Laterally attached liquid-crystalline polymers as stationary phases in reversed-phase high-performance liquid chromatography
III. Effect of the local anisotropic order on the separation of polycyclic aromatic hydrocarbons

F. Gritti[a,b], G. Félix[a,*], M.-F. Achard[b], F. Hardouin[b]

[a]ENSCPB Université Bordeaux I, Avenue Pey-Berland (BP 108), 33402 Talence, France
[b]CRPP-CNRS, Université Bordeaux I, Avenue du Dr Schweitzer, 33600 Pessac, France

Abstract

Specific stationary phases based upon non-liquid-crystalline polymers, liquid-crystalline molecules and side-on fixed liquid-crystalline polymers (SO-LCP) have been synthesized for use as silica modified stationary phases in high-performance liquid chromatography (HPLC). The mesogenic side group of the SO-LCP was composed of three phenyl ring benzoate type with terminal alkoxy chains and was laterally linked to a polysiloxane backbone via an alkyl ester spacer arm. This study demonstrated that the shape recognition of stationary phases based upon SO-LCP towards the length-to-breath ratio (L/B) was strongly connected to the existence of a local liquid-crystalline order into the pores of silica gel, warranting the interest of the collective organization of mesomorphic materials in liquid chromatography. Furthermore, the chromatographic performances depended on the kind of anisotropic order and it was more advantageous to use smectic side-on liquid-crystalline polymer than nematic and obviously non-liquid-crystalline ones. Finally, for a series of polymers having the same mesomorphism, the larger the temperature stability range of the mesophase, the more pronounced the local order effect and the higher the shape recognition.   © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Liquid crystalline stationary phases; Liquid crystals; Polynuclear aromatic hydrocarbons

1. Introduction

Liquid crystals have been widely applied in gas chromatography since Kelker described them for the first time in 1963 [1]. It has become indisputable that the liquid-crystalline stationary phases yielded separation based upon differences in solute molecular shape (length-to-breath ratio and planarity) independently of any specific energetic interactions [2–6].

The specificity of gas chromatography is the large mean thickness of the liquid phase layer covering the support surface. Typically, mean thickness of 100 nm is applied in capillary columns so the properties characteristic of the liquid crystal observed in the bulk are also manifested at the support surface. In this case, gas chromatography can provide with a good accuracy phase transition temperatures because the solute's retention is well sensitive to the thermodynamic state of the liquid phase. It is thus explained by the solubility difference of the solute in the mesophase and the isotropic liquid [7]. The solute's

*Corresponding author. Tel.: +33-55-684-6568; fax: +33-55-684-6633.

shape recognition is also correlated to the solubility's difference of shape isomers in liquid-crystal bulk. Thus, Janini and Ubeid correlated the separation effect obtained on liquid-crystalline stationary phases to the thermodynamics of solute dissolution in the corresponding liquid-crystalline bulk [8].

When the thickness of the liquid phase diminished progressively with lower amount of stationary phase on the support, a difference appears between transition temperatures determined chromatographically and those found by thermo-optic method for liquid-crystalline as well as for non-liquid-crystalline stationary phases [9–11]. In this situation, the part of retention volume imputable to the liquid–gas interface [12] increases at the expense of the part of retention volume imputable to the volume of liquid phase: adsorption mechanism becomes non negligible over dissolution mechanism.

As we are concerned, in high-performance liquid chromatography (HPLC), the porous silica gel usually used as solid support has a high specific surface (>50 m^2/g) and small pore size (<500 Å). On such a support, a coated organic stationary phase can be divided into two distinctive parts that are film or dispersed aggregates [13,14]. The thickness of the film is in most cases limited to a few monomolecular layers and neither the film nor the aggregates presents the same properties as the bulk phase. The thickness of the layer is very thin: for example, Klein and Springer [15,16] determined by elementary analysis the thickness of their coated liquid-crystalline polymer (LCP) films and found values of about 2 nm. Despite this small thickness, breaks in the temperature dependence of the capacity factor have been observed and the authors correlate these discontinuities with the transition temperatures of the pure LCP, even if they only occur for some solutes and at significantly lower temperatures. As a consequence, it is assumable that a liquid-crystalline mesophase still exists when the polymer is coated on irregularly solid particles of mean particle size of 10 μm with mean pore diameter of 500 Å as Springer et al. applied it in their study.

In this work, silica gel with smaller pores and particle sizes were used, thus increasing the interface effects and the degree of confinement imposed to the liquid crystal which was in this study akin to laterally attached liquid-crystalline polymers (SO-

LCP). As previously shown [17–20], these stationary phases show excellent planarity and shape recognition for polycyclic aromatic hydrocarbon (PAH) solutes in reversed-phase liquid chromatography (RP-HPLC). The debatable points of this paper are two-fold: (a) is the specific molecular organization of liquid-crystalline polymers still conserved when they are coated on a silica support and so reduced to a very thin film? (b) finally, is there any correlation between chromatographic data of based liquid-crystalline stationary phases and the bulk thermo-optical properties of these materials?

2. Experimental

2.1. Organic solvents and reagents

HPLC-grade methanol was purchased from ICS Nationale (Eysines, France) and used to prepare the mobile phase. Water was doubly distilled. Each stationary phase has been tested on polycyclic aromatic hydrocarbons purchased from Aldrich–Sigma and on the standard reference material SRM869a purchased from Promochem (Molsheim, France) as described in Fig. 1. Polyhydromethylsiloxane (PHMS67) was purchased from ABCR (Karlsruhe, Germany). All other chemical compounds needed for the material synthesis were purchased from Aldrich–Sigma. The silica gel (Kromasil, 5 μm, 200 Å pore size, 220 m^2/g) from Akzo Nobel was first activated at 180°C for 24 h.

