Synthesis, Characterization and Enhanced Selectivity in RP-HPLC of Polar Carbonyl Group Embedded Poly (Vinyl Octadecanoate) Grafted Stationary Phase by Simple Heterogeneous "Graft from" Technique

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A new high performance liquid chromatograpgy (HPLC) stationary phase that possesses an internal carbonyl functional group is synthesized by heterogeneous "graft from" method. This new stationary phase, poly (vinyl octadecanoate) grafted silica (**Sil-2**) is then characterized by different physico-chemical methods such as diffuse reflectance infrared fourier transform, suspension state ^{1}H NMR, solid state ^{13}C CP/MAS NMR, ^{29}Si CP/MAS NMR, elemental analysis and thermogravimetric analysis. Chromatographic properties of **Sil-2** were evaluated under reversed phase condition by separating polycyclic aromatic hydrocarbons (PAHs) and comparing the chromatographic results with those on polymeric as well as monomeric octadecylated silica stationary phases.

Key Words: "Graft from" technique, High performance liquid chromatography, Enhanced selectivity, Polycyclic aromatic hydrocarbons (PAHs)

Introduction

The excellent physical properties of porous silica with low levels of metallic impurities and its availability in a large range of pore diameters and specific surface areas have allowed silica to become the most widely used support for reversed phase liquid chromatography. Thus chemically modified porous silica with organic interactive layers packing materials are the foundation of modern high performance liquid chromatography and a popular approach for achieving novel solute selectivity.[1-5] A recent trend in RPLC has been the introduction of stationary phases containing a polar functional group in addition to the nonpolar RP ligand.[6-11] In general, two types of RP stationary phases have been developed: polar endcapped phases, in which a polar group is used as an endcapping reagent after derivatization with a nonpolar ligand, and polar embedded group phases, in which a polar functional group is located within the alkyl chain itself. Amide,[12-14] carbamate[15,16] and urea[17,18] are three of the embedded polar groups described in the literature that have been thoroughly investigated under reversed phase conditions. Recently, Horak *et al*. have attempted to interpret the retention characteristics of hybrid reversed phases with diverse interaction sites such as the chromatographic separations of reversed phase containing sulfide-group.[19,20]

Work on the immobilization of organic molecules on inorganic supports like silica has been intensively revived in the last few decades,[21,22] with much attention to the establishment of a new covalent bond on the desired surface.[23,24] In principle, the formation of polymer-grafted inorganic particles can be approached in two ways. 1) The "grafting to" technique[25] consists of the synthesis of end-functionalized polymers followed by the immobilization of these polymers onto the surface through anchoring groups. The "grafting to" method is experimentally

simple but it has a limitation, namely the difficulty in achieving high grafting density because of the steric crowding of the surface by the already grafted polymers and (2) on the contrary, in the "graft from" technique, polymer chains grow in situ from initiator molecules that have been pregrafted to the surface of inorganic particles.[26,27] The "grafting from" approach is considered to give higher densities because only monomer molecules have to diffuse to the active species. Among the "graft from" techniques, the ordinary one is nominated as the heterogeneous method,[29] which consists in reacting activated silica with a precursor silylating agent containing trialkoxysilyl groups. Subsequent reactions can expand the organic covalent chain with a variety of organic functions able to act with increased activity as a chelating agent, which can be used for a variety of purposes.[30,31]

The aim of the present study is to report the immobilization of poly (vinyl octadecanoate) which contains an embedded functional carbonyl group in the polymer structure on the silica surface by simple heterogeneous "graft from" method. This immobilized phase is then properly characterized by spectroscopic methods as well as thermal technique and the chromatographic behavior of the new phase was evaluated by using different size and shaped PAHs as elute. This new phase is further compared with commercially available polymeric octadecylated silica (ODS-p) and monomeric octadecylated silica (ODS-m) which do not have any polar functional group present in the ligand. We hope this new phase will find promising applications in HPLC.

