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Comparison of Chromatographic Performance for L-Phenylalanine-Derived Organic Phases on Silica by "Grafting from" and "Grafting to" Strategies

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Abstract

L-phenylalanine-derived polymerizable organogel, *N*'-octadecyl-N^{α}-(4-vinyl)-benzoyl-L-phenylalanineamide (4) has been prepared according to the procedure described elsewhere. Compound 4 was successfully polymerized by surface initiated atom transfer radical polymerization (ATRP) from the initiator grafted silica particles (sil-poly4). It was also telomerized with 3-mercaptopropyltrimethoxysilane (MPS) and the telomer (T4) was grafted on to silica (sil-T4). TGA and elemental analysis measurement revealed that higher amount of polymer can graft by ATRP process than that of "grafting to" strategy. The results of ^{13}C CP/MAS NMR measurement showed that the *N*-alkyl chain of the grafted polymers for both sil-poly4 and sil-T4 remained as less ordered *gauche* conformational form on silica surface and no inversion to *trans* form was occurred until temperature is increased up to 50˚C. The retention of alkylbenzene samples showed that sil-poly4 prepared by "grafting from" method yielded extremely higher retention than conventional C_{18} phase however, sil-T4 prepared by conventional "grafting to" method showed lower retention than C_{18} phase. Aspects of molecular recognition were evaluated by the retention studies of a series of polycyclic aromatic hydrocarbons (PAHs) and aromatic positional isomers. We have observed sil-T4 yielded slightly higher selectivity for PAHs than sil-poly4 regardless the fact that it has low surface coverage and lower hydrophobic interactions. The enhanced selectivity observed for sil-T4 than C_{18} phases and sil-poly4 can be explained by the π - π interactions between the guest PAHs and carbonyl groups present in the polymer chain. In addition the aromatic moieties of compound 4 that aggregates through π - π interactions also contribute to the separation of PAHs for both sil-poly4 and sil-T4. The minimal π - π interactions between the carbonyl groups and guest molecules for sil-poly4 probably due to the presence of long chain initiator which restrict the polymer to form order thin layer over silica surface.

Keywords: Polymerizable Organogel, Atom Transfer Radical Polymerization, Grafting from, Grafting to, HPLC, Molecular Recognition

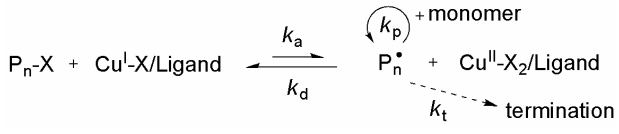
1. Introduction

Polymeric thin films are of increasing importance because of their applications to nonlinear optical materials [1], microfabrication [2], biocompatible medical implants [3], and capillaries for electrophoresis [4-7]. In all applications, controlling the degree of the polymerization is an issue in the preparation of these films, and the inability to control growth of polymer films has diminished their applicability to porous silica gel for liquid chromatographic stationary phases. Stationary phases prepared by surface modification of porous silica with organic interactive layers are the foundation of modern liquid chromatography [8]. Preparation of bonded stationary phases based on silica has been carried out almost exclusively by the "grafting to" technique, by attachment of alkyl chains (typically octadecyl) to porous silica particles through silane coupling reactions. This method is to

graft polymers to the surface by forming a covalent bond between the polymer and the surface. This has been accomplished in many ways, including the use of thiol-derivatized polymer for grafting to gold [9], the grafting of peptides [10] and dendritic polymers [11] through an amide linkage to the surface, and attaching a silane coupling agent to the terminus of a polymer to allow grafting to a silica surface [12,13]. Steric interactions limit the density of the polymer that often leads to low grafting density and low film thickness, as the functional moieties must diffuse through the existing polymer film to reach the reactive sites on the surface [14].

The major difference between the conventional radical polymerization techniques and the CRP is the lifetime of the propagating radicals. While radicals derived from thermal decomposition of conventional radical polymerization initiators (AIBN-type) may undergo termination reactions within a few seconds the lifetime of the propagating radicals can be extended to several hours in controlled processes [15]. Owing to the low radical concentration maintained by equilibrium between the dormant initiator and the propagating radicals (macroinitiator) in CRP the probability of the termination reactions is a negligible providing polymer with low polydispersity (M_w/M_n). Among the CRP increasing attention has been placed to atom transfer radical polymerization (ATRP) since its discovery by Matyjaszewski and Sawamoto [16,17], because this method does not require precise experimental conditions like other controlled polymerization processes. The reaction between the activator complex (often CuBr chelated by *N*-donor ligands) and the dormant initiator results in the formation of propagating radical and deactivator complex via reversible halogen atom transfer reaction (**Scheme 1**).

A low stationary concentration of propagating radical ($< 10^{-8}$ M) is maintained by the dynamic equilibrium which is established after a short period of time (in a few seconds). Using surface-initiated ATRP does not only improve the grafting density of the inorganic particles but may provide polymer chains with controlled molecular weight and molecular weight distribution. While this approach affords the advantages of controlled polymerization, it still suffers from the pH instability of conventional silica-based separation materials because the initial bond is silicon-carbon ether. Further, since most ATRP initiators used also contain an ester linkage as part of the initiator moiety, the pH stability will be even lower.



Scheme 1. Basic mechanism of ATRP process.

Self-assembled systems such as lipid membrane aggregates can provide a highly ordered microenvironment leading to unique host-guest chemistry exceeding the functions of the original lipid [18]. Dialkyl L-glutamide-derived lipids form nanotubes [19], nanohelices [20-22], and nanofibers [23] based on bilayer structures in water or in organic solvents and on the fact that intermolecular hydrogen bonding among the amide moieties contributes to self-assembly. We have reported [24,25] the application of double alkyl self-assembling L-glutamide derivative for HPLC that was prepared by covalent linkage with silica. Up to date there is no report on the synthesis of polymerizable organogel from L-phenylalanine and their application in separation science especially in high performance liquid chromatographic stationary phases. In this study we will present the synthesis, self-assembling properties of octadecyl L-phenylalanine derived polymerizable organogel and its application for separation science especially in high performance liquid chromatography. The grafted particles were prepared by surface initiated ATRP (grafting from) and conventional "grafting to" strategies. The complete characterization of the grafted silica particles in both approaches were done and compared with each other. Evaluation of chromatographic performance has been done for the separation of poly aromatic hydrocarbons and aromatic positional isomers and will be discussed in the following sections.