2.2. Apparatus

The mesomorphic properties of the precursors and corresponding liquid-crystalline polymers are characterized by differential scanning calorimetry (DSC) on a Perkin-Elmer DSC7 apparatus and optical microscopy. Observations by scanning electron microscopy (SEM) were performed on a Hitachi 4500 apparatus equipped with a field emission gun (FEG) at 3 kV with a previous metallization. HPLC was carried out using a modular apparatus equipped with a PU-980 Model gradient pump, a UV-975 UV–Vis detector, a LG-980-02 Ternary Gradient Unit mobile phase mixer, a DG-980-50 3-line degasser from Jasco, a Rheodyne 7725 injector (assembled with a
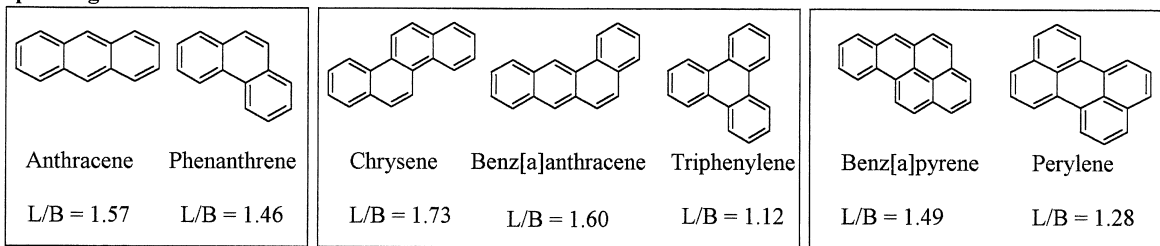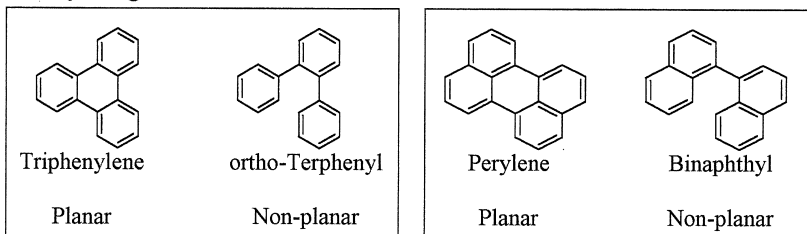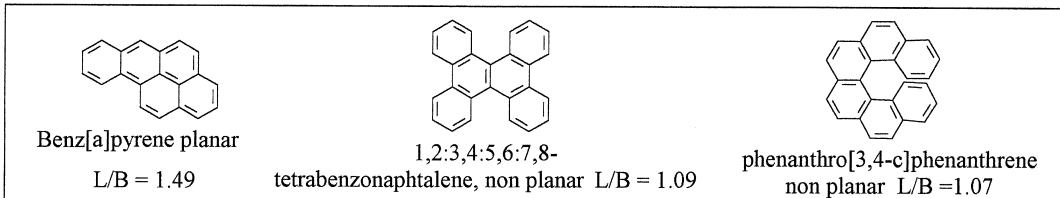
Shape recognition

Anthracene — L/B = 1.57
Phenanthrene — L/B = 1.46

Chrysene — L/B = 1.73
Benz[a]anthracene — L/B = 1.60
Triphenylene — L/B = 1.12

Benz[a]pyrene — L/B = 1.49
Perylene — L/B = 1.28

Planarity recognition

Triphenylene — Planar
ortho-Terphenyl — Non-planar

Perylene — Planar
Binaphthyl — Non-planar

Test SRM 869a

Benz[a]pyrene planar — L/B = 1.49

1,2:3,4:5,6:7,8-tetrabenzonaphtalene, non planar L/B = 1.09

phenanthro[3,4-c]phenanthrene non planar L/B =1.07

Fig. 1. PAHs solutes used in this study.

20 µl sample loop). The deflection mark due to injection was used as the void volume marker. Reversed-phase conditions using mixtures of methanol–water (90:10, v/v) and (80:20, v/v) were chosen for the chromatographic measurements carried out on the SRM869a solute's mixture and on the other PAHs, respectively. In each case, a flow rate of 1 ml/min was applied. All chromatograms were obtained at room temperature. A GradCil Model column heater (Cluzeau Infolabo, Sainte-Foy-la-Grande, France) was used for evaluating the temperature dependence of the capacity factor.

2.3. Preparation of the liquid-crystalline polymers (LCP)

The LCP used in this work belong to the family of the side chain liquid-crystalline polymers where the mesogenic rod-like units are laterally attached to a flexible polysiloxane backbone via a flexible spacer

('side-on fixation' SO-LCP). As previously shown [17,18], this lateral fixation of the side groups leads to higher chromatographic performances than the longitudinal fixation. The liquid crystal homopolysiloxanes are prepared through a classical hydrosilylation reaction [21,22] between the Si–H functions of the commercial polyhydromethylsiloxane and the unsaturated termination of the spacer arm of the side group. Fig. 2 shows the general formula of the resulting polymers $P_{n.m.m}$ (Y). The side groups are of the three-phenyl rings type with terminal alkoxy chains, n and m are the number of carbons of the lateral alkyl ester spacer arm and alkoxy terminal chains respectively; Y is either a –COO– or a –CH$_2$O– linking group. The synthesis of the benzoate side groups (Y=–COO–) has been described previously [18,23].