Experimental

Vinyl octadecanoate was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and used after purification and remov-

ing polymer inhibitor. Porous silica particles (YMC-GEL) were purchased from YMC Co. Ltd. (Kyoto, Japan) whose average diameter, pore size and surface area are 5 μm, 120 Å and 300 m^2 g-1, respectively. All silica particles were heated for 12 h at 400 °C before use and then allowed to cool to room temperature under nitrogen. 3-Mercaptopropyltrimethoxysilane (MPS), used as a silylating agent, was purchased from Azmax Co. Azobisisobutyronitrile (AIBN) was purified by recrystallization from methanol and was used as an initiator. Toluene was purchased from Nacalai Tesque (Kyoto, Japan) and used after distillation. All other reagents used were of analytical grade. The polymeric octadecylsilylated silica (ODS-p) column (Shodex C$_{18}$ P, particle size 5 μm, pore size 12 nm, surface area 300 m^2 g-1 with end cap of the unreacted silanol group) containing 17.5% C was obtained from Shodex (Tokyo, Japan). Monomeric ODS (ODS-m) (Inertsil ODS-3, 250 mm × 4.6 mm I.D.) were purchased from GL-Science (Tokyo, Japan).

IR measurements were conducted on a Jasco (Japan)FTIR-4 100 Plus instrument with the accessory DR PRO410-M for DRIFT measurement. TGA was performed on a Seiko EXSTAR 6000 TG/DTA 6300. Elemental analyses were carried out on a Perkin-Elmer CHNSO 2400 apparatus (USA). Solid (^{13}C CP/MAS and ^{29}Si CP/MAS) NMR spectra were measured by a Varian Unity Inova AS400 instrument at a static magnetic field of 9.4 T by using a solid probe for CP/MAS NMR spectroscopy at a spin rate of 4000 - 4500 Hz. ^{29}Si CP/MAS NMR spectra were collected with the same instrument. The chromatographic system consisted of a Jasco PU-1580 intelligent HPLC pump, a rheodyne sample injector with a 20 μL loop, and a Jasco multiwavelength UV detector MD 2010 plus. The column temperature was maintained by using a column jacket that had a circulator with a heating and a cooling system. A personal computer connected to the detector with Jasco-Borwin (Ver 1.5) software was used for system control and data analysis. Separations were performed with HPLC grade methanol/water (80:20) as mobile phase at a flow rate of 1.00 mL/min. Measurement of the retention factor (k) was carried out under isocratic elution conditions. The separation factor (α) is the ratio of the retention factor of two solutes that are being analyzed. The retention time of D$_2$O was used as the void volume (t_0) marker (the absorption of D$_2$O was measured at 400 nm, which is actually considered as injection shock).

To produce Sil-1 (Scheme 1), 4.0 g of properly dried porous
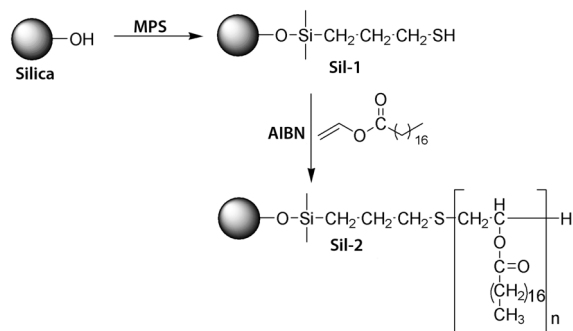
Scheme 1. Schematic illustrations of the preparation and immobilization of Sil-1 and **Sil-2**

silica was suspended in 40 mL of dry toluene. 1.2 mL (6.42 mmol) of MPS was added drop wise to the suspension and the mixture was mechanically stirred under solvent reflux for 72 h. After filtering, the solid was washed with toluene, chloroform, methanol and acetone consecutively. After 24 h vacuum drying the amount of carbon and hydrogen were determined to be 2.80 and 1.15% respectively by elemental analysis. Successive washing with chloroform as a good solvent showed no significant change in elemental analysis result.

Then Sil-1 was suspended in 40 mL of ethanol With vinyl octadecanoate and AIBN were added to the suspension and it was stirred for 20 minutes under an atmosphere of N$_2$ gas. The temperature was gradually raised to 60 °C and stirring continue for 24 h under N$_2$ atmosphere. After cooling, the solid denominated by **Sil-2** was filtered, washed consecutively with ethanol, toluene, chloroform and acetone. Finally the solid was dried in vacuum for 24 h. The total synthesis process is outlined in Scheme 1.

