



# Molecular-shape selective high-performance liquid chromatography: Stabilization effect of polymer main chain by alternating copolymerization

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## ABSTRACT

This work aims to clarify that stabilization or increased rigidity of polymer main chains as an organic stationary phase can lead the selectivity enhancement in high-performance liquid chromatography (HPLC). For this purpose, the alternating copolymer of octadecyl acrylate (ODA) with a cyclic monomer (*N*-octadecylmaleimide, OMI) as a rigid segment was synthesized and compared with the ODA homopolymer. Both of the polymer-grafted silicas (Sil-poly(ODA-*alt*-OMI) and Sil-poly(ODA), respectively) were prepared by radical polymerization on 3-mercaptopropyltrimethoxysilane-modified silica. The characterizations were carried out by elemental analyses, diffuse reflectance infrared Fourier transform (DRIFT), and solid-state <sup>13</sup>C cross-polarization magic angle spinning nuclear magnetic resonance (CP-MAS-NMR) spectroscopies. Chromatographic behaviors were evaluated by the retention studies of shape-constrained isomers of polycyclic aromatic hydrocarbons (PAHs), isomers of tocopherol and carotenoids. Higher molecular-linearity selectivity toward PAHs was obtained on Sil-poy(ODA-*alt*-OMI) regardless of temperature changes but at temperature below 40 °C, Sil-poly(ODA) showed better planarity selectivity than that of Sil-poy(ODA-*alt*-OMI). As a result, the higher separation ability toward tocopherols and carotenoids was obtained on Sil-poy(ODA-*alt*-OMI). These results indicate that the stabilization in the polymer main chain by alternating copolymerization and the stabilization in the side chains by a disordered-to-ordered phase transition were effective to enhance the molecular-shape selectivity.

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## 1. Introduction

Copolymerizations of various monomers have been studied for the preparation of new materials with new properties by incorporating two different monomers having diverse chemical and/or physical properties in the same structure [1,2]. Alternating copolymerization of *N*-substituted maleimides with electron-donating monomers is a facile technique for the preparation of copolymers with a high molecular weight in a high yield and modification of their thermal and mechanical behaviors [3–5]. The specialty of the alternating copolymer containing *N*-substituted maleimide repeating units is that they are semiflexible polymers due to the steric hindrance and the restricted rotation along the main chain [6,7]. On the other hand, very few semiflexible-chain polymers are known in the group of synthetic vinyl polymers. It is because the main chain of vinyl polymers consists of successive carbon-to-carbon single bonds, the substituents on which can rotate freely around

them. However, vinyl polymer of octadecyl acrylate (ODA) showed ordered structure due to side chain ordering at lower temperature (below the phase transition, 30 °C) or crystalline state and disordered state at high temperature [8].

We have reported the use of poly(ODA)-grafted silica (Sil-ODA<sub>n</sub>), containing multiple carbonyl groups, as a stationary phase for reversed-phase high-performance liquid chromatography (RP-HPLC) [8]. At lower temperature (below 30 °C) Sil-ODA<sub>n</sub> showed unique separation behavior and very high selectivity toward polycyclic aromatic hydrocarbons (PAHs) [9–13]. Our detailed investigations showed that the highly ordered structure in Sil-ODA<sub>n</sub> induced the orientation of carbonyl groups, which work as a carbonyl- $\pi$  interaction source with solute molecules. We have also found that aligned carbonyl groups at lower temperatures are effective for recognition of the length and planarity of PAHs through multiple carbonyl- $\pi$  interactions [14,15]. Nevertheless, some limitations still remain on the separation of the shape-constrained isomers of large molecules including carotenoids, tocopherols, etc., which are not separable with the above-mentioned stationary phase. Sil-ODA<sub>n</sub> also revealed lower molecular-shape selectivity at higher temperature.

On the other hand, most HPLC separations are performed using octadecylsilylated silica phases (ODSS) or C30 bonded

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phases [16–19]. C30 bonded phase was developed to increase the molecular-shape selectivity [17,18]. Depending on the bonding chemistry, ODS columns can be divided into two types: monomeric and polymeric. Generally, the solutes separation and molecular-shape selectivity achieved using polymeric stationary phases are better than those obtained using monomeric ones [20,21]. To enhance the molecular-shape selectivity, recently Kühnle et al. developed novel stationary phases by bonding sparsely distributed end-capping reagents to silica prior to synthesis of polymeric ODS phases. They intended to produce cavities within the stationary phase with defined characteristics, spectroscopic characterization of the silica surface and corresponding shape selectivity properties [22]. The separation behavior of alkyl-chain-modified silica columns is mainly based on the hydrophobic effect, which has limitations in the separation of shape-constrained isomers or compounds having similar hydrophobicities and polarities [23]. Therefore, substantial attempt has been expended toward developing an understanding of shape selective interactions between the alkyl-chain-bonded stationary phase and the analyte. From careful investigations on the retention behavior of shape-constrained PAH sample probes [22], it has been concluded that the polymeric phases form a rigid ligand structure on the support, explained as a kind of “slot-like” structure. Hence the molecular-shape selectivity of polymeric phases could be explained by the retention model (Slot Model). However, fundamental aspects of molecular recognition are not well-understood for alkyl-chain-bonded stationary phases.

We have reported that alternating copolymer-grafted silica (Sil-poly(octadecyl acrylate-*alt*-*N*-octadecylmaleimide)) showed the specific selective retention with discriminating molecular shapes of PAHs, tocopherols and carotenoids [24]. In this work, we wish to clarify that stabilization of polymer main chains as an organic stationary phase can lead the selectivity enhancement in HPLC. Here we describe the effectiveness of the semiflexible alternating copolymer phase with oriented carbonyl groups along the copolymer main chain in the separation of shape-constrained isomers and compare the results with relatively flexible homopolymer-grafted phase. Side chain rigid (at lower temperature) homopolymer-grafted silica (Sil-poly(ODA)) and main chain rigid or semiflexible alternating copolymer-grafted silica (Sil-poly(ODA-*alt*-OMI)) were synthesized for their application as an HPLC packing materials (Fig. 1). Our previously reported poly(ODA) modified silica column and polymeric ODS (ODS-p) were used as references. The interaction mechanism based on the chemical structures of the organic phases is also described.