2. Experimental Section

2.1. Materials and Methods

Triethylamine (Wako, 99+ %) was distilled over potassium hydroxide before use. L (-) phenylalanine (Wako, 99+ %), stearylamine (Wako 99%), *N*-carbobenzoxychloride (Z-Cl) (Wako, assay min 95%), diethyl phosphorocyanidate (DEPC) (Wako, assay min 90%) palladium carbon (Pd 5%, Wako) were used as received. Trichlorosilane (TCA, 97%), 2-bromoisobutyryl bromide (Aldrich, 98%), ω-undecene-1-ol (Aldrich, 98%), platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane (Karstedt catalyst) (Aldrich, 0.1 M in xylene), 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA) (Wako, 98.0%) and copper(I) bromide (Aldrich, 99.999%) were used as received. Toluene (Wako, 99+ %) and diethyl ether (Wako, 99.5+ %) were distilled from sodium/benzophenone and stored under nitrogen when not used. Porous silica particles (YMC-GEL) were purchased from YMC (Kyoto, Japan) whose average diameter, pore size and surface area are 5 μm, 12 nm, and 300 $m^2 \cdot g^{-1}$ respectively. HPLC grade methanol as well as PAHs samples was obtained from Nacalai Tesque (Japan). Analytical thin-layer chro-

matography (TLC) was performed on 0.25 mm silica gel plates, and silica gel column chromatography was carried out with silica gel 60 (Wakogel C-300, Silica Gel). The other chemicals used in this work were obtained from the commercial sources and used without further purification. An ordinary commercial monomeric C_{18} column (Inertsil, ODS 3, column size 250 mm × 4.6 i.d. with particle size 5.5 μm, pore size 10 nm, and surface area of silica particles 450 $m^2 \cdot g^{-1}$ with 13.8% C in the bonded octadecyl phase from G. L. Science, Tokyo, Japan) and a polymeric C_{18} column (Shodex, C18 P, particle size 5 μm, pore size 10 nm, surface area 300 $m^2 \cdot g^{-1}$ with end cap of the unreacted silanol group, containing 17.5% C purchased from Shodex, Tokyo, Japan) were used as references for chromatographic analysis.

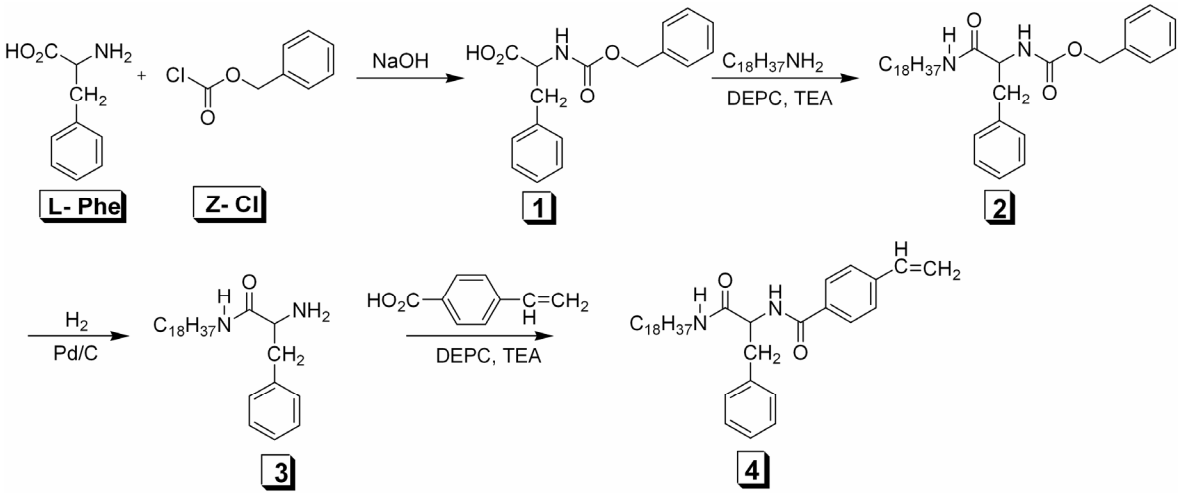
Synthesis of L-phenylalanine derived polymerizable organogel *N*'-octadecyl-N^{α}-(4-vinyl)-benzoyl-L-phenylalanineamide (4) has been done according to the process described in Rahman *et al.* [26] and is given in synthesis **Scheme 2**. IR measurements were conducted on a JASCO (Japan) FT/IR-4100 Plus instrument in KBr. For DRIFT measurement accessory DR PRO410-M (Jasco, Japan) was used. Thermogravimetic analyses were performed on a Seiko EXSTAR 6000 TG/DTA 6300 thermobalance in static air from 30˚C to 800˚C at a heating rate 10˚C/min. For characterization of synthesis ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA400 (Japan) instrument was used. Chemical shifts (δ) of ^1H, ^{13}C expressed in parts per million (ppm) with use of the internal standards Me_4Si (δ = 0.00 ppm) respectively. Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded on a PerSeptive Biosystems Voyager-DE STR spectrometer. Elemental analyses were carried out on a Perkin–Elmer CHNS/O 2400 apparatus. UV/Vis spectra were measured on a JASCO V-560 spectrophotometer using quartz cell of 1 cm width.

2.2. TGA Measurements

Thermogravimetic analyses were performed on a Seiko EXSTAR 6000 TG/DTA 6300 thermobalance in static air from 30˚C to 800˚C at a heating rate 10˚C/min.

2.3. ^{13}C-CP/MAS NMR, ^{29}Si-CP/MAS NMR and Suspended-State ^1H NMR Measurement

NMR spectra was measured by Varian UnityInova AS400 at a static magnetic field of 9.4 T using nanoprobe GHX for suspension-state NMR and solid probe for CP/MAS NMR as spin rate of 2000 - 3500 Hz for suspension-state NMR and 4000 - 4500 Hz for solid-state NMR. The samples for suspension-state ^1H NMR were made by using 10 mg of sil-poly4 and sil-T4 in 100 μL of CD_3OD including 0.03% tetramethylsilane. ^1H NMR spectra were measured at 20˚C - 50˚C at every 5˚C interval using a GHX Varian AS400 nanoprobe. The parameters used for measurement were delay time 1.5 s, pulse width 2.2, transient numbers 32, and spectral width 6000 Hz. Water was suppressed using a presaturation pulse sequence with saturation delay of 1.5 s and saturation power of 2 db. For assigning peaks, after determination of pulse width of 90˚ simple RELAY COSY (correlation spectroscopy test) was done and the chemical shifts of the terminal methyl and methylene proton of alkyl chain were determined. For solid-state ^{13}C CP/MAS, the NMR measuring parameters are spectral width 50000 Hz, proton pulse width PW 90 = 11.6 μs, contact time for cross polarization 5 ms, and delay before acquisition was 2 s. High-power proton decoupling of 63 db with fine attenuation of dipole *r* = 2500 was used only during detection periods. ^{29}Si Cross-polarization magic angle spinning (CP-MAS) NMR spectra were collected with the



Scheme 2. Synthesis of L-phenylalanine-derived self-assembled monomer.

same instrument. Representative samples of 200 - 250 mg were spun at 3500 Hz using 7 mm double bearing ZrO_2 rotors. The spectra were obtained with a cross-polarization contact time of 5 ms. The pulse interval time was 1.5 s. The transmitter frequencies of ^{29}Si and ^{1}H were 59.59 MHz and 300.13, respectively. Typically, 1.5 k FIDs with an acquisition time of 30 ms were accumulated in 1 kilobytes (kb) data points and zero-filling to 8 kb prior to Fourier transformation. The line broadening used was 30 Hz and the spectral width for all spectra was about 25 kHz.