Precursors with Y=CH$_2$O were synthesized according to the procedure described below for the 2,5-di(4-butoxybenzyloxy)benzoate 9-decenyl: In a
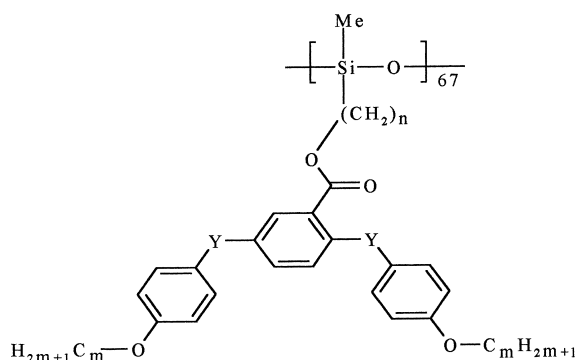
Fig. 2. General formula of the side-on fixed polysiloxanes.

round-bottom flask 1.8 g (10 mmol) of 4-butoxybenzyl alcohol was dissolved in 25 ml acetonitrile. To this solution 1.49 g (20 mmol) of potassium chloride was added and the suspension obtained was vigorously stirred. 2.55 ml of trimethylchlorosilane were added dropwise and the reaction mixture was allowed to stay at room temperature for 1 h. Acetonitrile was then evaporated and the crude mixture was redisolved in 50 ml diethyl ether. The potassium chloride salt was then filtered and the organic phase was successively washed with 25 ml distilled water and 25 ml aqueous solution saturated with sodium chloride. The organic phase was then dried on sodium sulfate, filtered and evaporated. The product was chromatographed on silica gel (70–230 mesh) with diethylether–hexane (50:50, v/v) as eluent to yield pure 4-butoxybenzyl chloride.

In a round-bottom flask equipped with a refrigerant 2.09 g (10.5 mmol) of 4-butoxybenzyl chloride, 1.46 g (5 mmol) of 2,5-dihydroxybenzoate-9-decenyl, 1.45 g (10.5 mmol) of potassium carbonate and 3.87 g (10.5 mmol) of tetrabutylammonium iodide is added to 50 ml acetone. The reaction mixture is allowed to stay at reflux temperature for 12 h. The solution mixture was passed through a silica gel column (70–230 mesh) in order to separate the product from the tetrabutylammonium iodide salt. The product was chromatographed on silica gel (70–230 mesh) with dichloromethane–hexane (70:30, v/v) as eluent. The product was recrystallized from pure ethanol.

At last, a new cholesteric mesogenic unit based upon cholesterol has been synthesized. Only the last step of the precursor's synthesis has been changed and is described as follows:

In a round-bottom flask equipped with a refrigerant 0.992 g (2.2 mmol) of cholesteryl chloroformate, 0.292 g (1 mmol) of 2,5-dihydroxybenzoate-9-decenyl, 320 μl (2.2 mmol) of triethylamine were dissolved in 100 ml of dichloromethane. The reactive mixture was allowed to stay at 60°C under stirring for 48 h. The crude mixture was concentrated and chromatographed on silica gel (70–230 meshs) with hexane–dichloromethane (60:40, v/v) as eluent. The pure liquid-crystalline product was recrystallized in pure methanol.

Table 1 gives the phase transition temperatures of the $P_{n.m.m}$ (Y) polymers used in this study. Depend-

Table 1
Phase transition temperatures of polymers[a]

Y	n	m	Polymers	Transition temperatures (°C)
COO	4	4	$P_{4.4.4}$	g 36 N 130 I
COO	4	8	$P_{4.8.8}$	g 24 N 93 I
COO	4	12	$P_{4.12.12}$	g 27 N 87 I
COO	10	0	$P_{10.0.0}$	Non-liquid-crystalline
COO	10	2	$P_{10.2.2}$	g 21 N 109 I
COO	10	4	$P_{10.4.4}$	g 17 N 99 I
COO	10	6	$P_{10.6.6}$	g 15 Sc 34 N 96 I
COO	10	8	$P_{10.8.8}$	g 13 Sc 54 N 93 I
COO	10	10	$P_{10.10.10}$	g 10 Sc 62 N 88 I
COO	10	12	$P_{10.12.12}$	g 8 Sc 63 N 81 I
CH_2O	10	0	$P_{10.0.0(CH2O)}$	Non-liquid-crystalline
CH_2O	10	4	$P_{10.4.4(CH2O)}$	Non-liquid-crystalline
OCOO	10	Cholesteryl	$P_{10.chol.chol}$	g 82 N* 134 I

[a] g, glassy; Sc, smectic C; N, nematic; N*, cholesteric; I, isotropic.

ing on the chemical formula, the obtained polymers are either non-liquid-crystalline or liquid-crystalline with a nematic, cholesteric, and/or smectic C phase.

2.4. Preparation of the stationary phases

The synthesis of the low-molecular-mass (LMW) liquid crystal bonded silica ($M_{4.4.4}$) and the preparation of the polymer coated silica as well as the knowledge of repetitive mesogenic unit per gram of silica was checked and calculated from carbon elemental analysis as described previously [18]. The concentration characteristics of each stationary phase are summarized in Table 2.

Despite our efforts to obtain identical mesogenic group concentration for each column, non-negligible differences have sometimes been observed. Thus, we compared columns with respect to the normalized retention factor calculated for a same mesogenic unit concentration per gram of silica since the retention factor is directly proportional to the polymer loading [17,20].

The monomeric C_{18} stationary phase (Bischoff) was purchased from ICS Nationale (Eysines, France).

