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Thermotropic laterally attached liquid crystalline polymers

I. New stationary phases for high-performance liquid chromatography

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Abstract

Up to now thermotropic liquid crystalline side chain polymers have been seldom used as stationary phases in high-performance liquid chromatography (HPLC). The preparation of a new class of surface modified silica gels is reported. They are obtained by coating on the silica support liquid crystalline polysiloxanes with mesogenic side groups laterally attached to the polymer backbone through a flexible spacer. Their chromatographic behavior in reversed-phase HPLC is described for the separation of polycyclic aromatic hydrocarbons. The results show excellent planarity and rod shape recognition capabilities. Comparisons with low-molecular-mass liquid crystalline-bonded silica and longitudinally attached liquid crystalline polymer-coated stationary phase are also reported. Finally, comparisons to commercially available C₁₈ phases are described for the separation of complex mixtures. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Molecular shape recognition; Liquid crystals; Polynuclear aromatic hydrocarbons; Liquid crystalline polymers

1. Introduction

Liquid crystals were introduced as stationary phases for the first time in 1963 in gas chromatography (GC) separations [1] and many applications have been developed for difficult separation problems towards geometric and optical isomers, taking advantage of the strong shape selectivity of liquid crystal stationary phases [2,3].

In contrast to the almost 400 works published in GC, use of liquid crystalline molecules in high-performance liquid chromatography (HPLC) is still

rare and only some 15 references [4–17] concerning a few liquid crystalline stationary phases have been reported so far. Preparation of liquid crystalline stationary phases for HPLC involves a surface modification of a solid support such as porous silica by coating or bonding either a low-molecular-mass (LMW) liquid crystal, or a liquid crystalline polymer (LCP). First examples were obtained by coating LMW liquid crystals on silica [4–6]. Nevertheless, this kind of material leads to a short column life time due to solubility and low resistance to shear forces of the liquid crystal under the mobile phase solvent conditions. Thus it was necessary for the LMW liquid crystal to be chemically bonded to the support

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[7–12]. In 1989 Pesek and Siouffi [7] reported a suitable method in which the liquid crystalline unit was first converted into a silanization reagent for which chemical coupling on silanol groups of silica was achieved. Recently, new synthetic pathways for chemical bonding of a LMW liquid crystal were proposed [12]. As above mentioned silica surface can also be modified by coating high molar mass liquid crystals such as LCPs. Klein and Springer [16,17] prepared such LCP stationary phases using polyacrylates with mesogenic side groups of the phenyl benzoate type which are rather stable in the mobile phase composition used (cyclohexane/*n*-heptane). Thus, chemical bonding of LCPs on the silica surface is not as imperative as for LMW liquid crystals. Nevertheless, one notes that chemical bonding of a LCP on the silica should be required to avoid restriction in eluent and temperature choices, and to improve column life times. Up to now no example of LCP-bonded stationary phases has been reported.

In the subfield of liquid crystalline systems, the corresponding molecules possess an anisotropic shape, either disk-like or rod-like. Only rod-like mesogenic molecules have been used for chromatographic applications. These rod-like units are longitudinally attached through a flexible spacer to the silica [7–15] or to the polymer backbone [16,17] yielding an axial fixation (Fig. 1a). As shown in Fig. 1b, another type of fixation exists: it corresponds to a lateral fixation where rod-like molecules are laterally attached to the polymer backbone giving rise to

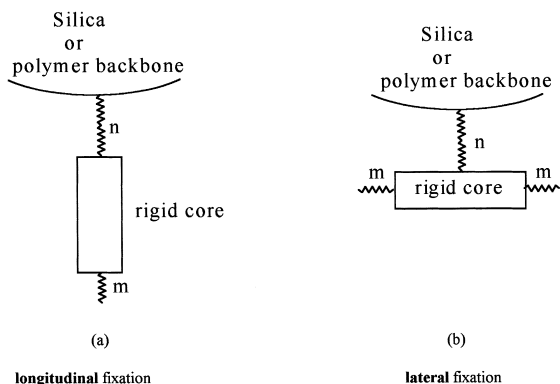


Fig. 1. Schematic representation of longitudinal (a) and lateral (b) fixations of a mesogenic group (schematized as a rectangle) on a support (either polymer or silica).

laterally attached LCP which constitute another class of side chain LCPs [18]. In this study, we use the concept of lateral fixation to get either chemically bonded LMW silica or LCP-coated silica to give new HPLC stationary phases.

The first part of this study is devoted to comparing chromatographic behaviors of a laterally bonded LMW liquid crystal stationary phase to the corresponding laterally attached LCP-coated one. The influence of the LCP architecture will be discussed: chromatographic results on “lateral LCP” coated silica are compared to those of their “longitudinal” homologues.

2. Experimental

2.1. Preparation of liquid crystalline stationary phases

2.1.1. Liquid crystalline chemicals

Materials used in this study are either LMW liquid crystals (M) or liquid crystalline polymers (P). The same mesogenic units M are chemically bonded either to the silica or to a polysiloxane backbone giving rise in this latter case to a LCP.

2.1.1.1. LMW liquid crystals, M

As shown in Fig. 2, liquid crystalline units M are of the three phenyl rings benzoate type, with terminal aliphatic chains (m). They are vinyl derivatives from the unsaturated termination of the spacer arm (n). They are labeled M n.m.m if the spacer has a lateral fixation on the rigid core and M n.m for an axial one.

The synthesis of the lateral M n.m.m units has been previously described [19].

The preparation of the longitudinally M n.m homologues is required in order to study the influence of the type of fixation on the chromatographic results. The synthetic pathway is summarized in Fig. 3.

Preparation of the lateral chain m containing organic moiety 1. In a round-bottom flask 4-alkoxybenzoic acid (17.8 mmol) and 4-(benzyloxy)phenol (19.6 mmol) are dissolved in 100 ml dichloromethane. *N,N'*-Dicyclohexylcarbodiimide (DCC) (19.6 mmol) and 4-dimethylaminopyridine (DMAP) (1.8 mmol) are added to the mixture at room temperature.