2.4. HPLC Measurement

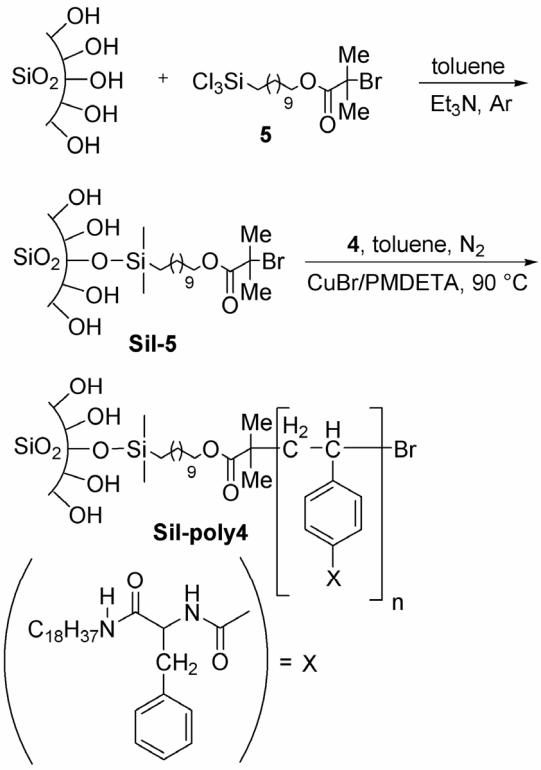
The chromatographic system consists of a Gulliver PU-980 intelligent HPLC pump with a Rheodyne sample injector having 20 μL loops. A Jasco multi-wavelength UV detector MD 2010 plus was used. The column temperature was maintained by using a column jacket with a circulator having a heating and a cooling system. A personal computer connected to the detector with Jasco-Borwin (Ver 1.5) software was used for system control and data analysis. As the sensitivity of UV detector is high, 5 μL of sample solution was used for each injection. To avoid overloading effects, special attention was given in this study to the selection of optimum experimental conditions. Chromatographic measurements were performed using HPLC grade methanol and water mixture (90:10) as mobile phase at a flow rate 1.00 mL·min^{-1}. The retention factor (k) measurement was done 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_2O was used as the void volume (t_0) marker (the absorption for D_2O was measured at 400 nm, which actually considered as injection shock). All data points were derived from at least triplicate measurements; with retention time (t_R) value varying ±1%. Water/1-octanol partition coefficient (P) was measured by the retention studies with octadecylated silica, ODS (monomeric) (Inertsil ODS, i.d. 250 mm × 4.6 mm, GL Science, Tokyo, Japan): log P = 3.579 + 4.207 log k (r) 0.999 997) [25].

2.5. Immobilization of ATRP Initiator on Silica Surface

5 g silica was suspended in 30 mL toluene in a round bottomed flask and 2.150 g (4.72 mmol) (11-(2-bromo-2-methyl)propionyloxy)undecyltrichlorosilane (5) was added and the suspension was rotated for five minutes. Then 1.44 g Et_3N (14.2 mmol) was added and the rotation was continued under inert atmosphere for 24 hours. Silica particles were separated washed with toluene, methanol, water, methanol and diethyl ether (each three times) and were stored at room temperature before polymerization.

2.6. General Procedure for "Grafting from" Method

4.1 g initiator-grafted silica (sil-5), 3.94 g (7.2 mmol) of 4 and 0.536 g (3.1 mmol) PMDETA was suspended in 17 mL dry toluene and purged with nitrogen. 0.085 g (2.02 mmol) CuBr was added and the mixture was degassed by three-freeze-pump thaw cycles. The flask was then placed in an oil bath with a preset temperature of 90˚C and rotate with slow velocity was maintained for 24 hours. The reaction mixture was let cool to room temperature, filtered and washed with hot toluene, hot chloroform and methanol repeatedly. For separation of the remaining catalyst particles were placed to a round bottomed flask, suspended in the mixture of methanol and aqueous solution of K_2EDTA (0.25 M) and the flask was rotated at 40˚C for 6 hours. After filtration silica particles were washed with water, methanol, and diethyl ether and dried under vacuum and finally packed in 25 cm and 4.6 mm i.d. stainless steel column for HPLC measurement. The synthesis scheme of surface-initiated ATRP process of compound 4 is given in reaction **Scheme 3**.



Scheme 3. Surface-initiated ATRP process of compound 4 from silica.

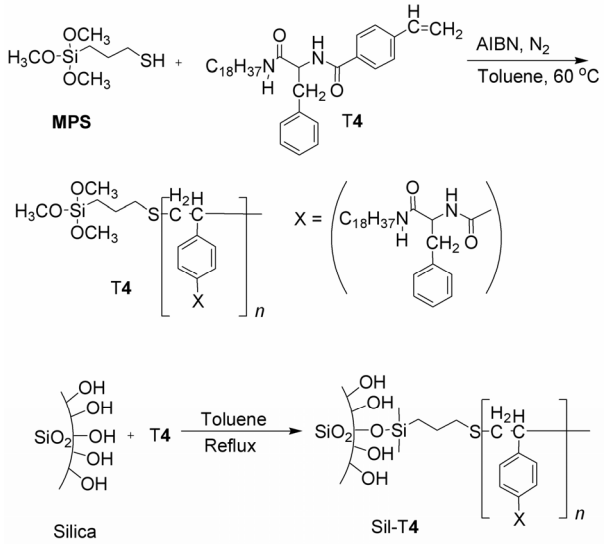
2.7. Procedure for "Grafting to" Method

4.0 g (7.32 mmol) of compound 4 was dissolved in 50 ml dry toluene by heating and 0.15 g (0.73 mmol) of 3-mercaptopropyltrimethoxysilane (MPS) and 40 mg of initiator AIBN was added into the solution and purged with N_2 gas. The mixture was degassed by three-freeze-pump thaw cycles. It was then placed in oil bath and heated at 60˚C for 24 hrs to complete the polymerization reaction. The reaction mixture was concentrated and dissolved in minimum amount of chloroform and re-precipitated from methanol. The white crystal appeared were filtered washed with methanol several times and dried under vacuum to obtain T4. For grafting on to silica surface 4.0 g of T4 and 4.0 g silica were taken in a 100 mL three-necked flask and 40 mL dry toluene was added. The suspension was refluxed for 72 hours to complete the grafting process. After filtration the silica particles were washed with hot toluene, methanol, and chloroform and again with methanol, and diethyl ether and dried under vacuum to get sil-T4. The synthesis scheme of telomerization and grafting processes are shown in **Scheme 4**.

