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Laterally attached liquid crystalline polymers as stationary phases in reversed-phase high-performance liquid chromatography V. Study of retention mechanism using linear solvation energy relationships

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Abstract

A linear solvation energy relationship model was used to characterize the retention behavior of a stationary phase based upon a nematic side-on liquid crystalline polymer (SOLCP) in reversed-phase liquid chromatography. The set of solutes was constituted of a high variety of compounds whose molecular sizes were considerably smaller than the mesogenic unit size. The results showed good statistical fits for these retention data in 65:35, 75:25 and 85:15 (v/v) methanol–water mobile phases. Both the cavity term and excess molar refraction are the most important favorable retention-governing parameters, whereas the solute hydrogen bond acceptor basicity is the most unfavorable retention parameter. Hydrophobicity and $\pi-\pi$ interactions decrease strongly when the percentage of methanol increases, leading to an important retention decrease despite the fact that the hydrogen bond interaction weakens as the organic solvent is added. The shape recognition ability of this side-on liquid crystalline stationary phase on polycyclic aromatic hydrocarbon solutes is partly explained by the solutes' high polarizability due to the presence of π-electrons. However, the solute polarizability is not sufficient and a stationary phase's ''structure effect'' must to be taken into account for the shape discrimination observed. The strong interaction between liquid crystal molecules caused likely a adsorption retention mechanism rather than a partition mechanism.

Keywords: Stationary phases, LC; Retention mechanisms; Linear solvation energy relationships; Liquid crystalline polymers

1. Introduction

The retention phenomenon in high-performance liquid chromatography (HPLC) depends simultaneously on various intermolecular interactions between the solute and the stationary phase, the solute and the mobile phase and the stationary and mobile phase. Solute–solvent interactions were largely studied by Kamlet et al. [1] and Abraham [2] using the solvatochromism theory based on a perturbation treatment using a reaction field model [3–5]. They developed ''linear solvation energy relationships'' (LSERs) in 1979 [6–8] which allowed one to link physico–chemical solute properties (formation con-

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stants, enthalpies of solution, solubilities and others) to particular solvent interaction parameters such as π–π interactions, hydrogen bond accepting and donating character, polarity/polarizability and ''cavity term'' related to the size of the solute molecule [1,9,10].

When two immiscible solvents are considered, it is possible to study the partition coefficient (as the solute property) between the two solvents, observing the relative strength of a first interaction between one solvent and the solute over the second interaction between the other solvent and the same solute. Thus, the LSER model could also have been applied in liquid [11,12] or in gas chromatography [13,14] considering the first solvent as the stationary phase and the second as the mobile phase. The equivalent of the partition coefficient as the studied solute property becomes, in chromatography, the retention factor expressing the relative strength of the solute–stationary phase interaction over the solute–mobile phase interaction. Consequently in reversed-phase (RP) HPLC, the third interaction term (mobile phase–stationary phase) is not taken into account in the model. However, it can be neglected in RP-HPLC because the stationary phase is an apolar phase whereas the mobile phase is a polar phase corresponding to the situation of two immiscible solvents.

Because no satisfactory model exists that can be used to predict the chromatographic retention data of solute in a fixed mobile–stationary system, the LSER method was thus largely and successfully used to characterize the retention properties of a great variety of stationary phases used in reversed-phase mode: ODS stationary phases [15,16], diol silica [17], cyanopropyl silica [18], trioxydimethylene, thiophenylpropyl and phenyl thiosulfoxypropyl silicas [19], phenyldimethyl, diphenylmethyl, pentafluorophenyldimethyl silica and tridecafluorooctyldimethyl silica [20], macroreticular porous polymer beads [21] or polyethylene silica and zirconia stationary phases [22].

Based on the LSER approach, the free energy of retention in HPLC can be correlated with various fundamental solute parameters using the following relation [23]:

$$\log k = crR_2 + s\pi_2 + a\sum\alpha_2^{\mathrm{H}} + b\sum\beta_2^{\mathrm{H}} + vV_2/100$$

where k is the retention factor of the solute (the solute property) for the fixed stationary and mobile phases. The log k term is the sum of five fundamental interaction terms between the solute and the stationary–mobile phase. Each term described one particular interaction and is the product of a descriptor for the stationary–mobile phase system (c, r, s, a, b and v) and the corresponding solvation parameters for the solute (terms with the subscript 2). The solute parameters are described as follows: R_2 is an excess molar refraction determined from the compound refractive index [24] and represents the tendency of the solute to interact with a solvent phase through π- or n-electron pairs. π_2^{H} is the solute dipolarity/polarizability [25,26] that cannot be described separately and evaluates the ability of the solute to take part in dipole–dipole or dipole-induced interactions. $\Sigma\alpha_2^{\mathrm{H}}$ is the solute summation hydrogen bond acidity [27] and measures the ability of the solute to release its overall hydrogen atoms to form hydrogen bonds with the solvent. Inversely, $\Sigma\beta_2^{\mathrm{H}}$ is the solute summation hydrogen bonds basicity [28] and measures the ability of the solute to take part in the formation of hydrogen bonds by attracting hydrogen atoms from the solvent. $V_2/100$ is the McGowan characteristic volume that can be easily calculated for any solute when its molecular structure is known [29] and represents both the required energy to create a cavity to accommodate the solute and the dispersion interaction between the solute and the solvent. All these solute parameters are positive and data for a large number of compounds have been published by Abraham et al. [35].