2.5. Column packing

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

3. Results and discussion

3.1. Characterization of the liquid-crystalline polymer coated silica

Scanning electron micrographs were performed on virgin silica particles on the one hand and on coated silica particles by $P_{10.4.4}$ LCP on the other hand with a polymer loading of 240 mmol/g of mesomorphic unit per gram of silica. Micrographs showed that in a major part the coated silica particles were similar to the virgin ones. Despite the presence of few aggregates, the polymer had to be reduced to a thin layer invisible in SEM whose best resolution was reached for dimension corresponding to the pore size, i.e. about 200 Å.

As a conclusion, the liquid-crystalline polymer entered the pores of the silica gel particle to form a thin thermally stable film strongly adsorbed or stayed at the surface of the particle to generate some aggregates which blocked up the entry to inner pores. It also appeared that the surface covering was not complete. Indeed, for a mesomorphic concen-

Table 2
Characterization of the stationary phases

Stationary phases	Repetitive unit mass (g/mol)	%C Elemental Analysis	[M]/g silica (μmol/g)
Coated $P_{4.4.4}$	622	6.65	181
Coated $P_{4.8.8}$	734	10.51	246
Coated $P_{4.12.12}$	846	10.91	215
Coated $P_{10.0.0}$	562	8.22	243
Coated $P_{10.2.2}$	650	8.58	189
Coated $P_{10.4.4}$	706	9.77	238
Coated $P_{10.6.6}$	762	9.25	202
Coated $P_{10.8.8}$	818	10.99	226
Coated $P_{10.10.10}$	874	11.08	210
Coated $P_{10.12.12}$	930	10.91	191
Coated $P_{10.0.0(CH2O)}$	534	5.50	155
Coated $P_{10.4.4(CH2O)}$	678	7.43	173
Coated $P_{10.chol.chol}$	1179	15.78	241
Bonded $M_{4.4.4}$	619	8.90	244

tration of 240 μmol/g, assuming a polymer density of 1.2 g/cm^3, the film thickness would be of 6 Å. It is largely inferior to the characteristic size of the polymer chain perpendicularly to the mesogens, determined by small-angle neutron scattering [24] (apparent radius of gyration: $R_\perp \approx 20$ Å). Consequently, the polymer covered only a part of the whole surface. This conclusion has been confirmed by nitrogen absorption measurements that show that the half silica surface was coated by the polymer.

It was of major interest to know if this liquid-crystalline film adsorbed on porous silica particles still presented the same properties as for the bulk liquid-crystalline compound. For this purpose, the $P_{10.8.8}$ polymer was chosen because it could be smectic or nematic according to temperature so that two temperature transitions could be seen by DSC measurements. In Fig. 3 is displayed the thermal behavior of $P_{10.8.8}$ (12 mg) and revealed as expected two peaks at about 54°C and 93°C corresponding to the smectic-nematic and nematic-isotropic transition temperatures, respectively. Half the quantity of $P_{10.8.8}$

(6 mg) was introduced into pores of the virgin silica through the coating procedure. The thermal analysis of this coated stationary phase did not display any detectable exothermic peak and its thermogram looked like this of the virgin silica in a proportional way. This DSC result showed that the long distance ordering present in the bulk polymer vanishes. The possible persistence of a collective ordering into silica pores would be limited necessarily at short range imposed by the small pore's size (200 Å).

This short range ordering could be seen indirectly using a PAH probe in RP-HPLC. Chrysene was chosen because of its high anisotropic shape. The temperature-dependent chromatographic measurements were carried out on the $P_{10.8.8}$ column (Fig. 4) by heating from 35°C up to 110°C thus covering the transition temperatures of the polymer. Variations in the capacity factor revealed an indisputable break in the linear dependence and a slightly k jump at about 80°C and 45°C, respectively. Being sure these anomalies were attributable to the structures of the coated SC-LCP, the temperature-dependent measure-
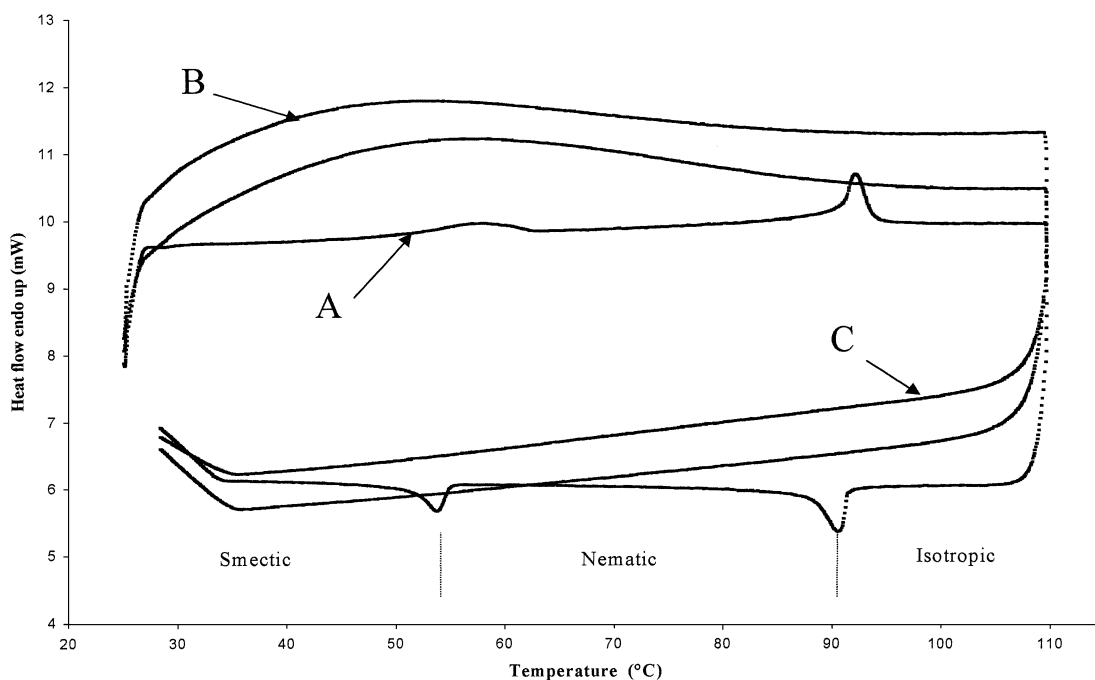

