Preparation of high selective HPLC packing materials based on alternating copolymer-grafted silica

Abul K. Mallik, Tsuyoshi Sawada, Makoto Takafuji, Hirotaka Ihara*

Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto, Japan

*Corresponding author
H. Ihara
Department of Applied Chemistry and Biochemistry
Kumamoto University
2-39-1 Kurokami, Kumamoto 860-8555, Japan
Tel. & Fax. +81-96-342-3661
E-mail: ihara@kumamoto-u.ac.jp

ABSTRACT

Three alternating copolymer-grafted silica stationary phases with different monomers (styrene (ST), *N*-methylmaleimide (MMI), *N*-octadecylmaleimide (OMI), and octadecyl acrylate (ODA)) were newly prepared to be used in reversed-phase high-performance liquid chromatography (RP-HPLC). The alternating copolymer-grafted silicas (Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI)) were characterized by elemental analysis, thermogravimetric analysis (TGA), diffuse reflectance infrared Fourier transform (DRIFT), and ^{13}C cross polarization magic angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectroscopy. Aspects of molecular shape selectivity were evaluated for three different columns with Standard Reference Material (SRM) 869b, Column Selectivity Test Mixture for Liquid Chromatography. Selectivity for isomer separations was enhanced for the stationary phase prepared with Sil-poly(ODA-*alt*-OMI), which was enable to separate 16 PAHs (SRM 1647e) in an isocratic elution. Sil-poly(ODA-*alt*-OMI) showed best molecular-shape selectivity and separation ability among the other phases investigated. In this paper, the effectiveness of this phase is also demonstrated by the separation of tocopherol isomers.

Key words: Alternating copolymer based stationary phase; Shape selectivity; High-performance liquid chromatography; Polycyclic aromatic hydrocarbons; Tocopherols

1 Introduction

Modification of silica packing materials with organic interactive layers remains a popular approach to solve many separation challenges in high-performance liquid chromatography (HPLC) [1–5]. At present time HPLC is one of the most widely used analytical methods because of its good separation power and wide applicability [6–8]. Most progress in reversed-phase (RP)-HPLC separation has been achieved due to the introduction of various silica-based stationary phase materials. New organic phases are being introduced [9–13] and demands for the new types of stationary phase are still increasing and scientists are interested in the synthesis of the phases, which can provide unique separations. Most of the HPLC separations are performed using octadecylsilica phases (ODSs) or alkyl chains-bonded silica columns [14–17]. Depending on the bonding chemistry ODS columns can be divided into two types, monomeric and polymeric. Generally, better separations of solutes or molecular shape selectivity can be achieved by polymeric stationary phases than monomeric one [18, 19]. However, the separation behavior alkyl chains-modified silica column is based on the hydrophobic effect, which leads the problems in the separation of compounds having similar hydrophobicity and polarity [20]. Therefore, there is a need for the development of new kind of stationary phases, which can enter into further interactions besides hydrophobic effects, resulting in the separation of complex isomeric mixtures. On the basis of this assumption, we have designed and synthesized weak interaction sites (π-electron) containing organic phases for high selective RP-HPLC. For example, we reported about the use of poly(octadecyl acrylate)-grafted silica (Sil-ODA$_n$), a lipid membrane analogue containing weak interaction sites, as stationary phase [21]. Sil-ODA$_n$ showed unique separation behavior with ordered-to-disordered phase transitions of long alkyl chains and extremely high selectivity toward polycyclic aromatic hydrocarbons (PAHs) in the ordered (crystalline) state [22–25]. Our detailed investigations showed that the highly ordered structure in Sil-ODA$_n$ induced the orientation of carbonyl groups that work as a π–π interaction source with solute molecules. We have also found that the aligned carbonyl groups are effective for recognition of length and planarity of PAHs through multiple π–π interactions [26, 27]. Similarly, we have reported that π-electron containing dioctadecyl ʟ-glutamide type lipid membrane analogue has been used as organic phase for HPLC that showed extremely enhanced selectivity for PAHs through multiple π–π interactions [28]. However, the separation of the shape-constrained isomers of large molecules like tocopherol was not possible with the above mentioned stationary phases.

In this paper, we report a relatively new type of HPLC stationary phases based on alternating copolymerization on silica. The aim of this work is to integrate the weak interaction sites along the polymer main chain to enhance the aspect ratio of interaction for multiple interactions with the solutes. Alternating copolymerization with maleimide type

monomer also increase the rigidity of the polymer main chain. Alternating copolymerization mediated by electron donor-acceptor interactions remains a popular approach for achieving special materials with different functionalities in a wide range of scientific fields including nanotechnology [29] and drug delivery [30]. Very recently, we first time reported the application of alternating copolymer-grafted silica in the HPLC separation [31]. Alternating copolymer can be obtained by the copolymerization of an electron-rich (donor type) monomer and an electron-deficient (acceptor type) monomer through the formation of charge transfer complexes (CTCs) [32, 33]. The selectivity results of the three alternating copolymer-grafted silica packing materials will also be compared and discussed in the following sections.

2 Materials and methods

2.1 Chemicals and reagents

Styrene (ST) monomer was purchased from Wako (Osaka, Japan) and used after removing polymerization inhibitor. N-Octadecylamine was purchased from Sigma (St. Louis, MO, USA). Maleic anhydride and methylmaleimide (MMI) were purchased from TCI (Kyoto, Japan). Octadecyl acrylate (ODA) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and used after removing polymerization inhibitor. Standard Reference Material (SRM) 869b, Column Selectivity Test Mixture for Liquid Chromatography, and SRM 1647e, Priority Pollutant Polycyclic Aromatic Hydrocarbons, were obtained from the Standard Reference Materials Program (NIST, Gaithersburg, MD). The tocopherol isomers were obtained from Calbiochem (Darmstadt, Germany). 3-Mercaptopropyltrimethoxysilane (MPS) was purchased from Azmax (Chiba, Japan). A YMC silica (YMC SIL-120-S5 having diameter 5 μm, pore size, 12 nm, and surface area 300 m^2g^{-1} (YMC-gel, Kyoto, Japan) was used in all cases. HPLC grade methanol was obtained from Wako (Osaka, Japan). All PAHs were commercially available and used without further purification.

2.2 Proton NMR

^{1}H NMR were recorded with a JEOL JNM-LA400 (Japan) instrument at 400 MHz in CDCl$_3$ solutions at 25˚C. Chemical shifts (δ) of ^{1}H, expressed in parts per million (ppm) with use of the internal standards Me$_4$Si (δ = 0.00 ppm).

2.3 FT-IR, DRIFT and Elemental analysis

FT-IR measurements were conducted with JASCO FT/IR-4100 (Japan). For DRIFT

measurement accessory DR PRO410-M (JASCO, Japan) was used. Elemental analyses were carried out on a Yanaco CHN Corder MT-6 Apparatus (Japan).

2.4 Thermogravimetric analysis (TGA)

Thermogravimetric analyses (TGA) were performed on a Seiko EXSTAR 6000 TG/DTA 6300 thermobalance in static air from 30 to 800˚C at a heating rate of 10˚C min^{-1}.

2.5 Solid-state ^{13}C CP/MAS NMR

Adamantane was used for adjusting the magic angle before each experiment. NMR frequency referencing was performed by adjusting carbon peak of adamantine to 38.5 ppm. Representative samples of 200-250 mg were spun at 4000-4500 Hz using 7 mm double bearing ZrO_2 rotors. Solid-state ^{13}C CP/MAS NMR spectra were measured at temperatures: 20, 25, 30, 35, 40, 45, and 50˚C using a line-broadening factor of 5 (l_b = 5). Other important parameters were relaxation delay 4.0 s, pulse 85.5 degrees, acquisition time 0.05 s, spectral width 40282.0 Hz. High power proton decoupling of 63 db with fine attenuation of dipole r = 2500 was used only during detection periods.

2.6 Instrumentation and chromatographic conditions

The chromatographic system consists of a Gulliver PU-1580 intelligent HPLC pump a Rheodyne sample injector having 20 μL loop. The chromatograph included a JASCO 1580 pump and a JASCO MD-1510 UV-vis photodiode array detector. As the sensitivity of UV detector is high, 5 μL sample was injected through a Reodyne Model 7125 injector. The column temperature was maintained by using a column jacket with a circulator having heating and cooling system. A personal computer connected to the detector with JASCO-Borwin (Ver 1.5) software was used for system control and data analysis. Methanol was used as a mobile phase for the separation of SRM 869b at a temperature of 15˚C. Separations of SRM 1647e and other PAHs were carried out using a 90:10 (v/v) methanol/water mobile phase at 15˚C and 20˚C, respectively. The separation of tocopherol isomers was performed using 90:10 (v/v) methanol/water at 35˚C. All separations were at a flow rate of 1 mL/min. UV detection for the separation of SRM 869b and 16 PAHs (SRM 1647e) was at 254 nm and for tocopherol isomers was at 285 nm. 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 chromatography was done under isocratic elution conditions. 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). All data points were

derived from at least triplicate measurements; with retention time (t_R) value varying ±1%. Water/1-octanol partition coefficient (log P) was measured by the retention studies with octadecylsilylated silica, C_{18} (monomeric) (Inertsil, ODS, column size 250 × 4.6 mm i.d., G. L. Sciences, Tokyo, Japan): log $P = $ 3.579 + 4.207 log k ($r = 0.999997$) [34].

Alternating copolymers, poly(styrene-*alt*-*N*-methylmaleimide)-grafted silica (Sil-poly(ST-*alt*-MMI), poly(styrene-*alt*-*N*-octadecylmaleimide)-grafted silica (Sil-poly(ST-*alt*-OMI), and poly(octadecyl acrylate-*alt*-*N*-octadecylmaleimide)-grafted silica (Sil-poly(ODA-*alt*-OMI) stationary phases were synthesized, characterized, and packed into stainless steel column (150 mm × 4.6 mm i.d.). A YMC silica (YMC SIL-120-S5 having diameter 5 μm, pore size 12 nm, and surface area 300 m^2 g^{-1}) was used. For comparing chromatographic results, we have used two commercial monomeric and polymeric ODS (ODS-m and ODS-p respectively) columns. The monomeric ODS column (Inertsil, ODS 3, column size 250 mm × 4.6 mm i.d. with particle size 5.5 μm, pore size 10 nm, and surface area of silica particles 450 m^2 g^{-1}) was purchased from G. L. Sciences (Tokyo, Japan). This contains 13.8% C in the bonded octadecyl phase. The polymeric ODS column (250 mm × 4.6 mm i.d., Shodex, C18 P, particle size 5 μm, pore size 10 nm, and 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).