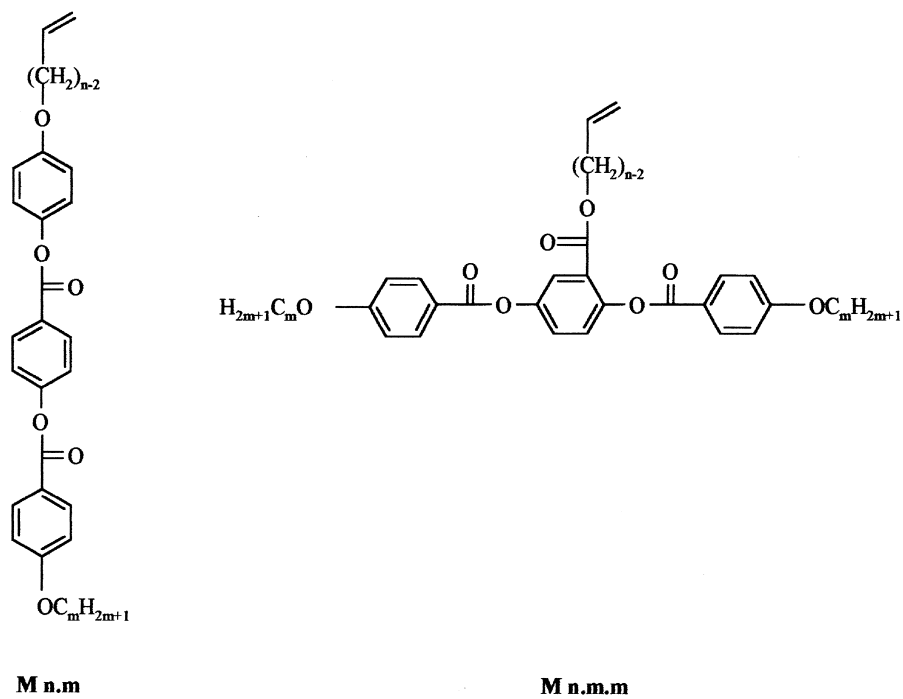


Fig. 2. Schematic representation of an axial and a lateral positions of the spacer arm on to the rigid core rising to **M n.m** (a) and **M n.m.m** (b), respectively.

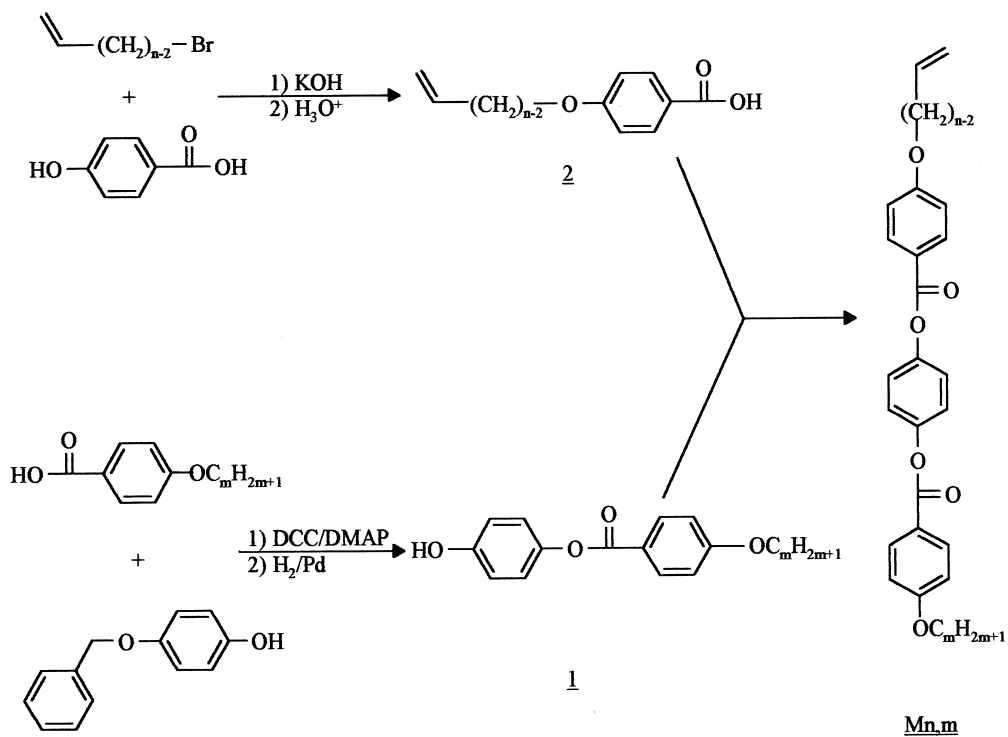


Fig. 3. Synthetic scheme for synthesis of mesomorphic **M n.m**.

The reactive mixture was allowed to stand at room temperature under stirring for 24 h. The work-up was filtered in order to remove hydrated DCC from crude mixture. Evaporation to dryness and recrystallization from ethanol give the pure product (about 16 mmol). The product was dried under vacuum at 80°C. Leaving off the protective benzyl group was then realized by hydrogenation with palladium on activated carbon as a catalyst. The resulting crude alcohol was purified from boiling ethanol to give the pure material with nearly 100% yield.

Preparation of the spacer containing organic moiety 2. 4-Ethylhydroxybenzoate (80 mmol) was placed in a two-necked round-bottom flask and dissolved in 100 ml absolute ethanol. KOH (80 mmol) was then added to the homogenous mixture. As reflux temperature is reached, an ethanolic solution of 1-bromoalcene (88 mmol) was added under vigorous stirring. Reflux temperature was maintained for 24 h. Cooling down to room temperature, evaporation of ethanol, dissolution in distilled water, gave an aqueous basic phase (pH 12). As neutralization with concentrated HCl (12 M) was reached, a crude white precipitate was observed and isolated by filtration. Purification from boiling ethanol was carried out. The pure product was dried under vacuum at 80°C.

Condensation between the organic moieties 1 and 2. In a round-bottom flask the acid 1 (7.8 mmol) and the phenol derivative 2 (8.5 mmol) were dissolved in 100 ml CH₂Cl₂. DCC (8.5 mmol) and DMAP (0.8 mmol) are added to the mixture at room temperature. The reactive mixture was allowed to stay at room temperature under stirring for 24 h. The work-up was filtered to remove the hydrated DCC from the crude mixture. Evaporation to dryness and two recrystallizations from ethanol gave the pure product (about 7 mmol). The product was dried under vacuum at 80°C, and its mesomorphic properties studied: crystal · 118°C · nematic · 208°C · isotropic liquid.

