

Self-Assembled Organic Phase for RP/HPLC

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Abstract

Molecular ordering through self-assembling of organic stationary phase is very useful for increasing selectivity in high-performance liquid chromatography (HPLC). Herein, we introduce comb-shaped polymers as self-assembled organic stationary phase for HPLC so that the selectivity can be robustly enhanced by increasing polymer chain rigidity as well as grafting density. For example, comb-shaped poly(octadecyl acrylate)-grafted silica (prepared by *grafting-to* and *grafting-from* methods) columns showed very high selectivity toward polycyclic aromatic hydrocarbon (PAH) solutes with regard to grafting technique onto silica. A comparison of the results obtained with synthetic columns and with commercial octadecylsilylated silica (ODS) revealed the effectiveness of tailor-made columns. Potential application of polypeptide lipid-grafted type stationary phases for molecular recognition has also been described.

INTRODUCTION

Liquid chromatography has become an indispensable tool for both routine analysis and research in the pharmaceutical, biomedical, and biotechnology industries. On an analytical level reversed-phase high-performance liquid chromatography (RP-HPLC) is the most widespread technique, probably owing to the broad applicability of that mode of separation to a wide range of compounds and sample matrices. The majority of bonded phases employed in RP-HPLC are still of the reversed-phase *n*-alkyl type, mostly octadecylsilylated silica (ODS) or C18 and C8. The development of new chemically bonded stationary phases for reversed-phase liquid chromatography (RPLC), engineered for solving specific separation problems, has led to improved analyses of a broad range of compounds. Refinements in approaches used to characterize chemically modified surfaces have resulted in increased understanding of stationary-phase morphology, which in turn has permitted development of novel stationary phases with properties tailored for specific applications. Usually, retention and selectivity of solutes increases with increasing grafting density of the organic phase on silica.^[1,2] Molecular ordering of the organic phase also increases the selectivity.^[3,4] On the basis of these viewpoints, polymeric phases are reasonable tools as organic stationary phases, and polymer immobilization process can also play an important role in increasing the grafting

density as well as improving molecular ordering. Generally, the surfaces of inorganic materials are functionalized with polymer chains by using either the *grafting-to* or the *grafting-from* method. *Grafting-to* method has some limitations while there are distinct advantages in the control of polymerization degree; a polymer with a terminal reactive group can be obtained by one-step telomerization, and usual spectroscopy is applicable for determination of the chemical structure before immobilization onto silica. The main disadvantage of this method is low grafting densities resulting from steric crowding of the reactive sites.^[5] On the other hand, the *grafting-from* technique results in significantly higher grafting density because the steric barrier to incoming polymers imposed by the in situ grafted chains does not limit the access of smaller monomer molecules to the active initiation sites.^[6-8] Though *grafting-to* is still a good method for the preparation of HPLC stationary phases, increasing attention has been devoted to *grafting-from* techniques, especially for high retentivity and selectivity.^[9-11] One of the most extensively used *grafting-from* method is atom transfer radical polymerization (ATRP)^[12,13] from initiator-grafted inorganic particle surface. In this entry, we introduce high-density polymeric organic phases with π -electron-containing moieties as stationary phases for selectivity enhancement using surface-initiated ATRP (*grafting-from*) technique. Further, it is also described how the concentration of carbonyl π -electrons, through peptide-derived low

molecular compounds and polymers, brings about extremely high selectivity enhancement in HPLC.