The descriptors c, r, s, a, b and v are a unique set for a fixed combination of the stationary liquid chromatographic phase and the mobile phase. They are calculated by fitting the experimental retention data of a large set of solutes by multiple linear regression of the LSER equation. Except for the regression intercept c, these fitting parameters can be expressed as a difference between two terms: considering the X interaction, they are related to the solute–stationary phase interaction (x_sX_2) on the one hand, and to the solute–mobile phase interaction (x_mX_2) on the other hand:

$$\log k = c + (r_s - r_m)R_2 + (s_s - s_m)\pi_2 + (a_s - a_m)\sum\alpha_2^{\mathrm{H}} + (b_s - b_m)\sum\beta_2^{\mathrm{H}} + (v_s - v_m)V_x/100$$

The s and m subscripts refer to the stationary and mobile phase properties, respectively. For example a negative value obtained for the $a_s - a_m$ coefficient would mean that the hydrogen bond basicity of the stationary phase is weaker than that of the mobile phase and so contributes to diminish the log *k* value. Inversely, a positive $s_s - s_m$ fitting coefficient would indicate that the dipolarity/polarizability interaction of the solute is stronger with the stationary phase than the mobile phase and would favor solute retention.

As a result, the different fitting coefficients can be regarded as follows: *c* is the regression intercept that should be negative because it corresponds to the ratio of the stationary and mobile phase volumes (<1). The *r* coefficient is the tendency of the phase to interact with solute π- and n-electron pairs and indicates the polarizability of the stationary phase; *s* represents the phase dipolarity/polarizability; the *a* coefficient measures the phase hydrogen bond basicity (as acidic solutes will interact with basic phases) and *b* the phase hydrogen bond acidity. Finally, the *v* coefficient characterizes the phase hydrophobicity.

Herein, we use the LSER equation to examine the retention-governing interactions in RP-HPLC of a stationary phase based upon a side-on fixed liquid crystalline polymer (SOLCP) stationary phase whose performance has been previously studied [31–34]. The fitting parameters will be compared to those obtained on classical ODS stationary phases by other authors and the effect of the mobile phase composition on the regression coefficients will be also studied.

The shape recognition of this phase was then considered as a function of the solute polarizability and its probable retention mechanism was compared to that of ODS phases.

2. Experimental

2.1. Materials

2.1.1. Chemicals

A HPLC-grade methanol solvent, used as the mobile phase, was obtained from ICS (France). Water was doubly distilled.

The 21 solutes used in the multiple linear regression are listed in Table 1 with their solvation parameters as given by Abraham et al. [35].

Table 1
Solute descriptors used in the solvation parameter model

	Probe solute	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	$V_2/100$
1	*n*-Hexane	0.000	0.00	0.00	0.00	0.9540
2	Hept-1-ene	0.092	0.08	0.00	0.07	1.0519
3	Oct-1-ene	0.094	0.08	0.00	0.07	1.1928
4	Diethyl phthalate	0.729	1.40	0.00	0.88	1.7100
5	Bromobenzene	0.882	0.73	0.00	0.09	0.8914
6	Benzonitrile	0.742	1.11	0.00	0.33	0.8711
7	4-Nitroaniline	1.220	1.91	0.42	0.38	0.9904
8	Benzamide	0.990	1.50	0.49	0.67	0.9728
9	Phenol	0.805	0.89	0.60	0.31	0.7751
10	2,6-Dimethylphenol	0.840	0.79	0.39	0.39	1.0569
11	4-Ethylphenol	0.800	0.90	0.55	0.36	1.0569
12	3-Methoxyphenol	0.879	1.17	0.59	0.38	0.9747
13	3-Nitrophenol	1.050	1.57	0.79	0.23	0.9493
14	Thiophene	0.687	0.56	0.00	0.15	0.6411
15	Benzene	0.610	0.52	0.00	0.14	0.7164
16	Acenaphthene	1.604	1.04	0.00	0.20	1.2586
17	Phenanthrene	2.055	1.29	0.00	0.26	1.4540
18	Anthracene	2.290	1.34	0.00	0.26	1.4540
19	Fluorene	1.588	1.03	0.00	0.20	1.3565
20	Naphthalene	1.340	0.92	0.00	0.20	1.0854
21	1-Methoxynaphthalene	1.700	0.99	0.00	0.37	1.2850

The silica gel (Kromasil, 5 μm diameter, 200 Å pore size, 220 m^2/g,) was a gift from Akzo Nobel (Bohus, Sweden).