2.1.1.2. Liquid crystalline polymers

The LCPs used were polysiloxanes. They were prepared through a classical hydrosilylation reaction [20,21] involving a commercial polyhydrogenosiloxane chain containing 70 silane units (ABCR, Germany) and the vinyl terminations of the spacer arm present in the mesogenic units M n.m.m or M n.m. Names of polysiloxanes refer to their own molecular parameters: P n.m.m whether they are laterally attached, or P n.m for axially attached ones. Table 1 gives the phase transition temperatures determined by differential scanning calorimetry for the synthesized materials. The nematic–isotropic transition temperatures (T_{IN}) are within the range 110–260°C.

These products were identified by ¹H NMR spectroscopy on a Bruker 200 Hz spectrometer. The mesomorphic properties were characterized by usual methods: DSC on a Perkin-Elmer DSC7 apparatus and optical microscopy.

2.1.2. Preparation of stationary phases

The silica support was Kromasil 5 μm, 200 Å pore size manufactured by Akzo Nobel, Sweden. Silica was dried and activated under vacuum at 180°C for 24 h before organic derivatization.

Efforts were made to prepare stationary phases with same liquid crystalline unit coverage which meant identical mesomorphic unit concentrations, in order to reduce the number of experimental parameters that would influence chromatographic retentions and shape discriminations. A unit is defined as the smallest repetitive unit present on silica. Concentrations of mesomorphic units on silica [M] are calculated from elemental analysis, as follows:

$$[M] \text{ (mmol/g)} = \frac{10^3 \% C}{12N_C - M_w \% C} \quad (1)$$

$$[M] \text{ (}\mu\text{mmol/m}^2\text{)} = Sp[M] \text{ (nmol/g)} \quad (2)$$

Table 1

Phase transition temperatures (in °C) of the liquid crystalline molecules either low molecular mass (M) or polymers (P)

M 4.4.4	Crystal	100	Nematic	113	Isotropic		
P 4.4.4	Glassy state	36	Nematic	130	Isotropic		
P 10.4.4.	Glassy state	17	Nematic	99	Isotropic		
P 4.8	Glassy state	90	Smectic	220	Nematic	260	Isotropic

Table 2
Characterization of the liquid crystalline stationary phases

Liquid crystalline phases	M 4.4.4 ^a	P 4.4.4 ^b	P 4.8 ^b	P 10.4.4 ^b
% C	9.26	9.14	8.12	9.77
[M] (mmol/g)	0.240	0.236	0.234	0.237
[M] ($\mu\text{mol}/\text{m}^2$)	1.20	1.15	1.15	1.19

Low-molecular-mass liquid crystal-bonded silica through organosilicon linkage^a, LCP-coated on silica^b.

where % C is the percentage of carbon contained in the considered stationary phase, N_C is the number of carbons in one unit; M_w , the molecular mass in g/mol; S_p , the specific surface of silica ($200 \text{ m}^2/\text{g}$). As shown in Table 2 the three stationary phases show the same mesomorphic concentration.

It is interesting to note that surface coverages are lower than those of conventional stationary phases for which C_{18} bonding ranges from 2 to $7 \mu\text{mol}/\text{m}^2$ [22–25].

2.1.3. LMW bonded silica

The synthetic way used first involves the synthesis of an intermediate silanization reagent [7,9]: mesomorphic unit M (5 mmol) dissolved in 5 ml of dried toluene was placed in a two-necked round-bottom flask equipped with a reflux condenser. Glassware was kept under a nitrogen atmosphere. Dimethylchlorosilane (9 mmol) was added to the reaction flask through septum to avoid moisture in the reaction mixture. After 5 min, hexachloroplatinic acid (~5 mg) was added, and the reaction was heated to 60°C for three days, under nitrogen. The obtained silane was added to silica (5 g) in dry toluene. The reaction mixture was allowed to stand under reflux for 48 h. The resulting silica was filtered off and washed with toluene, dichloroethane and acetone give the final bonded silica.

2.1.4. LCP-coated silica

The synthesized LCPs were physically coated on the silica surface. For this purpose, an organic solution of the polymer was prepared in 20 ml of tetrahydrofuran (THF). The amount of LCP was chosen to fix the concentration of the mesomorphic units to $0.25 \text{ mmol}/\text{g}$ silica ($1.25 \mu\text{mol}/\text{m}^2$). This solution was added to 3 g silica under vigorous stirring in order to obtain an homogenous suspen-

sion. THF was then removed under vacuum and the resulting coated silica was dried before characterization and use.

2.2. Chromatographic measurements

2.2.1. Column preparation

The stationary phases were packed in stainless steel tubes ($150 \times 4.6 \text{ mm}$ I.D.) by a slurry method. Packing was carried out at room temperature under a pressure of 400 bars, using a mixture of methanol–2-propanol (25:5, v/v) as the suspension medium and methanol as the pressure fluid.

2.2.2. Chromatographic measurements






Chromatographic studies were done with a modular HPLC apparatus which consists of an equipment produced by Jasco (a PU-980 Model gradient pump module, a UV-975 UV-Vis detector set at 254 nm) and a Gradcil Model column heater produced by Cluseau, France. The dead time was obtained with 2-propanol as the non-retained compound. All solvents used were of HPLC grade. Reversed-phase conditions using a methanol–water mixture (70:30) are chosen for chromatographic studies and the typical flow-rate was 1 ml/min. Under these mobile phase conditions, chromatographic studies as a function of temperature are limited and Van't Hoff plots [$\ln k' = f(1/T)$] are then restricted to the range $T = 20\text{--}80^\circ\text{C}$ and thus isotrope–nematic transition temperatures cannot be reached in the considered chromatographic mode. A commercially available Kromasil ($5 \mu\text{m}$, 100 \AA pore size) C_{18} bonded stationary phase was used as a reference to evaluate efficiencies and chromatographic properties of the prepared stationary phases.



Column efficiency, relative to peak broadening was calculated as follows for a solute molecule through the typical plate number per meter of the column: $N_{\text{eff}} = 5.54[t'_R/\delta'_l]/0.15$, with t'_R the reduced retention time and δ'_l the half-peak width.