## 2. Experimental

### 2.1. Materials and methods

Homopolymer and alternating copolymer, poly(octadecyl acrylate)-grafted silica (Sil-poly(ODA)) and poly(octadecyl acrylate-*alt*-*N*-octadecylmaleimide)-grafted silica (Sil-poly(ODA-*alt*-OMI)) stationary phases, respectively were synthesized, characterized, and packed into a stainless steel columns (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<sup>2</sup> g<sup>-1</sup>) obtained from Kyoto (Japan) was used in all the cases. In contrast, we used commercial polymeric ODS column (Shodex, C18 P 4D, column size 150 mm × 4.6 mm I.D., a 5 μm particle size, a 10 nm pore size, and surface area 320 m<sup>2</sup> g<sup>-1</sup>) containing 17% C obtained from Shodex (Tokyo, Japan) for the comparison of chromatographic results. Maleic anhydride was purchased from TCI (Tokyo, Japan). Octadecyl acrylate (ODA) was obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and used after removing polymerization inhibitor.

Octadecylamine and *trans*-β-carotene were obtained from Aldrich (Tokyo, Japan). The tocopherol isomers were purchased from Calbiochem (Darmstadt, Germany). For the sample preparation, *trans*-β-carotene was photoisomerized based on a literature procedure [25]. 3-Mercaptopropyltrimethoxysilane was purchased from Azmax (Chiba, Japan). HPLC grade methanol was obtained from Wako (Osaka, Japan). All PAHs were commercially available and used without further purification.

### 2.2. Solid-state NMR spectroscopy

All solid-state NMR spectra were measured by using Varian Unity<sup>Inova</sup> AS400 at static magnetic field of 9.4 T using GHX Varian 7 mm VT CP-MAS probe for solid-state <sup>13</sup>C-CP-MAS-NMR. Spin rate of 4000–4500 Hz for solid-state <sup>13</sup>C-CP-MAS-NMR measurements was used.

### 2.3. FT-IR, DRIFT, and <sup>1</sup>H NMR spectroscopy

FT-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. For characterization of synthesis <sup>1</sup>H NMR spectra were recorded on a JEOL JNM-LA400 (Japan) instrument.

### 2.4. HPLC measurement

The chromatographic system 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 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. The retention factor (*k*) measurement was done under isocratic elution conditions. The separation factor ( $\alpha$ ) was given by the ratio of retention factors. The chromatography was done under isocratic elution conditions. The retention time of D<sub>2</sub>O was used as the void volume (*t*<sub>0</sub>) marker.

Water/1-octanol partition coefficient (log *P*) was determined by retention factor with octadecylsilylated silica, ODS (Inertsil ODS-3, 250 mm mm × 4.6 mm I.D., GL Science, Tokyo, Japan): log *P* = 3.759 + 4.207 log *k* (*r* = 0.99997) [14].

### 2.5. Synthesis of stationary phases

Poly(octadecyl acrylate)-grafted silica (Sil-poly(ODA)) (YMC silica gel, 5 μm diameter, pore size 12 nm, surface area 300 m<sup>2</sup> g<sup>-1</sup> containing 15.70% C in the bonded ligand) was synthesized and characterized [8]. The copolymer-grafted silica obtained from octadecyl acrylate and *N*-octadecylmaleimide (Sil-poly(ODA-*alt*-OMI)) was according to our previously reported method [24] containing similar amount of carbon (15.68% C). In both the cases, same diameter, pore size and surface area silica particles were used. The organic phases were characterized by elemental analyses, DRIFT and solid-state <sup>13</sup>C-CP-MAS NMR spectroscopy. The immobilization of organic phases on silica was confirmed by DRIFT spectroscopy. Sil-poly(ODA) demonstrated characteristics signals at 2922, 2852, and 1732. The signals at 2922 and 2852 cm<sup>-1</sup> arise from C–H stretches. The band at 1732 arises from ester carbonyl stretching. Similar peaks were obtained for alternating copolymer-grafted silica (Sil-poly(ODA-*alt*-OMI)). In addition to Sil-poly(ODA-*alt*-OMI) the peaks near 1773 and 1700 cm<sup>-1</sup> are attributed to the symmetrical C=O stretch and the asymmetrical C=O stretch of the imide linkage of the OMI moiety.