Results and Discussion

IR Analysis. The grafting of the silylating agent and polymer onto silica was qualitatively confirmed by FTIR and NMR spectroscopies. The FTIR spectra shows (figure not shown) characteristic vibrations of the carbonyl ((1737 cm^{-1}) and the aliphatic groups (2931 and 2859 cm^{-1}) of **Sil-2**.
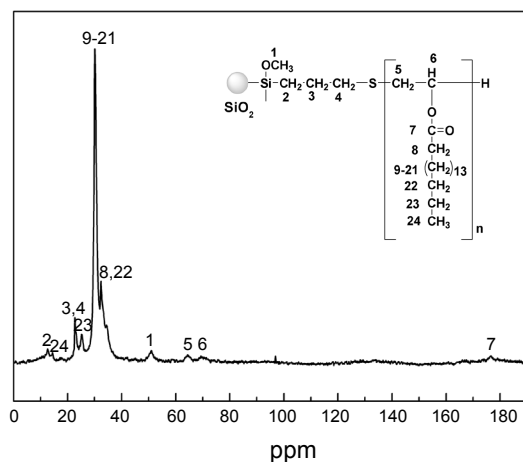
Surface Coverage and Thermogravimetric Analysis. Surface coverage of **Sil-2** was calculated on the basis of elemental analysis result and as per earlier reported method.[32] As shown from Table 1, the calculated grafting density of polymer of **Sil-2** was 2.63 μmol/m^2 while the phase grafting density of polymeric octadecylated silica (ODS-p) and monomeric octadecylated silica (ODS-m) were 3.40 μmol/m^2 and 1.72 μmol/m^2 respectively.[33]

Thermogravimetric analysis (TGA) of silica gel showed an initial mass loss of 4.5% in the 0 to 155 °C range, which is attributed to the physically adsorbed water on the surface while the remaining mass loss upto 900 °C is related to the condensation of free silanol groups on the surface to form siloxane groups.[34] For the compound referring to Sil-1 immobilized surface, a first mass loss of 4.0% from 0 to 125 °C is due to physically adsorbed water and the second mass loss of about 8.0% in the range of 213 °C to 650 °C is due to the decomposition of the organic group covalently bonded on the silica surface. The immobilized polymer **Sil-2** showed the first mass loss of 1.7% between 0 to 80 °C, which is related to the physically adsorbed water, as happens with untreated silica gel. The characteristic second and third mass losses of 13.6% and 7.3% in the range of 205 to 311 °C and from 324 to 650 °C, respectively, are related to the grafted organic groups on silica surface. Carbon percentage calculated from TGA analysis was found to be little lower than calculated by our previously reported method.[32] However this finding may be acceptable as elemental analysis result may overestimate the concentration of hydrogen and carbon arising from the ligand.[35]

NMR Studies. In suspension state ^{1}H NMR spectroscopy, It was reported that restricted alkyl chain mobility decrease NMR intensity while more flexible alkyl chain increase the NMR

Table 1. Elemental analysis, TGA data and grafting density of Sil-1 and **Sil-2**

	Elemental Analysis			TGA	Surface coverage calculated by C%	
	C%	H%	N%	Immobilization, wt %	Amount of immobilization, wt %	Polymer density, $\mu molm^{-2}$
Sil-1	2.8	1.15	0	4.85	5.83	0.28
Sil-2	15.65	3.00	0	18.5	19.17	2.63

Figure 1. Solid state ^{13}C CP/MAS NMR spectrum for **Sil-2**.

Figure 2. Typical log *p* vs log *k* curve for polycyclic aromatic hydrocarbons (PAH's) and alkylbenzenes in **Sil-2**, ODS-p and ODS-m. Mobile phase: methanol/water (80/20), flow rate: 1.0 mL/min, temperature: 25 °C.