2.1.2. Liquid crystalline bonded stationary phase

The synthesis and the characterization of the bonded LCP silica has been described previously [34,36,37]. The concentration of mesogenic unit per gram of silica was checked to 207 μmol/g.

2.2. HPLC experiments

2.2.1. Column packing

The liquid crystal stationary phase was packed in a stainless steel column (150×4.6 mm I.D.) using a Haskel pneumatic amplification pump. The packing was carried out under a pressure of 400 bar with methanol as the pressure fluid and a mixture of methanol–cyclohexanol (25:5, v/v) as the suspension medium fluid.

2.2.2. Apparatus

HPLC was carried out using a modular HPLC apparatus equipped with a Rheodyne 7725 injector (assembled with a 20-μl sample loop), a PU-980 Model gradient pump, a UV-975 UV–Vis detector, an LG-980-02 ternary gradient unit mobile phase mixer and a DG-980-50 three-line degasser from Jasco. Reversed-phase conditions, using a mixture of methanol–water, were chosen for all chromatographic measurements at a flow of 0.5 ml/min.

3. Results and discussion

Before applying the LSER model to the liquid crystalline bonded stationary phase, some statistical precautions must be taken such as the number of solutes having to be higher than the number of solute descriptors. In our case, the number of data points is more than four times the number of solute parameters (5). However, these 21 solutes for five parameters are the lowest acceptable limit for the regression calculation. The descriptors of the chosen compounds must also not be colinear. Table 2 shows the variance covariance matrix of the solutes and indicates that no strong correlation exists between each pair among the five descriptors. However, a slight correlation could be advanced for (π_2^H, R_2), (π_2^H, $\Sigma\beta_2^H$) and (R_2, V_2) pairs with 0.648, 0.650 and 0.494 correlation coefficients, respectively. π_2^H and R_2 are not completely independent because they both reflect the solute polarizability, sensitive to the presence of π-electrons. The (π_2^H, R_2) correlation is clearer when the solutes do not possess any dipolar moment. π_2^H and $\Sigma\beta_2^H$ are also slightly dependent because they are similarly influenced by the presence of heteroatoms like oxygen or nitrogen. These electonegative atoms induce a higher hydrogen bond basicity character and generally a greater dipolar moment for the solute. Finally, R_2 and V_2 are slightly similar because the more double bonds there are, the bigger the volume of the solute.

Table 2
Correlation coefficient matrix of solute descriptors

	R_2	π_2^H	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	$V_2/100$
R_2	1				
π_2^H	0.648	1			
$\Sigma\alpha_2^H$	−0.070	0.434	1		
$\Sigma\beta_2^H$	0.188	0.650	0.311	1	
$V_2/100$	0.494	0.286	−0.319	0.397	1

This choice of solutes is acceptable because the correlation coefficient never exceeds 0.65.

As a liquid crystalline phase is being studied, some specific interactions (such as the shape recognition) have to be taken into account when calculating the solute retention. However, shape recognition is much less pronounced for small solutes and mainly observed for big polycyclic aromatic hydrocarbon (PAH) solutes with more than four condensed aromatic cycles. Herein, the solutes are considered small because their sizes are largely inferior to the dimension of the mesogenic unit attached to the polysiloxane chain. In this sense, it seems acceptable to neglect the specific shape interaction.

3.1. Comparison of LSER results between liquid crystalline and classical ODS stationary phases

3.1.1. ODS stationary phase

The ODS stationary phases have already been studied by several authors. Application of the LSER equation gives similar results from one C_{18} stationary phase to another in methanol–water mobile

phases. Abraham et al. [15] illustrated these observations in a great variety of C_{18} phases for methanol–water and acetonitrile–water (70:30, v/v) mixtures.