2.7 Calculation

The dipole moments of aromatic protons of β- and γ-tocopherols were calculated by Hyperchem Ver 5.1 with molecular mechanics (until the energy changes were below 0.001 kcal/mol) and following semi-empirical AM1 method.

2.8 Preparation of Stationary Phases Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI)

2.8.1 Synthesis of *N*-octadecylmaleimide (OMI)
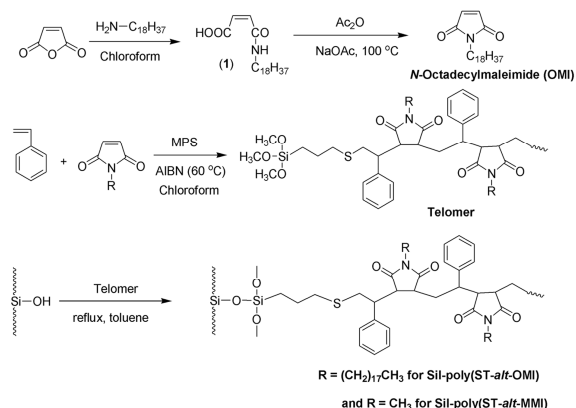
Scheme 1. Synthesis of *N*-octadecylmaleimide monomer and preparation of stationary phases Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI). Telomerizations were carried out with terminal reaction groups followed by immobilization onto silica.

The monomer *N*-octadecylmaleimide (OMI) was synthesized according to the previously reported method [35] and the reaction scheme is shown in Scheme 1. A solution of *N*-octadecylamine (6.496 g, 6.14 mmol) in chloroform (50 mL) was added dropwise to a solution of maleic anhydride (9.904 g, 36.74 mmol) in chloroform (100 mL), and the mixture was stirred for 30 min. The mixture was vacuum filtered, and the solid was washed with CHCl$_3$ (100 mL) and hexane (100 mL) to yield **1** as a white solid: yield 14.13 g (86%); mp 102–104˚C; ν_{max} (KBr) / cm^{-1} 3247 (br), 3077 (br), 2919, 2857, 1709, 1645, 1586, 1528, 1469, 1405, 1294, 1154; ^{1}H NMR (400 MHz, DMSO) δ 0.84–0.87 (t, 3H); 1.15–1.48 (m, 30H); 2.49–2.51 (q, 2H); 3.14–3.19 (t, 2H); 6.22–6.25 (d, 1H); 6.38–6.42 (d, 1H).

A mixture of **1** (10.820 g, 29.40 mmol), sodium acetate (4.100 g, 50.00 mmol), and acetic anhydride (200 mL) was heated to 100˚C for 1 h. Then the mixture was cooled to room temperature and poured into an ice/water slurry. The obtained solid was vacuum filtered and purified by column chromatography (CHCl$_3$, silica gel) to get *N*-octadecylmaleimide (OMI) as a white solid: yield 7.46 g (50%); mp 72–73˚C; ν_{max} (KBr) / cm^{-1} 2919, 2848, 1771, 1697, 1405; ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.86–0.90 (t, 3H); 1.25 (m, 32H); 1.55–1.59 (m, 6H); 3.48–3.52 (t, 2H); 6.68 (s, 2H).

2.8.2 Preparation of Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI)

The copolymer of styrene and *N*-methylmaleimide with terminal reactive group was synthesized by modifying our previous method [36] and then immobilized onto silica (Scheme 1). The typical synthetic procedure is as follows: styrene (4.00 g, 38.40 mmol), *N*-methylmaleimide (4.26 g, 38.40 mmol), 3-mercaptopropyltrimethoxysilane (0.84 g, 4.27 mmol), and 100 mg 2,2'–azobisisobutyronitrile (AIBN) initiator was taken in a round bottomed flask. The solution was degassed with three freeze-pump-thaw cycle and then heated for 6 h at 60˚C to complete the polymerization. After the formation of the polymer, the copolymer was dissolved in minimum amount of chloroform and re-precipitated from diethyl ether. Similar precipitation was repeated three times and the collected powders were successively washed with diethyl ether and dried under vacuum to obtain poly(ST-*alt*-MMI). The resultant poly(ST-*alt*-MMI) was grafted onto silica by using the terminal trimethoxysilyl group: 4.00 g of porous silica and 4.00 g of poly(ST-*alt*-MMI) were taken in a 100 mL three-necked flask and mixed in 40 mL of dry toluene and then was stirred gently at 60˚C for 3 days. The silica was collected and washed successively with toluene, chloroform and methanol. To remove non-grafted polymer soxhlet extraction was carried out with chloroform

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for 24 h and again washed with methanol and finally with diethyl ether to obtain alternating copolymer-grafted silica (Sil-poly(ST-*alt*-MMI)). The same procedure was used to prepare Sil-poly(ST-*alt*-MMI) as shown in Scheme 1.

2.8.3 Preparation of Sil-poly(ODA-*alt*-OMI)

To obtain the similar grafting amount of organic phase onto silica to Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI) we used surface-initiated radical-chain transfer reaction method to prepare Sil-poly(ODA-*alt*-OMI) as shown in Scheme 2. It was difficult to get similar amount of grafting with the telomerization followed by immobilization method for the bulk copolymer (poly(ST-*alt*-OMI)) due to steric hindrance. The preparation method is described as follows: dried silica gel (4.00 g) was placed to a dried flask and dispersed in dry toluene (30 mL). Then the MPS (1.2 g, 6.11 mmol) was added and the reaction mixture was refluxed for 72 h. The suspension was filtered and the solid was washed with toluene, methanol, water, methanol and diethyl ether successively to obtain the MPS-modified silica (Sil-MPS). After drying the particles were characterized by elemental analysis.
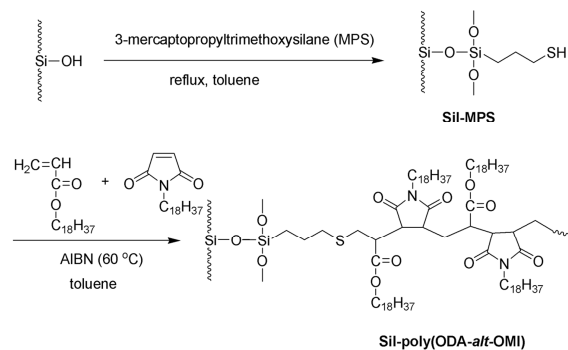
Scheme 2. Preparation of Sil-poly(ODA-*alt*-OMI). Copolymerization was carried out from MPS-modified silica via surface-initiated radical chain-transfer reaction.

To a reaction flask containing MPS-modified silica (3.50 g) a mixture of ODA (4.65 g, 14.32 mmol), OMI (5.00 g, 14.32 mmol), 100 mg of radical initiator AIBN and dry toluene (10 mL) was added. The polymerization mixture was gently stirred for 24 h at 60˚C. The slightly yellow-white reaction mixture was filtered and washed successively with toluene, chloroform, and methanol. To remove non-grafted polymer soxhlet extraction was carried out with chloroform for 24 h and again washed with methanol and finally with diethyl ether and dried in vacuo to obtain Sil-poly(ODA-*alt*-OMI) as shown in Scheme 2.

3 Results and discussion

The aim of this work is to integrate the weak interaction sites along the polymer main chain

by alternating copolymerization to enhance the aspect ratio of interaction for multiple interactions with the solutes. Three different alternating copolymer-grafted silica stationary phases were prepared according to Scheme 1 and 2 to compare the separation performance and elucidate the retention mechanism. The alternating copolymer-grafted silica phases, Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) were characterized by elemental analysis, TGA, DRIFT, ^{1}H NMR, and solid-state ^{13}C CP/MAS NMR spectroscopy. Carbon, hydrogen, and nitrogen elemental analyses and the grafting amount by TGA of the bonded silica phases are summarized in Table 1.

Table 1. Elemental analysis and TGA data of Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) stationary phases

	C (%)	H (%)	N (%)	C/N	Grafting (%) (TGA)
Sil-poly(ST-*alt*-MMI)	12.39	1.82	1.11	11.16	16.71
Sil-poly(ST-*alt*-OMI)	14.61	2.57	0.57	25.63	17.61
Sil-poly(ODA-*alt*-OMI)	15.68	3.52	0.45	35.00	20.13

From the elemental analysis results, the surface coverage of the bonded phases was calculated according to the previously reported method [37]. The surface coverage for Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) were calculated to be 6.24, 4.30, and 2.66 µmol m^{-2} respectively. The degree of immobilization of organic phase onto silica was also measured by TGA (Table 1). Typical TGA curves for the bare silica, Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) are depicted in the Supporting Information (Figure S1).