3. Results and Discussion

3.1. Application of Self-Assembling Organogel to Use in HPLC Stationary Phases

We have developed poly (octadecyl acrylate) derivatives as lipid membrane analogues for HPLC stationary phase in which the ordered structure induced the orientation of the carbonyl groups that work as π - π interaction sources with solute molecules. The aligned carbonyl groups were found effective for molecular recognition through multiple π - π interactions [27]. Dialkyl L-glutamide-derived lipids form nano-tubes, -helices, and -fibers based on bilayer structures in water and in organic solvents, furthermore on the fact that intermolecular hydrogen bonding among the amide moieties contribute to self-assembly [27,28]. L-glutamide-derived self-assembling organogel (without a polymerizable head group) has been directly immobilized onto silica surface though covalent linkage for reversed phase high performance liquid chromatography. We have observed higher molecular shape selectivity which was further enhanced by the multiple π - π interaction with ordered carbonyl groups present in the glutamide-derived self-assembling organogel. The dialkyl glutamide derived self-assembling molecule was rigidly immobilized and form a condensed layer over silica surface which make the carbonyl group in a ordered state favorable for multiple π - π interactions. The inter- and/or intramolecular hydrogen bonding promote this assembly, that was considered as a driving force for multiple carbonyl π-benzene π interactions [24,25]. In the L-phenylalanine-derivative (4) similar self-assem- bling system was observed in which the cohesion was mainly attributed to the hydrogen bonding among the amide moieties, and the π - π interactions among the phenyl groups would be advantageous for further non- covalent interactions between the stationary phase and the guest molecules. We anticipate that these possible interactions would be recipient for HPLC separation our attention shifted towards the polymerization of compound 4 on silica surface by "grafting from" method with surface-initiated ATRP and "grafting to" technique.



Scheme 4. "**Grafting to**" **process for the preparation stationary phase from compound 4.**

3.2. Grafting Density and Surface Coverage

The molar amount of octadecyl moieties per 1 g silica (M) can be calculated as according to following equation (from carbon percentages of the polymer grafted silica particles) as mentioned in our previous paper [28]

$$M\left(\mathrm{mol\,g^{-1}}\right) = 10^6\left(\mathrm{P}_C/100\right)/12n \qquad (1)$$

where P_C is the percentage of carbon element according to elemental analysis, and n is the number of carbon atoms present per molecule of monomer (in this case n = 36, since the monomer contain 36 carbons).

The amount of polymer grafted on to silica (P_w) can be calculated as:

$$P_W = m \times 10^{-4} M \qquad (2)$$

m is the molecular mass of each molecule of grafted monomer.

In addition surface coverage (N) can be calculated as:

$$N\left(\text{mol g}^{-2}\right) = M\Big/\left\{S\left[\left(100-P_{\text{W}}\right)/100\right]\right\} = 10^{6}P_{\text{C}}\Big/\left[12nS\left(100-P_{W}\right)\right] \quad (3)$$

where S is surface area of bare silica (in the present case the S = 300 $m^2 \cdot g^{-1}$).

The elemental analysis data of grafted silica particles in both approaches are given in **Table 1**. In sil-poly4 30.4% C included carbon from initiator and % C generated from the polymer is 20.1%. Using above equations we have calculated grafting density and surface coverage from the elemental analysis data of sil-poly4 and sil-T4. We have acquired that sil-poly4 yielded about 25.44% grafting density having surface coverage 2.08 μmol·m^{-2}. On the other hand sil-T4 showed only 19.8% grafting density and 1.45 μmol·m^{-2} surface coverage which is more than 28.5% lower than that yielded by surface initiated ATRP process.

3.3. Thermogravimetric Analysis (TGA)

The organic content of the grafted silica particles has been determined by thermogravimetric analysis. TGA runs were conducted at a constant heating rate of 10˚C /min in air using an empty crucible as reference. Heating process was carried out up to 800˚C that has been demonstrated to be sufficiently high to degrade all surface bonded organosilanes [29], and the resulted thermograms of bare silica, Sil-5, Sil-T4, Sil-poly4, T4 are shown in **Figure 1**.

All weight retention profiles were observed to reach a plateau at 650˚C confirming that there is no organic material remained on silica at 800˚C. Considering the TGA curve of bare silica particles as reference the weight of the immobilized initiator can be calculated as 9.6% which was translated to grafting density an average 0.61 initiator per nm^2. Similarly, the thermogravimetric analysis revealed that 25.5% poly4 is attached onto silica surface if the weight retention of sil-5 was considered as reference at 800˚C. Comparison between the weight retention of bare silica particles and sil-T4 showed 17.5% of grafted telomer that is only 69% of the immobilization obtained by SI ATRP. Interestingly we have observed that the results of grafting percentage and surface coverage calculated from elemental analysis almost correspond to the results obtained by TGA measurement. This also proves that extremely higher amount of organic phase can be grafted by "grafting from" method than that of "grafting to" process.

3.4. DRIFT-IR Measurements

Diffuse reflectance Fourier transform infrared spectroscopy (DRIFT IR) is a suitable method to study the effectiveness of both immobilization of organic molecules onto silica surface and surface initiated polymerization. DRIFT IR and FTIR spectra were recorded in the range of 3750 - 1370 cm^{-1} at room temperature using 4 cm^{-1} resolution and number of scans of 64. Comparison of DRIFT IR spectra of bare silica, 5 initiator-grafted silica, poly4-grafted silica, T4 telomer-grafted silica furthermore FTIR spectrum of T4 telomer are presented in **Figure 2**.

Table 1. Elemental analysis data of polymer and initiator grafted silica particles.

	% C	% H	% N
Sil-5	9.30	1.98	0
Sil-poly4	30.40	4.52	1.83
Sil-T4	15.22	2.41	0.92

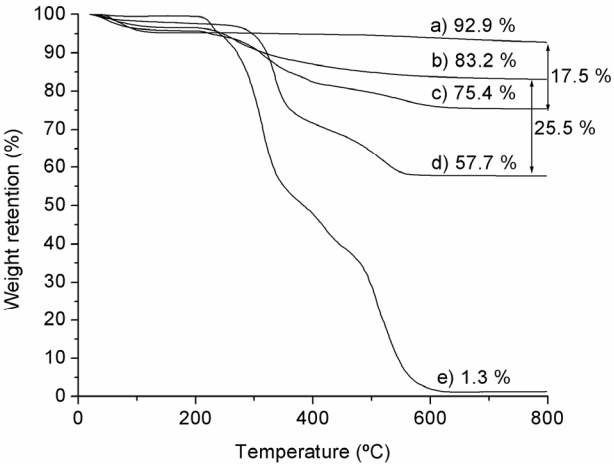


Figure 1. TGA curves of a) bare silica, b) Sil-5, c) Sil-T4, d) Sil-poly4, e) T4.

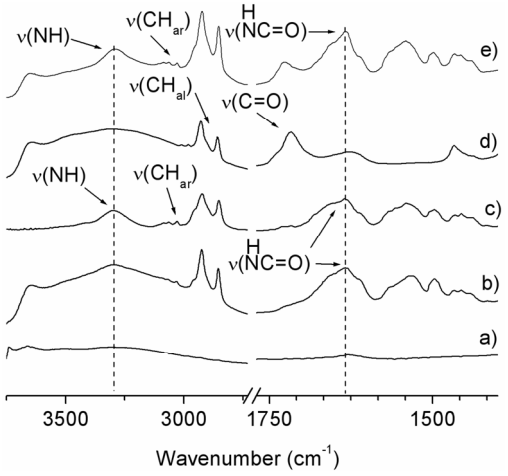


Figure 2. DRIFT IR spectrum of a) bare silica, b) Sil-T4, c) T4, d) Sil-5, e) Sil-poly4.