The *v* and *r* coefficients of the linear regression are always positive and *v* is, in every case, at least five times greater than *r*. This indicates that both the cavity term and dispersion interaction (vV_2) are more intense in the stationary phase than in the mobile phase and that the interaction through π- or n-electron pairs (rR_2) is not very influential. This is not astonishing due to the presence of octadecyl chains alone in the stationary phase. They possess no π-electron pairs and so are poorly polarizable. The three other regression coefficients *s*, *a* and *b* are all negative showing more favorable hydrogen bond and dipolar interactions of the solute with the methanol–water mobile phase than with the stationary phase. The *s* and *a* coefficients are at least two times lower than *b*. The latter parameter has the highest magnitude because the O–H protons of the mobile phase are much more labile than those of the ODS stationary phase (methylene CH_2 groups) to establish hydrogen bond interactions ($b\Sigma\beta_2^H$ hydrogen bond acidity of the stationary–mobile system). The *a* term appears less negative than *b*, probably due to the weak hydrogen bond acceptor character of water ($\Sigma\beta_2^H$ of water is 0.35) compared to its hydrogen bond donor character ($\Sigma\alpha_2^H$ of water is 0.82). Finally, in accordance with the authors' results, it is not surprising to find that the dipolarity/polarizability interactions are stronger with the polar mobile phase than with the rather apolar C_{18} stationary phase. It is worth noting that the sign and magnitude of each regression coefficient obtained for C_{18} columns in the reversed mobile phase, are in accordance with the chemical nature of the stationary–mobile phase system.

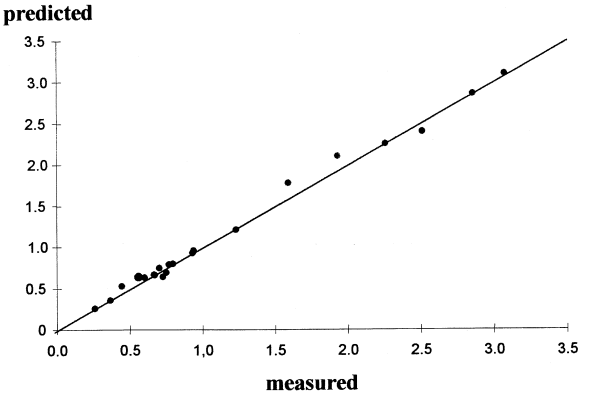

Fig. 1. Correlation between logarithms of retention factor, log *k*, of a set of 21 test solutes observed experimentally and predicted from the LSER model. Stationary phase: bonded SOLCP $P_{10.4.4}$. Mobile phase: MeOH–water (65:35, v/v), 0.5 ml/min. Room temperature.

3.1.2. Liquid crystalline stationary phase

The LSER regression coefficients obtained on the liquid crystalline bonded stationary phase are presented in Table 3 compared to those of a C_{18} stationary phase. Firstly, it is important to note the good correlation (Fig. 1) between the experimental data and the LSER model calculations; the correlation coefficients always exceed 0.992. The sign of each coefficient is the same for the ODS stationary phases confirming the apolar character of the liquid crystalline compound bonded onto the silica surface.

The *s*, *a* and *b* coefficients remain negative revealing the higher affinity of the methanol–water mobile phase for the solute with respect to the dipolarity/polarizability and hydrogen bond interactions.

The *v* value is positive highlighting the hydrophobic character of the LCP phase.

Table 3
LSER coefficients obtained with C_{18} and SOLCP stationary phases for different mobile phase compositions

Column	Eluent, MeOH–water (%, v/v)	*c*	*r*	*s*	*a*	*b*	*v*	*r*	*n*
Nucleosil 5-C_{18} [a]	65:35	0.13	0.20	−0.34	−0.37	−1.13	1.10	0.991	35
	75:25	0.09	0.15	−0.28	−0.29	−0.77	0.76	0.992	32
	80:20	0.09	0.12	−0.23	−0.25	−0.65	0.62	0.990	33
Kromasil SOLCP	65:35	−0.56	1.12	−0.30	−0.53	−1.18	1.25	0.995	21
	75:25	−0.42	0.80	−0.19	−0.35	−0.85	0.84	0.995	21
	85:15	−0.24	0.52	−0.08	−0.27	−0.56	0.50	0.992	21

[a] Values obtained from Ref. [16].

The main change concerns the *r* coefficient, which becomes largely positive and reaches the same magnitude as for the *v* coefficient. π–π interactions with the solute are specific to the LCP phase because it contains aromatic rings (three per mesogenic unit) and carbonyl groups (also three per mesogenic unit). It also indicates a higher polarizability of the LCP over the ODS stationary phase, coherent with the presence of more diffuse π-electrons compared to the less polarizable σ-electrons of the C_{18} chains.

It is also worth noting that the magnitudes of the *a* and *b* coefficients describing the hydrogen bond (HB) basicity and the HB acidity of the stationary–mobile phase system, respectively, remain similar for the LCP and the ODS stationary phases. It means that the hydrogen bond basicity and acidity interactions with solutes are not drastically modified from an LCP to an ODS stationary phase because they are always largely favored in the methanol–water solvent.

Predicting the sign of the *s* coefficient is more difficult because its corresponding interaction takes into account both the dipolarity, favored in the polar methanol–water mobile phase, and the polarizability, favored in the LCP stationary phase. It may be the reason why the *s* term contributes less (slightly negative) to the retention of the solute.