The formation of alternating structures could be identified due to the fact that *N*-alkylmaleimides cannot only be polymerized without a donor monomer like styrene or ODA and the 1:1 monomer composition was confirmed by an elemental analysis and ^{1}H NMR spectroscopy. The elemental analysis results indicate that the copolymer grafted onto silica surface. Furthermore, type of the copolymer can be estimated from the C/N ratio, because one monomer (styrene or ODA) has no nitrogen atom. The initial composition of the monomers for each polymerization was 1:1 and C/N = 11.08, 25.68, and 36.00 for Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) respectively. After immobilization onto silica or copolymerization from silica almost same C/N were obtained as shown in Table 1. It indicates that 1:1 monomer composition was obtained in both the cases. The formation of alternating copolymer of the monomer styrene and *N*-substituted maleimide has already been proved by some groups [38]. The telomers of poly(ST-*alt*-MMI) and poly(ST-*alt*-OMI) were also characterized by ^{1}H NMR and the characteristic peaks are shown

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in the Supporting Information (Figure S2 (A) and (B)). For further confirmation of alternating nature of Sil-poly(ODA-*alt*-OMI), we prepared the alternating copolymer of ODA and OMI by one step telomerization with 3-mercaptopropyltrimethoxysilane. After purification of the telomere, we measured ^{1}H NMR in CDCl$_3$ to identify the copolymer and to confirm the 1:1 composition of the monomers. The characteristic peaks (Supporting Information Figure S2(C)), which clearly indicates the formation of the copolymer. In addition, the peak area of 7 and 13 (Supporting Information Figure S2(C)) is almost same, revealing 1:1 composition of the monomers.
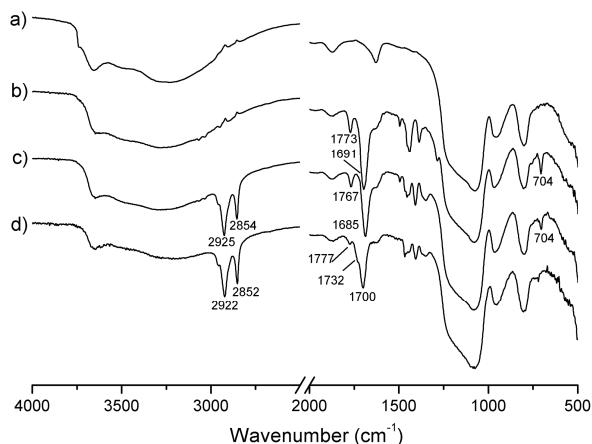
Figure 1. DRIFT spectra of (a) bare silica, (b) Sil-poly(ST-*alt*-MMI), (c) Sil-poly(ST-*alt*-OMI), and (d) Sil-poly(ODA-*alt*-OMI).

Immobilizations of copolymers onto silica were also confirmed by DRIFT spectroscopy. Figure 1 shows the DRIFT spectra, for (a) bare silica, (b) Sil-poly(ST-*alt*-MMI), (c) Sil-poly(ST-*alt*-OMI), and (d) Sil-poly(ODA-*alt*-OMI). In spectra (b), (c), and (d) the peaks near 1767–1777 and 1685–1700 cm^{-1} attribute to symmetrical C=O stretch and asymmetrical C=O stretch of the imide linkage of the copolymers respectively. In spectra (b) and (c) the peaks near 704 cm^{-1} arise from monosubstituted benzene rings that come from styrene unit in the polymers. The signals near 2922–2925 and 2852–2854 cm^{-1} arise from C–H stretches of the long alkyl chains in spectra (c) and (d). The band at 1732 (partially overlapped) in the spectrum (d) arises from ester carbonyl stretching of ODA. These results clearly indicate that the copolymers were grafted onto silica, which are in good agreement with the elemental analyses and TGA data. Almost similar amount of organic phases were found in each stationary phases.

Tailor-made stationary phases, Sil-poly(ST-*alt*-OMI), and (d) Sil-poly(ODA-*alt*-OMI) contain long alkyl chains. To observe the conformation of the alkyl chains of these phases, solid-state ^{13}C CP/MAS NMR were measured. It gives useful information about the chemical composition of the polymer grafted silica and offers evidence about the conformation and dynamics of immobilized alkyl chains [39]. Under the condition of magic angle spinning and
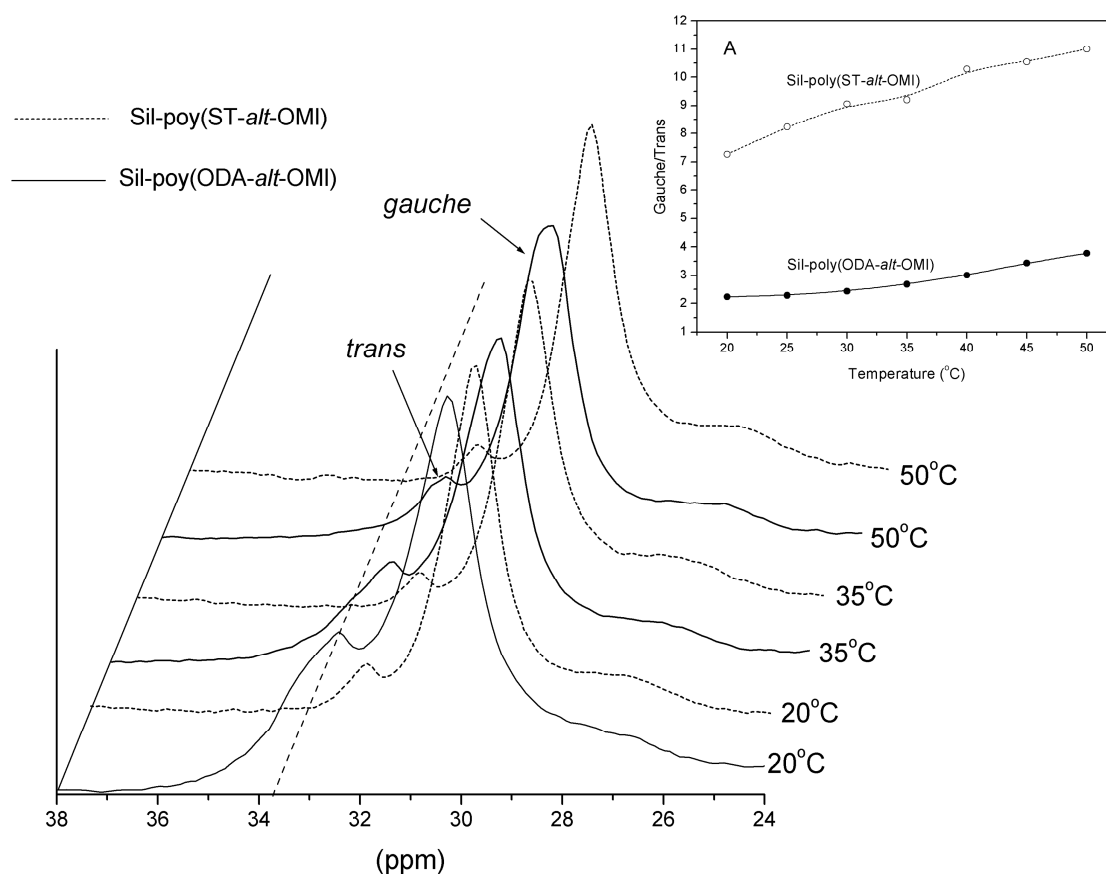
Figure 2. Variation of *gauche* to *trans* conformation of octadecyl moieties of Sil-poly(ST-*alt*-OMI) and Sil-poly(ODA-*alt*-OMI) with temperature (partial solid-state ^{13}C CP/MAS NMR spectra). (A) Comparison of gauche to trans conformation ratio between octadecyl chains of Sil-poly(ST-*alt*-OMI) and Sil-poly(ODA-*alt*-OMI).

dipolar coupling of protons, the chemical shift of methylene groups in ^{13}C CP/MAS NMR spectroscopy depends largely on the conformation of alkyl chains. Solid-state ^{13}C CP/MAS NMR spectra were acquired at variable temperature (20–50˚C) as shown in Figure 2. It is well known that the ^{13}C signals for alkyl chains is observed at two resonances, one is at 32.6 ppm attributed to *trans* conformation, indicating crystalline and rigid state, and the other at 30.0 ppm corresponding to *gauche* conformation, indicating disordered and mobile state [40]. Solid-state ^{13}C CP/MAS NMR spectroscopy reveals that in both Sil-poly(ODA-*alt*-OMI) and Sil-poly(ST-*alt*-OMI) the conformation of alkyl chains -$(CH_2)_n$- can mainly be attributed to *gauche* with a low content of *trans* conformation [39], while a slight temperature-dependent trans-gauche transition was observed (Figure 2). However, *gauche* conformation is much more dominated in Sil-poly(ST-*alt*-OMI) than Sil-poly(ODA-*alt*-OMI) as shown in Figure 2 (A), which confirmed the less orderness of alkyl chains in Sil-poly(ST-*alt*-OMI) than
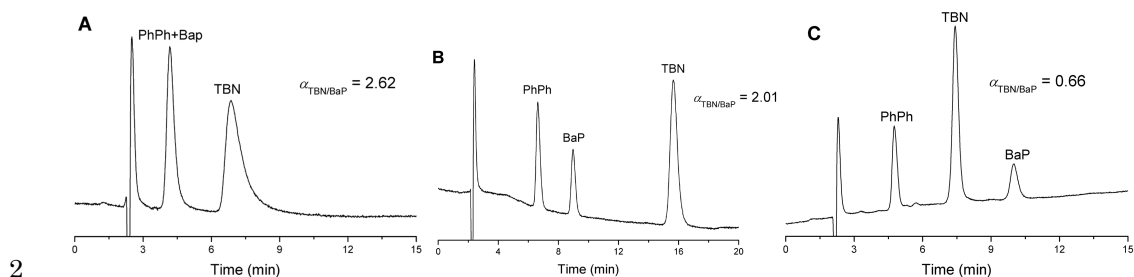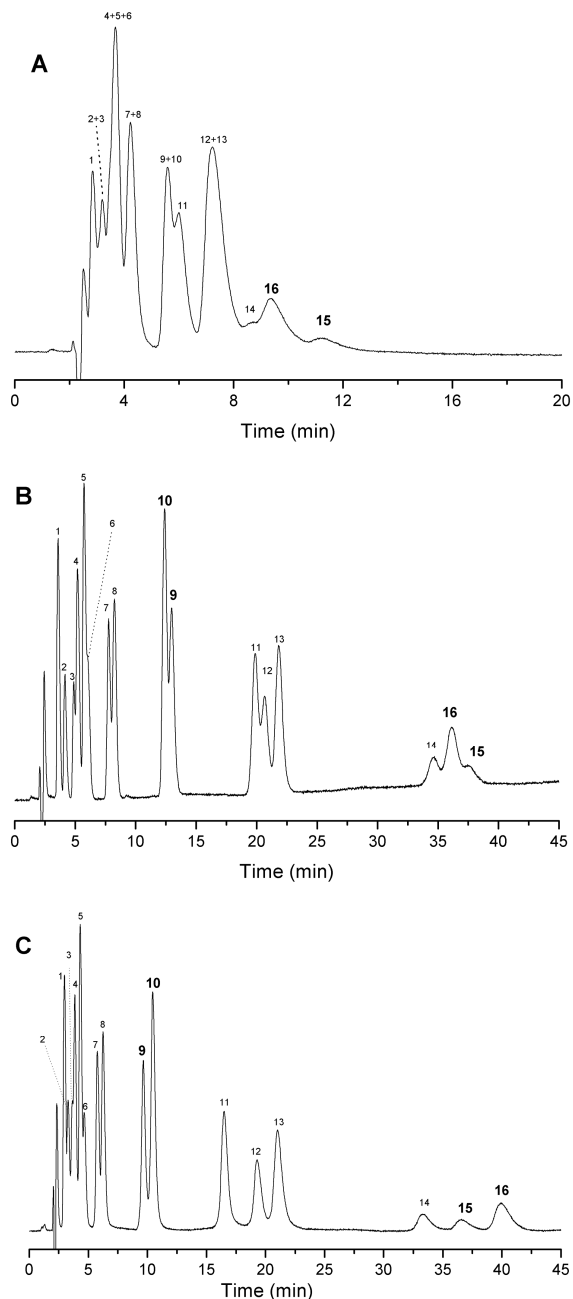
Sil-poly(ODA-*alt*-OMI).