2.2.3. Choice of solutes

Retention of polycyclic aromatic hydrocarbons (PAHs) on conventional bonded octadecylsilane (C_{18}) stationary phases have been widely reported,

Table 3
Polyaromatic molecules chosen as probes

Isomeric three rings		Isomeric four rings		
				
Phenanthrene	Anthracene	Triphenylene	Benz[a]anthracene	Chrysene
L/B = 1.46	L/B = 1.57	L/B = 1.12	L/B = 1.60	L/B = 1.73

Molecules for planarity recognition	
	
ortho-Terphenyle	Triphenylene
Non-planar	Planar

SRM 869 « Column Selectivity Test Mixture »		
		
BaP	TBN	PhPh

and reversed-phase HPLC on these phases has become the common mode for their separations [23,24]. PAHs probe samples (from Aldrich) were dissolved in 2-propanol. Molecular discrimination will be evaluated towards selected isomeric PAHs such as anthracene/phenanthrene ($M_w = 178$) and chrysene/benz[*a*]anthracene ($M_w = 228$) for rod shape recognition. Triphenylene and *o*-terphenyl were taken for planarity recognition. These solutes provide excellent probes for studying shape recognition. Shape, size and molecular structure of the studied PAHs are summarized in Table 3.

Furthermore, a simple test has been used to assess column shape selectivity towards PAHs. This test is based on the retention of three PAHs solutes (see Table 3): benz[*a*]pyrene (BaP), phenanthrophenanthrene (PhPh) and tetrabenzonaphthalene (TBN). This material is available at the National Institute of Standards and Technology: Standard Reference Material 869, "Column Selectivity Test Mixture for Liquid Chromatography (Polycyclic Aromatic Hydrocarbons)" (Standard Reference Materials Program, NIST, Gaithersburg, MD, USA).

3. Results and discussion

Chromatographic performances of each stationary phase will be studied in terms of molecular recognition by using same the PAH solute test mixtures to evaluate the influence of the kind of fixation of liquid crystalline materials on the separation capabilities. It is worth noting that in these reversed-phase chromatographic conditions, we observed a good stability of the stationary phases and reproducible results over two years of investigations.

3.1. Comparison between laterally bonded LMW liquid crystalline silica and lateral LCP coated silica

Two approaches are reported for laterally attached liquid-crystalline-based stationary phases, depending on the way the mesomorphic units are introduced on the chromatographic support. In the first way the liquid crystalline units M 4.4.4 are laterally bonded to silica through an organosilane reagent ("LWM bonded silica"). In the second method, the same M4.4.4 units are fixed to a polysiloxane backbone (degree of polymerization: $D_{pn} = 70$) giving rise to a LCP (P 4.4.4) which is then coated to the silica support ("lateral LCP coated silica").

Retention data for six PAHs with these phases are summarized in Table 4. Despite similar mesogenic units concentrations, we first note that the M 4.4.4 bonded stationary phase ($[M] = 0.240$ mmol/g of silica), shows lower retention factors towards PAHs solutes than the P 4.4.4 coated silica ($[M] = 0.234$ mmol/g) except for *o*-terphenyl for which same retention factors are obtained on both stationary phases ($k' = 2.49$).

For PAHs, the most useful molecular descriptor is the length to breath L/B ratio which provides an indication of the overall two-dimensional shape of the molecule and as first observed in GC on liquid crystalline stationary phases, retention increases as L/B increases [24]. These observations have been extended to reverse LC with C_{18} stationary phases [25] and LMW bonded phase [11].

In the case of M 4.4.4 and P 4.4.4 stationary phases, it is clearly observed that solutes are retained with respect to their L/B value. Thus, we can note in

Table 4
Retention factor k' for the M 4.4.4 bonded phase and the polymer P 4.4.4 coated silica

Liquid crystalline phases		M 4.4.4 bonded silica	P 4.4.4 coated silica
Compounds	L/B		
Phenanthrene	1.46	2.37	4.13
Anthracene	1.57	2.84	5.82
Triphenylene	1.12	9.53	15.80
Benz[<i>a</i>]anthracene	1.60	13.02	23.25
Chrysene	1.76	13.17	27.15
<i>o</i> -Terphenyle (non-planar)		2.49	2.48

Mobile phase: methanol–water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 25°C.

Table 4 that the elution order for an isomeric test mixture is correlated to the L/B value of each solute and that the elution factor k' increases with increasing this ratio. For example, on the P 4.4.4 coated stationary phase, phenanthrene, with an L/B value of 1.46, is eluted faster ($k'=4.13$) than the anisotropic anthracene with $L/B=1.57$ (for which $k'=5.82$). For tetracyclic isomeric PAHs ($M_r=228$), the elution order of triphenylene ($k'=15.80$), benz[*a*]anthracene ($k'=23.25$) and chrysene ($k'=27.15$) is also related to their respective L/B values: $L/B=1.12$, 1.60 and 1.73.

In addition to larger retention factors, greater shape selectivities (Table 5) are obtained on the P 4.4.4 LCP coated silica compared to M 4.4.4 bonded one. Shape discriminations with respect to phenanthrene/anthracene and chrysene/benz[*a*]anthracene are higher on the P 4.4.4 polymer coated silica ($\alpha=1.41$ and 1.17, respectively) than on the liquid-crystalline M 4.4.4 bonded stationary phase ($\alpha=1.20$ and $\alpha=1.00$ corresponding to co-elution).

Finally, planarity recognition towards triphenylene/*o*-terphenyl is twice as good on the LCP coated phase ($\alpha=6.37$) than on its homologous LMW bonded silica ($\alpha=3.83$).

Since the two stationary phases have same mesogenic contents, these comparisons suggest that shape recognition is highly dependent on the average distance between mesomorphic units introduced on the silica surface. Indeed, Si–OH reactive groups on silica are randomly distributed [26] and the average distance between two M 4.4.4 bonded moieties is estimated from surface coverage to about 12 Å. Conversely, in a side-chain LCP, the position of the mesomorphic units on the polysiloxane backbone is imposed by the Si–O–Si bond length (~3.5 Å). This proximity favors mesogen–mesogen interactions and generates a local anisotropic order on the resulting coated stationary phase. This seems to be an important condition for high shape recognition ability. Martire et al. [27–30] developed a theory based on statistical thermodynamics which predicts that shape selectivity increases as the stationary phase order increases. In this sense, observations reported here clearly indicate that the P 4.4.4 coated stationary phase is more ordered than the M 4.4.4 bonded one: on the P 4.4.4 coated stationary phase the small distance between the points of fixation of the mesomorphic units on the polysiloxane backbone favors the anisotropic interactions and the orienta-

Table 5
Selectivity (α) obtained for three different isomeric compounds on the M 4.4.4 bonded phase and the polymer P 4.4.4 coated silica

Compounds	Liquid crystalline phases	
	M 4.4.4 bonded silica	P 4.4.4 coated silica
Anthracene/phenanthrene	1.19	1.41
Chrysene/benz[<i>a</i>]anthracene	1.00	1.17
Triphenylene/ <i>o</i> -terphenyle	3.83	6.37

Mobile phase: methanol–water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 25°C.

tional order between the mesogenic units. Anisotropic rod-shaped solutes, corresponding to high L/B values, are retained more strongly than less anisotropic ones. This close proximity of the mesogens which imposes an anisotropic packing at least at short range order. This phenomenon is thus probably responsible of the high shape recognition of the LCP coated stationary phase.