SELF-ASSEMBLED PHASES FOR MOLECULAR RECOGNITION

Biological organisms are constructed by molecular building blocks, and these molecules are assembled spontaneously through various intermolecular interactions.^[14,15] Self-assembling systems provide supramolecular functions further than those of unit segment molecules in a solution state such as lipid membranes, proteins, and nucleic acids. For instance, self-assembled systems such as lipid membrane aggregates can provide a highly ordered microenvironment leading to a unique host-guest chemistry exceeding the functions of the original lipid. Thus, there are many reports on biomimetic approaches to reproduce lipid membrane functions with totally synthetic lipids.^[16–18] One of the successful results is seen in L-glutamide-derived amphiphiles. For example, dialkyl L-glutamide-derived amphiphilic lipids form nanotubes,^[19] nanohelices,^[20] and nanofibers^[21] based on bilayer structures in water and on the fact that intermolecular hydrogen bonding among the amide moieties not only contributes self-assembly^[20] but also shows a very unique secondary chirality with extremely strong circular dichroism (CD) signals.^[20] Another unique self-organization has been realized by lipophilic derivatives of L-glutamide even in organic solvents.^[22] These low-molecular compounds can form nanofibrillar aggregates in organic solvents to make a gel through a three-dimensional (3-D) network formation. Hence, this phenomenon is often called as “molecular gelation,” but the functions are rather more similar to those of lipid bilayer membranes than to those of conventional polymer gels.

This knowledge encourages us to apply biomimetic membranes for molecular recognition systems such as HPLC. However, lipid membrane systems suffer from a serious problem owing to their instability in organic solvents. To overcome this problem, we have reported the use of poly(octadecyl acrylate)-grafted silica (Sil-ODA_n) as a lipid membrane analogue for stationary phases in RP-HPLC.^[23] Poly(octadecyl acrylate) cannot

form bilayer structures in water but forms “nanogels” which undergo temperature-responsive phase transition between ordered and disordered structures like lipid membrane systems. Sil-ODA_n showed unique separation behaviors with ordered-to-disordered phase transitions of long alkyl chains. In particular, very high selectivity toward polycyclic aromatic hydrocarbons (PAHs) was observed in the ordered (crystalline) state.^[24,25] 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]

GRAFTING OF COMB-SHAPED POLYMER AS AN ORDERED ORGANIC PHASE

Polymer grafting onto a silica surface is a very useful approach to introduce highly ordered organic phases. Three main routes are usually reported for chemically attaching a polymer to a surface: 1) the *grafting-to* method, where end functionalized polymers react with appropriate surface sites; 2) the *grafting-from* method, where chains grow in situ from preformed surface-grafted initiators; and 3) surface copolymerization through a covalently linked monomer.

Polymer *Grafting-to* Method

Single-anchor grafting

A comb-shaped polymer as a lipid membrane analogue is readily prepared by radical telomerization of octadecyl acrylate. By using 3-mercaptopropyl trimethoxysilane (MPS) as a telogen, not only the degree of polymerization (*n*) can be controlled by adjusting the initial molar ratio of the monomer to telogen, but also the reactive terminal group can be introduced at one end of the polymer main chain. Thus, poly(octadecyl acrylate)-grafted silica (Sil-ODA_n) is prepared by mixing the polymer with appropriate porous silica gels (Fig. 1).^[27]

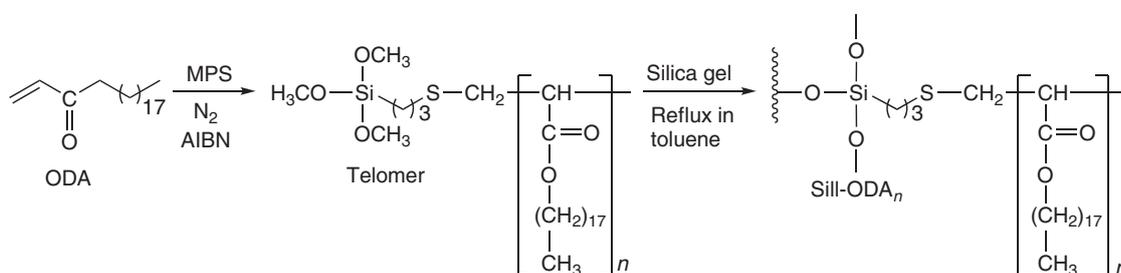


Fig. 1 Synthesis process for polymeric organic phase by one-step telomerization followed by immobilization onto silica through the terminal methoxysilyl group (grafting-to method).