Figure 3. Separation of SRM 869b on (A) Sil-poly(ST-*alt*-MMI), (B) Sil-poly(ST-*alt*-OMI), and (C) Sil-poly(ODA-*alt*-OMI). Chromatographic conditions: injection volume: 5 μL, mobile phase: methanol, flow rate: 1 mL/min, UV detection: 254 nm, column temperature: 15˚C.

The chromatographic performance of each column was evaluated with SRM 869b, Column Selectivity Test Mixture for Liquid Chromatography [41-43]. SRM 869b contains benzo[*a*]pyrene (BaP), phenanthro[3,4-*c*]phenanthrene (PhPh) and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN). It is suggested that the elution order of this test mixture could be used to screen unknown phases, and for column selectivity towards more complex PAHs mixture. Generally, late elution of BaP relative to TBN indicates enhanced column selectivity towards geometric isomers. The elution order on monomeric phases is usually BaP≤PhPh<TBN, and polymeric phases, PhPh<TBN≤BaP. To characterize the phase selectivity, the retention factor (*k*) ratios of TBN and BaP ($\alpha_{TBN/BaP}$) are used. The value of $\alpha_{TBN/BaP}$ <1 is assigned for polymeric phases, and $\alpha_{TBN/BaP}$ ≥1.7 for monomeric phases. Furthermore, densely loaded monomeric phases or lightly loaded polymeric phases were defined as 'intermediate' ODS phases. Columns show the elution order of PhPh<BaP<TBN, and 1< $\alpha_{TBN/BaP}$ <1.7 are termed 'intermediate'.

Separations of SRM 869b were carried out on each of the stationary phases as shown in Figure 3. In each case, the selectivity coefficient $\alpha_{TBN/BaP}$ was found to change as a function of changing co-monomers. The greatest shape recognition ability with polymeric behavior is indicated ($\alpha_{TBN/BaP}$ = 0.66) for the column prepared with poly(ODA-*alt*-OMI), which contain concentrated weak interaction sites (π-electron containing carbonyl groups) and more ordered alkyl chains compared to other phase. The other phases prepared with poly(ST-*alt*-MMI) and poly(ST-*alt*-OMI) showed monomeric like behavior ($\alpha_{TBN/BaP}$ = 2.62 and $\alpha_{TBN/BaP}$ = 2.01 respectively). Sil-poly(ST-*alt*-MMI) without long alkyl chains showed least molecular shpe-selectivity. The three phases prepared with alternating copolymer-grafted silica have nearly identical carbon loadings but differ significantly in selectivity characteristics.

Separations of a mixture of 16 EPA priority pollutant PAHs (SRM 1647e) are shown in Figure 4. Values of $\alpha_{TBN/BaP}$ determined with SRM 869b have been shown to be useful in

predicting selectivity of ODS columns toward PAHs. A similar relationship was observed for the alternating copolymer-grafted silica phases. Complete separation of 16 PAHs was observed with Sil-poly(ODA-*alt*-OMI) ($\alpha_{TBN/BaP}$ = 0.66) in isocratic elution, which represented one of the most important separation

Figure 4. Separation of the 16 Priority Pollutants PAHs (SRM 1647e) on (A) Sil-poly(ST-*alt*-MMI), (B) Sil-poly(ST-*alt*-OMI), and (C) Sil-poly(ODA-*alt*-OMI). Chromatographic conditions: injection volume: 5 μL, mobile phase: methanol/water (90:10 v/v), flow rate: 1 mL/min, UV detection: 254 nm, column temperature: 15˚C. Peak identification: 1 – naphthalene, 2 – acenaphthylene, 3 – acenaphthene, 4 – fluorene, 5 –

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phenanthrene, 6 – anthracene, 7 – fluoranthene, 8 – pyrene, 9 – benzo[*a*]anthracene, 10 – chrysene, 11 – benzo[*b*]fluoranthene, 12 – benzo[*k*]fluoranthene, 13 – benzo[*a*]pyrene, 14 – dibenz[*a,h*]anthracene, 15 – bezo[*ghi*]perylene, and 16 – indeno[1,2,3-*cd*]pyrene.

challenges in isocratic elution. Generally, it is analyzed in a gradient elution mode on polymeric ODS phases because separation is not possible, in isocratic mode, on such phases due to wide retention range of these PAHs [41-43]. Though, isocratic elution is much more attractive for various reasons such as: less complex apparatus, better inter-laboratory reproducibility and increased column life. On the other hand, larger values for $\alpha_{TBN/BaP}$ are indicative of reduced shape selectivity (as observed in monomeric ODS columns), and several PAHs coelute with Sil-poly(ST-*alt*-MMI) ($\alpha_{TBN/BaP}$ = 2.62). Sil-poly(ST-*alt*-OMI) containing long alkyl chain showed very good separation ability for the SRM 1647e, though the $\alpha_{TBN/BaP}$ value is relatively large (2.01) (Figure 4B). However, the elution order of indeno[1,2,3-*cd*]pyrene followed by bezo[*ghi*]perylene is observed for Sil-poly(ST-*alt*-MMI), which is also common for monomeric ODS columns.

    Enhancement of molecular-shape selectivity is important for the separation of structurally similar compounds, including natural products and PAHs. Several size and shape parameters for PAHs were introduced for systematic investigations on retention behavior. The *F* number is a molecular size descriptor proposed by Hurtubise et al. [44], which is defined as follows: *F* = (number of double bonds) + (number of primary and secondary carbons) – 0.5 (number of nonaromatic rings). The selectivity for the two-dimensional shape has been

Table 2. Retention and separation factors of PAHs for Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) stationary phases

Solute	Planar or non-planar	L/B or slendernes or linearlity	Sil-poly(ST-*alt*-MMI)		Sil-poly(ST-*alt*-OMI)		Sil-poly(ODA-*alt*-OMI)	
			k	α	*k*	α	*k*	α
Benzene	planar	1.109	0.12		0.37		0.21	
				2.16		2.11		2.14
Naphthalene	planar	1.24	0.26		0.78		0.45	
				2.30		2.56		2.64
Anthracene	planar	1.57	0.60		1.99		1.19	
				2.02		2.41		2.74
Chrysene	planar	1.73	1.21		4.79		3.26	
				1.14		1.17		1.95
Naphthacene	planar	1.90	1.38		5.60		6.35	
				1.15		1.34		3.00
Triphenylene	planar	1.12	1.20		4.18		2.12	

				2.79		3.07		3.72
o-Terphenyl	non-planar	1.11	0.43		1.36		0.57	
				2.06		2.29		1.89
m-Terphenyl	non-planar	1.47	0.89		3.11		1.08	
				1.02		1.01		1.68
p-Terphenyl	almost planar	2.34	0.91		3.15		1.81	
Dibenzo[*a,h*]anthracene	planar	1.82	3.10		10.90		8.71	
				1.03		1.10		1.57
Dibenzo[*a,c*]anthracene	planar	1.24	3.00		9.95		5.54	

Mobile phase: methanol-water (90:10, v/v). Column temperature: 20˚C. Flow rate: 1.00 mL min^{-1}.

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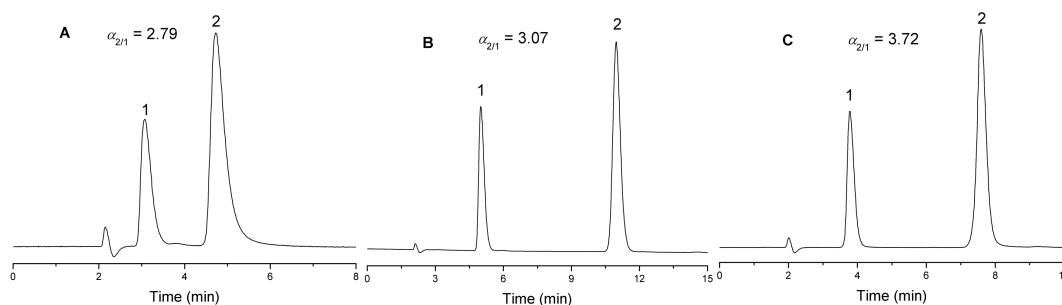
Figure 5. Separation of *o*-terphenyl and triphenylene on (A) Sil-poly(ST-*alt*-MMI), (B) Sil-poly(ST-*alt*-OMI), and (C) Sil-poly(ODA-*alt*-OMI). Chromatographic conditions: injection volume: 5 μL, mobile phase: methanol/water (90:10 v/v), flow rate: 1 mL/min, UV detection: 265 nm, column temperature: 20˚C. Peak identification: 1 – *o*-terphenyl and 2 – triphenylene.

