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Liquid crystalline polymers as stationary phases IV. Chemical bonding and immobilization of the polymer on silica characterization by solid-state nuclear magnetic resonance spectroscopy

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Abstract

Chemical bonding reaction and immobilization through low energy radiation (heating) have been investigated to fix a side-chain liquid crystalline polymer (SC-LCP) on silica particles in order to use the resulting modified silica in normal-phase HPLC. Highly stable chromatographic stationary phases are observed under excellent polymer solvent flow conditions (THF) for both methods and better column efficiencies are also exhibited towards PAHs' separation compared to the classical coated stationary phase. The characterization of these new stationary phases and the rationale for improved column stability have been investigated by solid state ^{13}C and ^{29}Si CP/MAS NMR spectroscopy. It is clearly shown that the chemical bonding is achieved by the classical hydrosilylation reaction between PHMS chains and vinyl modified silica. The bonded polymer is likely a copolymer than a homopolymer. The immobilization of the SC-LCP by heating results in the breaking of Si–O–Si bonds of the polysiloxane chain after the attack of the silica surface silanols. Applications to fullerenes and carotenes separation of these bonded stationary phases are compared to the separation power of a classical monomeric C_{18} stationary phase in NP-HPLC as *n*-hexane–toluene or methyl-tertiobutyl ether–methanol mixtures.

Keywords: Stationary phases, LC; Chemical bonding; Immobilization; Liquid crystalline polymers

1. Introduction

Continuing our work on new stationary phases based upon side-on fixed liquid crystalline polymer in RP-HPLC [1–5], it has been decided to enlarge their applications from reversed-phase to normal-phase conditions. By this way it will be easier to use appropriate mobile phase condition, compatible with the solute solubility to obtain good separations. Up to now the use of our based liquid crystalline polymer stationary phases were only limited to reversed mobile phase conditions (like MeOH–H_2O

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or MeCN–H_2O mixtures). This was due to the fact that the liquid crystalline polymer was simply coated onto the surface of the silica particle. With normal-phase, the coating procedure was doomed to failure due to the bleeding of the column. With the aim of increasing the column lifetime, it was necessary to link the liquid crystalline polymer onto the silica particle (chemical bonding) or to make it insoluble towards normal mobile phase by producing inter-chain bonds directly on the silica surface (immobilization).

The chemical bonding method of low molecular mass liquid crystal was first developed through the organochlorosilane pathway that allows to convert the silanol functions of the silica into the desired chemical function in the presence of a linear carbon spacer [6–10]. A more recent bonding method via the formation of a hydride intermediate on silica (Si–OH→Si–H) and hydrosilylation has also been successful [11,12]. Some authors also prepared bonded liquid crystal stationary phases from amino-propylsilica [13,14].

Herein we proposed a new synthetic route to achieve the linkage of a liquid crystalline polysiloxane to the silica surface [2] on the one hand, and an immobilization procedure of the liquid crystalline polymer directly onto the silica surface on the other hand. Immobilization could be initiated by free radicals whose production requires a low energy (heat) [15], by chemical initiators (azo-compounds [16] peroxides [17] and ozone [18]) or by high energy radiation such as electrons [19] or gamma radiation from Cobalt-60 [20]. The chemical agent route was not chosen because addition of external reagents and undesirable by-products might alter the intrinsic chromatographic behavior of the column. The possibility of using the gamma radiation method was also excluded because of the presence of low energetic ester functions in the mesogenic unit that might give away under γ-radiation treatment. Thus, we studied here the liquid crystal polymer immobilization by initiating free radicals through low radiation energy that simply consisted in heating the classical coated stationary phase.

As it will be seen below, interesting results were observed, and a molecular explanation put forward, on the basis of solid state NMR. Indeed, magic angle spinning (MAS) NMR has been proven to be a suitable technique in the study of surface chemistry of silica gels. After the pioneering work of Maciel and coworkers [21–23], the solid state NMR technique was largely applied for characterization of new stationary phases for HPLC [24–29] and silicone NMR under proton cross-polarization (CP) conditions was shown to be able to discriminate against ^{29}Si far removed from protons. NMR signals of ^{29}Si bound to OH, CH_3, CH_2, etc... could easily be distinguished and have been used herein as indicators of reaction pathways.

The purposes of this paper are thus fourfold: (i) Prove the column stability of both new stationary phases; (ii) Compare their chromatographic ability with the corresponding coated stationary phase in RP-HPLC; (iii) Characterize these new stationary phases by ^{13}C and ^{29}Si solid state NMR; (iv) Apply to mixtures separation in NP-HPLC.

2. Experimental section

2.1. Chemicals

The polyhydrogenomethylsiloxane chain (PHMS 67) was purchased from ABCR (Karlsruhe, Germany). All other chemicals required for the synthesis of the liquid crystal polymer and the PAHs solutes were obtained from Aldrich-Sigma (L'Isle d'Abeau Chesnes, France).

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

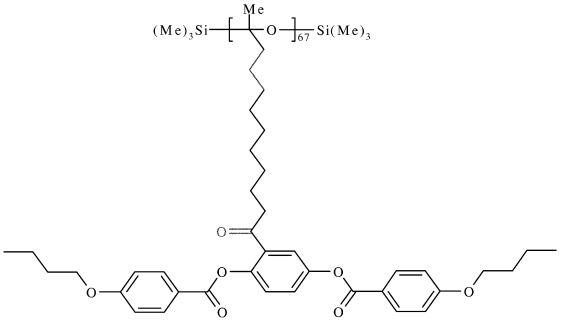
HPLC grade methanol was purchased from ICS.

2.2. Liquid crystalline polymers (SO-LCP)

The LCPs used in this work belong to the family of side chain liquid-crystalline polymers where the mesogenic rod-like units are laterally attached to a flexible polysiloxane backbone via a flexible spacer. The mesogenic groups are of three-phenyl ring benzoate type with terminal alkoxy chains. They are labeled $M_{n.m.m}$ where n and m are the number of carbons of the lateral alkyl ester spacer arm and alkoxy terminal chains respectively. The synthetic

method of the mesogenic compounds $M_{n.m.m}$ has been described previously [1,30].