The average degree of polymerization (n) is estimated by the proton ratio, based on the terminal methoxy group using proton nuclear magnetic resonance (H-NMR) spectroscopy. Immobilization of ODA $_n$ is usually carried out by mixing with silica at reflux temperature for 72 hr in toluene. Silica gels with different pore sizes and diameters can be employed for the grafting process. Usually, silica gels with an average diameter of 5 μm , a pore size of 120 \AA , and a specific surface area of 300 m^2/g are considered to be suitable for HPLC. The resultant polymer-grafted silicas indicate a distinct phase transition that can be assigned to a crystal-to-isotropic transition (Fig. 2a). This phase transition temperature is observed even in methanol, ethanol, and acetonitrile, and in their aqueous mixtures acting as usual mobile phases in RP-HPLC, although the peak-top temperature (T_c) somewhat decreases owing to solvation.^[28]

The column packed with Sil-ODA $_n$ shows very unique separation in RP-HPLC. Especially the uniqueness is emphasized when the solutes are PAHs. An extremely high separation factor, as compared with conventional ODS columns, is observed at temperatures below T_c : e.g., the separation factor $\alpha_{\text{pentacene/chrysene}}$ is 17.6 and 1.6 for Sil-ODA $_n$ and conventional ODS columns respectively. To explain this unusual selectivity, we have proposed the multiple π - π interaction mechanism between PAHs and carbonyl groups of acrylate moieties in the ordered state.^[27] This interaction is quite possible according to our previous calculations and experiments: 1) Fig. 3a shows the temperature dependencies of the separation factor α for geometrical isomers of stilbene. The poly(methyl acrylate) phase is less hydrophobic than Sil-ODA $_n$ and ODS, as well as in a disordered state because of the absence of any long-chain alkyl groups, but the selectivity is distinctly higher than that in ODS. 2) A carbonyl- π -benzene- π interaction was simulated by the ab initio study.

When HCHO and benzene was chosen as a model complex, the potential-energy curves indicated that the binding energy in HCHO-benzene was much larger (1.87 kcal/mol)^[28-30] than that in benzene-benzene (0.49 kcal/mol) in plane-to-plane stacking.^[31] 3) The selectivity with geometrical isomers from various substituted azobenzene compounds was investigated.^[26] As a result, it was found that the separation factor between the *trans*- and *cis*-isomers was remarkably dependent on the electron-donating property of the substituent group. This strongly suggests that bonded ODA $_n$ works as an electron-acceptor and a π - π interaction is brought about by a carbonyl- π moiety in ODA $_n$. This estimation was confirmed by the observation that addition of acetone as a carbonyl group-containing solvent to a mobile phase reduced the selectivity remarkably, whereas the presence of 2-propanol had almost no effect. Acetone works as a sort of an inhibitor for ODA $_n$ -PAHs interactions.

These observations prompt us to propose multiple π - π interactions between carbonyl groups and PAHs. ODA $_n$, which is in an ordered state, interacts better with planar and slender PAHs such as *trans*-stilbene,^[32] naphthalene, and pentacene^[27] than with non-planar and bulky substances such as *cis*-stilbene^[32] and *o*-terphenyl,^[23] and less slender substances such as triphenylene^[23] and coronene.^[33] However, these unique selectivities distinctly decrease when ODA $_n$ is in a disordered state, but rather similar to that of ODS. As shown in Fig. 3b, it is highly probable that multiple π - π interactions are more effective both in an ordered state and for planar and slender substances, but not in a disordered state.