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further studied with a molecular shape descriptor, length-to-breadth (L/B) ratio. The parameter was proposed by Wise et al. [45] and Kaliszan et al. [46] and has been defined as the length-to-breadth ratio of the two-dimensional shape of a molecule, and it quantitatively classifies *rodlike* molecules and *squarelike* molecules. The combination of these size and shape parameters, *F* and L/B, has been successfully introduced for the characterization of ODS phases [47]. Tanaka et al. [48] established a commonly used method to determine molecular-shape selectivity. In this work, two compounds containing the same number of carbon atoms and π-electrons but having different molecular shapes (non-planar and planar), *o*-terphenyl (*F* = 9, L/B = 1.11) and triphenylene (*F* = 9, L/B = 1.12) were used as probes. In

a typical monomeric ODS phase, the selectivity $\alpha_{\text{triphenylene}/o\text{-terphenyl}}$ ranges from 1.0 to 1.7, where as the value on a polymeric ODS phase lies between 2.0 to 2.7. However, a selectivity $\alpha_{\text{triphenylene}/o\text{-terphenyl}} \geq 2.0$ is an indication of significant solute planarity recognition suggested by Jinno et al. [49, 50]. On the other hand, all the alternating copolymer-grafted silica phases, Sil-poly(ST-*alt*-MMI), Sil-poly(ST-*alt*-OMI), and Sil-poly(ODA-*alt*-OMI) demonstrated remarkably higher selectivity ($\alpha_{\text{triphenylene}/o\text{-terphenyl}}$ = 2.79, 3.07, and 3.72 respectively) than conventional ODSs (Figure 5). We also investigated two molecules of same number of carbon atoms and π-electrons (dibenzo[*a,c*]anthracene and dibenzo[*a,h*]anthracene) but differ in molecular length. It is found that Sil-poly(ODA-*alt*-OMI)
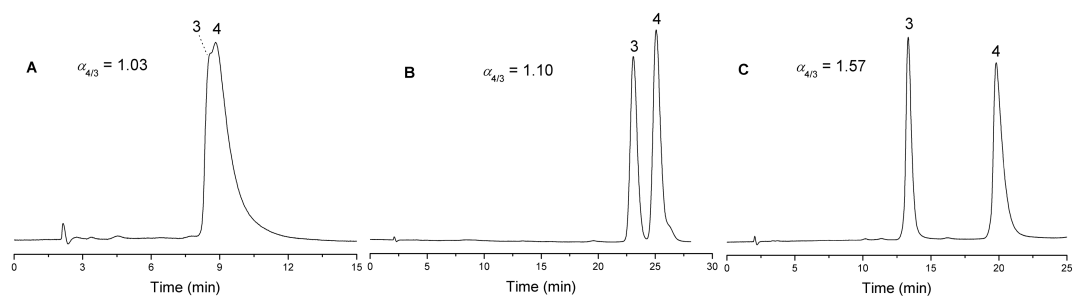
Figure 6. Separation of dibenzo[*a,c*]anthracene and dibenzo[*a,h*]anthracene on (A) Sil-poly(ST-*alt*-MMI), (B) Sil-poly(ST-*alt*-OMI), and (C) Sil-poly(ODA-*alt*-OMI). Chromatographic conditions: injection volume: 5 μL, mobile phase: methanol/water (90:10 v/v), flow rate: 1 mL/min, UV detection: 285 nm, column temperature: 20°C. Peak identification: 1 – dibenzo[*a,c*]anthracene and 2 – dibenzo[*a,h*]anthracene.

($\alpha_{\text{dibenzo[}a,h\text{]anthracene/dibenzo[}a,c\text{]anthracene}}$ = 1.57) can recognize not only molecular planarity but also molecular linearity or slenderness better than other phases investigated; for instance, Sil-poly(ST-*alt*-MMI) ($\alpha_{\text{dibenzo[}a,h\text{]anthracene/dibenzo[}a,c\text{]anthracene}}$ = 1.03) and Sil-poly(ST-*alt*-OMI) ($\alpha_{\text{dibenzo[}a,h\text{]anthracene/dibenzo[}a,c\text{]anthracene}}$ = 1.10) as shown in Figure 6. Table 2 includes the significant results for the other sample sets of planar/non-planar and linear/non-linear. These results indicate that all the phases are reasonably sensitive to molecular planarity and Sil-poly(ODA-*alt*-OMI) phase is very sensitive to molecular linearity or aspect ratio than Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI) phases (Figure 6). Enhanced molecular recognition ability of alternating copolymer-grafted silica stationary phases, especially for Sil-poly(ODA-*alt*-OMI) will be discussed in the following sections. The other phases, Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI) can be used as references to elucidate the interaction mechanism of Sil-poly(ODA-*alt*-OMI).

Generally, the molecular-shape selectivity in the ODS phase increases with increasing carbon loading [51] due to slight increase of alkyl chain ordering, but not for direct interaction with guest molecules [28]. However, Sil-poly(ODA-*alt*-OMI) showed very good molecular-shape selectivity, regardless of the fact that the alkyl chains of
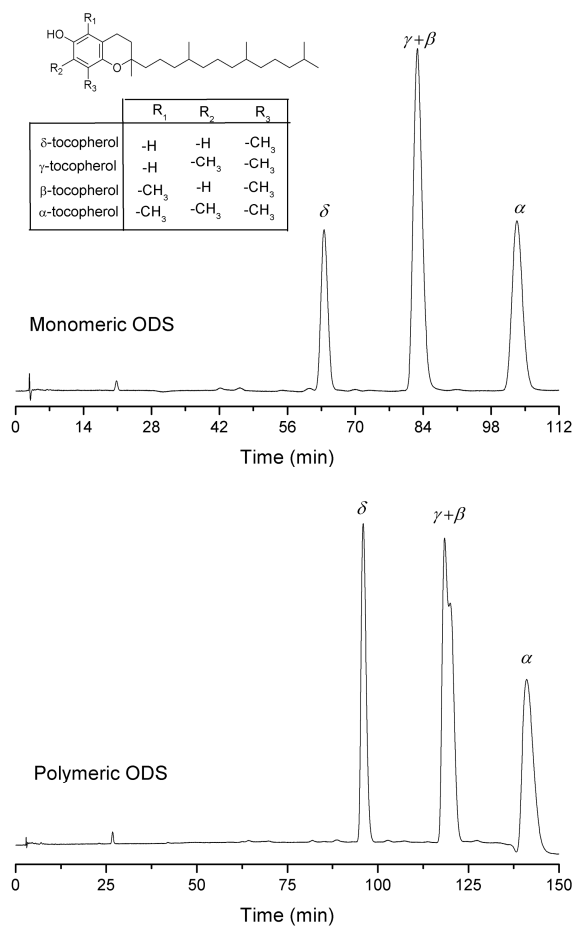
Sil-poly(ODA-*alt*-OMI) are not ordered completely like polymeric ODS phases [28]. Therefore, the direct interaction of solutes with carbonyl groups from the poly(ODA-*alt*-OMI) would be expected for these high separation selectivities. For example, the higher molecular-shape selectivity of Sil-poly(ODA-*alt*-OMI) cannot be explained by the hydrophobic effect with solute molecules. Conventional alkyl phases (ODS) can recognize the hydrophobicity of solutes and this hydrophobicity is measured by the methylene group selectivity of the stationary phases. This reflects the possibility of the phase being able to separate two molecules that differ only in methylene groups, e.g., amylbenzene and butylbenzene or ethylbenzene or toluene. The retention mode as well as the extent of hydrophobic interaction among the solutes and the packing materials in HPLC can be determined by retention studies of alkylbenzenes as solutes [52]. The correlation between log k and log P for Sil-poly(ODA-*alt*-OMI) and polymeric ODS phase showed that the retention mode of Sil-poly(ODA-*alt*-OMI), a reversed-phase mode to that of ODS phase as shown in the Supporting Information (Figure S3). It was observed that log k and log P plots of alkylbenzenes and PAHs in polymeric ODS were parallel and almost coincided with each other, providing evidence that ODS phase can recognize only the hydrophobicity of analytes. On the other hand, it has been found that Sil-poly(ODA-*alt*-OMI) showed higher retention for PAHs compared to its values for alkylbenzenes (Figure S3) such as the log P of naphthacene (5.71) is smaller than that of octylbenzene (6.29), while log k value of naphthacene (0.92) was higher than that of octylbenzene (0.25). The increase of log k for PAHs was accompanied by selectivity enhancement which provides specific interactive sites for PAHs that can recognize aromaticity besides molecular hydrophobicity. Therefore, other interactions are certainly involved for higher molecular-shape selectivity of Sil-poly(ODA-*alt*-OMI) toward PAHs. Previously, we have reported about the carbonyl-π interaction in our calculation works [53]. This interaction in a model complex of HCHO-benzene is much stronger (1.87 kcal mol^{-1}) than a CH_4-benzene interaction (0.53 kcal mol^{-1}) and a plane-to-plane interaction between two benzenes (0.49 kcal mol^{-1}) [53]. We also reported about carbonyl-π interaction to homopolymers from octadecyl acrylate (ODA). When ODA_n was grafted onto silica and evaluated by the retention time of PAHs, enhanced selectivity was observed, especially at crystalline temperatures of ODA_n than for ODS columns [26, 27]. The carbonyl groups in Sil-ODA_n are polarized to δ$^+$ (carbon) and δ$^-$ (oxygen) [53]. These polarized atoms can work as an electrostatic source of π–π in which carbon atoms act as electron acceptors and interact with π-electrons containing guest molecules. Since ODA_n has a crystal-to-isotropic phase transition, the carbonyl groups in the stationary phases interact with aromatic elutes through π–π interactions and a multiple carbonyl-π interaction with PAHs becomes possible through the ordering of carbonyl groups (ordered alkyl chains) at crystalline temperatures.
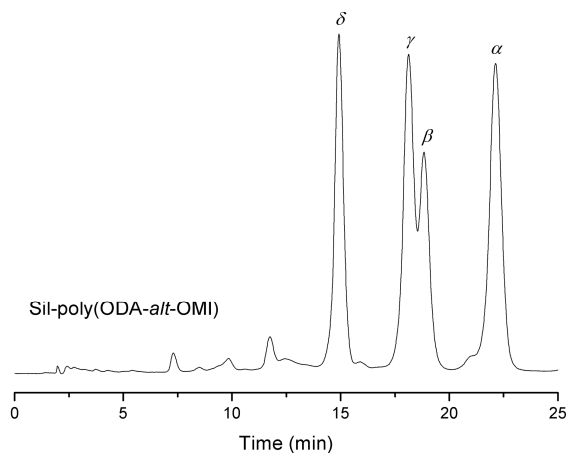
On the other hand, poly(ODA-*alt*-OMI) has no such phase transition as well as a slight ordering of the alkyl chains (low content of *trans* conformation), but the alternating

copolymerization containing a rigid maleimide ring two long alkyl chains can definitely provide a rigid and balanced polymer main chain that leads to the linear ordering of carbonyl groups derived from both maleimide and acrylate. Moreover, a maleimide ring and an acrylate moiety concentrate the carbonyl groups and an alternating nature can integrate and balance (alternating long alkyl chains help to maintain steadiness of the polymer main chain) the carbonyl groups along the polymer main chain, leading to an increase in the interaction area for linear and planar PAHs with a high molecular aspect ratio. As a result, multiple carbonyl-π interaction is more pronounced to linear planar molecules than to non-linear planar molecules (Table 2), and therefore to enhance molecular linearity selectivity significantly for PAHs.