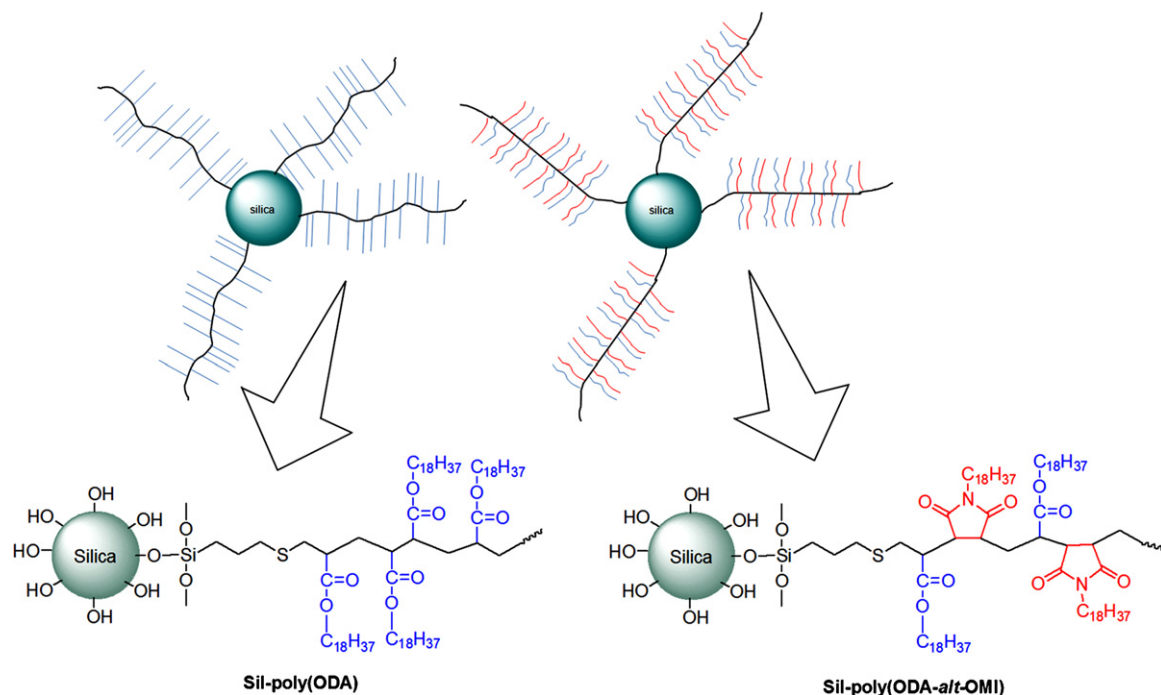


Fig. 1. Schematic illustration of the chemical structures of packing materials.

### 3. Results and discussion

#### 3.1. Solid-state $^{13}\text{C}$ -CP-MAS-NMR

The useful information about the chemical composition of the polymer-grafted silica can be obtained by solid-state  $^{13}\text{C}$ -CP-MAS-NMR spectroscopy. It also offers evidence about the conformation and dynamics of immobilized alkyl chains [26]. The  $^{13}\text{C}$ -CP-MAS-NMR measurements were carried out at different temperatures from 25 to 50 °C to investigate the conformations of the long alkyl chains for both of the grafted organic phases. It is reported that the  $^{13}\text{C}$  signal for  $(\text{CH}_2)_n$  groups 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 attributed to *gauche* conformation, indicating disordered and mobile state [27,28].

We observed that *trans* conformation is dominated in Sil-poly(ODA) at crystalline state or lower temperature, while *gauche* conformation is dominated in Sil-poly(ODA-*alt*-OMI). Fig. 2 shows *trans* to *gauche* ratio of the organic phases at variable temperature. It indicates that Sil-poly(ODA) shows ordered to disordered phase transition with increasing temperature and at high temperature it becomes disordered completely. On the other hand, alkyl chains in Sil-poly(ODA-*alt*-OMI) remain disordered even at lower temperature but at high temperature (above 40 °C), it can keep more ordered state than in Sil-poly(ODA) (Fig. 2). This may be due to the semiflexible nature of the maleimide-based alternating copolymer.

#### 3.2. Retention mode

The retention mode of an organic phase in HPLC can be evaluated by comparing the retention behaviors of alkylbenzenes and PAHs [11,29]. Conventional ODS or alkyl phases can recognize the hydrophobicity of solutes in HPLC 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 and toluene [30]. Fig. 3 shows the correlation between  $\log k$  (capacity factor) and  $\log P$

(water/1-octanol partition coefficient) of ODS, Sil-poly(ODA) and Sil-poly(ODA-*alt*-OMI) phases. The relationship between  $\log k$  and  $\log P$  is used to determine the hydrophobicity recognition ability or retention mode of a stationary phase. From the slope of  $\log k$  vs.  $\log P$  three phases showed similar hydrophobicity or retention mode (Fig. 3). However, it was observed that  $\log k$  and  $\log P$  plots of alkylbenzenes and PAHs in ODS were parallel and almost coincided with each other, providing evidence that ODS phase can

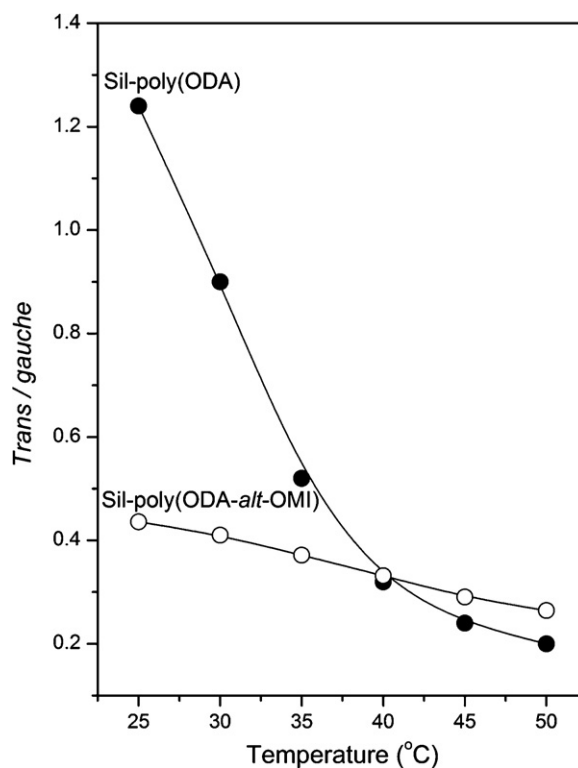
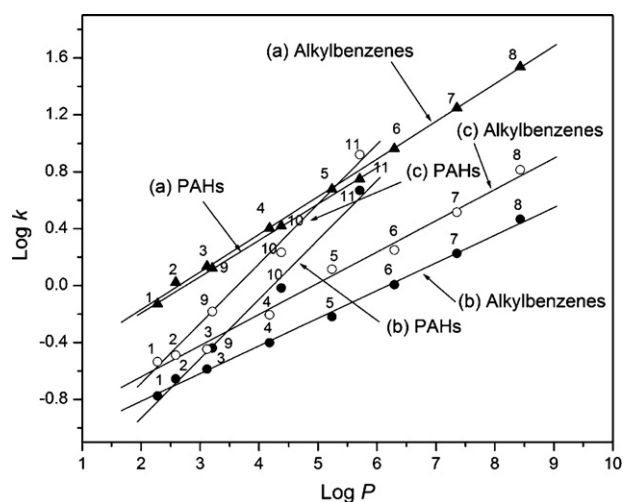


Fig. 2. *Trans* to *gauche* ratio of the stationary phases with variable temperature.