Multianchor grafting

A reduction in the molecular mobility of a polymer chain as an organic stationary phase would lead to an increase in the selectivity in HPLC. Porphyrin derivative-bonded phases show a unique shape selectivity by retaining planar PAHs.^[34] Similar molecular-planarity selectivity is also observed in cholesteryl-10-undecenoate-^[35] and 4,4'-dipentyl-diphenyl-bonded phases.^[36] These phases contain rigid structures, and hence the limited mobility in their organic phases contributes to the shape selectivity. Similar phenomenon of selectivity increase can be observed in the comparison of the retention behaviors of polymeric and monomeric ODSs.^[37]

From this viewpoint, poly(octadecyl acrylate) having the plural reactive groups in the side chain has been synthesized and immobilized onto silica (Fig. 2b). The polymer is obtained by a one-step cotelomerization with ODA and methacryloxypropyl trimethoxysilane (MAPTS). The monomer composition of the resultant copolymer is readily adjusted by the initial molar ratio and then estimated by ¹H-NMR spectroscopy. Sil-co-ODA $_n$ prepared by multi-anchoring showed remarkably higher selectivity for PAHs with different molecular planarity (thickness) than for the corresponding Sil-ODA $_n$ prepared by single anchoring although no significant difference was detected for planar

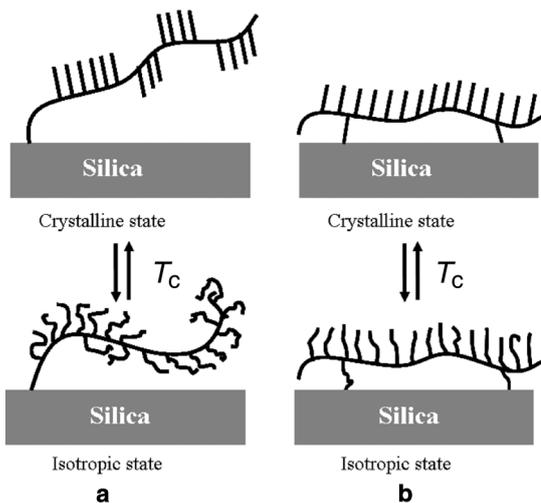


Fig. 2 Schematic illustrations of the phase transition behavior of silica-supported organic phases (a) Sil-ODA $_n$ (single-anchor) (b) Sil-co-ODA $_n$ (multianchor).