Fig. 3. DSC traces of pure $P_{10.8.8}$ liquid-crystalline polymer (A), the coated silica with half an equivalent quantity of $P_{10.8.8}$ (B) and the virgin silica (C). Heating and cooling at 4°C.min^{-1}.
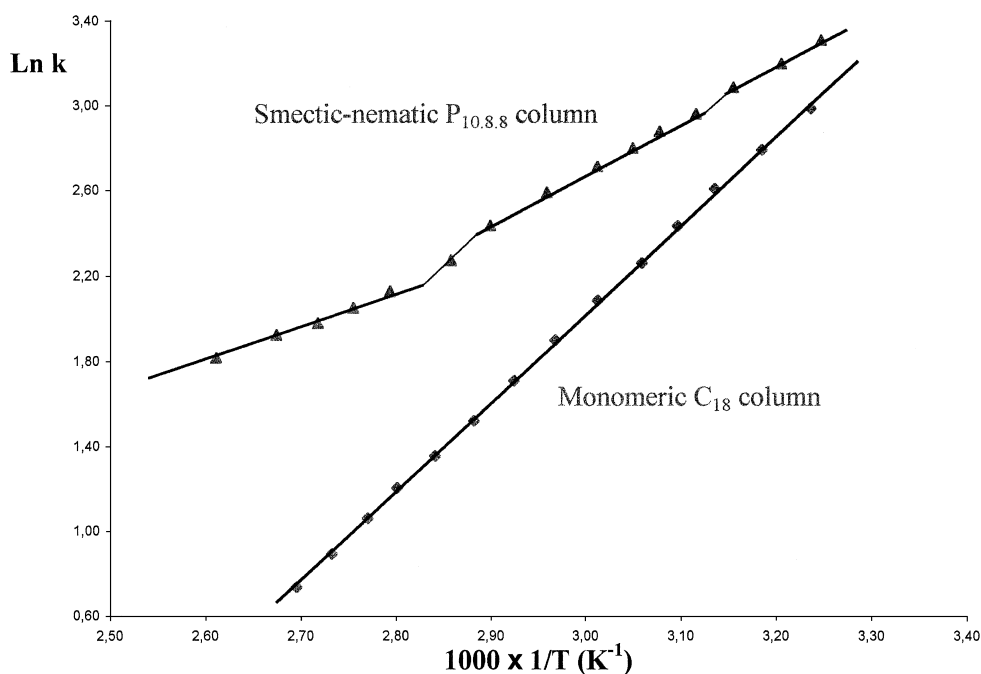
Fig. 4. Temperature-depending measurement on the coated smectic-nematic $P_{10.8.8}$ and a commercial monomeric C_{18} columns. Mobile phase: acetonitrile–water (55:45, v/v), 1 cm^3 min^{-1}. UV detection, $\lambda = 254$ nm. min.

ment was also carried out on a non-liquid-crystalline commercial monomeric C_{18} column. As expected, results showed a linear dependence over the temperature range studied. Thus, these temperatures were in relation with the transition temperatures of the pure liquid-crystalline side-chain polymer. Furthermore, both are about ten degrees lower than in the pure polymer owing to the plasticizing effect of the mobile phase as Klein and Springer mentioned it on their liquid-crystalline side-chain polyacrylates [15,16].

At this step, it was likely that the bulk mesophase of the liquid-crystalline polymer was not preserved when the polymer was coated on the silica surface. Nevertheless, although no macroscopic liquid-crystalline phase seemed to persist on silica, mesogenic units necessarily stay in interaction because they are regularly fixed at a short distance on the polysiloxane backbone: as a consequence, a liquid-crystalline order, at least at short range, still exists in a thin polymer layer. As we can see in the following, this local anisotropic order is sufficient to have specific effects on retention mechanism.

3.2. Influence of a "local anisotropic order" on the chromatographic performances

One can note that two conditions are required to generate a liquid-crystalline order: first the shape and the formula of the molecules must be adapted and second, these molecules must freely interact to develop the appropriate intermolecular interactions. Thus, to demonstrate the influence of the liquid-crystalline order on the chromatographic separations, we choose: firstly to suppress the mesophase by changing the molecular formula of the side groups ($P_{10.0.0}$, $P_{10.0.0(CH2O)}$, $P_{10.4.4(CH2O)}$) and to compare their chromatographic performances to those of a stationary phase based on a nematic polymer ($P_{10.4.4}$), and secondly to unfavour the anisotropic order by increasing the distance between the mesogenic groups using side groups statistically bonded to the silica ($M_{4.4.4}$) to be compared to a polymer coated silica ($P_{4.4.4}$).

The formula of the side groups is changed in order to obtain either a liquid-crystalline polymer ($P_{10.4.4}$) or a non-liquid-crystalline one ($P_{10.0.0}$) by taking the

aliphatic tails away. As previously shown, $P_{10.4.4}$ based stationary phase demonstrates a good shape recognition with respect to the *L/B* ratio for the PAHs containing three, four or five condensed aromatic cycles [20]. Conversely, the $P_{10.0.0}$ column has no shape recognition as shown in Table 3.