As a conclusion of these results obtained from the LSER study, it appears that this bonded SOLCP–MeOH–water stationary–mobile phase system is equivalent to the ODS–MeOH–water system except it is more polarizable as revealed by the large, positive *r* regression coefficient. The LCP phase could thus be identified with a so-called ''polarizable C_{18}'' phase when characterized by the LSER model.

3.1.3. Mobile phase composition effect

The variation of the mobile phase composition was also studied. The evolution of the regression coefficient for methanol volume percentages of 65, 75 and 85% is represented in Fig. 2 for the liquid crystalline stationary phase. Whatever the methanol percentage is, the sign of each coefficient remains the same.

The magnitude of the positive coefficients (*v* and *r*) regularly decreases as the amount of organic solvent increases. The decreases in *v* and *r* are caused by lower cohesivity (resulting in lower cavity formation energy) and higher polarizability of the mobile phase, respectively.

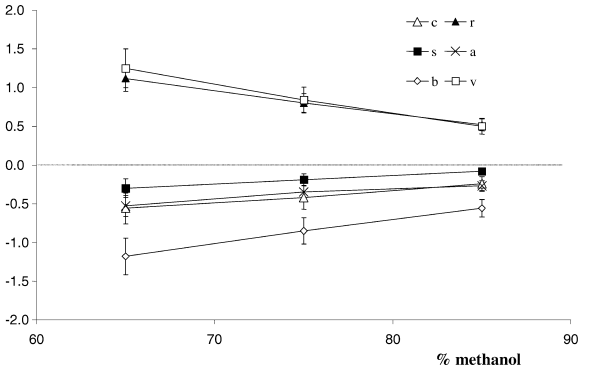

Fig. 2. Variation of the LSER regression coefficients with mobile phase composition.

The dipolarity/polarizability and hydrogen bond coefficients (*s*, *a* and *b*) become less negative. This is obviously caused by the lower polarity of the mobile phase. Furthermore, the mobile phase contains less labile O–H protons (resulting in lower hydrogen bond acidity) and the single oxygen atom of methanol has a weaker electronic density than the water molecule (also resulting in lower hydrogen bond basicity). Despite this, these three coefficients remain negative.

Finally, the decrease of *v* and *r* on the one hand and the increase of *s*, *a* and *b* on the other hand, result in a retention factor decrease confirming the importance of both hydrophobicity and π–π interactions in the retention mechanism of the small test solutes.

3.2. SOLCP shape recognition mechanism

3.2.1. Importance of π-electrons polarizability

The shape recognition of the classical ODS stationary phases has been largely studied regarding monomeric, intermediate and polymeric phases [39–41]. Sander and Wise proposed an empirical interpretation of the shape recognition of these alkyl chains based upon a ''slot model'' [42]: the accessible spaces between the alkyl chains (brush-like appearance) are considered as slots into which the solute molecules penetrate. These slots are considered as selective: planar molecules fit between the

akyl chains more easily than non-planar molecules and long narrow molecules fit between chains more readily than square-shaped molecules. The shape recognition mechanism is thus based only on the steric hindrance regarding the geometry of the solute molecules and the accessible space between C_{18} alkyl chains.

If the vertical, long and flexible alkyl C_{18} chains are substituted by rod-like liquid crystal molecules, the space between liquid crystalline molecules becomes inaccessible due to both the high density of liquid crystal molecules and the strong interactions between them. As already shown, by studying the stationary phase based upon a side-end liquid crystal polymer [31], it results in a solute exclusion from the stationary phase, fast elution and no shape discrimination. Hence, PAH solutes cannot interact with the polarizable π-electron of the stationary phase but only with the short and poorly selective terminal alkyl chains (C_8). Here, the ''slot model'' cannot be applied to the based side-end liquid crystalline polymer stationary phases because the solutes are not sensitive to the intermesogen molecular organization (rich in π-electrons) because of the strong interaction between the mesogens.

Conversely, with a side-on liquid crystal polymer whose polysiloxane chain is stretched in the direction of the mesogenic groups, which form a jacket around it [38], large retention times and good shape recognition were observed. This jacket structure allows access for the solutes and favors the mesogen–solute interactions particularly by means of π-electrons of the rod-like liquid crystal molecules. This is because of the probable ''horizontal'' direction of the mesogen on the support silica surface. It would suggest that the anchoring of the mesogenic unit on the silica surface is directly related to the way it is attached to the polysiloxane backbone, either longitudinally (vertical orientation) or laterally (horizontal orientation). It does not depend on the polymer polymorphism, because the smectic longitudinally-attached LCP $P_{4.8}$ does not retain PAH solutes [31], whereas the corresponding and still smectic laterally-attached LCP $P_{10.8.8}$ strongly interacts with the PAH and is very selective toward shape isomers. (The $P_{n.m}$ and $P_{n.m.m}$ symbols are the names of the previously used side-end and side-on liquid crystal polymers, respectively. In both cases, *n* and *m* are the number of carbon atoms of the spacer arm and the aliphatic tails).