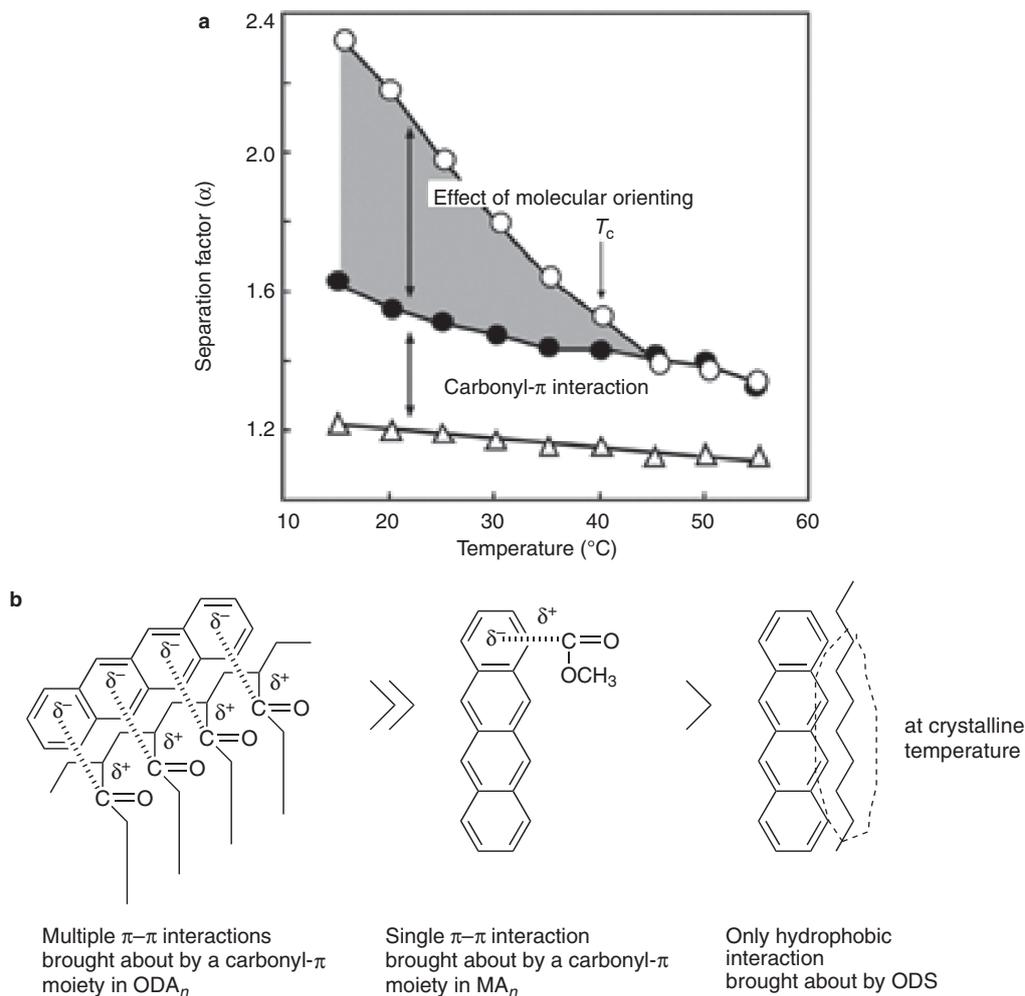


Fig. 3 (a) Temperature dependencies of the separation factors of *cis*- and *trans*-stilbene with columns: Sil-ODA_n (open circles), Sil-MA_n (solid circles), and ODS (open triangles). Mobile phase: methanol:water (70:30). Sil-MA_n: poly(methyl acrylate)-grafted silica. (b) Schematic illustrations of interaction mechanism between solutes and stationary phases.

PAHs.^[32] This cannot be explained only by the increase of side-chain ordering, because it seems that there is no significant difference in the phase transition behavior between Sil-ODA_n and Sil-co-ODA_n in the mobile phase, as detected by differential scanning calorimetry (DSC) and suspension-state ¹H-NMR.^[32] Therefore, we focus on the rigidity of the polymer main chain in Sil-co-ODA_n. It is certain that the mobility of the polymer main chain is relatively restricted by multianchoring effect in co-ODA_n (Fig. 2b). This must be accompanied also by suppression of the mobility around the carbonyl groups for main interaction sites with PAHs. Therefore, it is estimated that the higher selectivity of Sil-co-ODA_n can be attributed to the carbonyl groups on the rigid main chain, which are immobilized by both molecular ordering of the long-chain alkyl groups and multianchoring effect. Especially, the rigidity in Sil-co-ODA_n reduces the interaction with bulky PAHs such as *cis*-stilbene and *o*-terphenyl. Based on these facts it is concluded that the molecular-shape selectivity observed

with Sil-ODA_n is derived from multiple carbonyl- π interactions, which is promoted by the ordering of the octadecyl groups in ODA_n at temperatures below the phase transition temperature (T_c). In the same way, this interaction would be included as a main driving force in the shape selectivity observed with Sil-co-ODA_n.

Grafting-from Method for High-Density Polymeric Phase

The grafting-from technique involves the immobilization of initiators onto the substrate followed by in situ surface-initiated polymerization to generate a tethered polymeric phase. This approach has generally become the most attractive way to prepare thick, covalently tethered polymer brushes with a high grafting density. A variety of synthesis methods such as radical chain transfer reaction,^[38] reverse ATRP,^[39] living anionic surface-initiated polymerization,^[40] ATRP,^[41,42] dispersion polymerization,^[43] and

reversible addition fragmentation chain transfer (RAFT) polymerization have been proposed for the preparation of polymer brushes. ATRP is one of the well-developed controlled living radical polymerization, and has been attracting much attention as a new route to well-defined polymers with low polydispersities and high grafting density.