Nevertheless, if the solute–stationary phase molecular interactions are favored for anisotropic rod-shaped and planar solutes, it is not the case with non-planar molecules: indeed, as above mentioned, the bulky tri-dimensional *o*-terphenyl solute is quickly excluded and has identical retention times on both M 4.4.4 bonded and P 4.4.4 coated silica. Thus, it is more difficult for laterally attached mesogens to interact with non-planar solute than with planar ones.

Thus, the results reported here clearly demonstrate that these new laterally attached LCP coated stationary phases possess high selectivity towards structural isomers and good ability for planarity and shape recognition. In addition, the P 4.4.4 coated phase is higher in molecular shape recognition capability than the M 4.4.4 bonded one although the surface coverage value in mesogenic units is kept the same. So, since liquid crystalline polymer coating gives better results than the direct fixation of the low molar mass liquid crystal on the silica, we will now focus on LCP coated stationary phases, in particular to determine the influence of the lateral fixation on the discrimination ability.

3.2. Influence of LCP architecture lateral or longitudinal fixations

Two different polymer architectures are now compared. They result from lateral and longitudinal fixations of mesomorphic units on the polymer backbone (see Fig. 1).

Stationary phases are prepared through coating two LCPs with the same three phenyl rings rigid core and the same aliphatic content (spacer plus lateral chains). So we compare longitudinal P 4.8 to lateral P 4.4.4 coated stationary phases. Results from elemental analysis indicate same concentrations in terms of mesomorphic units ($[M]=0.234, 0.236$ mmol/g). Chromatographic data are listed in Table 6.

Table 6

Influence of the polymer architecture (longitudinally attached P 4.8 and axially attached P 4.4.4) on the retention factor k' of the resulting coated stationary phases

Compounds	L/B	Liquid crystalline phases	
		P 4.8	P 4.4.4
Phenanthrene	1.46	$\cong 1$	4.13
Anthracene	1.57	$\cong 1$	5.82
Triphenylene	1.12	2.79	15.80
Benz[<i>a</i>]anthracene	1.60	4.02	23.25
Chrysene	1.76	4.91	27.15
<i>o</i> -Terphenyle		0.99	2.48

Mobile phase: methanol–water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 25°C.

First we note that faster elutions are observed with longitudinal P 4.8 coated silica. For example, retention factor of chrysene is $k'_{\text{chrysene}}=7.91$ while on the P 4.4.4 this same solute is longer retained: $k'_{\text{chrysene}}=27.15$. As shown in Fig. 4, no shape discrimination towards phenanthrene/anthracene is observed with P 4.8 phase. Four rings PAHs isomers are more or less separated according to their L/B values. Moreover, selectivity (Table 7) in terms of planarity recognition towards triphenylene/*o*-ter-

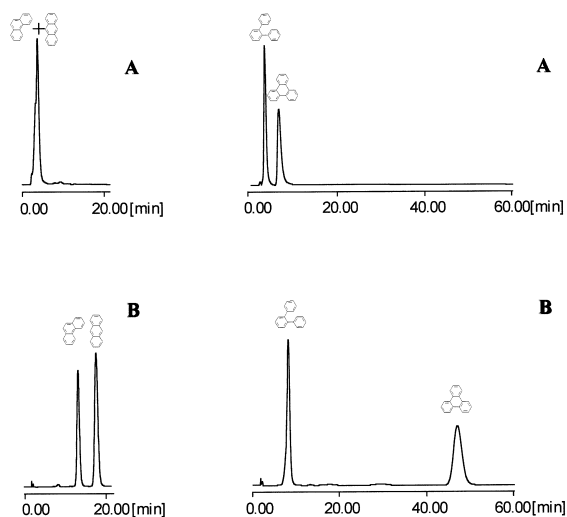


Fig. 4. Molecular shape discrimination towards anthracene/phenanthrene (left side) for rod-shape recognition and triphenylene/*o*-terphenyl (right side) for planarity recognition, on (A) longitudinal P 4.8 and (B) on lateral P 4.4.4 LCP coated silica. Mobile phase: methanol–water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 25°C.

Table 7

Influence of the polymer architecture (longitudinally attached P 4.8 and laterally attached P 4.4.4) on the selectivity α obtained for three mixtures

Compounds	Liquid crystalline phases	
	P 4.8	P 4.4.4
Anthracene/phenanthrene	$\cong 1$	1.41
Chrysene/benz[a]anthracene	1.22	1.17
Triphenylene/ <i>o</i> -terphenyle	2.80	6.37

Mobile phase: methanol–water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 25°C.

phenyl is significantly higher for the lateral P 4.4.4 coating ($\alpha = 6.37$) than for longitudinal P 4.8 coated silica ($\alpha = 2.80$).

So, major differences are observed between these two classes of LCP coated phases. Since they bear comparable mesogens, the chromatographic performances thus appear highly dependent on the architecture of the coated polymer.