In this paper, we have studied the $P_{10.4.4}$ bonded polymer that presents a jacket structure and so allows the solute to interact with the π-electrons of the aromatic rings of the mesogen molecule. So the specific structure of the side-on liquid crystal polymer would confirm the LSER results, showing that the rR_2 polarizability term induced by the diffuse π-electrons of the mesogenic group, plays a major role in the retention mechanism in addition to the usual vV_2 hydrophobicity term interaction. Fig. 3 shows some solute properties on log *k* values with a 75% methanol mobile phase for the LCP bonded stationary phase. This graph clearly exhibits the large influence of the hydrophobic and π–π interactions (positive terms) consistent with the chemical structure of the LCP. It also proves that the elution order is no longer correlated to the shape of the solute as shown by the shorter elution time of the elongated 4-ethylphenol solute over the square-shaped 2,6-dimethylphenol solute. In this case, the shape interaction may be in competition with stronger interactions such as the HB acidity of the solute (difference in $\Sigma\alpha_2^H$).

Fig. 3 reveals that the shape recognition ability of the LCP phase can be partly explained by the solute

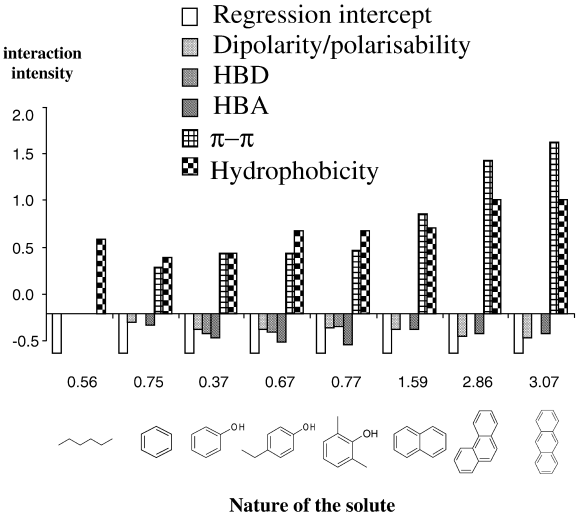

Fig. 3. Contribution of intermolecular interactions to retention of the bonded side-on LCP on different solutes. The number above the molecule represents its log *k* value determined experimentally.

polarizability (R_2 descriptor). For example, although the LSER model contains no fundamental interactions terms, directly dependent on the purely geometrical length-to-breadth ratio (L/B) of planar molecules, it is able to distinguish the retention time difference between phenanthrene and anthracene. The main difference comes from the rR_2 interaction term which is 1.644 and 1.832 for phenanthrene and anthracene solutes, respectively. Thus, anthracene is retained longer because the distribution its π-electrons repartition causes a higher solute polarizability than phenanthrene (2.290 and 2.055 R_2 values, respectively). The variation of these R_2 values increase or decrease in the same way as the molecular polarizability of each isomer ($25.93 \cdot 10^{-30}$ vs. $24.70 \cdot 10^{-30}$ m^3 for α values [30]). It is therefore possible to affirm that the bigger the solute polarizability is, the longer its retention time.

3.2.2. Limits of the π-electrons polarizability for liquid crystal stationary phase

However, the polarizability effect is not sufficient to explain the shape discrimination of some bigger PAH isomers. For example, it does not account for the separation of benz[*a*]anthracene and chrysene whose values of polarizability are very similar ($32.86 \cdot 10^{-30}$ and $33.06 \cdot 10^{-30}$ m^3, respectively). Another specific form of interaction based upon the shape of the solute (L/B ratio), independent of the molecular polarizability should play an important role in the mechanism separation of shape isomers.