	R₁	R₂	R₃
δ-tocopherol	-H	-H	-CH₃
γ-tocopherol	-H	-CH₃	-CH₃
β-tocopherol	-CH₃	-H	-CH₃
α-tocopherol	-CH₃	-CH₃	-CH₃

Figure 7. Separation of the isomers of tocopherol on monomeric ODS, polymeric ODS, and Sil-poly(ODA-*alt*-OMI). Chromatographic conditions: injection volume: 5 μL, mobile phase: methanol/water (90:10 v/v), flow rate: 1 mL/min, UV detection: 285 nm, column temperature: 35˚C.

Other phases, Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI) reasonably showed less molecular-shape selectivity (linearity as well as planarity) than Sil-poly(ODA-*alt*-OMI) phase. These phases have less concentrated carbonyl groups and there are no concentrated alternating octadecyl chains to help to increase the rigidity (balance) of the polymer main chain as well as carbonyl groups. In Sil-poly(ST-*alt*-OMI) one monomer contains long alkyl chains and showed better selectivity than Sil-poly(ST-*alt*-MMI). However, the alkyl chains are completely disordered in Sil-poly(ST-*alt*-OMI) compared to Sil-poly(ODA-*alt*-OMI) as shown in Figure 2. All the phases showed comparatively good molecular planarity selectivity (Figure 5), regardless of the rigidity difference of the polymer main chain. This is may be due to higher interaction aspect ratio is possible with planar compound (e.g., triphenylene) than non-planar compound (e.g., *o*-terphenyl), even though the polymer main chain is not straight or rigid. Sil-poly(ODA-*alt*-OMI) showed slightly better planarity selectivity than Sil-poly(ST-*alt*-MMI) and Sil-poly(ST-*alt*-OMI) is due to concentrated carbonyl groups.

Finally, Sil-poly(ODA-*alt*-OMI) was applied for the separation of the isomers of tocopherol (vitamin E). Among the α-, β-, γ-, and δ- isomers of tocopherol, the separation of β- and γ-tocopherols is the most challenging issue due to their structural similarity. The baseline separation of these two homologues especially for β - and γ-isomers could not be achieved in conventional RP-HPLC, and is completely impossible on ODS phases [15, 54, 55]. The separations of tocopherol isomers with Sil-poly(ODA-*alt*-OMI), monomeric and polymeric ODS phases are shown in Figure 7, which clearly represents the separation ability of Sil-poly(ODA-*alt*-OMI). Sil-poly(ODA-*alt*-OMI) can separate the isomers within 25 min, while monomeric or polymeric ODS cannot separate even in more than 100 min. For the

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separation of β - and γ-isomers of tocopherol, a slight difference in their dipoles is very important for Sil-poly(ODA-*alt*-OMI). The calculated dipole moments of β- and γ-tocopherols are 2.71 and 2.24 respectively. Therefore, it is logical to assume that poly(ODA-*alt*-OMI) as a carbonyl group-rich organic phase can recognize this difference, and that the polymeric structure can then promote multiple carbonyl-π (dipole-dipole) interactions. In contrast, no such effect can be expected on ODS phases.

4 Concluding remarks

In this paper, synthesis, characterization and the application of alternating copolymer-based stationary phases for selectivity enhancement towards PAHs and isomers of tocopherol have been discussed. High molecular-shape selectivity was observed for stationary phase prepared with alternating copolymer of octadecyl acrylate and *N*-octadecylamaleimide (Sil-poly(ODA-*alt*-OMI)). Standard Reference Materials (SRM 869b and 1647e) were used for the chromatographic characterization of the newly developed alternating copolymer-based stationary phases. The interaction mechanism of high molecular-shape selectivity of Sil-poly(ODA-*alt*-OMI) has been elucidated by multiple π–π interaction with the arranged carbonyl groups along the polymer main chain. These results indicate that preparation of alternating copolymer-grafted silica stationary phases by combining appropriate monomers will be able to solve many separation challenges.

5 References

[1] Jinno, K., Nagoshi, T., Tanaka, N., Okamoto, M., Fetzer, J. C., Biggs, W. R., *J. Chromatogr.* 1987, *392*, 75–82.

[2] Jinno, K., Yamamoto, K., Ueda, T., Nagashima, H., Itoh, K., *J. Chromatogr.* 1992, *594*, 105–109.

[3] Saito, Y., Jinno, K., Pesek, J. J., Chen, Y. -L., Luehr, G., Archer, J., Fetzer, J. C., Biggs, W. R., *Chromatographia* 1994, *38*, 295–303.

[4] Strohschein, S., Pursch, M., Lubda, D., Albert, K., *Anal. Chem.* 1998, *70*, 13–18.

[5] Wegmann, J., Albert, K., Pursch, M., Sander, L. C., *Anal. Chem.* 2001, *73*, 1814–1820.

[6] Dorsey, J. G., Cooper, W. T., Siles, B. A., Foley, J. P., Barth, H. G., *Anal. Chem.* 1998, *70*, 591–644.

[7] LaCourse, W. R., *Anal. Chem.* 2000, *72*, 37–51.

[8] Issaq, H. J., *A Century of Separation Science*, Marcel Dekker, New York 2001.

[9] Neue, U. D., *J. Sep. Sci.* 2007, *30*, 1611–1627.

[10] Pesek, J., Cash, T., *Chromatographia* 1989, *27*, 559–564.

[11] Kirkland, J. J., Glajch, J. C., Farlee, R. D., *Anal. Chem.* 1989, *61*, 2–11.

[12] Welch, C., Pirkle, W. H., *J. Chromatogr.* 1993, *609*, 89–101.

[13] Kimata, K., Hosoya, K., Araki, T., Tanaka, N., *J. Org. Chem.* 1993, *58*, 282–283.

[14] Bell, C. M., Sander, L. C., Wise, S. A., *J. Chromatogr. A* 1997, *757*, 29–39.

[15] Strohschein, S., Pursch, M., Lubda, D., Albert, K., *Anal. Chem.* 1998, *70*, 13–18.

[16] Schauff, S., Friebolin, V., Grynbaum, M. D., Meyer, C., Albert, K., *Anal. Chem.* 2007, *79*, 8323–8326.

[17] Saito, Y., Ohta, H., Jinno, K., *J. Sep. Sci.* 2003, *26*, 225–241.

[18] Sander, L. C., Wise, S. A., *Anal. Chem.* 1984, *56*, 504–510.

[19] Sander, L. C., Wise, S. A., *Anal. Chem.* 1987, *59*, 2309–2313.

[20] Sander, L. C., Pursch, M., Wise, S. A., *Anal. Chem.* 1999, *71*, 4821–4830.

[21] Hirayam, C., Ihara, H., Mukai, T., *Macromolecules* 1992, *25*, 6375–6376.

[22] Fukumoto, T., Ihara, H., Sakaki, S., Shosenji, H., Hirayama, C., *J. Chromatogr. A* 1994, *672*, 237–241.

[23] Mallik, A. K., Rahman, M. M., Czaun, M., Takafuji, M., Ihara, H., *J. Chromatogr. A* 2008, *1187*, 119–127.

[24] Chowdhury, M. A. J., Ihara, H., Sagawa, T., Hirayama, C., *J. Chromatogr. A* 2000, *877*, 71–85.

[25] Ihara, H., Tanaka, H., Nagaoka, S., Sakaki, S., Hirayama, C., *J. Liq. Chromatogr.* 1996, *19*, 2967–2984.

[26] Ihara, H., Sagawa, T., Goto, Y., Nagaoka, S., *Polymer* 1999, *40*, 2555–2560.

[27] Ihara, H., Goto, Y., Sakurai, T., Takafuji, M., Sagawa, T., Nagaoka, S., *Chem. Lett.* 2001, 1252–1253.

[28] Rahman, M. M., Takafuji, M., Ansarian, H. R., Ihara, H., *Anal. Chem.* 2005, *77*, 6671–6681.

[29] Tian, Y., He, Q., Tao, C., Cui, Y., Ai, S., Li, J., *J. Nanosci. Nanaotechnol.* 2006, *6*, 2072–2076.

[30] Harrisson, S., Wooley, K. L., *Chem. Commun.* 2005, 3259–3260.

[31] Mallik, A. K., Sawada, T., Takafuji, M., Ihara, H., *Anal. Chem.* 2010, *82*, 3320–3328.

[32] Hill, D. J. T., O'Donnell, J. H., O'Sullivan, P. W., *Macromolecules* 1985, *18*, 9–17.

[33] Prementine, G. S., Jones, S. A., Tirrel, D. A., *Macromolecules* 1989, *22*, 770–775.

[34] Goto, Y., Nakashima, K., Mitsuishi, K., Takafuji, M., Sakaki, S., Ihara, H., *Chromatographia* 2002, *56*, 19–23.

[35] Vargas, M., Kriegel, R. -M., Collard, D. M., Schiraldi, D. A., *J. Polym. Sci. Part A: Polym. Chem.* 2002, *40*, 3256–3263.

[36] Mallik, A. K., Takafuji, M., Ihara, H., *J. Chromatogr. A* 2009, *1216*, 7433–7439.

[37] Qiu, H., Jiang, Q., Wei, Z., Wang, X., Liu, X., Jiang, S., *J. Chromatogr. A* 2007, *1163*, 63–69.

[38] Lazzara, T. D., van de Van, T. G. M., Whitehead, M. A., *Macromolecules* 2008, *41*, 6747–6751.

[39] Ansarian, H. R., Derakhsan, M., Rahman, M. M., Sakurai, T., Takafuji, M., Taniguchi, I., Ihara, H., *Anal. Chim. Acta* 2005, *547*, 179–187.

[40] Pursch, M., Strohschein, S., Händel, H., Albert, K., *Anal. Chem.* 1996, *68*, 386–393.

