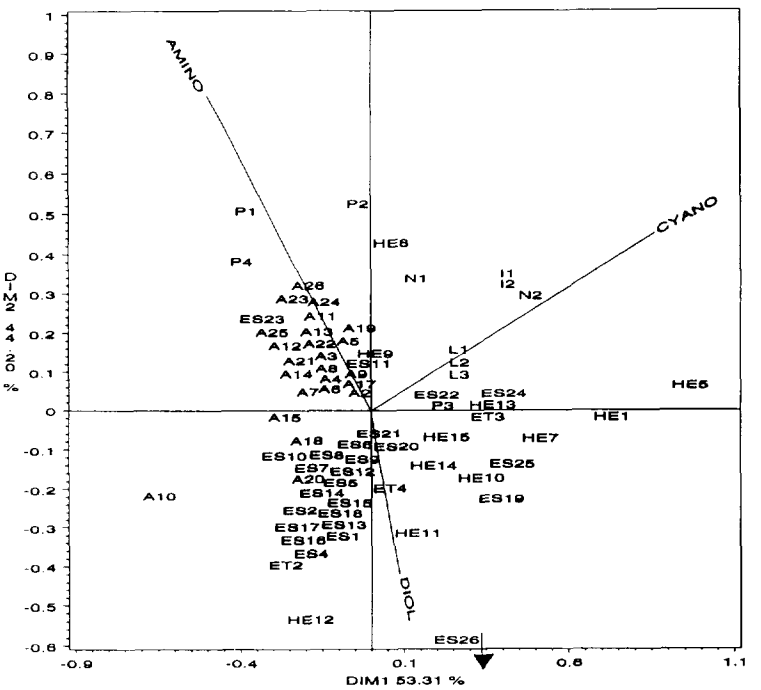

Fig. 7. Principal component analysis on log (k'/mean k') values of Table 3. Correlation coefficients (r): diol–cyano −0.18, diol–amino −0.80, cyano–amino −0.15. Explanation of compound codes: see Table 3. For better legibility of the plot, compound ES26 was shifted upwards. Its original coordinates are: 0.25, −1.02.

an other paper [20], these authors determined extended solubility parameters for the same three columns, and situated the stationary phases in a selectivity triangle. Again, cyano silica was found to be essentially dipolar, while amino and diol silica were basic and dipolar, with little difference.

4. Conclusions

The adsorption model has proven to successfully describe retention on polar bonded silicas, and allowed the quantification of the various phenomena intervening in the retention behaviour. Localization effects were observed on all three stationary phases, although they were less important on the cyano phase. From our results we conclude that restricted-access delocalization not only occurs on residual silanols, but also on the functional groups of the hydrocarbon chains attached to the silica. The order of solute retention corresponds approximately to that found for bare silica. Absolute average retention is weakest on cyano, and highest on diol. However, important differences exist in secondary solvent effects and localization strength, leading to differences in selectivity. The principal component analysis presented here confirms these selectivity effects. It shows particular affinity of diol silica for ethers and esters, while amino silica preferentially retains acidic compounds.

The diol bonded silica seems to be the most versatile phase, since low retention on cyano and the restriction of amino to non-carbonyl compounds may preclude some applications. Nevertheless, both amino and cyano phases can be attractive for on- or off-line multi-column prefractionation techniques, when advantage is

taken of selectivity effects for particular compounds.

Acknowledgements

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