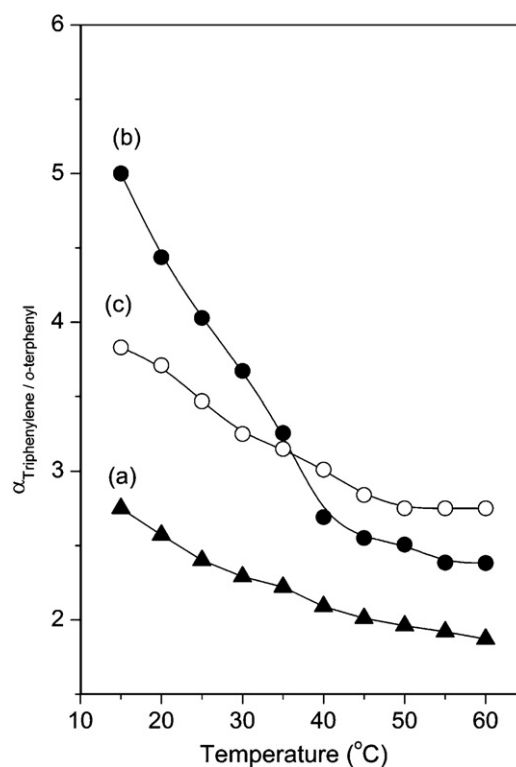
**Fig. 3.** Log  $k$  vs. log  $P$  for: (a) ODS, (b) Sil-poly(ODA), and (c) Sil-poly(ODA-*alt*-OMI) stationary phases. Elutes: 1, benzene; 2, toluene; 3, Et-benzene; 4, Bu-benzene; 5, hexylbenzene; 6, octylbenzene; 7, decylbenzene; 8, dodecylbenzene; 9, naphthalene; 10, anthracene; 11, naphthacene. Mobile phase: methanol–water = 90:10, column temperature: 30 °C, flow rate: 1.00 ml min<sup>-1</sup>.

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. For example, the log  $P$  of naphthacene (5.71) is smaller than that of octylbenzene (6.29), while log  $k$  value of naphthacene (0.92) is higher than that of octylbenzene (0.25). The increase of log  $k$  for PAHs was accompanied by selectivity enhancement which provides specific interactive sites as well as molecular hydrophobicity for PAHs.

### 3.3. Molecular-shape selectivity for PAHs in RP-HPLC

Molecular-shape selectivity is essentially powerful for the separation of shape-constrained isomers 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 and co-workers [31], which is defined as follows:  $F = (\text{number of double bonds}) + (\text{number of primary and secondary carbons}) - 0.5(\text{number of nonaromatic rings})$ . The selectivity for the two-dimensional shape has been further studied with a molecular shape descriptor, length-to-breadth ( $L/B$ ) ratio. The parameter was proposed by Wise et al. [32] and Kaliszán et al. [33] 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 octadecylsilylated silica (ODS) phases [34]. Tanaka and co-workers [30] established a commonly used method to determine molecular-shape selectivity. In this work, two compounds containing the same number of carbon atoms and  $\pi$ -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, while the value on a polymeric ODS phase lies between 2.0 and 2.7. There are some other types of commercial columns, which have higher selectivity values [35]. 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. [36,37].

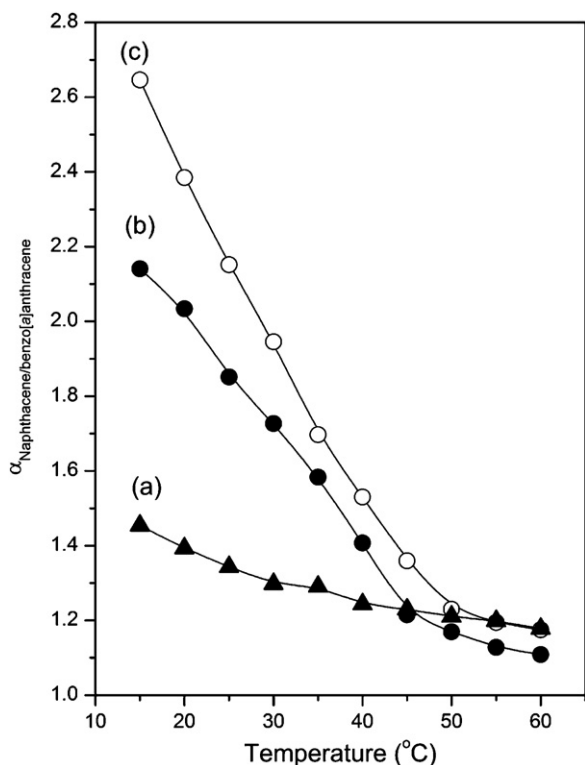
Fig. 4 shows that both Sil-poly(ODA) and Sil-Poly(ODA-*alt*-OMI) phases demonstrated remarkably higher planarity selectivity



**Fig. 4.** Temperature dependencies of the separation factors between triphenylene and *o*-terphenyl with: (a) ODS-p, (b) Sil-poly(ODA) and (c) Sil-poly(ODA-*alt*-OMI). Mobile phase: methanol–water = 90:10, flow rate: 1.00 ml min<sup>-1</sup>.