At this point, we have to keep in mind the different structures of these two classes of LCPs. Longitudinally attached LCPs mainly exhibit smectic phases (e.g., more ordered phases than nematic, with both orientational and layering order). This comb-like structure, resulting from mesogenic pendants longitudinally attached, can be first thought to be compared to the typical C_{18} stationary phase and the observed results on the longitudinal P 4.8 stationary phase can be interpreted with the Sander and Wise slot model proposed for planarity recognition on C_{18} [31]. In this model, the stationary phase is considered as slots into which planar solutes penetrate readily: rod-like molecules penetrate in an easier manner than less anisotropic molecules and non-planar solutes are excluded from slots. These slots have been thought as the space between the bonded alkyl chains. In the case of the longitudinal P 4.8 LCP (Fig. 5a), X-ray analysis shows that the average distance between two mesogens is about 4.5 Å and that the layer spacing in the smectic phase is 46 Å. As a consequence, all the PAHs with the average thickness 3.9 Å [32] cannot penetrate between the mesogenic side-chains pendants (e.g., in $[4.5 \times 46] \text{ \AA}^2$ slots) because of the steric hindrance due to aromatic π -electrons. This results in exclusion from stationary phase. The rare

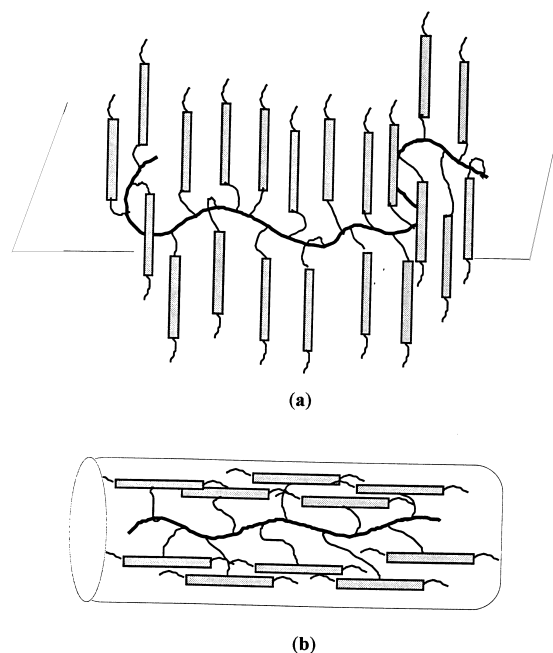


Fig. 5. Structure of a smectic phase for longitudinal LCP (a) and jacketed structure of the nematic phase for lateral LCP (b).

feasible solute–stationary phase interactions take place at the periphery of the polymer, resulting in fast elution and then no shape discrimination: thus k' weakly varies with L/B . The only significant discrimination concerns the planar/non-planar solutes: non-planar distorted solutes have much less interaction with accessible pendants of the stationary phase than their homologous planar compounds for which their planarity involve stronger interactions with the stationary phase. This explains why non-planar compounds are eluted more quickly.

Conversely, lateral P 4.4.4 LCP exhibits a nematic phase: SANS measurements [33] allowed a description of the structural arrangement in the nematic phase (or in the glassy nematic state). The polymer backbone is stretched in the direction of the mesogenic groups which form a jacket around it (Fig. 5b). This jacketed structure gives a good access to the solutes and favors the mesogen–solute interactions thus increasing the discriminations capability. In addition the rod-like shape of the mesogens retains more the rod-like solutes than the compact ones.

To summarize:

As just mentioned, the longitudinally attached LCP coating gives poor results for shape discrimination but is able to differentiate planar/non-planar solutes.

LMW lateral bonding to silica clearly shows better retention factors and better selectivities than those obtained on the longitudinally attached based stationary phase. It is difficult to quantitatively compare these data to results previously reported in the literature, since the mobile phase composition appears an important factor influencing the planarity recognition. Nevertheless we can note from, that comparable selectivity was obtained with LMW axially bonded silica, but with a three-times higher surface coverage ($3.5 \mu\text{mol}/\text{m}^2$) (Refs. [10,11]).

Finally, laterally fixed LCP coated silica possesses the required structure for shape recognition ability in terms of anisotropic as well as planarity recognitions. This type of liquid crystalline stationary phases is new since the mesogenic groups linked to the silica or to the polymer backbone cover the silica surface horizontally whereas the typical C_{18} or the axially fixed mesogens are attached vertically to the support.

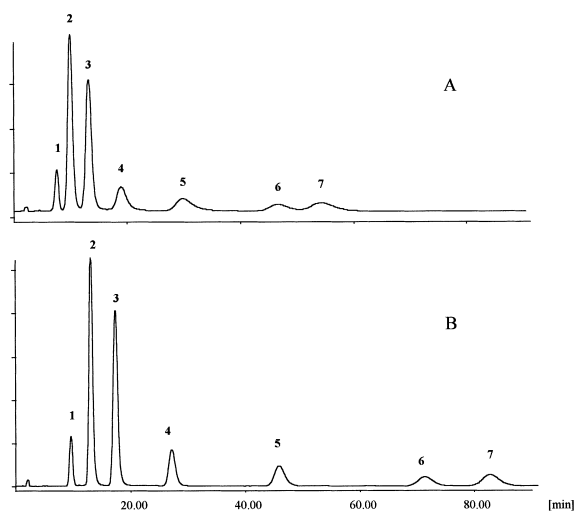


Fig. 6. Separation of a seven PAH test mixture on the lateral P 4.4.4 (A) and on the P 10.4.4 (B) coated stationary phases. Note that the quantitative differences between chromatograms A and Fig. 4 can be explained by more concentrated solutes and a little variation in mobile phase composition. Mobile phase: methanol–water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 25°C. Peaks: 1 = fluorene; 2 = phenanthrene; 3 = anthracene; 4 = pyrene; 5 = triphenylene; 6 = benz[*a*]anthracene; 7 = chrysene.

Furthermore, it is important to underline that this lateral P 4.4.4 stationary phase is only the first example of this kind of coated stationary phase and in this sense does not constitute an optimal case. Additional works are currently under in progress to evaluate the influence of different molecular parameters of the LCP on the chromatographic properties. It seems that the spacer length should have a significant influence. Indeed, when comparing results obtained on a seven PAH solute test mixture on the lateral P 4.4.4 and P 10.4.4 coated stationary phases, better efficiencies and selectivities are obtained when the long spacer is used. Chromatograms of both stationary phases are illustrated in Fig. 6 and the resulting column efficiencies are calculated on phenanthrene: $N_{\text{eff}} = 3750$ plates/m on P 4.4.4 (spacer $n = 4$) whereas $N_{\text{eff}} = 13\,030$ plates/m on P 10.4.4 (spacer $n = 10$). It appears that column efficiency is three-times higher when using the P 10.4.4 coated stationary phase.