The spectra of sil-T4 and T4 are nearly identical providing an evidence that fairly high amount of telomere has been grafted onto silica particles. Successful immobilization of 5 initiator is confirmed by the two strong bands at 2924 cm^{-1} and 2853 cm^{-1}, respectively attributed to the asymmetric and symmetric CH_2 stretching furthermore the band at 1717 cm^{-1} arising from carbonyl group of the ester moiety. Analysis of spectrum 2e) shows a broad band at 1633 cm^{-1} with a shoulder at 1656 cm^{-1} derived from the overlapped amide carbonyl stretching vibrations and a band at 3290 cm^{-1} due to the N-H stretching vibration indicating the presence of poly4 on silica surface. These findings clearly proved that silica particles could be coated using two approaches, namely grafting to technique and grafting from technique (SI ATRP).

3.5. Solid-state NMR Spectroscopic Measurements

^{29}Si CP/MAS NMR spectroscopy is well suited for assessing the surface reaction of silanes with silica. Resonances for the silica species appears from –90 to –110 ppm. The ^{29}Si CP/MAS NMR spectra of the polymer grafted silica for sil-poly4 and sil-T4 are shown in **Figure 3**. The ^{29}Si CP/ MAS NMR spectrum for bare silica and ATRP initiator grafted silica (sil-initiator) are also included for comparison. The two polymer grafted silica showed that large extent of silanol groups remain unfunctionalized by both grafting methods.

Figure 3 shows the differentiation between free silanol groups (Q^3) and geminal silanol groups (Q^2) besides the siloxane groups (Q^4) that are indicated by signals at –92, –102 and –111 ppm, respectively. In ATRP polymer-grafted silica the signal corresponding to residual geminal silanols (Q^2) is not seen while its intensity is very less in ATRP initiator grafted silica (sil-initiator), however this signal is quite intense for sil-T4 which was prepared by "grafting to" method. In the spectra after immobilization of ATRP initiator (b) and after polymerization (c) emphasize signals for T^n species that are related to the number of siloxane bonds. When the initiator was reacted with silica surface a large amount of cross-linked T^2 type silicon species (–57 ppm) was observed while polymer grafting increased cross-linked surface indicating by the appearance of T^3 signals (–65 ppm). The disappearance of signal for T^1 in the spectrum (b) and (c) is due to the reaction of the silanol with self-assembling monolayer formed by ATRP initiator [30]. For sil-T4 the signal corresponds to T^2 and T^3 are completely absent however, T^1 signal is slightly appeared which indicates that relatively higher amount of silanol groups remain unfunctionalized in sil-T4 than sil-poly4.

In liquid- or suspended-state NMR, only those molecules or parts of molecules are detectable that has very fast rotational motions. The suspension-state ^{1}H NMR of sil-poly4 and sil-T4 were measured from 25˚C to 50˚C. Neither half-height width (line width) of methylene groups nor spin-spin relaxation time (*T*2) showed any significant change with temperature (20˚C - 50˚C) for both sil-poly4 and sil-T4. We observed that intensity of the NMR peaks representing terminal methyl and methylene groups of octadecyl moieties increased slightly and detectable when a very high vertical scale was used for graphical presentation. These results indicate that the organic phase on the silica surface is in a solid state at room temperature.

Under the condition of magic angle spinning and dipolar coupling of protons, the chemical shift of methylene groups in ^{13}C CP/MAS NMR spectroscopy depends largely on the conformation of octadecyl chains [31]. For each central carbon atom in an octadecyl chain with the *trans* conformation, a chemical shift of around 33 ppm is expected. On the other hand, for conformations with rapid changes between *gauche* and *trans*, a chemical shift of about 30 ppm is expected [32]. Solid-state ^{13}C

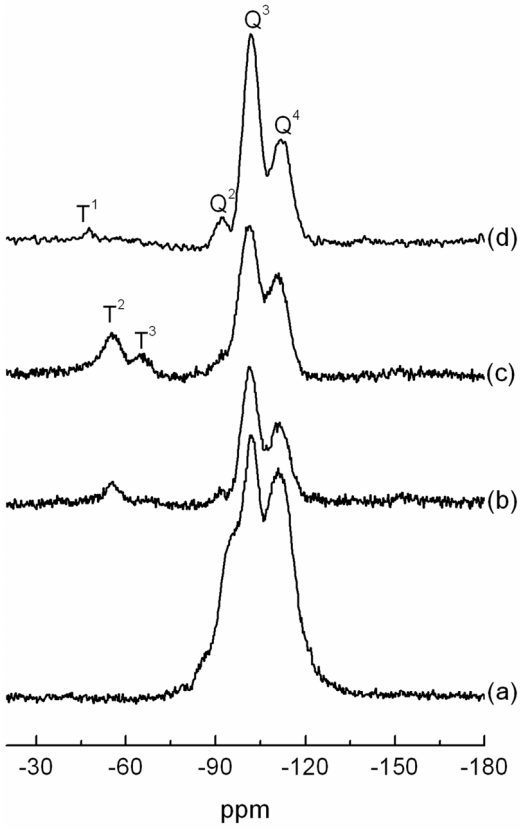


Figure 3. ^{29}Si CP/MAS NMR spectra (a) bare silica, (b) sil-ATRP initiator, (c) sil-poly4 and (d) sil-T4.

CP/MAS NMR spectra were measured for both sil-poly4 and sil-T4 at room temperature. We have observed an intense signal at 30.6 ppm which indicated that *N*-alkyl moieties appeared at less ordered *gauche* conformational form and its shoulder which is almost negligible at around 32.8 ppm results that there are some parts of the alkyl moiety representing *trans* or ordered form. However, in sil-T4 the *N*-alkyl moiety also appear by dominating *gauche* form at somewhat up field than sil-poly4 and at 30.0 ppm. In this case a shoulder at around 32.4 ppm which is more intense than sil-poly4 indicates it contains more ordered *N*-alkyl chain than sil-poly4.

3.6. Evaluation of Retention Mode

It is known that conventional reversed phase HPLC packing materials or C_{18} or alkyl phases can recognize the hydrophobicity of elutes and this hydrophobicity is measured by the methylene activity of the stationary phases.

For instance, Engelhardt [33] proposed using the selectivity factor (α) between ethyl benzene and toluene in a mobile phase of methanol/water mixture as an indicator of hydrophobicity. We observed sil-poly4 yielded slightly higher selectivity ($\alpha_{\text{ethylbenzene/toluene}} = 1.23$) than sil-T4 ($\alpha_{\text{ethylbenzene/toluene}} = 1.20$) for this sample set. Kimata *et al.*[51] suggested that hydrophobicity value be determined from the selectivity (α) between amylbenzene and butylbenzene in a mobile phase of methanol/water. We have also observed that sil-poly4 ($\alpha_{\text{amylbenzene/butylbenzene}} = 1.30$) showed higher selectivity than sil-T4 ($\alpha_{\text{amylbenzene/butylbenzene}} = 1.25$) for this test mixture too.