Thus, these results showed the higher shape and planarity recognition ability when using a ''liquid-crystalline'' stationary phase. However, we have to assure beforehand that the short terminal aliphatic chains do not significantly improve the shape recognition with non-mesomorphic polymers. Indeed, in RP-HPLC, it is well known that longer chain lengths contribute to an increase of shape recognition particularly for polymeric alkyl phase (C_8–C_{30}) [25].

The two non-liquid-crystalline polymers were compared [$P_{10.0.0(CH2O)}$ and $P_{10.4.4(CH2O)}$]. As for the $P_{10.0.0}$ stationary phase, $P_{10.0.0(CH2O)}$ possesses no shape recognition power towards PAHs (Table 3). Considering the $P_{10.4.4(CH2O)}$, a slight shape recognition was observed for some PAHs. As no ordering structure could exist with $P_{10.4.4(CH2O)}$, this slight improvement in separation ability was attributable to the presence of butyl group. But this shape recognition remained considerably lower than in the case of the nematic $P_{10.4.4}$ stationary phase (Table 3).

By comparison, it was noted that in the case of nematic polymers ($P_{4.4.4}$, $P_{4.8.8}$, $P_{4.12.12}$) the lengthening of the terminal aliphatic tails had no importance in the separation mechanism, and only expanded the

solutes' retention time by increase of the phases' hydrophobicity [20]. It is still in agreement with the opinion that the local anisotropic order generated by the liquid-crystalline polymer was predominant over such chemical changes of the aliphatic moieties in the separation's mechanism.

The comparison between a laterally bonded LMW silica and a coated LCP stationary phase [18] well exhibited the intrinsic role of the collective arrangement of liquid crystal molecules in the separation mechanism. The main difference between those was the average distance between the mesomorphic units. Despite similar mesogenic unit concentration on the silica surface, the chromatographic results clearly revealed longer retention factors and better shape recognition for the $P_{4.4.4}$ coated phase than for the $M_{4.4.4}$ bonded phase. For the polymeric phase $P_{4.4.4}$, both the proximity of the mesomorphic units and the flexibility of the polysiloxane backbone, absent in the bonded $M_{4.4.4}$ phase, favor the mesogen-mesogen interactions and generate a local liquid-crystalline order on the silica.

At this step, the question raised is to know if the nature of the local anisotropic order generated by liquid-crystalline polymers at the silica surface influences the chromatographic performances of the stationary phases. Indeed, as shown in Table 1, the side-on fixed polymers used in this study exhibit a nematic phase (i.e.; an orientational order of the mesogens), a cholesteric phase (an orientational order

Table 3

Selectivities (α) obtained for four stationary phases based on a nematic polymer ($P_{10.4.4}$) and three non-liquid-crystalline polymers [$P_{10.0.0}$, $P_{10.0.0(CH2O)}$ and $P_{10.4.4(CH2O)}$][a]

Stationary phases	$P_{10.4.4}$ Nematic polymer α	$P_{10.0.0}$ Non-liquid crystalline α	$P_{10.0.0(CH2O)}$ Non-liquid crystalline α	$P_{10.4.4(CH2O)}$ Non-liquid crystalline α
Shape recognition				
Anthracene/phenanthrene	1.26	1.00	1.00	1.16
Chrysene/triphenylene	1.64	1.00	1.00	1.20
Chrysene/benz[*a*]anthracene	1.17	1.00	1.00	1.00
Benzo[a]pyrene/perylene	1.39	1.00	1.00	1.14
Planar/non planar				
Triphenylene/*o*-terphenyl	5.34	3.16	4.08	4.83
Perylene/binaphthyl	7.67	3.30	3.37	4.19

[a] Mobile phase: MeOH–water (80:20, v/v), 1 cm^3 min^{-1}. Room temperature.

with an helicoidal geometry) and a smectic phase (i.e. a layered structure) which presents both orientational and translational order.

The interest was indeed to compare a "nematic" ($P_{10.4.4}$) and a "smectic" ($P_{10.8.8}$) based stationary phases in order to see the effect of the mesomorphic nature on chromatographic results.

For this comparison, the normalized retention factor to the mesomorphic unit concentration of 220 μmol/g was used. Firstly, if we do not enter into all the details in the chromatographic results of $P_{10.4.4}$ and $P_{10.8.8}$ columns, no qualitative difference was observed. The elution order of all the PAH molecules remained identical. It is surprising to note that retention times on the $P_{10.8.8}$ column were equivalent or shorter than on the $P_{10.4.4}$ one because stationary phases with a higher content of carbon content are usually more hydrophobic. Moreover, the nearly square shape solutes such as triphenylene or perylene, were more rapidly eluted on $P_{10.8.8}$ column. Thus, the shape discriminations with respect to chrysene/triphenylene and benzo[a]pyrene/perylene were higher on "smectic" coated silica ($\alpha = 1.87$ and 1.59, respectively) than on "nematic" coated silica ($\alpha = 1.64$ and 1.39) in a reduced experimental time. By comparison, the "nematic" stationary phases $P_{4.4.4}$ and $P_{4.8.8}$, with longer aliphatic tails (from 4 to 8 CH_2) expanded the retention times but did not change the selectivities. The mesomorphism's change of the polymer by varying from a pure nematic to a smectic-nematic polymer was more influential on the separation mechanism than the required chemical change by addition of methylene groups.

The selectivity of these planar isomers (benzo[a]pyrene and perylene) clearly increased from non-liquid-crystalline ($1.00 < \alpha < 1.14$) to nematic ($1.27 < \alpha < 1.39$), cholesteric ($\alpha = 1.41$) and smectic ($1.50 < \alpha < 1.73$) polymers based stationary phases.