Fig. 4 thus exhibits the chromatograms of nine PAH compounds obtained with a non-liquid crystalline stationary phase ($P_{10.0.0}$) on the one hand and the bonded liquid crystalline stationary phase ($P_{10.4.4}$) on the other hand, as used in the previous LSER study. These two phases possess the same mesogenic core (three-phenyl ring benzoate) and so have comparable polarizability. It is important to note that the higher the solute polarizability is, the longer the retention time observed on the $P_{10.0.0}$ stationary phase, as well as on the LCP $P_{10.4.4}$ (see Fig. 5): this still confirms the important role of solute polarizability in retention. Consequently, non-liquid crystalline stationary phases are able to recognize some shape isomers as long as the solutes have distinctive molecular polarizability. For example, fluoranthene and pyrene are resolved as well as phenanthrene and anthracene: indeed, fluoranthene and pyrene have the same molecular mass but different polarizability, $28.35 \cdot 10^{-30}$ and $29.34 \cdot 10^{-30}$ m^3, respectively. This is not the case as for the

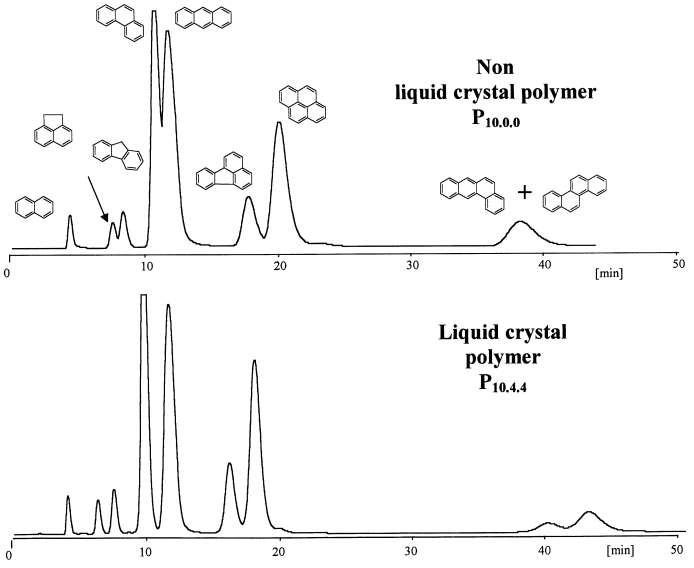

Fig. 4. Separation of PAH solutes of known polarizability on a non-liquid crystalline ($P_{10.0.0}$) and a liquid crystalline stationary phase ($P_{10.4.4}$).

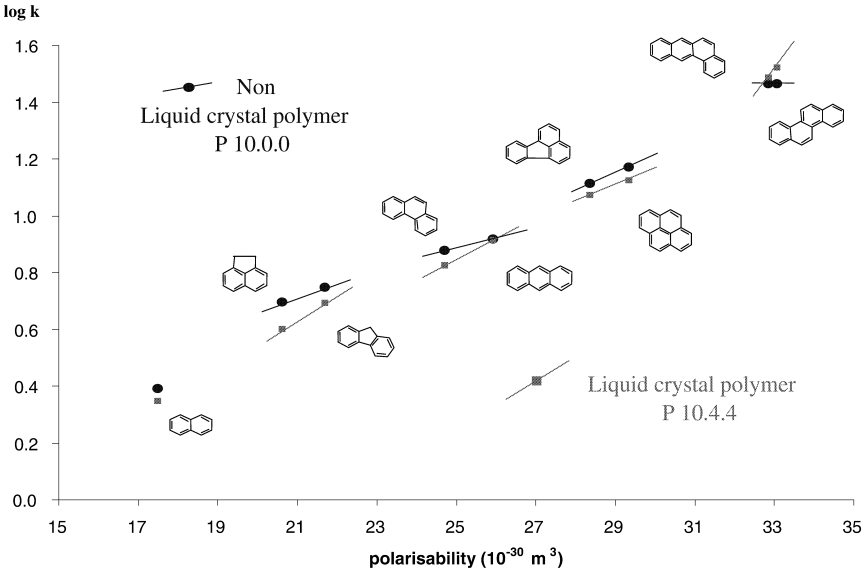

Fig. 5. Correlation between logarithm of retention factor, log k, and polarizability, α, of PAH solutes on $P_{10.0.0}$ and $P_{10.4.4}$ stationary phases.

benz[*a*]anthracene/chrysene pair ($32.86 \cdot 10^{-30}$ and $33.06 \cdot 10^{-30}$ m^3, respectively), no obvious separation occurs on the $P_{10.0.0}$ stationary phase. However, when the same mesogenic core generates a liquid crystalline compound after slight chemical change (addition of short and poor polarizable C_4 aliphatic tails at the ends of the core), the retention differences between shape isomers are increased if only their L/B values are greatly different. Concerning fluoranthene and pyrene molecules whose L/B values are very close (1.21 and 1.25, respectively), selectivity remains unchanged from a non-liquid crystal to a liquid crystalline stationary phase ($\alpha = 1.14$ and 1.15). Inversely, when the L/B variation of a solute pair becomes larger, an increase of selectivity is always observed (see Fig. 5): shape recognition with respect to the pairs acenaphthene ($L/B = 1.09$)/fluorene (1.52), phenanthrene (1.46)/anthracene (1.57) and benzo[*a*]anthracene (1.60)/chrysene (1.73) is always higher on the liquid crystalline $P_{10.4.4}$ stationary phase ($\alpha = 1.24$, 1.23 and 1.08) than on the non-liquid crystalline $P_{10.0.0}$ stationary phase ($\alpha = 1.12$, 1.10, and 1.00).