The retention mode as well as the extent of hydrophobic interaction between the elutes and the packing materials in HPLC can be determined by retention studies of alkylbenzenes as elutes [35-38]. **Figure 4** shows the relationship between log *k* and log *P* for sil-poly4, sil-T4 and C_{18}-M (monomeric C_{18}) phase. The figure clears us that both sil-poly4 and sil-T4 phases trailed the retention mode similar to that of conventional reversed-phase materials. As observed, sil-T4 showed extremely low retention for alkylbenzenes comparing to sil-poly4 and C_{18}. It was also observed that log *k* and log *P* plots of alkylbenzenes and PAHs in C_{18} are parallel and almost coincided with each other. This happened because monomeric C_{18} can recognize only the hydrophobicity of elutes. Nevertheless, both sil-poly4 and sil-T4 gives higher retention for PAHs compared to its values for alkylbenzenes. For instance, the log *P* of naphthacene (5.71) is much smaller than dodecylbenzene (8.43), but the log *k* value of naphthacene (0.68) is much higher than dodecylbenzene (0.53) in sil-T4. In contrast log *k* for dodecylbenzene (3.25) is higher than naphthacene (2.95) in sil-poly4 which divulged that sil-T4 can recognize molecular hydrophobicity inferior than sil-poly4. These results indicate that the sil-T4 phase provides specific interactive sites for PAHs that can recognize aromaticity relatively high regardless it has low surface coverage and less hydrophobic interactions than sil-poly4 and C_{18} phase. The chromatogram for four PAHs on sil-T4 is given in **Figure 5**.

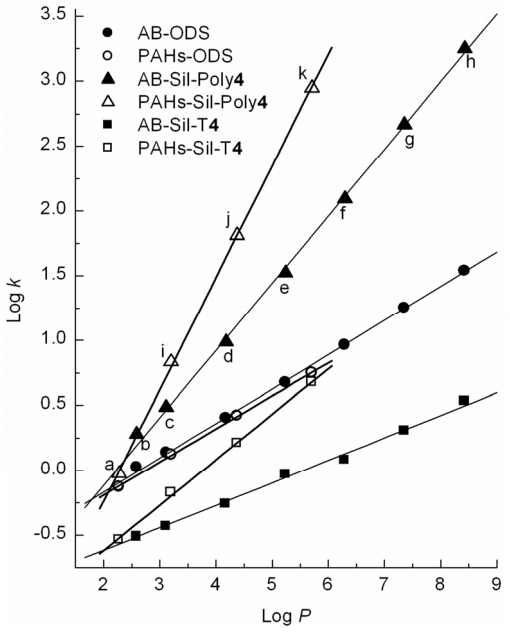


Figure 4. Relationships between log *k* and log *P* of different stationary phases. Mobile phase: methanol-water (90/10 v/v), column temperature: 30˚C. Elutes: a, benzene; b, toluene; c-h, ethyl-, butyl-, hexyl-, octyl-, decyl-, and dodecylbenzene; i, naphthalene; j, anthracene; and k, naphthacene.

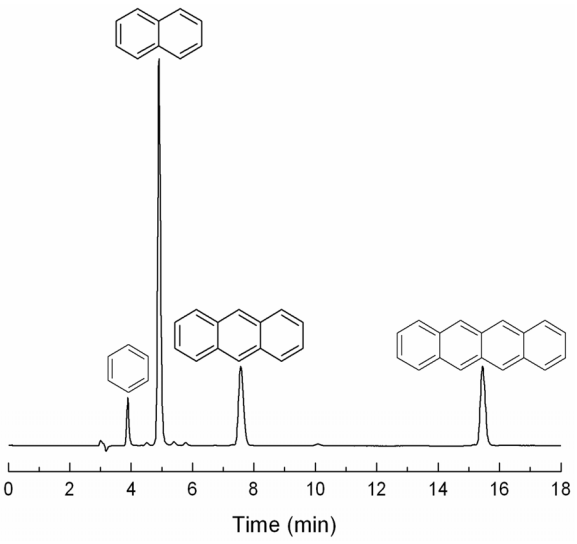


Figure 5. Chromatogram of four PAHs of sil-T4. Mobile phase: methanol/water (90/10), column temperature: 30 C, flow rate: 1.0 mL/min.

3.7. Separation Performance

The first chromatographic evaluation was performed using the Tanaka test mixture containing hydrophobic probes which give information about shape and methylene selectivity, ion-exchange capacities from acidic and neutral media and hydrogen bonding capacities [33]. **Figure 6** shows the chromatogram obtained for the above mentioned test mixture on sil-poly4 and sil-T4, we observed that all of the compounds were well separated by both phase. However, sil-T4 requires less than half time than that of sil-poly4 to get well resolved separation. It also observed that peak shape is rather better in sil-T4 than sil-poly4. This characterization protocol is a well-developed approach that is recommended to obtain information about functionality of the silylant reagent, and the methylene selectivity as well as to establish the repeatability and reproducibility of the separation behavior of commercially available stationary phases. The chromatogram (in **Figure 6**) shows the separation of two homologous alkyl benzenes and non-planar polyaromatic hydrocarbons (PAHs), where it was observed that all compounds are well resolved.

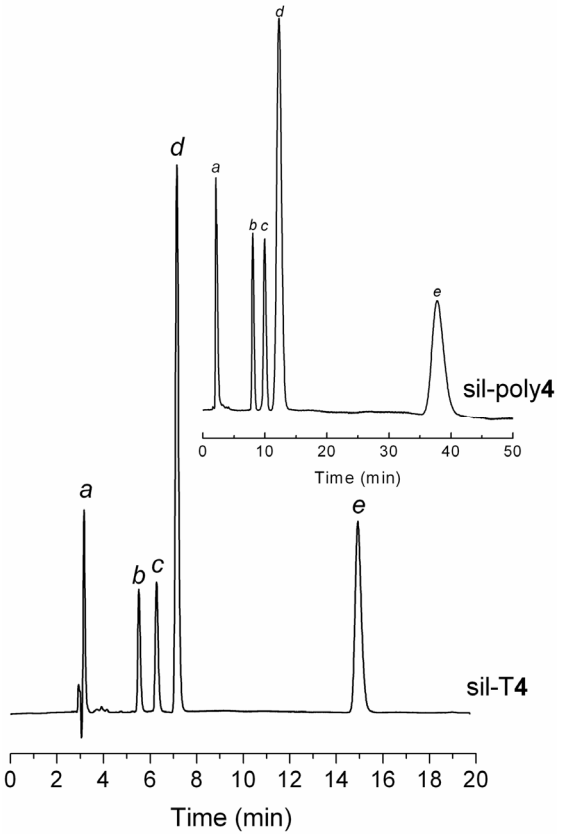


Figure 6. Separation of Tanaka test mixture by sil-poly4 and sil-T4. Mobile phase: methanol/ water (90/10), flow rate 1.0 mL/min, column temperature 30˚C.