than polymeric ODS phase (ODS-p). However, at lower temperature Sil-Poly(ODA) showed higher planarity selectivity than Sil-Poly(ODA-*alt*-OMI) and at higher temperature vice versa. These results may be due to the fact that at lower temperature the alkyl chains in Sil-poly(ODA) are very rigid and highly ordered as determined by solid-state <sup>13</sup>C-CP-MAS-NMR spectroscopy. In the case of Sil-poly(ODA), at lower temperature, ordered alkyl chains help to orient the carbonyl groups in ordered state. Therefore, it is difficult for non-planar compounds enter into the interaction sites (carbonyl groups) than planar compounds at lower temperature and elutes earlier and at higher temperature no such effect occurred due to disordered alkyl chains. Ordered carbonyl groups are believed to be responsible for multiple carbonyl– $\pi$  interactions with the solutes (Fig. S1, Supporting Information). For planar triphenylene, interaction aspect ratio is higher than non-planar *o*-terphenyl. The detailed interaction mechanism is given in Section 3.5. On the other hand, in the case of Sil-poly(ODA-*alt*-OMI) rigidity of the alkyl chains are not important and penetration effect is less due to disordered alkyl chains. Nevertheless, in Sil-poly(ODA-*alt*-OMI) polymer main chain (carbonyl groups) remain ordered state or stable and keeps carbonyl groups in ordered state to facilitate carbonyl– $\pi$  interactions even at higher temperature and thus showed better planarity- or shape-selectivity at higher temperature (Fig. S2, Supporting Information).

Second examination was carried out with two non-linear and linear PAHs. Naphthacene and benzo[*a*]anthracene both have the same number of carbon atoms and  $\pi$ -electrons, but differ in their molecular length and aspect ratio ( $L/B = 1.90$  and  $L/B = 1.60$ , respectively). In the case of molecular-linearity selectivity, we also observed very high linearity selectivity on Sil-poly(ODA) and Sil-poly(ODA-*alt*-OMI) compared to polymeric ODS column as shown in Fig. 5. Here, Sil-poly(ODA-*alt*-OMI) showed better selectivity than Sil-poly(ODA) even at lower temperature. This phenomenon can be explained again by the multiple carbonyl– $\pi$  interactions



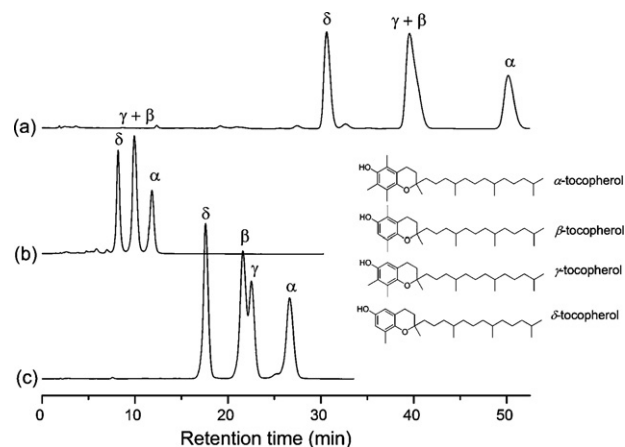
**Fig. 5.** Temperature dependencies of the separation factors between naphthacene and benzo[*a*]anthracene with: (a) ODS-p, (b) Sil-poly(ODA) and (c) Sil-Poly(ODA-*alt*-OMI). Mobile phase: methanol–water = 90:10, flow rate: 1.00 ml min<sup>-1</sup>.

with the linear molecules due to oriented carbonyl groups along the polymer main chain stabilized by alternating copolymerization of ODA and OMI [24]. At the same time, large interaction aspect ratio is possible for linear molecules than non-linear molecules (Fig. S3, Supporting Information).

### 3.4. Separation to tocopherol and carotenoid isomers

Due to very high planarity and linearity selectivities of Sil-poly(ODA) and Sil-poly(ODA-*alt*-OMI), we tried to apply these phases to separate some biologically important shape-constrained isomers such as vitamin E (tocopherol) and carotenoids that are difficult to separate by conventional and polymeric ODS phases. The vitamin E or tocopherols offer versatile health benefits and is important for mitochondrial electron-transport function in physiological systems. Due to their lipid soluble antioxidative properties, these compounds inhibit lipid peroxidation processes of polyunsaturated fatty acids and other compounds in cell membranes [38]. Antioxidant activity of tocopherols has been confirmed in bulk oil and emulsion model systems [39] as well as in shelf life and oxidograph tests [40]. Tocopherols protect humans from the oxidative stress mediated by active oxygen and nitrogen species. The protective role of vitamin E against atherosclerosis, cardiovascular diseases, cataracts, neural tube defects and cancer has been the subject of extensive studies [41]. Landrier et al. recently reported that both  $\alpha$ - and  $\gamma$ -tocopherols significantly decrease endogenous cholesterol synthesis and apo-AI-mediated cholesterol secretion in Caco-2 cells [42]. Therefore, the separation of tocopherol isomers is very essential. However, among the  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -isomers of tocopherol, the separation of  $\beta$ - and  $\gamma$ -tocopherols is the most challenging because of their structural similarity [43]. They differ only in the position of the methyl groups (Fig. 6).

The separations of tocopherol isomers with ODS-p, Sil-poly(ODA), and Sil-poly(ODA-*alt*-OMI) phases are shown in



**Fig. 6.** Separation of tocopherol isomers with: (a) ODS-p, (b) Sil-poly(ODA) and (c) Sil-Poly(ODA-*alt*-OMI). Mobile phase: methanol–water = 90:10, column temperature: 30 °C, flow rate: 1.00 ml min<sup>-1</sup>.

**Fig. 6.** This clearly shows the separation ability of alternating copolymer-grafted silica, Sil-poly(ODA-*alt*-OMI) compared to homopolymer-grafted silica phase (Sil-poly(ODA)) and polymeric ODS phase.