As we have mentioned, the main limitation of telomerization followed by grafting, or the grafting-to method, is the low grafting densities owing to steric crowding of reactive sites during grafting process.^[5] To overcome the limitations of the grafting-to technique, it is reasonable to use the grafting-from method to prepare poly(octadecyl acrylate)-grafted silica (Sil-gf-ODA_n) for HPLC packing material.^[10] In the surface-initiated ATRP process (grafting-from) the polymer chains grow from the initiators that have been previously anchored onto the inorganic particle surface. Consequently, the grafted chains do not hinder the diffusion of the small monomers to the reaction sites, so that well-defined polymer chains with higher graft density can be obtained.^[6,7,44]

The synthesis procedure for Sil-gf-ODA_n is as follows: the ATRP initiator is synthesized from 2-bromo-2-methyl propionic acid undecyl ester by hydrosilylation with trichlorosilane and then immobilized onto porous silica gel. The reaction between a trichlorosilane anchoring group and a surface OH group of silica results in the formation of a self-assembled monolayer on silica surface. The surface-initiated polymerization of ODA is carried out from the initiator-grafted silica using copper(I)bromide and *N,N,N',N''N'''*-pentamethyldiethylenetriamine (PMDETA) as catalyst precursors (Fig. 4). The polymer-grafted stationary phase was characterized by diffuse reflectance infrared Fourier transform (DRIFT), elemental analysis, suspension-state ¹H-NMR, solid-state ¹³C cross-polarization/magic angle spinning-nuclear magnetic resonance (CP/MAS-

NMR), and DSC measurements. The surface coverage of ATRP polymerized ODA, Sil-gf-ODA_n, was calculated on the basis of elemental analysis results according to our previously reported method.^[45] The grafting density of polymer chains on Sil-gf-ODA_n is 3.75 μmol/m² which is significantly higher than that obtained from Sil-ODA_n prepared by the grafting-to method (2.63 μmol/m²).^[10]

Characteristic features of high-density polymeric phase

The DSC thermogram of polymeric ODA (ODA_n) shows a sharp endothermic peak (*T*_c) in both the heating and the cooling processes. ODA_n provided an endothermic peak at 47°C (*T*_{c2}, peak-top temperature) with a shoulder at around 42°C (*T*_{c1}) in the heating process (Fig. 5). By polarization microscopic analysis *T*_{c1} and *T*_{c2} can be assigned as crystalline-to-liquid crystalline and liquid crystalline-to-isotropic phase transitions, respectively, as mentioned above. Similar phase transitions were also observed even after immobilization on silica (Sil-ODA_n), as in methanol where the peak-top temperature (phase transition temperature) decreased from 47°C to 38°C,^[45] which indicated that silica perturbs the molecular ordering. However, high-density Sil-gf-ODA_n yields less reduction in peak-top temperature which is in the range from 47°C to 43°C (Fig. 5). These results indicate that the polymer side chains in high-density Sil-gf-ODA_n remain ordered structures at higher temperatures, as compared to those in Sil-ODA_n.

Solid-state ¹³C CP/MAS-NMR spectroscopy is a powerful tool for evaluation of the chemical composition and conformational properties of chemically modified surfaces. It is reported that the ¹³C signal for (CH₂)_n groups are observed at two resonances: One is at 32.6 ppm