3.8. Selectivity for PAHs

The shape selectivity of a given stationary phase can be measured by the separation factor (α) value for pairs of test solutes having the same carbon number but different shape, such as perylene/1, 1'-binaphthyl, triphenylene/ *o*-terphenyl, chrysene/pyrene and *trans*-stilbene/*cis*- stilbene. On a typical monomeric C_{18} phase (C_{18}-M) the separation factor $\alpha_{\text{triphenylene/}o\text{-terphenyl}}$ ranges from 1.0 to 1.7, while the value on a polymeric C_{18} (C_{18}-P) phase lies between 2.0 to 2.7. Jinno *et al.* [39,40] suggested that a separation factor $\alpha_{\text{triphenylene/o-terphenyl}} \geq 2.0$ is an indication of significant solute planarity recognition.

Table 2 lists some of the α values measured on columns packed with sil-T4, sil-poly4 and C_{18} phases. It is clear that the sil-T4 and sil-poly4 phase with a selectivity factor $\alpha_{\text{triphenylene/ }o\text{-terphenyl}}$ of 3.30-3.20 possesses enhanced shape selectivity for PAHs. The selectivity factor $\alpha_{\text{perylene/ 1,1'-binaphthyl}}$ 16.5 and 13.5 on the sil-T4 and sil-poly4 further exemplifies this extremely high planarity recognition. Interestingly, for the isomeric aromatic pair, chrysene and pyrene, the sil-T4 phase yielded a selectivity factor of $\alpha_{\text{chrysene/pyrene}} = 1.55$ while sil-poly4 yielded slightly lower value 1.52 demonstrated that on both phases shape recognition includes not only planarity but also the geometry of solutes. As suggested from the data in **Table 2**, compared with C_{18} phases, sil-T4 phases provide a greater degree of shape selectivity. The retention data for PAHs and aromatic positional isomers for sil-poly4 and sil-T4 in two different mobile phase systems are given in **Table 3**.

3.9. Separation Mechanism

Under isoeluotropic conditions, the aqueous binary mobile phases of methanol, acetonitrile and tetrahydrofuran possess equal solvent strength and similar retention behavior on alkyl-based stationary phases [41]. If π - π interaction sites are present, the differences between methanol and acetonitrile become significant, as acetonitrile [42-44] shows π - π solvent interactions. Acetonitrile as an electron-rich organic modifier suppresses the π - π interaction between the solute and the aromatic π - π active moiety of the stationary phase through formation of electron donor-acceptor complexes with either the aromatic ligand or the solute, depending on whether the one or the other is the more electron-deficient counterpart. Non-functional planar and non-planar PAHs are commonly employed test solutes to demonstrate the presence of π - π interaction, since they only allow hydrophobic and π – π type interactions, without further interaction possibilities [45-48]. Retention factors of planar and non-planar PAHs for methanol and acetonitrile mobile

Table 2. Separation factors of PAH sample sets on different columns after adding acetone to the mobile phase.

	separation factor (α)							
	sil-T4[b]	sil-T4[c]	sil-poly4[b]	sil-poly4[c]	C_{18}-P[b]	C_{18}-P[c]	C_{18}-M[b]	C_{18}-M[c]
triphenylene/*o*-terphenyl	3.30	2.19	3.20	2.84	2.28	2.27	1.29	1.28
chrysene/pyrene	1.55	1.33	1.52	1.43	1.40	1.40	1.22	1.22
trans-stilbene/*cis*-stilbene	1.58	1.28	1.52	1.41	1.22	1.22	1.06	1.06

Mobile phase: [b]methanol/water (90/10), [c]methanol/acetone/water (70/20/10), column temperature: 30˚C and flow rate: 1.00 mL·min^{-1}

Table 3. Surface coverage and retention factors (*k*) of PAH isomers on different stationary phases.

	retention factor (*k*)					
	sil-T4[a]	sil-T4[b]	sil-poly4[a]	sil-poly4[b]	C_{18}-P[a]	C_{18}-M[a]
surface coverage (μmol/m^{-2})	1.43	1.43	2.21	2.21	3.40	1.72
benzene	0.2904	0.135	0.975	0.35	0.74	0.53
naphthalene	0.673	0.28	2.30	0.56	1.39	1.04
phenanthrane	1.40	0.564	5.48	0.90	2.58	2.21
anthracene	1.62	0.62	6.13	0.97	2.63	2.43
pyrene	2.68	0.96	10.28	1.40	3.76	3.76
triphenylene	3.50	1.17	14.42	2.20	4.57	4.76
benzo[a]anthracene	3.15	1.12	12.64	1.75	4.67	5.34
chrysene	3.63	1.20	14.67	2.01	4.80	5.36
naphthacene	4.76	1.42	19.01	2.24	5.50	6.93
benzo[e]pyrene[c]	6.00	1.96	26.84	2.97	0.67	0.78
benzo[a]pyrene[c]	6.30	1.96	27.05	2.97	0.70	0.84
perylene[c]	6.65	2.05	28.27	3.27	0.67	0.91
cis-stilbene[d]	0.88	0.38	3.22	0.42	2.10	1.59
trans-stilbene[d]	1.25	0.41	4.63	0.84	2.20	1.98
o-terphenyl	1.13	0.45	4.47	0.35	3.10	2.08
m-terphenyl	2.08	0.63	8.70	0.62	4.40	3.36
p-terphenyl	2.80	0.83	13.80	1.15	4.40	4.00

Column temperature: 30˚C, flow rate: 1.0 mL/min, mobile phase: [a]methanol/water (90/10), [b]acetonitrile/water (90/10), [c]ethanol (100%) and [d]methanol/water (70/30) for C_{18}-M and C_{18}-P.

phase were measured and are given in **Table 3**. Hence the planarity of a compound is of major importance because it defines the number of π-electrons that are eventually accessible for π - π interaction with the aromatic domains of the stationary phase. For instance, *o*-terphenyl and triphenylene possess both the same number of π-electrons. However, since the two aryl groups of *o*-terphenyl are in very close proximity, a repulsion of their π-electron clouds seems obvious and provides thereby the off-planarity of the molecule in a low energy conformation. The number of π-electrons, which effectively contribute to retention, is thereby reduced from 18 π-electrons to approximately 14 or even less. Consequently, *o*-terphenyl elutes on sil-poly4 and sil-T4 columns before anthracene and phenanthrene, while on C_{18} (both monomeric and polymeric) phases *o*-terphenyl elutes after these two three-ring isomers. Acetonitrile only reduces π - π interactions between solute and the aromatic moieties of the ligands, but does not completely eliminate them.