On the other hand, carotenoids constitute a group of compounds that continue to be studied for their possible ability to reduce the risk of developing certain types of cancer, and  $\beta$ -carotene has received the most attention in this regard [44]. However,  $\beta$ -carotene can occur as a variable mixture of *trans*- $\beta$ -carotene and several mono-, di-, and tri-*cis* isomers. These *cis* isomers have less provitamin A activity than *trans*- $\beta$ -carotene; therefore accurate determination of the *trans* form in the presence of *cis* isomers is nutritionally important.

The difficulty in the separation of these isomers is that they differ only in the characteristics of their molecular-shape, such as linearity or some bending as shown in Fig. 7.

Fig. 8 shows the chromatograms of all-E  $\beta$ -carotene isomers with ODS-p, Sil-poly(ODA) and Sil-poly(ODA-*alt*-OMI). We observed that Sil-poly(ODA-*alt*-OMI) had better separation ability of the critical isomer mixture than other phases. The peak of the most linear isomer (all-E) was identified by comparing the retention time of injected samples before and after isomerization. The other peaks were estimated according to the molecular linearity of the isomers (Fig. S4, Supporting Information). The separation of  $\beta$ -carotene isomers at very low temperature (5 °C) was only realized due to the very high molecular linearity selectivity of Sil-poly(ODA-*alt*-OMI), a result which agreed with the results shown in Fig. 5. At lower temperature, may be with rigid polymer main chain, slightly ordered alkyl chains synergistically enhance the degree of ordering of carbonyl groups in Sil-poly(ODA-*alt*-OMI) (solid-state <sup>13</sup>C-CP-MAS was not measured at 5 °C).

### 3.5. Molecular-recognition mechanism

There are chemical and physical property differences between Sil-poly(ODA) and Sil-poly(ODA-*alt*-OMI), which reflect on their selectivity differences. For example, alkyl chains in Sil-poly(ODA) can form ordered structure at lower temperature (below 30 °C) which help to orient carbonyl groups and enhance selectivity by multiple carbonyl- $\pi$  interactions. At a higher temperature it becomes more flexible than Sil-poly(ODA-*alt*-OMI) due to homopolymer structure and therefore, carbonyl groups become completely disordered (not suitable for multiple carbonyl- $\pi$  interactions). On the other hand, Sil-poly(ODA-*alt*-OMI) form semi-flexible structure due to incorporation of maleimide ring in the

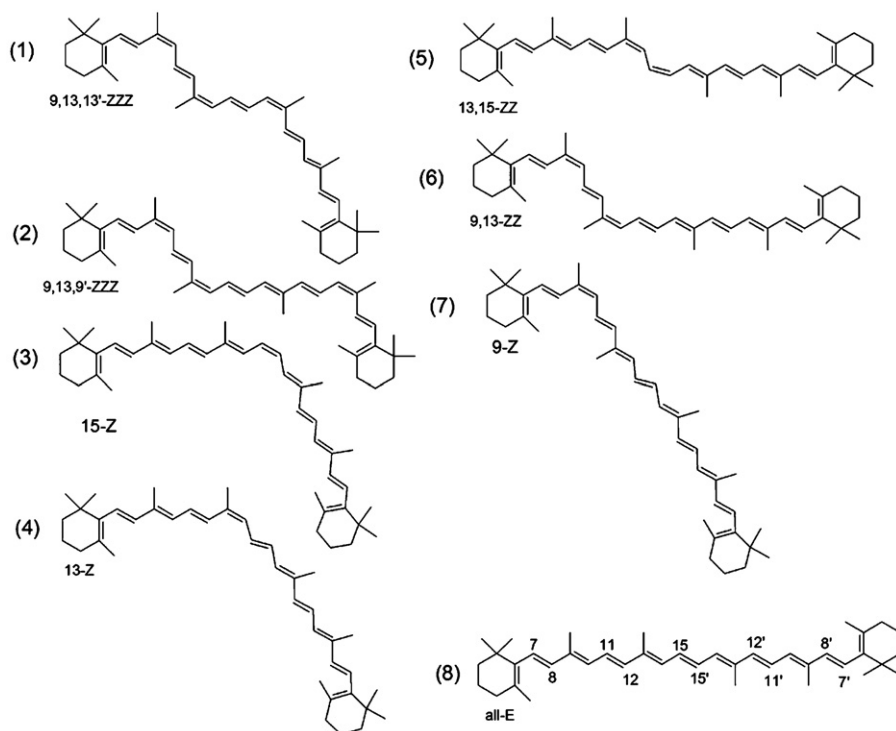


Fig. 7. Chemical structures of the  $\beta$ -carotene isomers.

polymer main chain even though alkyl chains are not ordered and this main chain rigidity (help to orient carbonyl groups) can retain up to higher temperature than Sil-poly(ODA) as determined by solid-state NMR (Fig. 2). Additionally, Sil-poly(ODA-*alt*-OMI) had concentrated carbonyl groups because OMI contain two carbonyl groups in a molecule and multiple carbonyl- $\pi$  interactions are favorable. Generally, the molecular-shape selectivity in the ODS phase increases with increasing carbon loading and alkyl chain length [20,21,23,45–48]. This phenomenon has been attributed to slight increase of alkyl chain ordering but not for direct interaction with guest molecules. Sil-poly(ODA-*alt*-OMI), however, showed better selectivity, regardless of the fact that not only does it have lower carbon loading (%C 15.68) than the ODS-p phase (%C

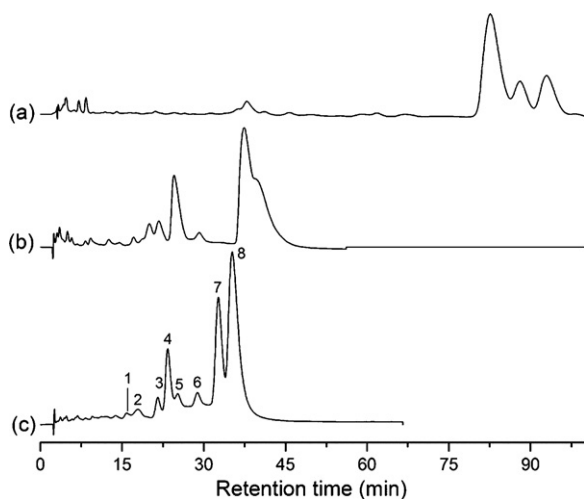


Fig. 8. Separation of  $\beta$ -carotene isomers with: (a) ODS-p, (b) Sil-poly(ODA) and (c) Sil-Poly(ODA-*alt*-OMI). Mobile phase: methanol, column temperature: 5 °C, flow rate: 1.00 ml min<sup>-1</sup>.