3.3. Selectivity of the different stationary phases towards SRM 869a test mixture

Using the SRM 869a test applied by Sander and Wise to differentiate monomeric, intermediate and polymeric C_{18} columns [26], the chromatographic responses of the different stationary phases appeared more strongly correlated to the nature of the anisotropic order on the silica surface.

For all stationary phases, the phenanthro-phenanthrene (PhPh) solute, (26 π electrons, non-planar, $L/B = 1.07$) was eluted first because of its large non-planarity and so was less retained on ordered phases. The comparison concerned benzo [a] pyrene (BaP), (20 π electrons, planar, $L/B = 1.49$) and tetrabenzonaphthalene (TBN), (26 π electrons, slightly non-planar, $L/B = 1.09$) where influence of solute geometrical factors as planarity and shape could compete with the classical π–π interactions. Usually, on monomeric or disordered stationary phases the elution order BaP$\leq$PhPh$<$TBN was observed with respect to first the number of π electrons and then to the planarity of solutes. With stationary phases based on nematic $P_{4.4.4}$ and $P_{4.8.8}$ polymers the elution orders PhPh$<$BaP$\cong$TBN and PhPh$<$BaP$<$TBN were noted respectively, indicating a higher sensitivity of these phases to the shape of the solute (L/B). Conversely, the elution orders of based $P_{10.4.4}$ and $P_{10.8.8}$ columns became PhPh$<$BaP$\cong$TBN and PhPh$<$TBN$<$BaP (Fig. 5) thus showing that the use of smectic-nematic polymer significantly altered the chromatographic data compared to the pure nematic one. Consequently, the more ordered the bulk polymer mesophase, the higher the sensitivity of the corresponding coated silica towards the solute' s planarity and shape. Thus

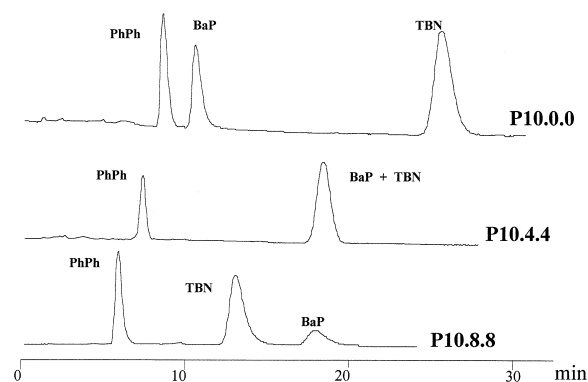
Fig. 5. Recognition of SRM test mixture on coated $P_{10.0.0}$, $P_{10.4.4}$ and $P_{10.8.8}$ polymers. Chromatograms are shown for the same concentration in mesogenic units, i.e., 220 μmol per gram of virgin silica. Mobile phase: MeOH–water (90:10, v/v), 1 cm^3 min^{-1}. UV detection, $\lambda = 254$ nm. Room temperature.

the nature of the local anisotropic order (nematic or smectic) intervened in the separation's mechanism and supplanted the hydrophobicity effect induced by longer aliphatic tails.

The preceding examples have shown that it may be possible to classify stationary phases based upon liquid-crystalline polymers as a function of the bulk polymer's polymorphism. As a check, test SRM 869a was applied to all the stationary phases including non-liquid-crystalline polymers, bonded liquid crystal precursors, and mesomorphic SO-LCPs. The selectivity coefficients $\alpha_{BaP/TBN}$ were represented on Fig. 6 for all the stationary phases. For the four non-liquid-crystalline polymers tested, $\alpha_{BaP/TBN}$ varied between 0.38 and 0.53. The same coefficient varied between 0.80 and 1.02 and between 1.30 and 1.87 for five pure nematic and four smectic-nematic polymers respectively. The cholesteric polymer stationary phase was also been tested with the SRM 869a test mixture and a $\alpha_{BaP/TBN}$ value of 1.08 was obtained. Not surprisingly, this new stationary phase behaved similarly to the family of nematic phases.

So, each class of polymer gave rise to a distinct selectivity domain that allowed for determination with a high degree of confidence, non-liquid-crys-

talline, nematic and smectic-nematic polymers side-on fixed. The more ordered the mesophase of the polymer, the higher $\alpha_{BaP/TBN}$ and, thus, greater sensitivity to a solute's geometry.

Furthermore, it was interesting to see that the bonded liquid-crystalline precursor stationary phase was intermediate between the non-liquid-crystalline and the nematic ones considering the $\alpha_{BaP/TBN}$ value. It suggested that a slightly ordered arrangement was generated.