signal intensity.[36] The suspension state ^{1}H NMR of **Sil-2** is thus measured from 20 to 50 °C and it was apparent that alkyl chain mobility is high enough irrespective of temperature variation. Relative NMR intensities of long alkyl chain was measured according to the formula described elsewhere[32] and it was shown that alkyl chain intensity increases steadily with increase of temperature. Solid-state ^{13}C CP-MAS NMR provides useful information of the chemical composition of modified surfaces, furthermore it reveals evidence about conformation and dynamics of immobilized alkyl groups. Looking at the spectra (Fig 1), the intense signal at 30.2 ppm representing the alkyl chains -(CH2)*n*- where *n* = 9 - 21, was attributed to *gauche* conformation. This chemical shift arises from the average of the chemical shifts of the *trans* and *gauche* conformations of the alkyl chains, showing that the alkyl chains on the silica surface are quite mobile at room temperature. However, the low field intensity of the signal at 32.5 ppm (C8 and C22) due to the *trans* conformation is very low. While *trans* conformations indicate rigid, ordered chains; the *gauche* conformations characterize mobile, amorphous regions. Furthermore, it is established that the octadecyl moieties of ODS-p generally exists in a highly ordered *trans*-conformational form (δ = 32.84 ppm) although ODS-m this subsisted with mobile *gauche* conformational state.

^{29}Si CP-MAS NMR has the ability to ascertain the surface chemistry of silica bonded phases prepared by different bonding synthetic strategies. ^{29}Si CP-MAS NMR spectroscopy can also reveals the functionality of the silanes. Inspection of the ^{29}Si CP/MAS NMR spectra of **Sil-2** reveals the presence of different types of silicon atom which differ in their coordination sphere as a result of the silylation step. The ^{29}Si CP/MAS NMR spectrum for the **Sil-2** modified silica showed two peaks at −111.5 and −101.9 ppm. These peaks are attributed to silicon in the
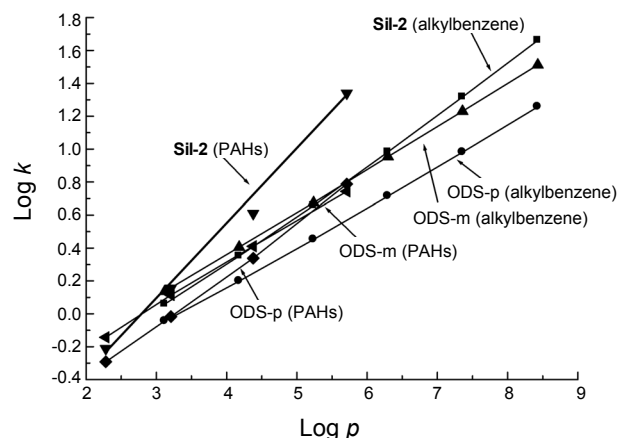
siloxane bonding environmental without hydroxyl groups and to isolate silanol groups respectively, signals that are also observed for the unmodified silica surface.[40] The peak at -57.3 ppm is related to the silicon atoms of the type: R-Si*-(OSi≡)$_2$-OH and finally the peak at −48.1 ppm is attributed to the silicon atoms of the type R-Si*-(OSi≡)(OH)$_2$. The lack of trifunctional silanes at around −65.0 ppm indicates a lesser degree of cross-linking which is in good agreement with the result of ^{13}C CP/MAS NMR spectroscopy.

Chromatographic Evaluation of Retention Mode. It is proved that conventional ODS stationary phases in HPLC can recognize the hydrophobicity of elutes only and this hydrophobicity is measured by the methylene activity of the stationary phase. The retention mode as well as the extent of hydrophobic interaction between the elutes and the stationary phase can be determined by retention studies of alkylbenzenes as elutes.[41,42] When alkylbenzenes were used as solutes in HPLC, **Sil-2** showed the same elution order as polymeric octadecylated silica (ODS-p). It was also confirmed that the retention factors (*k*) increased with increase of water content in the mobile phase. These results confirmed that **Sil-2** has the retention mode similar to RP-HPLC. The Specialty of **Sil-2** can be seen in the retention behavior of polycyclic aromatic hydrocarbons (PAHs). Fig. 2 showed the relationship between log *p* and log *k* of **Sil-2**, ODS-p and ODS-m for a series of alkylbenzenes and PAHs. **Sil-2** (as shown in Fig. 2) showed higher retention factors for PAHs than for alkylbenzenes. For example, log *P* of naphthacene (5.71) is smaller than that of octylbenzene (6.30) but log *k* of naphthacene is much higher (1.217) than that of the latter (0.983). The increase of log *k* for PAHs was accompanied by the selectivity enhancement. For example, α = 26.8 was obtained for naphthacene/

benzene in Sil-2 while $\alpha = 12.05$ in ODS-p and $\alpha = 7.69$ in ODS-m respectively. These results indicate that the **Sil-2** phase provides specific interactive sites for PAHs which can recognize aromaticity.