[41] Sander, L. C., Wise, S. A., SRM 869, Column Selectivity Test Mixture for Liquid Chromatography (Polycyclic Aromatic Hydrocarbons). Certificate of Analysis. NIST, Gaithersburg, MD, 1990.

[42] Sander, L. C., Wise, S. A., SRM 869a, Column Selectivity Test Mixture for Liquid Chromatography (Polycyclic Aromatic Hydrocarbons). Certificate of Analysis. NIST, Gaithersburg, MD, 1998.

[43] Sander, L. C., Wise, S. A., SRM 869b, Column Selectivity Test Mixture for Liquid Chromatography (Polycyclic Aromatic Hydrocarbons). Certificate of Analysis. NIST, Gaithersburg, MD, 2008.

[44] Schabron, J. F., Hurtubise, R. J., Silver, H. F., *Anal. Chem.* 1977, *49*, 2253–2260.

[45] Wise, S. A., Bonnet, W. J., Guenther, F. R., May, W. E., *J. Chromatogr. Sci.* 1981, *19*, 457–465.

[46] Kaliszan, R., Lamparczyk, H., *J. Chromatogr. Sci.* 1978, *16*, 246–248.

[47] Jinno, K., *Chromatographic Separations Based on Molecular Recognition*, Wiley-VCH, New York 1997.

[48] Kimata, K., Iwaguchi, K., Onishi, S., Jinno, K., Eksteen, R., Hosoya, K., Araki, M., Tanaka, N., *J. Chromatogr. Sci.* 1989, *27*, 721–728.

[49] Jinno, K., Yamamoto, K., Nagashima, H., Ueda, T., Itoh, K., *J. Chromatogr.* 1990, *517*, 193–207.

[50] Jinno, K., Ibuki, T., Tanaka, N., Okamoto, M., Fretzer, J. C., Biggs, W. R., Griffiths, P. R., Olinger, J. M., *J. Chromatogr.* 1989, *461*, 209–227.

[51] Wise, S. A., Sander, L. C., in: Jinno, K. (Ed.) *Chromatographic Separation Based on Molecular Recognition*, Wiley-VCH, New York 1996, pp. 1–64.

[52] Claessens, H. A., Van Straten, M. A., Cramers, C. A., Jezierska, M., Buszewski, B., *J. Chromatogr. A* 1998, *826*, 135–157.

[53] Sakaki, S., Kato, K., Miyazaki, T., Ohkubo, K., Ihara, H., Hirayama, C., *J. Chem. Soc. Faraday Trans* 1993, 9, 659–662.

[54] Abidi, S. L., *J. Chromatogr. A* 2000, *881*, 197–216.

[55] Henry, C. W., Fortier, C. A., Warner, I. M., *Anal. Chem.* 2001, *73*, 6077–6082.

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Sil-poly(ODA-*alt*-OMI). Chromatographic conditions: injection volume: 5 μL, mobile phase: methanol/water (90:10 v/v), flow rate: 1 mL/min, UV detection: 285 nm, column temperature: 35°C.
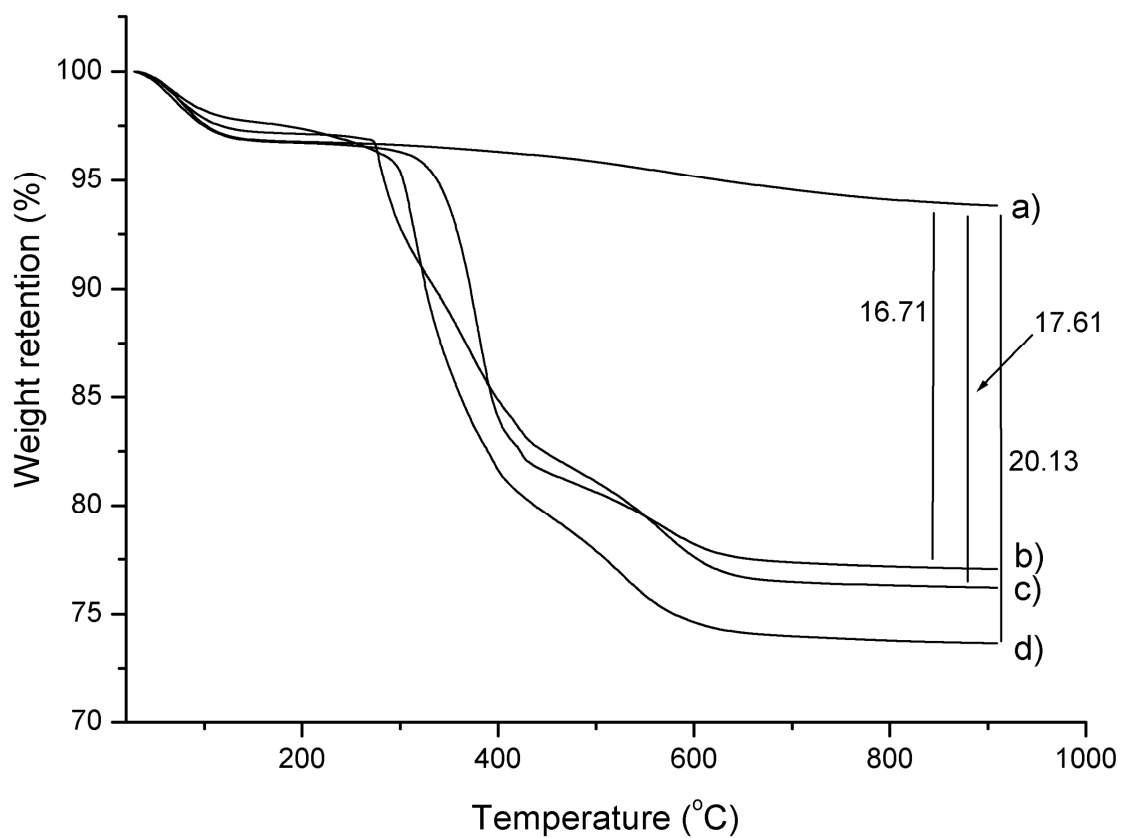
Figure for Supporting Information

Figure S1 TGA thermograms of the stationary phases; (a) bare silica, (b) Sil-poly(ST-*alt*-MMI), (c) Sil-poly(ST-*alt*-OMI), and (d) Sil-poly(ODA-*alt*-OMI).

Figure S2

Figure S3 Log *k* versus log *P* plots for polymeric ODS and Sil-poly(ODA-*alt*-OMI) stationary phases. Mobile phase: methanol/water (90:10 v/v). Column temperature: 30°C. Key: a, benzene; b, toluene; c, ethylbenzene; d, butylbenzene; e, hexylbenzene; f, octylbenzene; g, decylbenzene; h, dodecylbenzene; i, naphthalene; j, anthracene; k, naphthacene.