Retention due to π - π interaction has obviously a strong shape discriminative effect that is mainly based on the density and location of π-electrons within the molecular structure of the interacting aromatic species. Though acetonitrile reduces the shape selectivity on sil-poly4 and sil-T4 to some extent however, the shape selectivity yielded by these phases in acetonitrile are still higher than C_{18}-P regardless the fact that both sil-poly4 and sil-T4 have low surface coverage and lower order alkyl chain than C_{18}-P. This higher selectivity by both phases can be explained by π - π contribution from the

carbonyl groups present in the polymer chain and π-electron present in the guest aromatic moieties. We have reported previously that carbonyl groups in Sil-ODA$_n$ are polarized to δ^+ (carbon) and δ^- (oxygen) [49]. These polarized atoms can work as an electrostatic source of π - π interaction in which carbon atoms act as electron donors and interact with π-electrons containing guest molecules. The carbonyl groups in stationary phases interact with aromatic elutes through π - π interactions that are stronger (1.87 kcal·mol^{-1} in HCHO-benzene) than those of both CH-π (0.57 kcal·mol^{-1} in CH_4-benzene) and benzene π-benzene π (0.49 kcal·mol^{-1} in the plane-to-plane stacking), and the aligned carbonyl groups in Sil-ODA$_n$ are effective for enhancing higher selectivity toward PAHs. Based on that idea, we have clarified that the carbonyl groups in the polymer chain work as a π-electron interaction source for both sil-poly4 and sil-T4. The electrostatic carbonyl-π and π-electrons of guest PAHs benzene-π interactions worked more effectively in sil-T4 than sil-poly4 although both were prepared from same monomer but in different grafting processes. This phenomenon of π - π interactions was also supported by ^{13}C CP/MAS NMR spectral results, in which the alkyl chain in sil-poly4 and sil-T4 do not form an ordered *trans* conformation even at lower temperature unlike the polymeric C_{18} phase. It is known that higher shape selectivity can be obtained by the polymeric C_{18} phase since the highly ordered octadecyl chains enhance the selectivity. In support of this hypothesis, when we added acetone (an inhibitor for π - π interactions) to the mobile phase, the selectivity for both sil-poly4 and sil-T4 were found to decrease for the test PAHs sample sets (as shown in **Table 2**), however the intensities of reduction of the selectivities are much lower in sil-poly4 than in sil-T4. While no change of selectivity were found for either monomeric or polymeric C_{18} phases. The chromatogram for *o*-terphenyl and triphenylene with and without acetone on sil-T4 are shown in **Figure 7**. The contributions of π - π interactions were also investigated by using nitro substituted benzene sample sets. The selectivity between benzene-nitrobenzene can be used as a criterion of the contribution of retention from π-interactions in the reversed phase system.[67] It is known that in conventional RP-HPLC with C_8 or C_{18} (both monomeric and polymeric) stationary phases the higher nitrated compounds are eluted first [50]. In contrast, the higher nitrated compounds are eluted later for sil-T4 while sil-poly4 showed mixed behavior (as shown in **Table 4**) since it only shows higher retention for m-substituted benzene than benzene itself. For other nitro substituted benzene it showed similar retention behavior to that of C_{18} phase. This observed behavior for sil-T4 is an effect of specific interactions between the π-electron systems of the solutes on the one hand and the carbonyl groups of the stationary phase on the other hand.

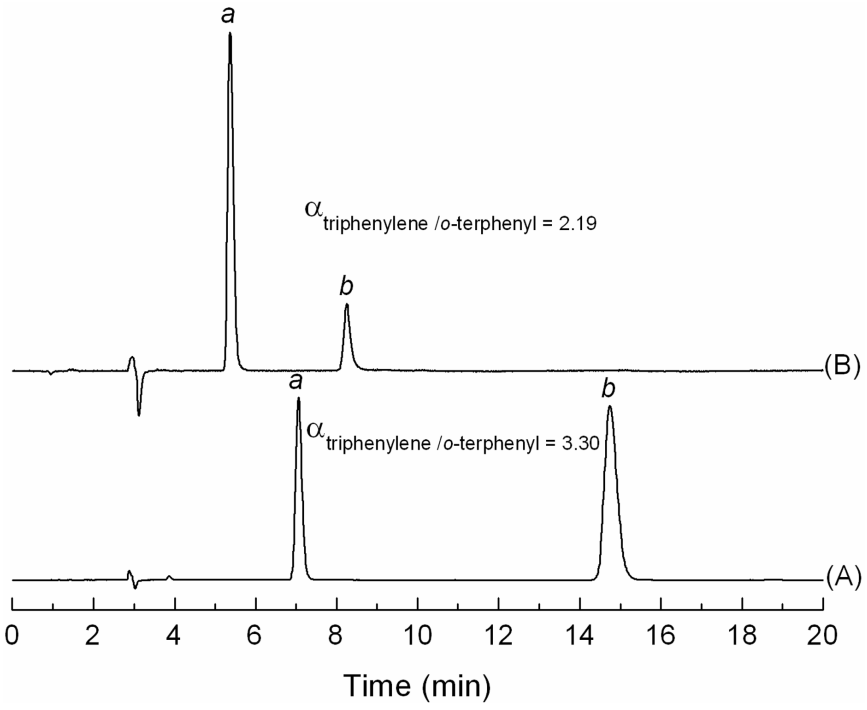


Figure 7. Chromatogram for (a) *o*-terphenyl and (b) triphenylene on sil-T4. (A) Mobile phase: methanol/water (90/10); (B) Mobile phase: methanol/acetone/water (70/20/10). Column temperature: 30˚C, flow rate 1.0 mL/min for both cases.

Table 4. Retention factors (*k*) and separation factors (*α*) for nitrobenzenes/benzene on sil-T4 and sil-poly4 revealing π-electron donating-accepting properties under RP conditions.

	sil-T4		Sil-poly4	
	Retention factor (*k*)	Separation factor (*α*)	Retention factor (*k*)	Separation factor (*α*)
benzene	0.290		0.9750	
		1.09		0.98
nitrobenzene	0.315		0.9584	
		1.14		0.80
o-dinitrobenzene	0.33		0.7850	
		1.24		0.98
p-dinitrobenzene	0.358		0.9530	
		1.35		1.13
m-dinitrobenzene	0.392		1.100	

Mobile phase: methanol/water (90/20), column temperature: 30˚C, flow rate: 1.00 mL/min, UV detection 254 nm.

The solutes are substituted with electron-drawing nitro-groups therefore; their aromatic ringsystems possess a low π-electron density. In contrast, both sil-T4 and sil-poly4 possess carbonyl groups and π-electron rich benzene ring in each molecule, which increases the π-electron density in the stationary phases, can act as an electron donor, while the nitrated solutes act as electron acceptors. These functionalities result in specific π - π interactions that can lead to donor-acceptor complexes. The stability of these complexes depends on the electron density of both the solutes and the stationary phase [52]. Since the electron density of the solutes decreases with increasing numbers of nitro groups, the intensity of π - π interaction also increases in the same order. As discussed above the carbonyl group in sil-T4 possibly formed a thin condensing layer over silica surface by forming hydrogen bonding among the amide moieties which favorable for multiple π - π interactions. Conversely due to the presence of long chain initiator in sil-poly4 the polymer might be unable to form such a condensed layer over silica surface and is less auspicious for multiple interactions and showed lower π - π interactions with guest PAHs samples than sil-T4.

4. Conclusions

L-phenylalanine based polymerizable organogel has been grafted on silica surface by "grafting from" and "grafting to" strategies. Elemental analysis and TGA measurements showed that extremely higher amount of organic phase can be grafted by surface-initiated ATRP process than "grafting to" method. Chromatographic results revealed that slightly higher selectivity for PAHs was obtained by the stationary phase prepared from "grafting to" technique although it has much lower grafting density than that prepared by "grafting from" technique. The new stationary phase prepared from amino acid derived polymeric oganogel and by "grafting to" method exhibits good separation properties for application in RP-HPLC.

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