The polysiloxanes are prepared through a classical hydrosilylation reaction [31,32] between the vinyl groups of the spacer arm of the $M_{n.m.m}$ precursors and the Si-H functions of the polyhydrogenosiloxane chain. The $P_{10.4.4}$ polymer used in this study has the following formula:



2.3. Preparation of the stationary phases

The silica gel was dried at 180°C under 0.01 Torr over 24 h. The concentration of polymer repetitive unit per g of virgin silica was checked and calculated from carbon elemental analysis as described previously [1].

2.3.1. Coated way

Silica was added to a tetrahydrofuran (THF) solution of polymer. A homogeneous suspension was obtained by vigorous stirring and THF was progressively removed under a slight vacuum (water pump) at room temperature. The coated silica was finally dried under 0.01 Torr at room temperature.

2.3.2. Immobilized way

Immobilization was achieved according to the method used by Pirkle [45] consisting in heating at 130°C for 24 h the coated silica under reduced pressure (0.01 Torr). By successive washing with hot THF, the unimmobilized polymer was extracted. About 30% weight of polymer was finally lost after this extensive washing.

2.3.3. Chemical bonding

The first step consisted in modifying partially the silica surface (1% of the overall surfaced Si–OH functions) by an organochlorosilane compound offering a terminal vinylic function. Secondly, the classical hydrosilylation reaction on PHMS 67 occurred with both the mesogenic unit and the functionalized silica (Fig. 1).

2.3.4. Synthesis of the modified silica (A)

68 μl (0.41 mmol) of 7-oct-1-enyldimethylchlorosilane were added to 17 g of silica in dry toluene. The reaction mixture was heated at reflux for 48 h. The grafted silica was then filtered and successively washed with toluene, xylene, dichloromethane, acetone, and then dried at 80°C under reduced pressure for 24 h.

2.3.5. Synthesis of the bonded LCP silica (B)

To a suspension of 4 g functionalized silica in 30 ml dry toluene were added 644 mg (1.0 mmol) of mesogenic unit $M_{10.4.4}$ and 140 μl (2.2 mmol Si–H) of PHMS 67. The reaction mixture was heated to 60°C. 300 μl of a solution of dichloro-dicyclopentadiene-platinum (II) (1 mg/ml) were added as catalyst to the suspension, kept at 60°C under a nitrogen atmosphere for 72 h. The bonded LCP silica was filtered and washed with toluene and THF. The residual platinum complex was removed during the successive washings of the bonded silica.

Concentration in mesogenic unit per g of silica,

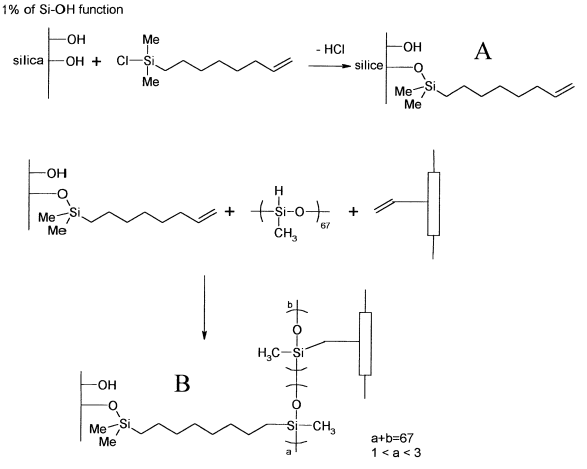


Fig. 1. Schematics for the synthesis of bonded liquid crystal polymer stationary phases: partially modified silica (A) and bonded liquid crystalline polymer (B).

was checked by carbon elemental analysis. This new stationary phase was synthesized five times to check the reproducibility of the method. A quite similar mesogen concentration has been found for the five columns [2]. Only a standard deviation of 0.30 is observed for an average carbon percentage of 8.79%.

2.4. Chromatographic experiments

2.4.1. Column packing

Each 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 bars with methanol as the pressure fluid and a mixture of methanol–cyclohexanol (25:5, v/v) as the suspension medium fluids.

2.4.2. 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, a LG-980-02 Ternary Gradient Unit mobile phase mixer and a DG-980-50 3-line degasser from Jasco. Reverse-phase condition using a mixture of methanol–water (80:20, v/v) was chosen for all chromatographic measurements at a flow-rate of 1 $ml \cdot min^{-1}$. HPLC grade methanol (purchased from ICS) was used to prepare the mobile phase. Water was doubly distilled. Normal-phase conditions were of *n*-hexane–toluene and methyl-tertiobutyl ether–methanol mixture.

2.4.3. Solutes

The stationary phases have been tested on various mixtures of polynuclear aromatic hydrocarbons (PAHs) solutes. Fullerenes and carotens have been used in normal-phase conditions.

2.5. NMR experiments

The ^{29}Si and ^{13}C solid-state NMR spectra were recorded on a Bruker DSX 500 spectrometer. The solid samples were spun in 4 mm diameter ZrO_2 rotors. TMS was used as a reference for the chemical shifts.

High-resolution solid-state ^{29}Si and ^{13}C NMR experiments were conducted at 99.36 and 125.76 MHz Larmor frequencies, respectively. Quadrature detection was accomplished using a radio-frequency coil that was doubly tuned for both ^{1}H and X (^{29}Si or ^{13}C) resonances. Ramped CP-MAS technique with ^{1}H decoupling during the acquisition period was applied. Variable contact time experiments were performed as described by Maciel et al. [21–23] and yielded CP relaxation times (T_{HSi}) of the order of a few ms. Hence, an operational Hartman-Hahn contact time of 5 ms was chosen for most ^{29}Si spectra. Protons $T_{1\rho}$ values were relatively short (hundredth ms) and protons spin-lattice relaxation times afforded pulse recycling delays from 1s to 10s. The number of scans ranged between 1 k and 16 k and the MAS spinning frequencies were fixed at 5 or 10 kHz.

The ^{29}Si liquid-state NMR spectrum of the PHMS chain was obtained on a Bruker DPX 200 operating at 39.74 MHz by making use of the INEPT pulse sequence.