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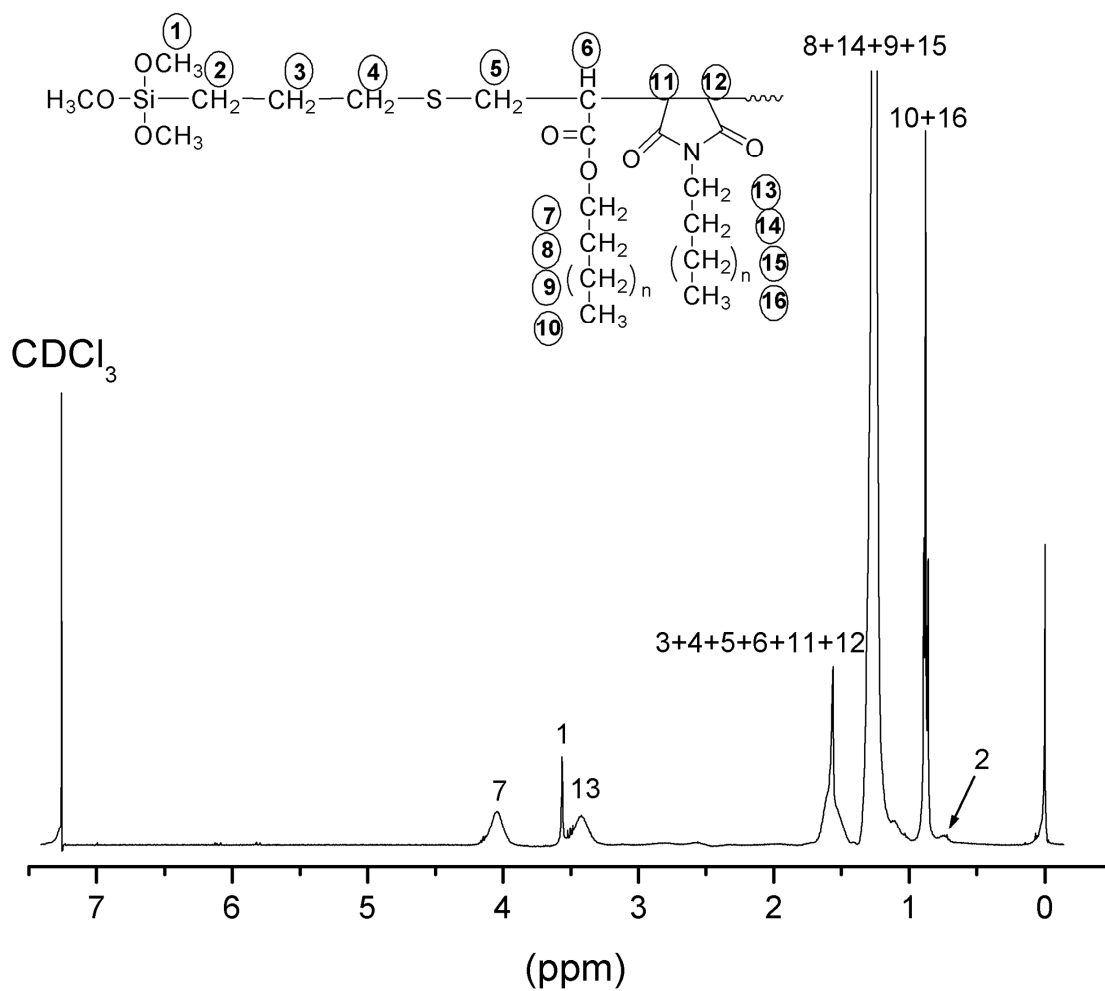**Figure S1.** TGA thermograms of the stationary phases; (a) bare silica, (b) Sil-poly(ST-*alt*-MMI), (c) Sil-poly(ST-*alt*-OMI), and (d) Sil-poly(ODA-*alt*-OMI).
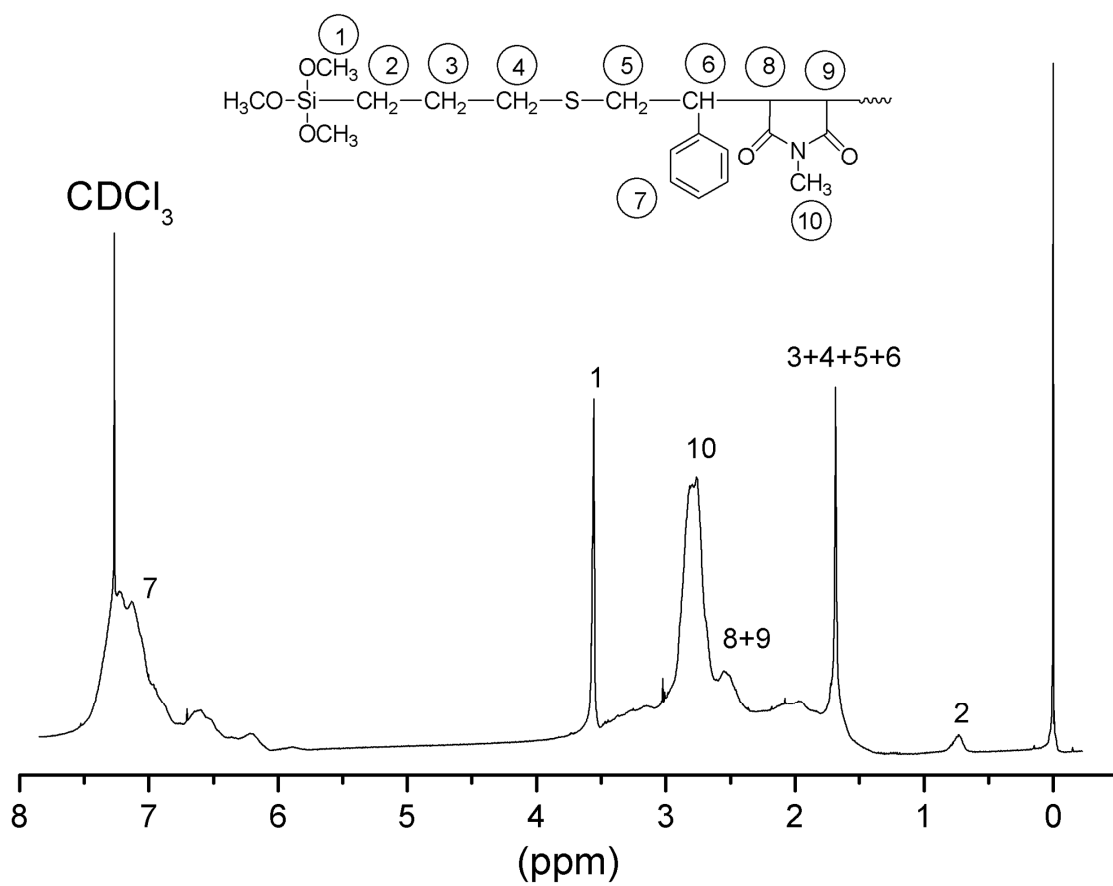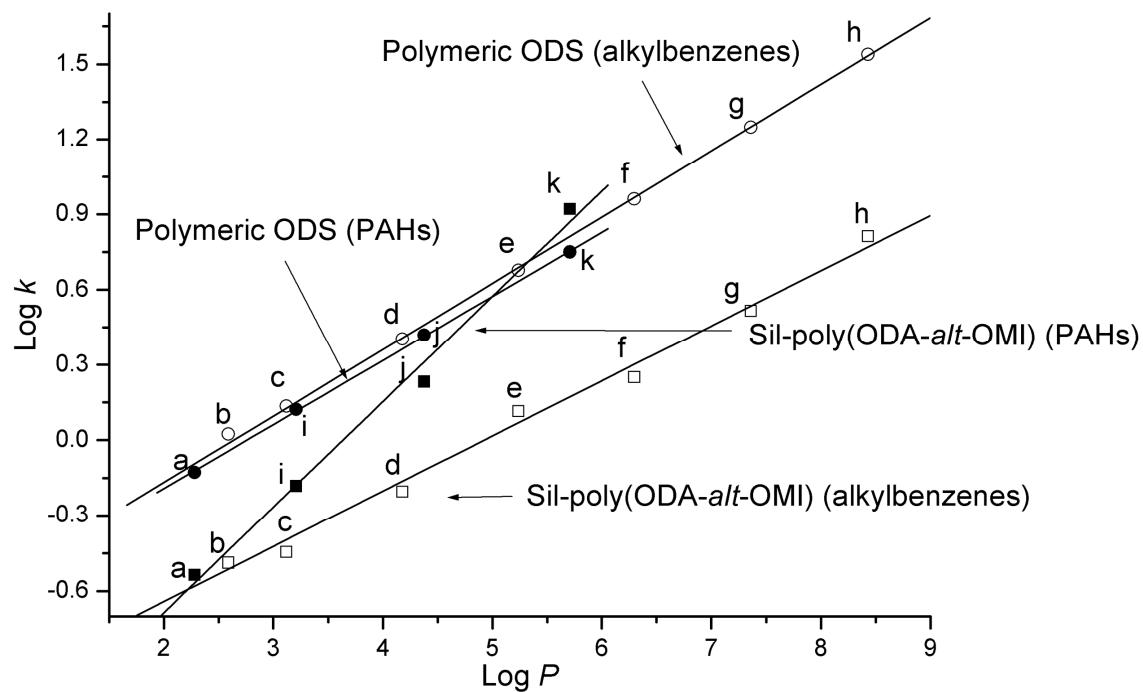
Figure S2 A) ^{1}H NMR spectrum of the end functionalized poly(ST-*alt*-MMI).
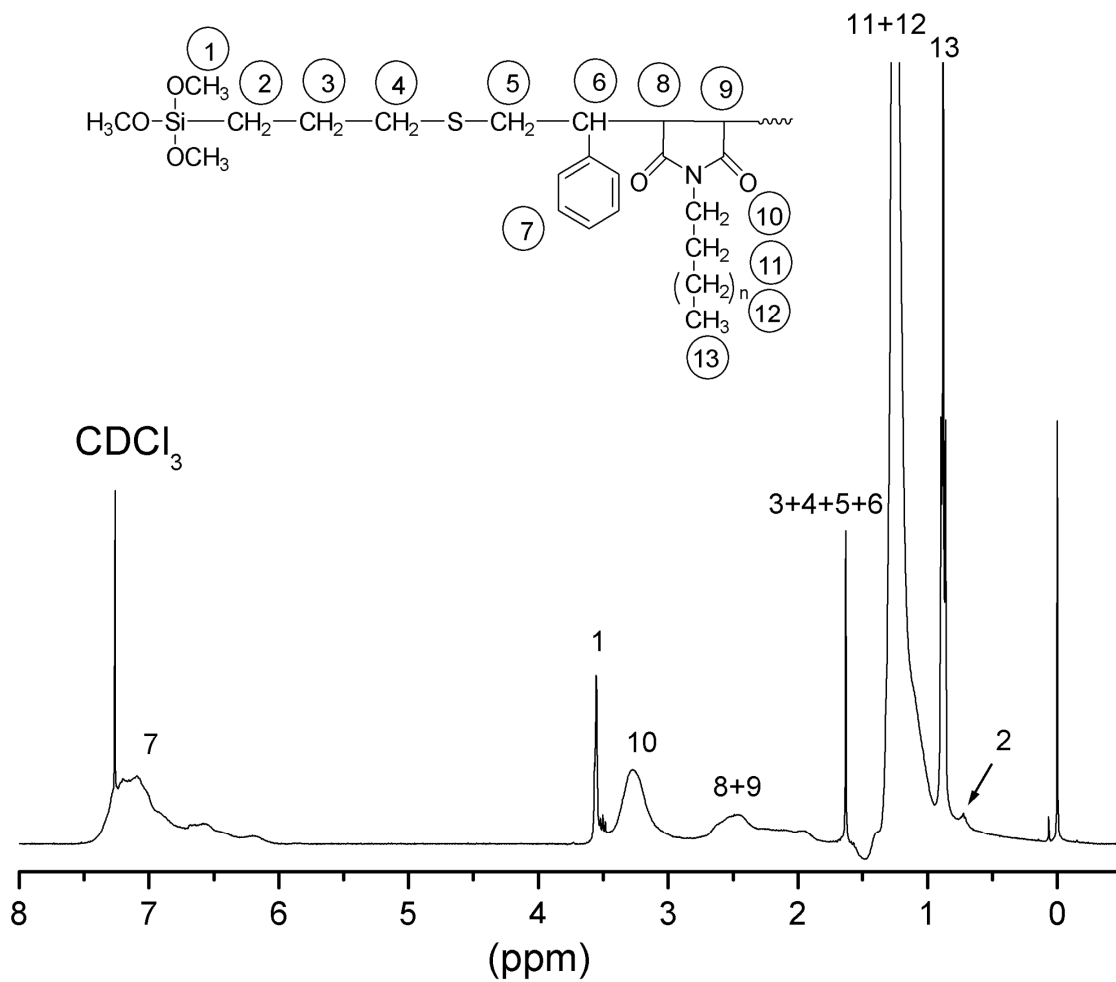
Figure S2 B) ^{1}H NMR spectrum of the end functionalized poly(ST-*alt*-OMI).

Figure S2 C) ^{1}H NMR spectrum of the end functionalized poly(ODA-*alt*-OMI).

Figure S3 Log *k* versus log *P* plots for polymeric ODS and Sil-poly(ODA-*alt*-OMI) stationary phases. Mobile phase: methanol/water (90:10 v/v). Column temperature: 30˚C. Key: a, benzene; b, toluene; c, ethylbenzene; d, butylbenzene; e, hexylbenzene; f, octylbenzene; g, decylbenzene; h, dodecylbenzene; i, naphthalene; j, anthracene; k, naphthacene.