The chromatographic evaluation was further performed by using the Tanaka test mixture[43] containing hydrophobic probes. Figure 3 shows the chromatogram obtained, in which it can be observed that all compounds were separated with good efficiencies and good peak shapes in **Sil-2**. This characterization protocol is a well-developed approach that is recommended to
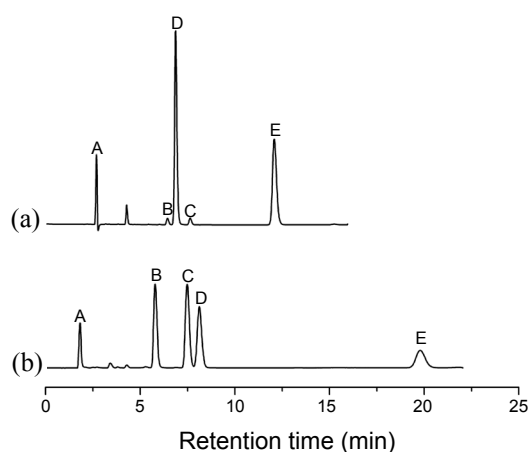

Figure 3. Typical chromatogram of Tanaka test mixture in ODS-p (a) and **Sil-2** (b). Solute: uracil (A), butylbenzene (B), pentylbenzene (C), *o*-terphenyl (D) and triphenylene (E); mobile phase: methanol/water (80/20); flow rate: 1.0 mL/min and temperature: 25 °C.

obtain information about the functionality of the silyl reagent and the methylene selectivity, as well as to establish the repeatability and reproducibility of the separation behavior of commercially available reversed phases. The most relevant properties, which are measured by the chromatographic parameters for the separation of compounds, are shape and methylene selectivities, hydrogen bonding, and ion-exchange capacities in neutral media.

Molecular Shape Selectivity. Molecular shape can often provide a basis for the separation of compounds with constrained molecular structure; however, shape selective columns are less effective for solutes with conformational freedom. Shape selectivity is exemplified by the capacity of a column to resolve isomers on the basis of molecular structure. PAH isomers typically elute in order of increasing length to breadth ratio (L/B), and nonplanar solutes elute before planar solutes with similar L/B. Contributions to shape recognition for ODS phases increases with (1) polymeric surface modification chemistry, (2) increasing bonding density, (3) increasing alkyl chain length, and (4) decreasing temperature. Different size and shaped PAHs were examined to evaluate the retention performance of the new stationary phase **Sil-2**. The retention of a series of PAHs isomers and aromatic positional isomers were done in order to asses the shape selectivity by **Sil-2** and the retention data were compared with the ODS-p as well as ODS-m.

To evaluate the planarity recognition capability of ODS phases, Tanaka *et al*. and Jinno *et al*. introduced the selectivity for two solutes, namely, *o*-terphenyl and triphenylene. Both *o*-terphenyl and triphenylene possess the same number of carbon atoms and π-electrons but the molecular planarity is com-

Table 2. Retention (k) and separation (α) factors for different PAHs with **Sil-2**, ODS-p and ODS-m at 25 °C

Selectivity	PAHs	Sil-2		ODS-p		ODS-m	
		k	α	k	α	k	α
Planarity	[a]*o*-terphenyl	3.66	2.81	1.76	2.39	2.95	1.52
	[a]Triphenylene	10.28		4.20		4.49	
	[a]*cis*-stilbene	2.37	1.52	1.40	1.24	2.19	1.07
	[a]*trans*-stilbene	3.60		1.73		2.35	
Isomeric	[a]*o*-terphenyl	3.66	1.87	1.76	1.60	2.95	1.48
	[a]*m*-terphenyl	6.85	2.50	2.81	1.91	4.34	1.48
	[a]*p*-terphenyl	9.15		3.36		4.34	
Linearity	[a]Triphenylene	10.28	1.28	4.20	1.11	4.49	1.02
	[a]Benz[a]anthracene	13.16	1.32	4.65	1.14	4.57	1.05
	[a]Chrysene	13.57	2.12	4.79	1.46	4.71	1.23
	[a]Naphthacene	21.79		6.14		5.54	
	[b]Dibenzo[a,c]anthracene	9.78	1.36	5.56	1.14	9.26	1.02
	[b]Dibenzo[a,h]anthracene	13.30		6.34		9.49	