Table 1
Measured carbon elemental analysis (±0.3%) of $P_{10.4.4}$ LCP after the column's rinse with 1500 ml THF for three kinds of stationary phases. Loadings are calculated and expressed in μmol of polymer repetitive unit per g of virgin silica

	Stationary phase coated $P_{10.4.4}$	Stationary phase bonded $P_{10.4.4}$	Stationary phase immobilized $P_{10.4.4}$
%C before THF rinse	9.8	9.1	10.4
Loading before THF rinse (μmol/g)	237	225	256
%C after THF rinse	6.1	8.7	7.8
Loading after THF rinse (μmol/g)	140	207	184

3. Results and discussion

3.1. Proof of stability columns using the immobilized and bonding ways

Before testing the new bonded and immobilized stationary phases, it was necessary to prove that the liquid crystal polymer was irreversibly confined into the 200 Å mean diameter pores. After successive washings of the modified silica powder, carbon elemental analysis made it indisputable that the major part of the LCP remained at the silica surface. However, to assure the complete stability of the column, the latter was rinsed with THF (very good solvent of the LCP) on the HPLC apparatus. This washing is more efficient than the classical polymer extraction because the solvent pressure can be increased into the silica gel pores with a higher controlled THF shear flow. The column bleeding is controlled by passing known PAHs' mixtures in RP-HPLC and by recording the chromatograms before and after the column's rinse as shown in Fig. 2. The elemental carbon analysis and the calculated LCP loadings are listed in Table 1 before and after the column rinse with 1500 ml THF.

A coated based upon the $P_{10.4.4}$ LCP stationary phase was chosen as the reference to assay the column stability: after the rinse with 1500 ml THF, retention times of sixteen PAHs were progressively shortened to seventy percent of their initial value (Fig. 2b).

Conversely to the coated column, the bonded LCP stationary phase exhibits only a very slight retention time decrease (−5%) after the first rinse of 1500 ml THF (Fig. 2d). The two following ones showed no further change thus proving the good column stability and validating the synthesis method of the LCP bonded silica. The column stability is confirmed by the carbon elemental analysis showing a slight decrease in the carbon rate (Table 1).

Equivalent rinses have been carried out for the immobilized LCP stationary phase (Fig. 3). The first generated a column bleeding (retention times variation of −20%) superior to the bonded column but the two last rinses, corresponding to a total of 1500 ml added THF, also showed no changes in PAHs' retention (Fig. 3b). Thus, despite a higher column bleeding, the immobilization method is as efficient as the chemical bonding regarding the stability of the column towards LCPs solvent mobile phase. Table 1 reveals that about 25% of the initial amount of polymer is desorbed from the silica surface.

In both cases, and because the LCP is irreversibly confined into silica gel pores, it will be allowed to

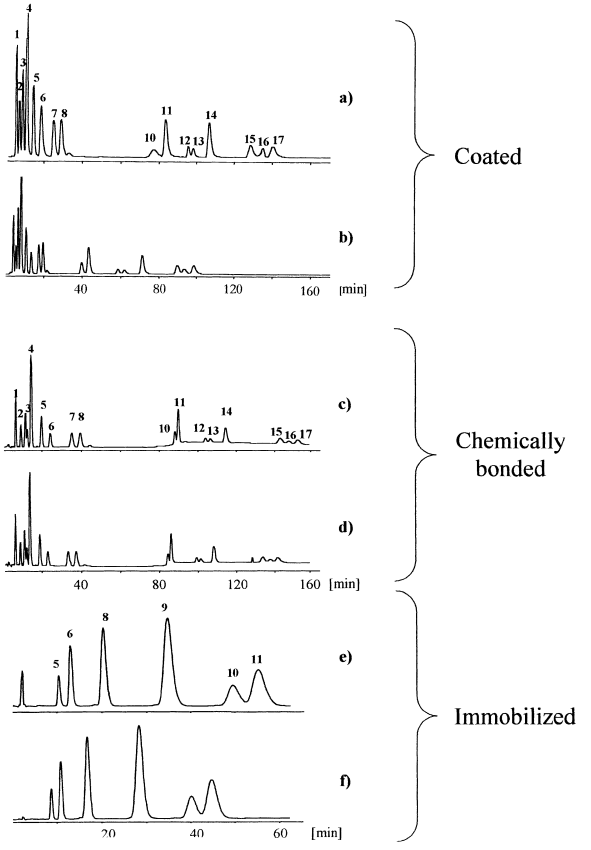


Fig. 2. LCP bleeding for the $P_{10.4.4}$ coated, bonded and immobilized stationary phases observed on a mixture of sixteen PAHs. (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) triphenylene; (10) benz[a]anthracene; (11) chrysene; (12) benz[b]fluoranthene; (13) benz[k]fluoranthene; (14) benzo[a]pyrene; (15) dibenz[a,h]anthracene; (16) indeno[1,2,3-cd]pyrene; (17) benzo[ghi]perylene. (a) coated stationary phase before THF rinsing; (b) coated stationary phase after 1500 ml THF rinsing; (c) bonded stationary phase before THF rinsing; (d) bonded stationary phase after 1500 ml THF rinsing. Mobile phase composition MeOH–H_2O: 0–70 min (65:35, v/v), 80–140 min (80:20, v/v) 1 ml·min^{-1}, T=298 K; (e) immobilized stationary phase before THF rinsing; (f) immobilized stationary phase after 1500 ml THF rinsing. Mobile phase composition: MeOH–H_2O (70:30, v/v) 1 ml·min^{-1}, T=298 K.