3.3. Comparison to a commercially available C_{18} stationary phase

The SRM 869 selectivity test mixture, based on the retention of three PAHs (Table 3): Ba, TBN and PhPh, provides a sensitive measure of the polymeric or monomeric character of an alkyl bonded stationary phase [22,23]. Strict chromatographic conditions, recommended by the Certificate of the NIST, are under mobile phase (acetonitrile–water, 85:15, 2 ml/min) and temperature ($T = 25 \pm 2^\circ\text{C}$). The selectivity factor $\alpha_{\text{TBN/BaP}}$ has been shown to correlate with PAH selectivity and phase type for either monomeric or polymeric (high surface coverage). Monomeric and polymeric alkyl phases differ in the stationary phase chemistry, leading to surface coverages ranging from 2 (monomeric) to $7 \mu\text{mol}/\text{m}^2$ (polymeric). Sander and Wise reported that monomeric C_{18} phases give a selectivity $\alpha_{\text{TBN/BaP}} > 1.7$ whereas polymeric C_{18} ones give $\alpha_{\text{TBN/BaP}} < 1.0$. Values such as $1.0 < \alpha_{\text{TBN/BaP}} < 1.7$ refer to light polymeric stationary phases or densely loaded monomeric stationary phases.

As shown in Fig. 7 the lateral P 10.4.4 coated stationary phase gives a selectivity value $\alpha_{\text{TBN/BaP}} = 1.20$ which indicates its “intermediate” character, despite its very low surface coverage $[M] = 1.19 \mu\text{mol}/\text{m}^2$. The C_{18} phase used as a reference can be

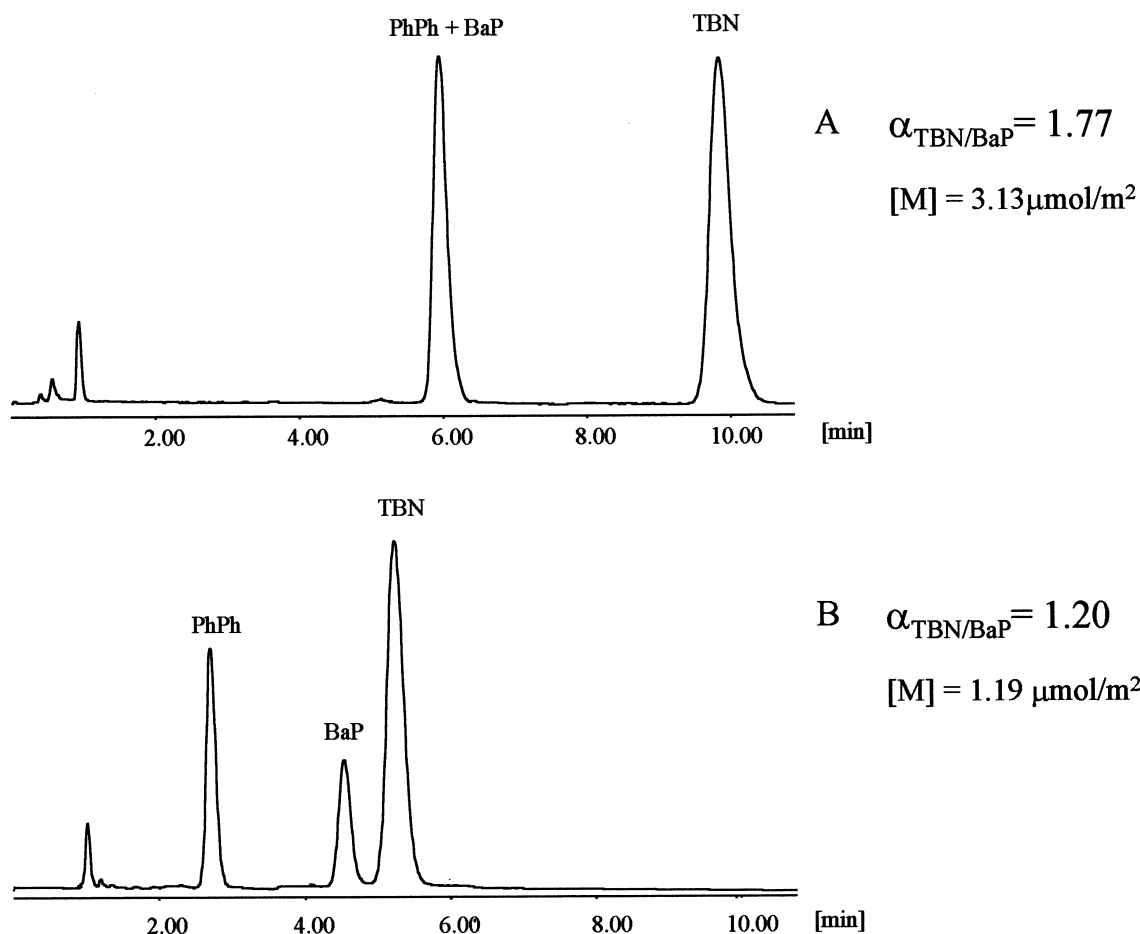


Fig. 7. Separation of SRM 869. “Column selectivity test mixture” on the commercial C_{18} phase (A) and on the lateral P 10.4.4 coated stationary phase (B). Mobile phase conditions, as described by NIST for Certification: MeCN–water (85:15, v/v), flow-rate: 2 ml/min, temperature = $25 \pm 2^\circ\text{C}$.

qualified as “monomeric” from its shape selectivity: $\alpha_{\text{TBN/BaP}} = 1.77$. Column efficiencies are calculated on TBN for both phases: $N_{\text{eff}} = 14\,570$ plates/m for the P 10.4.4 coated silica whereas $N_{\text{eff}} = 34\,000$ plates/m for the commercial C_{18} stationary phase. The latter provides better efficiencies which may be attributed to industrial column packing run at a higher pressure for example. Nevertheless the value obtained on P 10.4.4 is reasonable compared with efficiencies given for other C_{18} stationary phases [34].

The laterally attached LCP based stationary phases have been demonstrated in previous parts to be highly selective towards PAHs and we tried to perform separations of a more complex mixture

containing 16 PAHs identified by the US Environmental Protection Agency (EPA) as priority pollutants. Fig. 8 relates gradient elution for separation of the complex PAH mixture, carried out on two stationary phases, one of which (A) being the commercially available C_{18} presented as the reference. The other chromatogram (B) is relative to the separation obtained on the laterally attached P 10.4.4 based stationary phase. Analysis relative to these two stationary phases is achieved in 130 min. Retention order is observed to be the same on both commercially and mesomorphic polymer coated silica.