Finally, the chromatographic results showed that the more stable the nematic phase is (Table 1), the higher the selectivity between BaP and TBN will be (Fig. 6). A more pronounced local anisotropic ordering on the silica surface would occur when the stability of the bulk nematic phase increased. One could indeed assume two antagonist forces: the first describes the tendency of the SO-LCP to organize itself at the silica gel surface and the second is the adsorption forces which randomly fix the polysiloxane chain on the surface. This observation was also noted for the series of smectic-nematic polymers. The larger the stability of the smectic phase over the nematic one, the higher the selectivity $\alpha_{BaP/TBN}$ (Tables 1 and Fig. 6). Thus it becomes clear that the
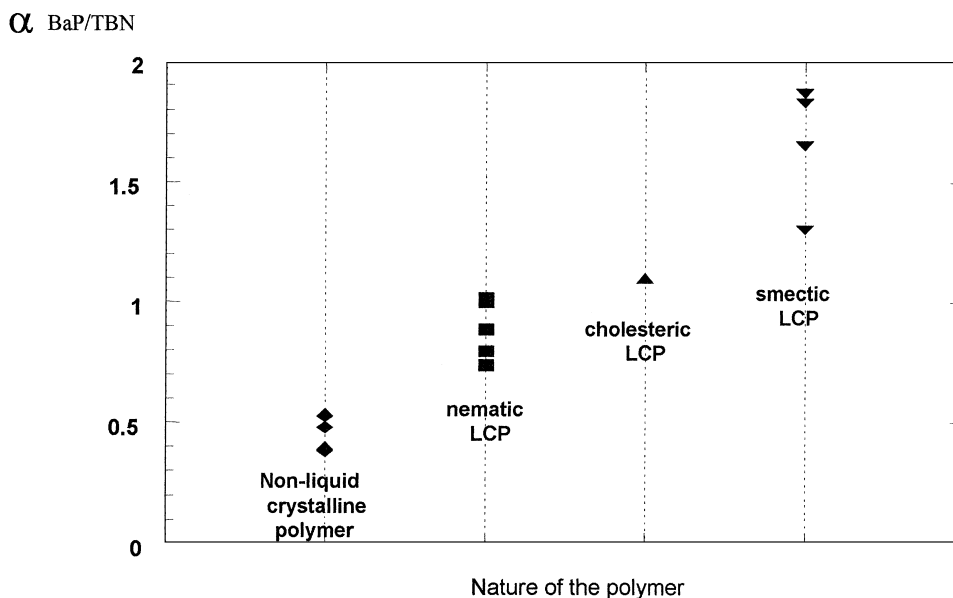

Fig. 6. Selectivity $\alpha_{BaP/TBN}$ obtained for the different stationary phases.

temperature range stability of the bulk mesophase influences the anisotropic order at the silica surface, which indirectly influences the solute separations.

4. Conclusion

As a final result, this study showed that a local liquid-crystalline order still exists on silica surface when side-on fixed mesomorphic polymers were used as coated stationary phases despite the loss of the bulk properties. This anisotropic order appears to be largely responsible for the separation of PAHs with respect to their planarity and length-to-breath ratio. Conversely, the use of the equivalent, bonded liquid-crystalline molecules exhibited lower separation ability because they generated a lower orientation order.

The more ordered the bulk liquid-crystalline mesophase, the more selective the corresponding coated stationary phase towards shape isomers. Thus, it is more beneficial to use smectic side-on liquid-crystalline polymer than nematic and non-liquid-crystalline ones.

Finally, the wider the mesophase range in nematic or smectic liquid crystals, the more pronounced the shape recognition because of the presence of a more ordered film on the silica surface.

Acknowledgements

References

[1] H. Kelker, Z. Anal. Chem. 198 (1963) 254.

[2] D.N. Kirk, P.M. Shaw, J. Chem. Soc., C, (1971) 3979.
[3] G. Chiavari, L. Pastorelli, Chromatographia 7 (1974) 30.
[4] D.E. Martire, P.A. Blasco, P.F. Carone, L.C. Chow, H. Vicini, J. Phys. Chem. 72 (1968) 3489.
[5] S. Popiel, Z. Witkiewicz, Biul. Wojsk. Akad. Tech. 2 (1983) 32.
[6] Z. Witkiewicz, S. Popiel, J. Chromatogr. 154 (1978) 60.
[7] H. Kelker, A. Verhelst, J. Chromatogr. Sci. 7 (1969) 79.
[8] G.M. Janini, M.T. Ubeid, J. Chromatogr. 236 (1982) 329.
[9] W. Marciniak, Z. Witkiewicz, J. Chromatogr. 207 (1981) 333.
[10] W. Marciniak, Z. Witkiewicz, Biul. Wojsk. Akad. Tech. 30 (1981) 123.
[11] J. Serpinet, Wiad. Chem. 30 (1976) 793.
[12] R.L. Martin, Anal. Chem. 33 (1961) 347.
[13] B.G. Belenky, A.I. Rusanov, L.D. Turkova, J. Chromatogr. 80 (1973) 147.
[14] C. Devillez, C. Eon, G. Guiochon, J. Coll. Interface Sci. 49 (1974) 232.
[15] B.H. Klein, J. Springer, J. Liq. Chromatogr. 14 (1991) 1519.
[16] B.H. Klein, J. Springer, J. Liq. Chromatogr. 14 (1991) 1539.
[17] I. Terrien, PhD Thesis, Université Bordeaux 1, No. 1911, 1998
[18] I. Terrien, G. Félix, M.-F. Achard, F. Hardouin, J. Chromatogr. A 810 (1998) 19.
[19] I. Terrien, G. Félix, M. Laguerre, M.-F. Achard, F. Hardouin, Mol. Cryst. Liq. Cryst. 331 (1999) 431.
[20] F. Gritti, G. Félix, M.-F. Achard, F. Hardouin, J. Chromatogr. A 897 (2000) 131.
[21] M. Mauzac, F. Hardouin, H. Richard, M.-F. Achard, G. Sigaud, H. Gasparoux, Eur. Polym. J. 22 (1986) 137.
[22] G. Gray, J.S. Hill, D. Lacey, Angew. Chem. Int. Educ. Engl. Adv. Mater. 28 (1989) 1120.
[23] P. Keller, F. Hardouin, M. Mauzac, M.-F. Achard, Mol. Cryst. Liq. Cryst. 155 (1988) 171.
[24] F. Hardouin, S. Mery, M.-F. Achard, L. Noirez, P. Keller, J. Phys. II France, 511 and erratum J. Phys. II France, 271 (1991).
[25] L.C. Sander, S.A. Wise, J. Chromatogr. A 656 (1993) 335.
[26] L.C. Sander, S.A. Wise, Anal. Chem. 56 (1987) 504.