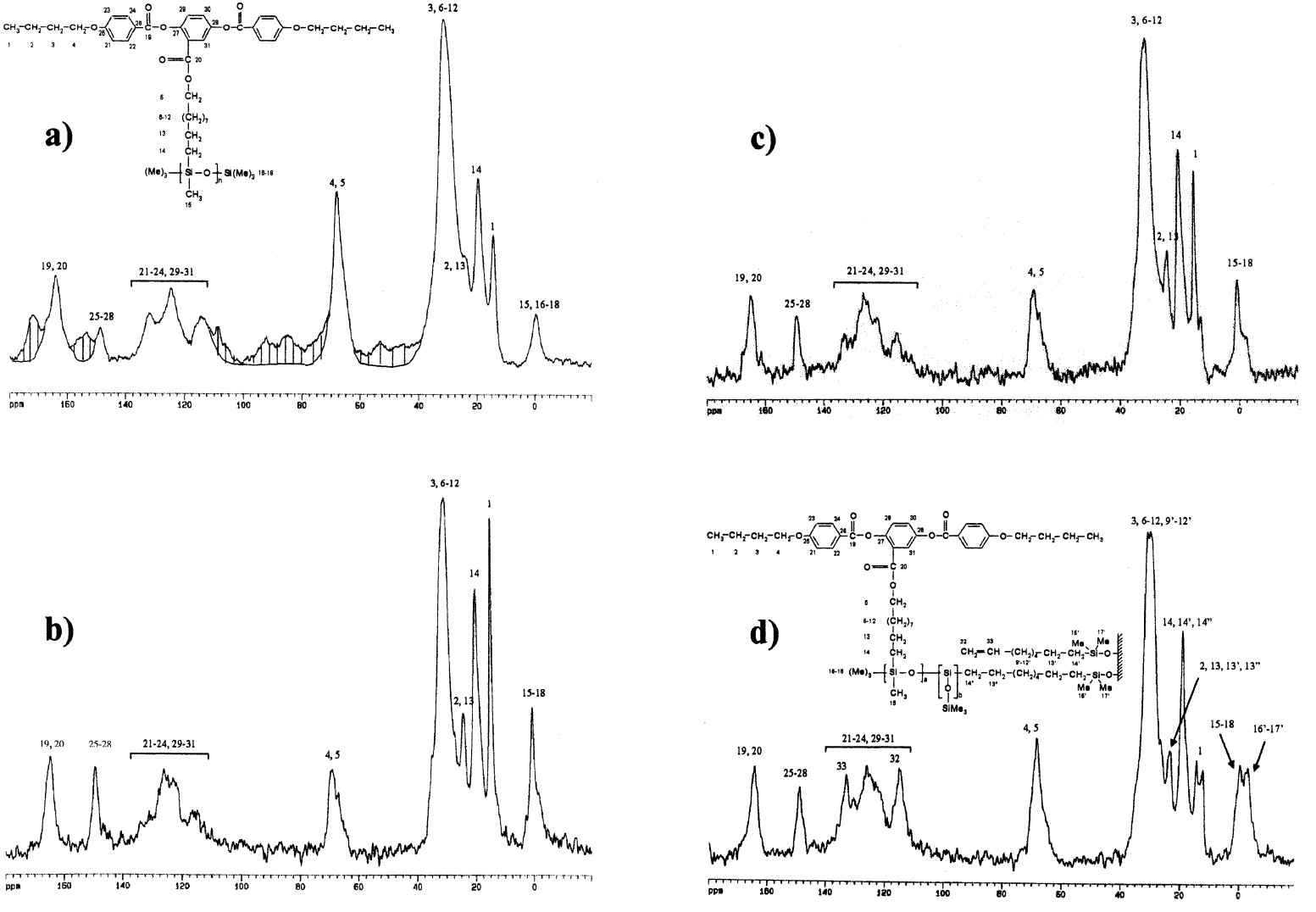


Fig. 3. ^{13}C CP/MAS NMR spectra, with reference to TMS (0 ppm): (a) Thin powder of pure LCP $P_{10.4.4}$ $-T=267$ K. MAS spinning rate=5 kHz. Hatched areas are due to the spinning side bands at low 5 kHz frequency. Spectral width: 50 kHz. Recycle delay: 10 s. Number of scans: 1024. Contact time: 1 ms. Numbers on spectrum represent assignment of groups in the schematized compound structure (insert in the left-high corner); (b) LCP coated silica, same as a) except $T=293$ K and MAS spinning rate of 10 kHz; (c) LCP immobilized silica $-T=293$ K. MAS spinning rate=10 kHz. Spectral width: 50 kHz. Relaxation delay: 3 s. Number of scans: 18432. Contact time: 5 ms. Numbers on spectrum represent assignment of groups in the schematized compound structure (insert in the upper left corner); (d) LCP bonded silica $-T=293$ K. MAS spinning rate=10 kHz. Spectral width: 50 kHz. Relaxation delay: 10 s. Number of scans: 8192. Contact time: 5 ms. Numbers on spectrum represent assignment of groups in the schematized compound structure (insert in the lower right corner).

use these new stationary phases in normal mobile phase conditions for specific separations.

3.2. Chromatographic ability of these new columns

The chromatographic ability of the new columns was compared to the reference LCP coated silica in RP-HPLC.

3.2.1. Coated silica

The column with a loading in mesogenic groups of 237 μmol/g silica was tested using the $P_{10.4.4}$ nematic SOLCP before the rinse process to serve as reference. As mentioned in a previous paper [4], while retention factors continually increased with more heavily loaded phases, selectivities stayed constant. The optimal separation required a minimal amount of interaction sites that was evaluated to 0.7 μmol/m^2 and selectivities remained unchanged beyond.

Concerning the column efficiency, it varied between 478 and 1928 for anthracene and benzo[a]pyrene respectively and as shown previously [4] it decreased at high-level loading.

3.2.2. Immobilized silica

Firstly, it was important to note that the immobilization method did not alter the shape recognition of the liquid-crystalline stationary phase on PAHs solutes. As chromatographic results, it appeared that selectivity did not reach a limiting value when the phase loading increased from 184 to 496 μmol/g contrary to the trend observed for the coated stationary phases [4]. As an example, shape discrimination with respect to anthracene/phenanthrene and benzo[a]pyrene/perylene continuously increased from 1.20 to 1.32 and 1.34 to 1.40, respectively. The immobilization process would thus result in a more efficient covering of the silica gel surface than the simply coated process. Thus, it could be of advantage to heavily load the silica gel when the latter has been subjected to a thermal treatment to obtain better separations. Furthermore, the shape selectivities obtained were similar to those measured on the coated phases and could be improved with higher immobilized polymer loading despite longer retention times.

Another opposite behavior to the coated columns was the efficiency's increase with higher immobilized polymer loading. For example, it jumped from 578 to 1501, 1633 to 2754 and 1650 to 3274 for anthracene, chrysene and benzo[a]pyrene respectively when the polymer loading varied from 184 to 496 μmol/g. This was in agreement with the fact that the silica gel surface coverage by the immobilized polymer was not yet complete at the level coating of 496 μmol/g: otherwise the resistance to mass transfer would have increased because of a higher polymer film thickness. The thermal treatment might thus improve the dispersal of the polymer into silica.