From these chromatograms, carried out under the same methanol–water gradient conditions, an interesting tendency is found for the laterally attached P

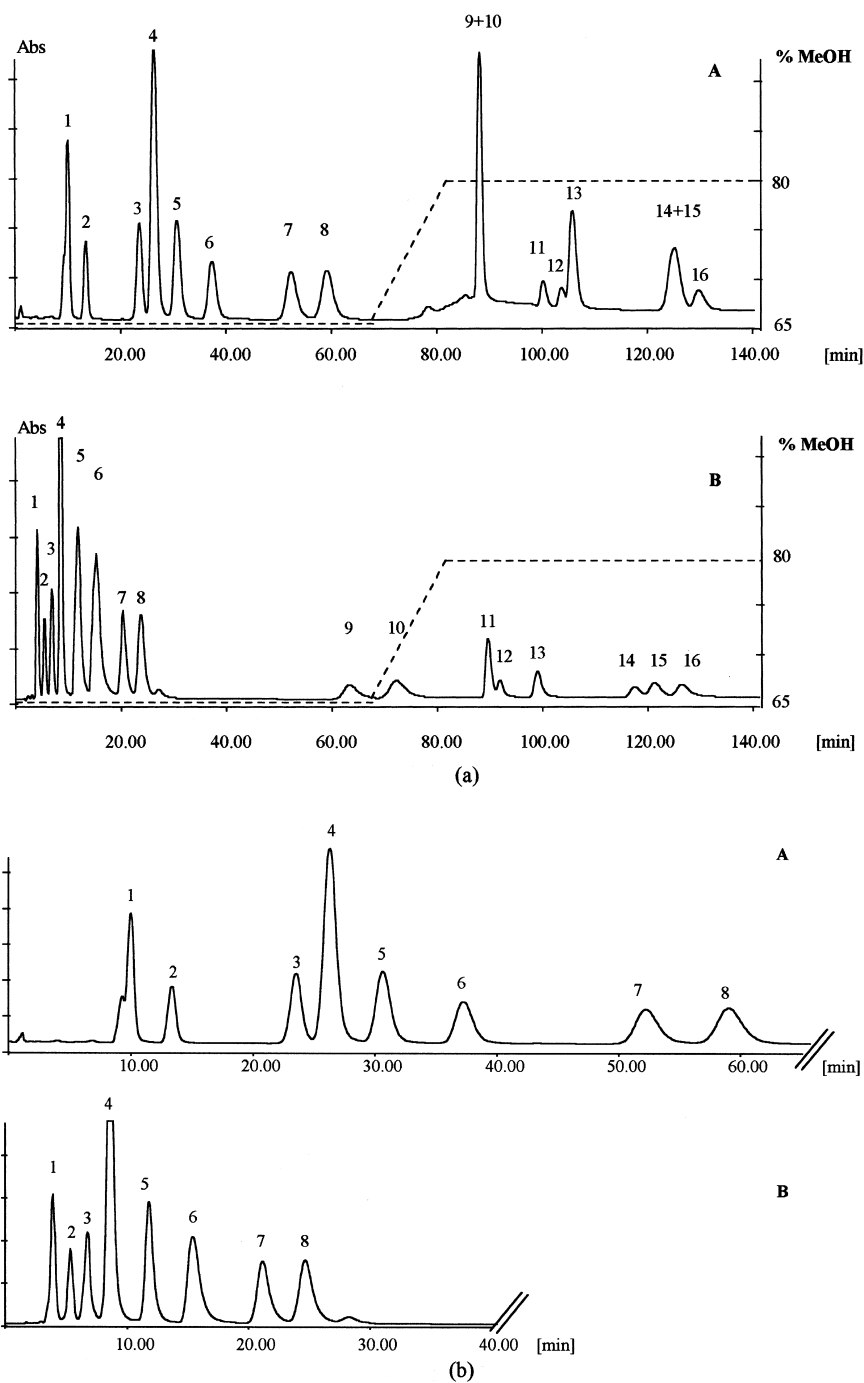


Fig. 8. (a) and (b) Separation ability towards a complex mixture of 16 PAHs on a reference Kromasil C_{18} commercial phase (A), and on the lateral P 10.4.4 coated stationary phase (B); mobile phase MeOH–water gradient mode: (65:35) 0–70 min, (80:20) 82–170 min; temperature: 25°C; Peaks: 1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = benz[*a*]anthracene; 10 = chrysene; 11 = benzo[*b*]fluoranthene; 12 = benzo[*k*]fluoranthene; 13 = benzo[*a*]pyrene; 14 = dibenz[*a,h*]anthracene; 15 = indeno[1,2,3-*cd*]pyrene; 16 = benzo[*ghi*]perylene.

10.4.4 based stationary phase: analysis shows that the lateral P 10.4.4 stationary phase possesses the entire ability for separation and resolution of the pollutants, the commercial C₁₈ does not separate benz[*a*]anthracene (9) to chrysene (10) on one hand, and dibenz[*a,h*]anthracene (14) to indeno[1,2,3-*cd*]pyrene (15) on the other hand.

4. Conclusions

Preliminary results reported here show that the shape selectivity is highly dependent on the type of fixation of the mesogenic units and that lateral fixation leads to considerably higher chromatographic performances than an axial fixation.

In addition, laterally attached LCP silica coatings result in stationary phases for which shape discrimination are better than the corresponding lateral LMW liquid crystalline bonded silica. This result is consistent with an higher local ordering into the stationary phase for LCP coatings. Unfortunately, under the mobile phase conditions, no correlation between non-linear Van't Hoff plots and phase transition temperature can be established in the accessible temperature range.

Finally, the laterally attached LCP coated stationary phases show very interesting combination between their poor coverage and their excellent planarity and anisotropic recognition capabilities. Laterally attached LCP coated silicas behave as an “intermediate selective C₁₈ bonded silica” and complete separation and resolution of a 16 PAH mixture was achieved.

Optimization of these phases in terms of molecular parameters of the lateral LCP is currently under study.

Additional chromatographic investigations are also being undertaken for separation of other kind of solutes such as isomeric estrogens or vitamins.

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