17) but also the alkyl chains of Sil-poly(ODA-*alt*-OMI) are not ordered completely and are rather flexible, as indicated by the NMR results. Therefore, direct interaction with carbonyl groups is proposed for poly(ODA-*alt*-OMI) and the multiple carbonyl- $\pi$  interaction with large interaction aspect ratio was enhanced by semiflexible alternating copolymer-grafted silica even at higher temperature (Figs. S2 and S3, Supporting Information). Previously we reported about multiple carbonyl- $\pi$  interactions for the Sil-poly(ODA) at lower temperature [15]. The higher selectivity of Sil-poly(ODA-*alt*-OMI) cannot be explained by the hydrophobic effect only with solute molecules or molecular Slot Model of ODS-p phase as we described in Sections 3.2 and 3.3. Hence, other interactions are involved with oriented carbonyl groups in the enhanced selectivity of Sil-poly(ODA-*alt*-OMI) toward PAHs and isomers of tocopherol and  $\beta$ -carotene. Recently, we reported about the oriented carbonyl groups interactions in a chirally ordered molecular gel forming compounds-grafted silica phase [49]. A carbonyl- $\pi$  interaction has been discussed in our previous calculation works [50]. This interaction in a model complex of HCHO-benzene is much stronger (1.87 kcal mol<sup>-1</sup>) than a CH<sub>4</sub>-benzene interaction (0.53 kcal mol<sup>-1</sup>) and a plane-to-plane interaction between two benzenes (0.49 kcal mol<sup>-1</sup>) [50]. Selectivity enhancement through a carbonyl- $\pi$  interaction has been also discussed in relation to homopolymers from octadecyl acrylate poly(ODA). Previously, poly(ODA)-grafted silica (Sil-ODA<sub>n</sub>) was evaluated by the retention time of PAHs, the resultant selectivity was higher, especially at lower temperature or crystalline state ODA<sub>n</sub> than for ODS columns [14,15]. Here, we also found similar higher selectivity for Sil-poly(ODA) at lower temperature (Figs. 4 and 5). This is attributed to the fact that at a lower temperature, alkyl chains as well as carbonyl groups keep in ordered state, so that a multiple carbonyl- $\pi$  interaction with PAHs becomes possible. In contrast, in Sil-poly(ODA-*alt*-OMI) alkyl chains are not completely ordered even at lower temperature, but a slight ordering of the alkyl chain can keep at higher temperature (Fig. 2) and the semiflexible polymer main chain leads to the linear ordering of carbonyl groups

derived from both maleimide and acrylate. As a result, multiple carbonyl- $\pi$  interaction is more advantageous for molecular linearity selectivity even at lower temperature and planarity selectivity at higher temperature (Figs. 4 and 5) and, therefore, to enhance molecular linearity and planarity selectivity for PAHs as well as tocopherol and  $\beta$ -carotene isomers (Figs. 6 and 8). No such interaction is available for the ODS phases.

#### 4. Conclusion

In the present work, the merits of using alternating copolymer-based stationary phase for selectivity enhancement toward PAHs, tocopherols and carotenoids over homopolymer-based stationary phase have been discussed. Higher molecular-shape selectivities were obtained by using alternating copolymer-grafted phase than homopolymer-grafted silica stationary phase as well as polymeric ODS phase. Selectivity enhancement for the shape-constrained isomers attributed to the stabilization effect of the *N*-substituted maleimide-based alternating copolymer-based stationary phase, which can orient the weak interactions sites (integrated carbonyl groups) along the polymer main chain to facilitate multiple carbonyl- $\pi$  interactions even at higher temperature. Therefore, alternating copolymer with appropriate comonomer will be very helpful for selectivity tuning of analytes.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.11.061.