3.2.3. Bonding silica

The bonded LCP stationary phase, whose polymer loading is 207 μmol/g, presented selectivities inferior to those obtained with the coated or immobilized stationary phases. For example, shape discriminations with respect to phenanthrene/anthracene, chrysene/benzo[a]anthracene and benzo[a]pyrene/perylene were higher on the equivalent $P_{10.4.4}$ immobilized or coated silica ($\alpha = 1.26$, 1.15 and 1.37, respectively) than on the $P_{10.4.4}$ bonded silica ($\alpha =$ 1.13, 1.06 and 1.20, respectively) for similar polymer loading. In this sense, we could suggest that the bonding method (through the resulting existence of a liquid crystalline copolysiloxane induced by the Si–H function excess of the PHMS chains (about 2 equivalents)) slightly modified the mesogenic arrangement of the corresponding homopolymer leading to a ''less ordered'' stationary phase and to a weaker selectivity. Indeed, it was shown that coated liquid crystal copolysiloxanes resulted in lower selectivity compared to the relevant homopolysiloxane [4].

Despite the decrease in selectivity, the resolution remained quite equivalent to coated or immobilized silica because of a very interesting column efficiency. Indeed, on comparing columns of quite similar polymer loading (207, 184 and 237 μmol/g for the bonded, immobilized and coated silica respectively), the efficiency with respect to anthracene, chrysene and benzo[a]pyrene was higher on the bonded silica ($N = 989$, 2075 and 3198) than on the immobilized ($N = 578$, 1633 and 1650) and coated silica ($N = 478$, 1829 and 1928). This could be explained by a lower resistance to the mass transfer occurring with the bonded liquid crystalline polymer

as we already observed it on stationary phases coated by liquid crystal copolysiloxanes.

To summarize, as compared to selectivities obtained on coated and immobilized stationary phases, the bonded LCP phase seemed to possess a lower shape discrimination ability but a higher column efficiency resulting in equivalent resolution.

3.3. Characterization of the $P_{10.4.4}$ coated, immobilized and bonded silica by ^{29}Si and ^{13}C CP/MAS solid state NMR

3.3.1. ^{13}C CP/MAS solid state NMR results

Four CP/MAS solid state NMR experiments have been carried out on the following compounds:

3.3.2. $P_{10.4.4}$ pure liquid crystal polymer

The acquisition of the ^{13}C spectrum of the pure polymer (Fig. 3a) required a specific treatment: it was indeed necessary to obtain highly-dispersed solid polymer particles for good packing into the zirconia rotor. Temperature had to be fixed under the glassy-nematic transition temperature of the polymer (17°C) which was thus cooled at −6°C and crushed in thin powder. Even in these conditions the rotor could not spin faster than 5 kHz at −6°C. This low experiment temperature gives rise to slightly broadened peaks and to a set of small peaks (hatched areas) due to spinning side bands. Furthermore, the spectrum could be recorded at one contact time only, namely 10 ms, so the intensities of the peaks are not indicative of the relative abundance of the different carbon nuclei. Despite these experimental difficulties, the general positions on the molecule could be identified. The signal at 0 ppm corresponds to the methyl groups (15) attached to silicon atoms including the end-groups of the polysiloxane chain $Me_3Si(O–)$ (16–18) and the repetitive unit $MeSiO_2(CH_2–)$ (15). Methyl groups (1) of the C4 aliphatic chain on the mesogen unit appear at 14 ppm and the corresponding Me(CH2–) methylene group (2) is detected at 24 ppm. Concerning the C_{10} spacer arm, the methylene groups in α (14) and β (15) position of the silicon atom give lines at 20 and 24 ppm, respectively. All other methylene groups contribute to the most intense peak centered at 31 ppm. The medium-intense peak at 69 ppm is attributed to the three $CH_2(O–)$ groups (4,5) present in the mesogenic unit. The protonated aromatic carbons (21–24, 29–31) give signals between 110 and 140 ppm whereas unprotonated aromatics (25–28) generate a single peak at 149 ppm. Finally, the carbonyl position (19,20) is identified at 164 ppm, so every carbon atom present in the polymer has been assigned. It will allow us to check whether these positions remain after the coating, immobilizing or bonding processes of the polymer onto the silica gel surface. Table 2 summarizes the data.

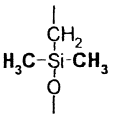
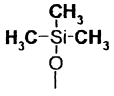
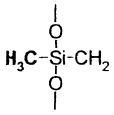
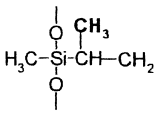
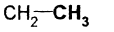
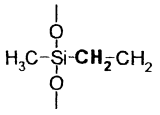
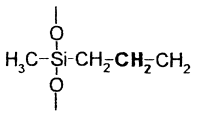
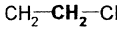
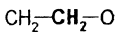
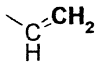
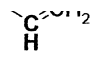
3.3.3. $P_{10.4.4}$ coated and immobilized silica

As the silica powder is composed of very small solid particles (mean-size diameter of 5 μm) it was possible to pack very well the rotor and to regulate the spinning rate up to 10 kHz. Experiments were carried out at room temperature. Sharper peaks were thus obtained and no spinning side bands occurred in ^{13}C spectra (Fig. 3b and 3c). The recognition of every carbon position above-mentioned is obvious showing that the carbon skeleton is well conserved during both, the coating or the immobilizing processes. The most important observation from both spectra is the high resemblance between them: it suggests that the immobilization of the LCP chain is not related to a chemical reaction involving one carbon atom environment. In other words, the mesogenic unit is not altered after immobilization. This is in nice agreement with the finding that the corresponding stationary phase gives similar chromatographic results than the coated phase.