Conditions: mobile phase: methanol/water (80/20) for PAHs (a) and (90/10) for PAHs (b). temperature: 25 °C, flow rate: 1.0 mL min^{-1} and UV detection: 254 nm.
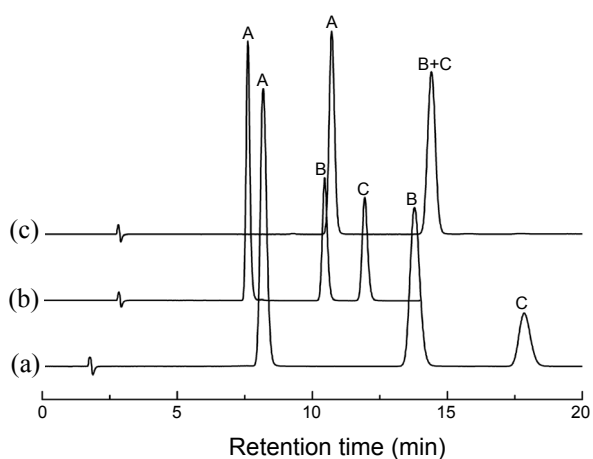
Figure 4. Chromatogram for a mixture of terphenyl isomers in ODS-m (a), ODS-p (b) and **Sil-2** (c). Solute: *o*-terphenyl (A), *m*-terphenyl (B) and *p*-terphenyl (C); mobile phase: methanol/water (80/20); flow rate: 1.0 mL/min and temperature: 25 °C.
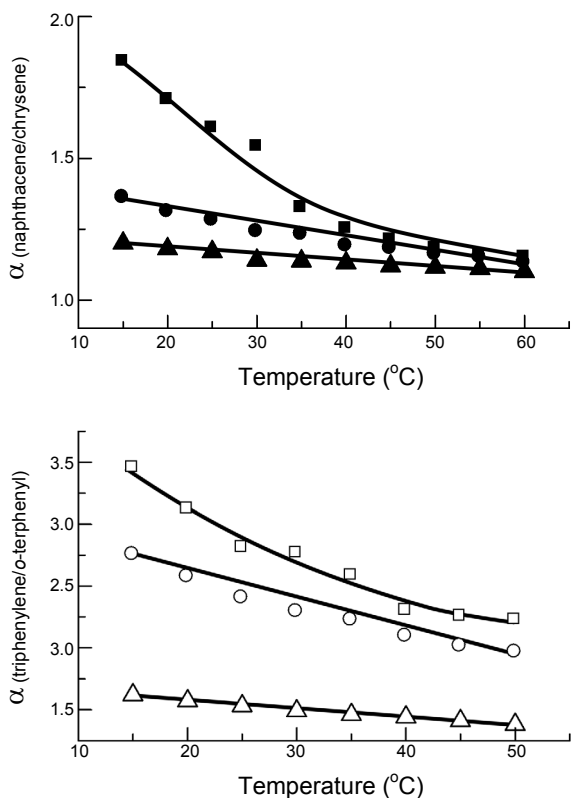

Figure 5. Temperature dependencies of the separation factors between naphthacene and chrysene and triphenylene and *o*-terphenyl with **Sil-2** (■, □), ODS-p (●, ○) and ODS-m (▲, △) respectively. Mobile phase: methanol/water (80/20); flow rate: 1.0 mL min⁻¹; temperature range: 10 - 50 °C.

pletely different.[42] Therefore the separation factor for them has been used as a good indicator to evaluate the molecular planarity selectivity. We observed that **Sil-2** ($\alpha_{triphenylene/o\text{-terphenyl}}$ = 2.64) showed enhanced selectivity as compared with ODS-p ($\alpha_{triphenylene/o\text{-terphenyl}}$ = 2.39) and ODS-m ($\alpha_{triphenylene/o\text{-terphenyl}}$ = 1.52). The results of various planar and non-planar sample sets

for **Sil-2**, ODS-p and ODS-m were also given in Table 2.