#### References

- [1] D.A. Tirrell, in: H. Mark, N.M. Bikales, C.G. Overberger, G. Menges (Eds.), *Encyclopedia of Polymer Sciences and Engineering*, vol. 4, 2nd ed., John Wiley & Sons, New York, 1985, p. 192.
- [2] A.K. Mallik, M. Takafuji, H. Ihara, *J. Chromatogr. A* 1216 (2009) 7433.
- [3] A. Matsumoto, T. Kubota, T. Otsu, *Macromolecules* 23 (1990) 4508.
- [4] J.R. Duke, M.A. Hoisington Jr., D.A. Langlois, B.C. Benicewicz, *Polymer* 39 (1998) 4369.
- [5] F. Yilmaz, L. Cianga, Y. Guner, L. Topppare, Y. Yagci, *Polymer* 45 (2004) 5.
- [6] A. Matsumoto, T. Kubota, T. Otsu, *Polym. Bull.* 24 (1990) 459.
- [7] A. Matsumoto, S. Umehara, H. Watanabe, T. Otsu, *J. Polym. Sci. Part B: Polym. Phys.* 31 (1993) 527.
- [8] C. Hirayama, H. Ihara, T. Mukai, *Macromolecules* 25 (1992) 6375.
- [9] T. Fukumoto, H. Ihara, S. Sakaki, H. Shosenji, C. Hirayama, *J. Chromatogr. A* 672 (1994) 237.
- [10] A.K. Mallik, M.M. Rahman, M. Czaun, M. Takafuji, H. Ihara, *Chem. Lett.* 36 (2007) 1460.
- [11] A.K. Mallik, M.M. Rahman, M. Czaun, M. Takafuji, H. Ihara, *J. Chromatogr. A* 1187 (2008) 119.
- [12] M.A.J. Chowdhury, H. Ihara, T. Sagawa, C. Hirayama, *J. Chromatogr. A* 877 (2000) 71.
- [13] H. Ihara, H. Tanaka, S. Nagaoka, S. Sakaki, C. Hirayama, *J. Liq. Chromatogr.* 19 (1996) 2967.
- [14] H. Ihara, T. Sagawa, Y. Goto, S. Nagaoka, *Polymer* 40 (1999) 2555.
- [15] H. Ihara, Y. Goto, T. Sakurai, M. Takafuji, T. Sagawa, S. Nagaoka, *Chem. Lett.* (2001) 1252.
- [16] C.M. Bell, L.C. Sander, S.A. Wise, *J. Chromatogr. A* 757 (1997) 29.
- [17] S. Strohschein, M. Pursch, D. Lubda, K. Albert, *Anal. Chem.* 70 (1998) 13.
- [18] S. Schauff, V. Friebohn, M.D. Grynbaum, C. Meyer, K. Albert, *Anal. Chem.* 79 (2007) 8323.
- [19] Y. Saito, H. Ohta, K. Jinno, *J. Sep. Sci.* 26 (2003) 225.
- [20] L.C. Sander, S.A. Wise, *Anal. Chem.* 56 (1984) 5044.
- [21] L.C. Sander, S.A. Wise, *Anal. Chem.* 59 (1987) 2309.
- [22] M. Kühnle, V. Friebohn, K. Albert, C.A. Rimmer, K.A. Lippa, L.C. Sander, *Anal. Chem.* 81 (2009) 10136.
- [23] L.C. Sander, M. Pursch, S.A. Wise, *Anal. Chem.* 71 (1999) 4821.
- [24] A.K. Mallik, T. Sawada, M. Takafuji, H. Ihara, *Anal. Chem.* 82 (2010) 3320.
- [25] L. Zechmeister, A. Polger, *J. Am. Chem. Soc.* 65 (1943) 1522.
- [26] H.R. Ansarian, M. Derakhshan, M.M. Rahman, T. Sakurai, M. Takafuji, I. Taniguchi, H. Ihara, *Anal. Chim. Acta* 547 (2005) 179.
- [27] M. Pursch, S. Strohschein, H. Handel, K. Albert, *Anal. Chem.* 68 (1996) 386.
- [28] A.E. Tonelli, F.C. Schiling, F.A. Bovey, *J. Am. Chem. Soc.* 106 (1984) 1157.
- [29] H. Ihara, W. Dong, T. Mimaki, M. Nishihara, T. Sakurai, M. Takafuji, S. Nagaoka, *J. Liq. Chromatogr.* 26 (2003) 2473.
- [30] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, *J. Chromatogr. Sci.* 27 (1989) 721.
- [31] J.F. Schborn, R.J. Hurtubise, H.F. Silver, *Anal. Chem.* 49 (1983) 2253.
- [32] S.A. Wise, W.J. Bonnet, F.R. Guenther, W.E. May, *J. Chromatogr. Sci.* 19 (1981) 248.
- [33] H. Kalisz, Lamparczyk, W.J. Bonnet, F.R. Guenther, W.E. May, *J. Chromatogr. Sci.* 16 (1978) 246.
- [34] K. Jinno (Ed.), *Chromatographic Separations Based on Molecular Recognition*, Wiley-VCH, New York, 1997.
- [35] M.R. Euerby, P. Petersson, *J. Chromatogr. A* 994 (2003) 13.
- [36] K. Jinno, K. Yamamoto, H. Nagashima, T. Ueda, K. Itoh, *J. Chromatogr.* 517 (1990) 193.
- [37] K. Jinno, T. Ibuki, N. Tanaka, M. Okamoto, J.C. Fretzer, W.R. Biggs, P.R. Griffiths, J.M. Olinger, *J. Chromatogr.* 461 (1989) 209.
- [38] J.K. Lang, H. Esterbauer, in: C. Vigo-Pelfrey (Ed.), *Membrane Lipid Oxidation*, vol. 3, CRC Press, Boca Raton, FL, 1990, p. 265.
- [39] K.H. Wagner, A. Kamal-Eldin, I. Elmadfa, *Ann. Nutr. Metab.* 48 (2004) 169.
- [40] M. Nogala-Kalucka, J. Korczak, K.-H. Wagner, I. Elmadfa, *Nahrung* 48 (2004) 34.
- [41] A. Azzì, A. Stocker, *Prog. Lipid Res.* 39 (2000) 231.
- [42] J.F. Landrier, E. Gouranton, E. Reboul, N. Cardinault, C.E. Yazidi, C.M. Desmoulin, M. Andre, M. Nowicki, M. Saudidi, P. Borel, *J. Nutr. Biochem.* 21 (2010) 1207.
- [43] S.L. Abidi, *J. Chromatogr. A* 881 (2000) 197.
- [44] R.G. Ziegler, *Ann. N.Y. Acad. Sci.* 691 (1993) 110.
- [45] K. Jinno, K. Tanabe, Y. Saito, H. Nagashima, *Analyst* 122 (1997) 787.
- [46] K.B. Sentell, J.G. Dorsey, *J. Chromatogr.* 461 (1989) 193.
- [47] K.B. Sentell, J.G. Dorsey, *Anal. Chem.* 61 (1989) 930.
- [48] N. Tanaka, K. Sakagami, J. Araki, *J. Chromatogr.* 16 (1978) 327.
- [49] A.K. Mallik, H. Qiu, T. Sawada, M. Takafuji, H. Ihara, *Chem. Commun.* 47 (2011) 10341.
- [50] S. Sakaki, K. Kato, T. Miyazaki, K. Ohkubo, H. Ihara, C. Hirayama, *J. Chem. Soc. Faraday Trans.* 89 (1993) 659.