3.3.4. $P_{10.4.4}$ bonded silica

Fig. 3 also shows the ^{13}C spectrum at room temperature of the $P_{10.4.4}$ bonded silica. As for the coated and immobilized phases, all the carbon positions of the polymer repetitive unit are still present on the spectrum but new resonances are detected. The differences observed are relevant of the bonding chemical procedure: first, the sudden emergence of two peaks at 115 and 133 ppm corresponds to the terminal vinylic groups of the silica's modifier which have not reacted with the Si–H functions of the polyhydromethylsiloxane chains. Despite the large excess of Si–H over –CH=CH2 surface modified functions, the presence of unreacted vinylic functions is still not surprising because as a polysiloxane chain becomes attached to silica, it limits the collision's

Table 2
^{13}C chemical shifts observed for the LCP chemical groups. In bold are carbons measured. Accuracy is of ±1 ppm

Species	δ ^{13}C (ppm from TMS)	
	Pure, coated and immobilized LCP	Bonded LCP
	–	−3
	−3	−3
	0	0
	12	12
	14	14
	20	20
	24	24
	24	24
Aliphatic $\mathbf{CH_2}$	25–37	25–37
	69	69
Protonated aromatics	110–140	110–140
	–	113
	–	133
Unprotonated aromatics	149	149
Carbonyl	164	164

probability between the two reactive groups. These resonances must not be mistaken with the vinylic groups of the liquid crystal monomers initially introduced because the successive washings at last removed them. Secondly, the methyl groups $(CH_3)_2Si(CH_2–)(O–)$ of the chemical modifier give rise to a signal at about −3 ppm sufficiently distinct from the methyl groups of the polysiloxane chain (0 ppm). Otherwise, the ^{13}C spectrum confirms the success of the bonding method, i.e. the bonding of both the PHMS chains (presence of methyl groups at 0 ppm) and the mesogenic unit (presence of all carbon chemical shifts of the mesogen molecule).

3.3.5. ^{29}Si CP/MAS solid state NMR results

Silicone NMR experiments have been carried out mainly to give insight on how the LCP is fixed onto silica after the immobilization procedure. All ^{29}Si chemical shifts observed on our samples are listed in Table 3.

3.3.6. PHMS and pure $P_{10.4.4}$ LCP

First, the ^{29}Si spectrum of the PHMS chain in $CDCl_3$ was carried out in order to check the presence of only $(CH_3)SiH(O–)_2$ and $(CH_3)_3Si(O–)$ groups at −35 ppm (D^H unit) and 10 ppm (M unit), respectively. The former gives rise to a highly positive peak and the later to a slightly negative peak (spectrum not shown). This proves that the chains are linear without any crosslinking between that would have generated a signal around −65 ppm (called T^3 signal). Then, the pure LCP was characterized (Fig. 4a): after the hydrosilylation reaction, the only substitution of $(CH_3)SiH(O–)_2$ by $(CH_3)Si(CH_2)(O–)_2$ was expected. This was indeed observed with emergence of the most intense peak at −20 ppm, called D, and the vanishing of the line at −35 ppm. In addition to the expected low-intensity peak at 10 ppm attributed to the silicium of the

Table 3
^{29}Si chemical shifts observed on the LCP modified silicas. M, D, D^H, D^{OH}, T^3, Q^2, Q^3 and Q^4 are the usual notations for the encountered ^{29}Si chemical shifts. Accuracy is of ±1 ppm

Species	 M	 D	 D^H	 D^{OH}	 T^3	 Q^2	 Q^3	Q^4
δ ^{29}Si (ppm from TMS)	9	−20	−35	−55	−64	−89	−98	−109

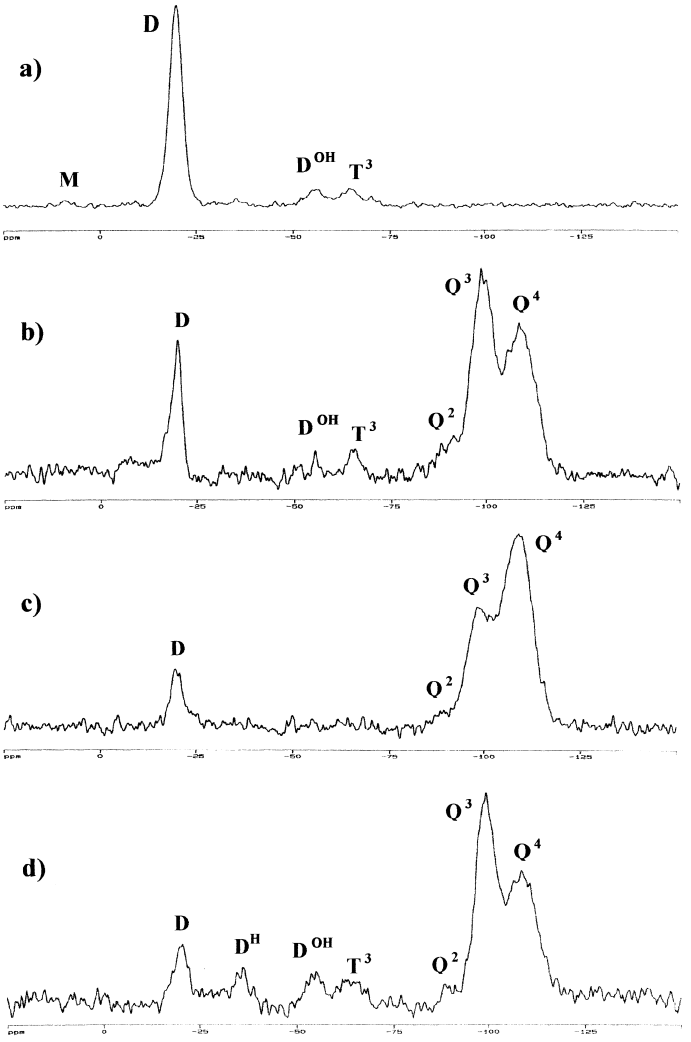


Fig. 4. ^{29}Si CP/MAS NMR spectra, with reference to TMS (0 ppm): (a) Thin powder of pure LCP $P_{10.4.4}$ $-T=267$ K. MAS spinning rate: 5 kHz. Spectral width: 50 kHz; recycle delay: 10 s. Number of scans: 4096. Contact time: 5 ms. Assignment's symbols on the spectrum are the usual notations for the encountered ^{29}Si chemical environments described in Table 3; (b) LCP coated silica, same as a) except $T=293$ K and MAS spinning rate = 10 kHz; (c) LCP immobilized silica, same as b) except relaxation delay of 30s; (d) LCP bonded silica, same as b) except number of scans = 8192.

terminal chain, two low-intensity peaks are observed in the so-called *T* region that are necessarily silicon atoms linked to three oxygen. Their chemical shifts are -55 and -64 ppm and can be attributed to $(CH_3)Si(OH)(O–)_2$ and $(CH_3)Si(O–)_3$ also labeled D^{OH} and T^3, respectively. These unexpected groups may be due to the use of a non-rigorously dry solvent required for the hydrosilylation reaction: the presence of water traces could transform some Si–H into Si–OH functions that might induce chain ramification.