As a consequence, the shape recognition of the bonded liquid crystalline phase is largely related to a purely geometrical factor of the solutes, independent of any differences in their molecular physico–chemical properties (polarizability, volume, dipolarity, hydrogen bond acidity and basicity).

Despite similar chromatographic results, the shape discrimination mechanism of a side-on liquid crystalline stationary phase is not comparable to the empirical ''slot model'' of Sander and Wise concerning the ODS stationary phases, because the intermesogen space has become inaccessible. However, as the usual intermolecular interactions do not discriminate shape isomers, we have to deduce that side-on liquid crystalline polymer materials develop a specific molecular arrangement onto the silica gel surface, generating selective interactions with respect to the geometry of the solute. This is coherent with the existence of a local anisotropic order, related to liquid crystal polymorphism (nematic, cholesteric, smectic) [33]. Thus, the shape recognition may be enhanced by an ''ordered structure effect'' of the stationary phase. It is not a question of slots generated by the space between two laterally-attached mesogenic parts (partition mechanism) but rather a specific interaction on a surface composed of oriented molecules (adsorption mechanism). The solutes do not penetrate the whole stationary phase but only interact with the selective structured surface by means of the π-electrons from the three phenylbenzoates core. It would thus be more a question of

adsorption than of a partition mechanism. The local anisotropic order of the mesogen may allow some $\pi-\pi$ interactions, whose intensity depends on the solutes' shape. As for isomers, the larger the anisotropic shape of the solute, the bigger the surface contact with the stationary phase, thus increasing the retention times. Fig. 6 describes empirical models proposed for longitudinally and laterally-attached liquid crystal polymer stationary phases, in accordance with the experimental observation obtained in RP-HPLC of PAH solutes:

- For ODS stationary phases, the solute penetrates in among the C_{18} alkyl chains. High and selective retention is observed as according to the ''slot model'' of Sander and Wise.
- For the longitudinally attached liquid crystal polymer, the solute cannot enter the polymer chains. Weak retention and poor selectivity are observed because the solutes only interact with the short C_8 alkyl chains of the mesogenic part. The side-end polymer structure might induce a homeotropic anchoring of the mesogens in relation to the silica surface.
- For the laterally-attached liquid crystal polymer, the solute can still not penetrate the polymer chain but access to the π-electrons of the mesogenic core becomes possible. The probe solute is also sensitive to the local anisotropic order marking the higher selectivity power of this kind of stationary phase over the longitudinally-attached LCP. In this case, the side-on polymer structure might generate a planar anchoring of the mesogen molecules.

It would thus be interesting in the future to develop a surface study of these coated liquid crystal polymers, to underline their probable specific intermolecular arrangement when they are reduced to a monolayer film.

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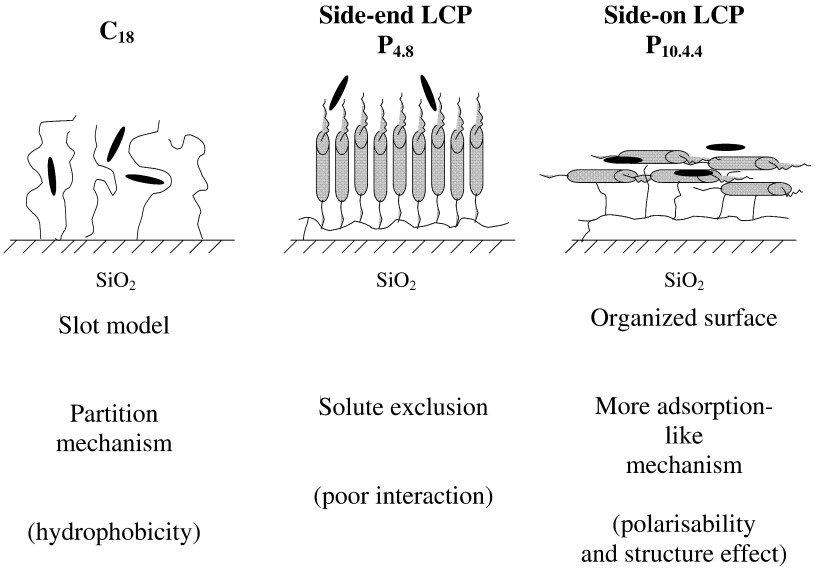

Fig. 6. Empirical models proposed to understand the retention of PAH solutes on longitudinally and laterally attached liquid crystal polymers phases compared to ODS columns.

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