Better selectivity of **Sil-2** can also be found in the separation of terphenyl-isomers. The three terphenyl-isomers differ strongly in the extent of their non-planarity.[44] The chromatograms of *o*-, *m*-, and *p*- terphenyl for **Sil-2**, ODS-p and ODS-m are shown in Fig. 4. *o*-Terphenyl possesses the highest deviation from planarity followed by *m*-terphenyl and *p*-terphenyl and similar was the retention order with **Sil-2** along with higher separation factor between the *p*-/*o*- terphenyls compared to the other reference columns (shown in Table 2). In case of geometrical isomers, **Sil-2** gave desirable result and the separation factor between *trans*- and *cis*-stilbene were also given in Table 2.

The molecular length selectivity or molecular slenderness can be evaluated by using four PAHs of four ring as solutes namely triphenylene, benz[a]anthracene, chrysene and naphthacene. All of them are planar compounds with same number of carbon atoms, π-electrons as well as same molecular weight but differ in length to breadth ratio (L/B ratio). It was noteworthy that **Sil-2** showed higher separation factor than other reference columns; for example, at ambient temperature $\alpha_{naphthacene/chrysene}$ = 1.44 for **Sil-2** while $\alpha_{naphthacene/chrysene}$ is 1.28 and 1.18 for ODS-p and ODS-m respectively. Higher selectivity can also be seen in the separation of five ring PAHs (Table 2).

It was reported that column temperature has been shown to have strong influence on shape selectivity in liquid chromatography.[45] Thus to understand the temperature dependencies on selectivity of **Sil-2**, ODS-p and ODS-m, the separation factor between a planar triphenylene and a non-planar *o*-terphenyl as well as naphthacene and chrysene were examined from 15 °C to 60 °C and the results are given in Fig. 5. It was observed that retention of both solute pairs decreases with increasing temperature. It was also demonstrated from the Fig. 5 that, **Sil-2** didn't show remarkable temperature dependencies on selectivity which eventually supports the NMR measurement result.

Discussion on Retention Mechanism. Generally the molecular shape selectivity in ODS phase increases with increasing carbon loading i.e. higher alkyl chain density exhibits higher molecular shape selectivity.[46,47] We have already mentioned that **Sil-2** showed enhanced selectivity towards PAHs and geometrical isomers compared to the other investigated columns regardless of the fact that **Sil-2** had lower alkyl chain density than polymeric ODS.[33] It was reported that the presence and availability of any π-π active functionality within the entirety of a RP-phase will have a great impact on the overall chromatographic selectivity of compounds with extended π-electron system. Thus a multiple carbonyl π-benzene π interaction mechanism was applied to explain unique selectivity of **Sil-2**. Our group had reported previously that carbonyl groups in Sil-ODAₙ are polarized to δ⁺ (carbon) and δ⁻ (oxygen).[48] Similarly in **Sil-2**, the carbonyl groups in the organic phase are polarized to δ⁺ (carbon) and δ⁻ (oxygen) and these polarized atoms can work as an electrostatic source of π-π interaction with π-electron containing guest molecules. As support of this, the selectivity between benzene-nitrobenzene was evaluated as it can be used as a criterion of the contribution of retention from π-interactions in the reversed phase system.[49] Pi-interactions are stronger in the case of nitrobenzene than with benzene, thus the value of selectivity of benzene-nitrobenzene is minimal for Sil-ODS packing

(no pi-interactions) and it is expected be rather high for **Sil-2**. It was found that $\alpha_{nitrobenzene/benzene}$ for ODS-m is 0.68 while the same for **Sil-2** is 0.93. This observed behavior is an effect of specific interaction between the π-electron systems of the solute and stationary phase.

Conclusion

A new type of stationary phase has been synthesized by heterogeneous "graft from" technique. The phase is characterized by spectroscopic methods and thermal analysis and its chromatographic behavior was investigated. Suspension state ^{1}H NMR suggests that the long alkyl chain mobility of the stationary phase is very high compared to conventional octadecylated phase; ^{13}C CP/MAS NMR revealed that the organic phase is in disordered state and remained so even at high temperature and ^{29}Si CP/MAS NMR suggests that degree of cross-linking is less during the reaction. Chromatographic results pointed out the enhanced selectivity of **Sil-2** over ODS-p and ODS-m for PAHs and geometrical isomers. It was also reveled that selectivity of **Sil-2** does not depend remarkably on temperature. The enhanced selectivity of **Sil-2** towards PAHs was may be brought by π-π interaction of the stationary phase and the guest molecule.

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