3.3.7. Virgin silica

The ^{29}Si spectrum (not shown) of the silica reveals the presence of the well-known Q^2, Q^3 and Q^4 species that correspond to the $(SiO–)_2Si(OH)_2$, $(SiO–)_3Si(OH)$ and $(SiO–)_4Si$ units giving rise to peaks at -89, -99 and -109 ppm, respectively, as

already reported by Maciel and Sindorf [21–23]. The Q^2 peak is greater than Q^4 and Q^3 whose areas are similar.

3.3.8. $P_{10.4.4}$ coated silica

As it could be expected, the ^{29}Si spectrum of the LCP coated silica (Fig. 4b) is simply the addition of both the pure LCP and the virgin silica spectra. Indeed, except for the M unit lost in the baseline noise, every previous peak, that is, D, D^{OH}, T^3, Q^2, Q^3 and Q^4 are still present.

3.3.9. $P_{10.4.4}$ immobilized silica

Concerning the LCP immobilized silica (Fig. 4c), the relative intensity of Q^3 and Q^4 units has been inversed. $(SiO–)_3Si(OH)$ species have been substituted by $(SiO–)_4Si$ during the immobilization: the immobilized polymer chains have thus necessarily reacted with the surface silanol groups which have been transformed into Q^4 groups. This statement is reinforced by the fact that no other specific silicon environment emerged and by the concomitant D intensity decrease (as compared to the silica signal) due to the 25% loss of LCP after immobilization. Thus, the immobilization treatment (heating at 120°C under reduced pressure) confirmed the Si–OH attack of the silica on Si–O bonds of the polysiloxane chains as already proposed by Schomburg et al. [33] (Fig. 5). The polysiloxane chain is broken into two parts: one is bound to the silica surface and the other becomes a silanol-terminated polysiloxane chain which then condensed to another silanol group and became also attached to the silica after water elimination favored by the applied vacuum. This phenomenon has been already proposed as silanol-terminated polysiloxanes that were used to deactivate silica surfaces [34]. This elementary step could recur on a previous bonded chain so that it is probable to generate double-bonded polymer chains. The proposed evolution of the silicon units for the two steps is thus as follows:

$$2D + 2Q^3 \xrightarrow{\text{Step 1}} M^{OH} + D + Q^3 + Q^4 \xrightarrow{\text{Step 2}} 2D + 2Q^4$$

The balance of the immobilization procedure thus results in the transformation of two Q^3 units into two Q^4 units with elimination of one water molecule. The probable successive bonding reactions might result in a very efficient surface covering, which is, certainly better that the simple polymer coating. Consequently, this way of bonding accounts for the unlimited selectivity value at high-level loading.

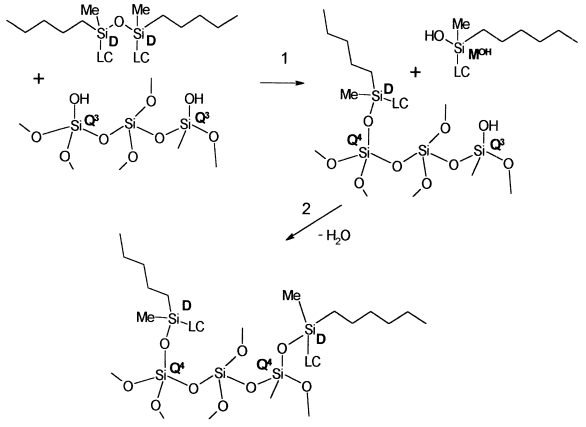


Fig. 5. Mechanism proposed for the polymer immobilization. (1) Break of the polysiloxane chain. (2) Condensation with water elimination. The chemical symbol LC represents the liquid crystalline side chain moiety.

3.3.10. $P_{10.4.4}$ bonded silica

The ^{29}Si spectrum of the $P_{10.4.4}$ bonded silica is shown in Fig. 4d. As only about one percent of the overall silanol groups of the silica have been modified, no drastic change in Q^3 and Q^4 intensity is observed in comparison to the virgin silica spectrum. Furthermore, the intensity of the D chemical shifts is comparable to these of D^H, D^{OH} and T chemical shifts confirming that the chemical bonding did not lead to the formation of the homopolymer but rather to a copolymer. The presence of remaining Si–H groups (D^H at -35 ppm) is not surprising because an excess (with respect to vinylic groups) of 1 Si–H equivalent was used for the hydrosilylation reaction. Highly reactive Si–H function is not allowed to rearrange to form inter-chains bonds because once attached to silica, polysiloxane chains lost their mobility to encounter another reactive function. As for the synthesis of the LCP out of silica, the in-situ reaction, which required the same dried solvent (toluene), also generated two peaks at -55 and -64 ppm corresponding to the previously seen D^{OH} and T^3 units respectively.

As a result, the bonding procedure leads to a

bonded liquid crystalline polysiloxane that can account for the better column efficiency as discussed in Section 2.3.

3.4. Application of the bonded liquid crystal stationary phases [2]

Bonded liquid crystalline phases could be useful for the separation of apolar solutes which require specific elution conditions. Herein, we tested the separation of a mixture of fullerenes (C_{60}, C_{70}, C_{76}, ...) already investigated by Pesek et al. [35] on low molecular mass liquid crystal and by Jinno et al. [36], Félix et al. [37] or Pirkle et al. [38] on various stationary phases. A mixture of carotenes previously studied on conventional C_{18} phases [39–44] was also tested. Both mixtures were subjected to chromatography with a *n*-hexane–toluene or a MeOH/MTBE mobile phase. These mobile phases justify the use of a bonded stationary phase because they are likely to dissolve the LCP used in this study. The resulting chromatograms compared to a monomeric C_{18} phase are shown in Figs. 6 and 7. Considering the fullerenes' separation, the heavier the solute is the longer its retention on the bonded LCP phase. It is not only a question of hydrophobic interaction because the apolar character of the mobile phase largely attenuates its intensity as the chromatograms obtained with the C_{18} column showed it with a weak retention without separation. It is more likely due to the differences in polarisability and shape between the C_N molecules. Indeed, the bigger *N* is the higher

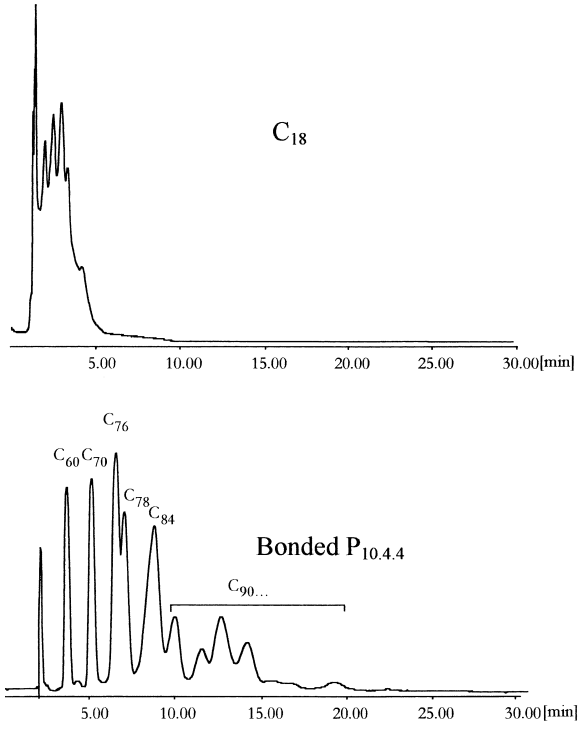


Fig. 6. Separation of a fullerene mixture on a monomeric C_{18} silica and on the bonded $P_{10.4.4}$ silica stationary phases. Mobile phase composition *n*-hexane–toluene (90:10, v/v); elution rate of 1 ml·min^{-1} at 25°C; detection at $\lambda = 254$ nm.

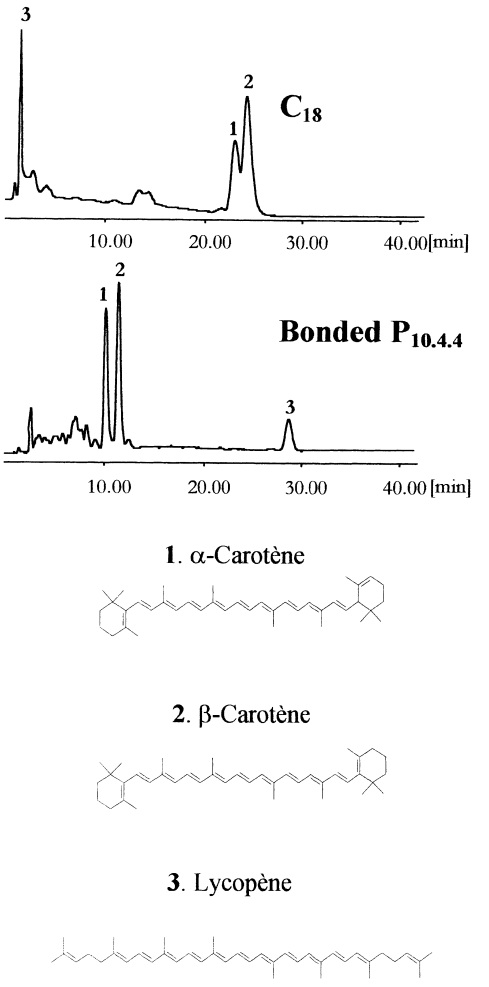


Fig. 7. Separation of a carotenoid mixture on a monomeric C_{18} silica and on the bonded $P_{10.4.4}$ silica stationary phases. Mobile phase composition MeOH–MTBE (98:2 to 90:10, v/v) during 20 min; elution rate of 1 ml·min^{-1} at 25°C; detection at $\lambda = 450$ nm.

the polarisability (more voluminous) and the more elongated shape the solute has. As the LCP is a rich π-electron phase and sensitive to the shape (length-to-breadth ratio) of the solute, the separation obtained on the bonded LCP phase is much larger than that of the C_{18} phase.

We also tested the separation of carotenes and observed that the separation power is slightly better on the LCP than on the C_{18} phase. First, the separation of α and β-carotenes is similar on both phases probably due to a planar-non planar effect. The β-caroten is indeed more planar than the α isomer (α hybridized sp^2 carbon for the β isomer instead of a sp^3 hybridization for the α isomer), which leads to a longer retention time. Secondly, as for the fullerenes, the elution order of lycopene on the LCP phase (α<β<L) could be explained by the higher engaged π-electron number (26 against 22) and its particularly elongated shape. On the C_{18} phase, the lycopenes solute is rapidly eluted (L<α<β) because of the monomeric character of the stationary phase. On a polymeric one, the order (α<β<L) would also be observed [44] as well as for the bonded LCP phase.

4. Conclusion

The chemical bonding and the immobilization of a liquid crystalline polymer on silica gel were achieved and led to stationary phases with very long lifetime in NP-HPLC. They globally result in similar chromatographic performances regarding resolution but differ in terms of selectivity and efficiency. The former generates higher numbers of theoretical plates and the latter afforded better selectivities. Solid state CP/MAS NMR, used as a technique in the study of surface chemistry of silica gel, allowed characterizing these new stationary phases. The ^{13}C solid state CP/MAS NMR revealed in both cases that the liquid crystalline moiety was kept unchanged compared to the pure liquid crystalline polymer. The ^{29}Si solid state CP/MAS NMR confirmed that the in situ chemical bonding reaction generated a bonded liquid crystalline copolymer. It also underlined that the immobilization procedure by heating resulted in the deactivation of the silanol groups at the silica gel surface. These latter attacked the polysiloxane chain and fixed it irreversibly on the surface.

As a consequence, these newly developed columns offered solutions to solve separations in NP-HPLC by using the good shape recognition ability of liquid-crystalline stationary phases.

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