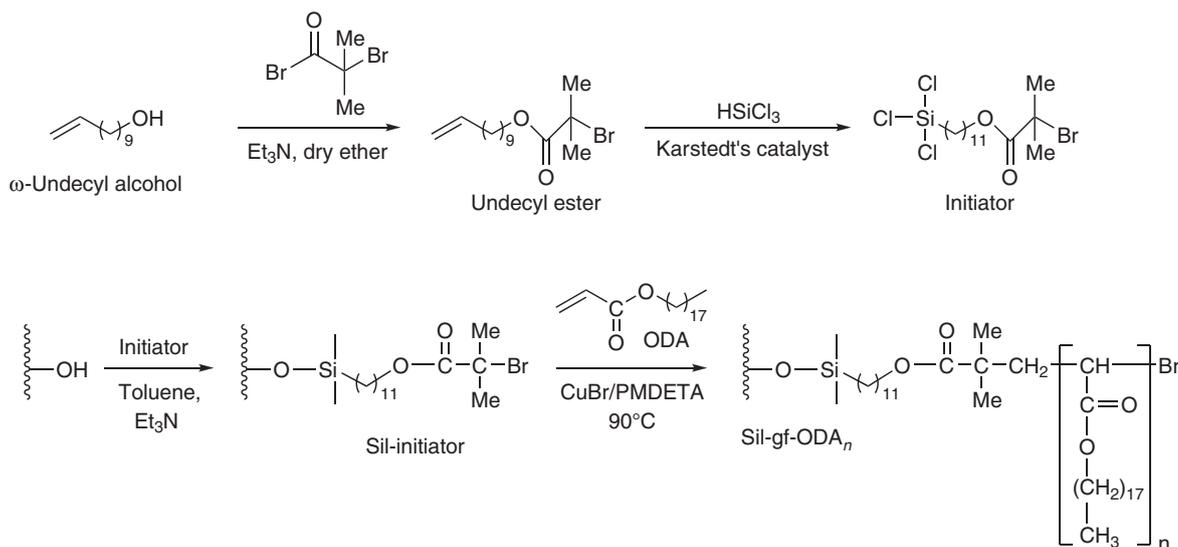


Fig. 4 Synthesis process of preparing polymeric organic phase by surface-initiated atom transfer radical polymerization (grafting-from method).

attributed to a *trans* conformation, indicating a crystalline and rigid state, and the other is at 30.0 ppm attributed to a *gauche* conformation, indicating disordered and mobile state.^[46,47] Solid-state ¹³C CP/MAS-NMR measurements were carried out at different temperatures from 25°C to 50°C for Sil-gf-ODA_n and Sil-ODA_n (Fig. 5). Fig. 6 shows transformation of (CH₂)_n chains from ordered to disordered states (*trans* to *gauche*) as the temperature is increased. However, comparing the two approaches, Sil-gf-ODA_n is more dominated by *trans* conformation than Sil-ODA_n immobilized by the grafting-to method (Fig. 6A). The above result of improved order and rigidity of alkyl chains in Sil-gf-ODA_n than in Sil-ODA_n agrees with the DSC observation, and it is an important characteristic to understand the separation behavior of organic layers grafted onto the silica surface.

Retention mechanism

As discussed in the section "Single-anchor grafting," in high-density ODA_n prepared by the grafting-from method, a main interaction source for molecular recognition is derived from a carbonyl- π moiety and not from molecular hydrophobicity. Fig. 7 shows the correlation between log *k* and log *P* for Sil-gf-ODA_n, Sil-ODA_n, and polymeric ODS

(ODS-p) phases. These results indicate that Sil-gf-ODA_n and Sil-ODA_n have a retention mode in RP-HPLC similar to that of an ODS-p phase. Plots of log *k* vs. log *P* for alkylbenzenes and PAHs almost superimpose each other for ODS-p; however, a significant deviation is observed for PAHs and alkylbenzenes for Sil-ODA_n and Sil-gf-ODA_n demonstrating the existence of other possible interaction sites besides molecular hydrophobicity, which can be attributed to π - π interaction between a solute and a stationary phase. This is the most important feature of ODA_n-grafted silica. Furthermore, it is observed that Sil-gf-ODA_n showed higher retention for PAHs as compared to alkylbenzenes. For example, log *P* of naphthacene (5.71) is smaller than that of octylbenzene (6.30), while log *k* of naphthacene (1.53) is higher than that of octylbenzene (0.93). The increase of log *k* for PAHs is accompanied by selectivity enhancement which provides specific interactive sites for PAHs that can recognize aromaticity besides molecular hydrophobicity. For instance, $\alpha_{\text{naphthacene/benzene}} = 33.0$ for Sil-gf-ODA_n, whereas Sil-ODA_n and ODS-p yielded values of 26.8 and 10.8, respectively (Fig. 8).

Molecular-shape selectivity

Table 1 shows selectivity results for a series of PAHs on ODA_n-grafted silicas prepared from two different grafting

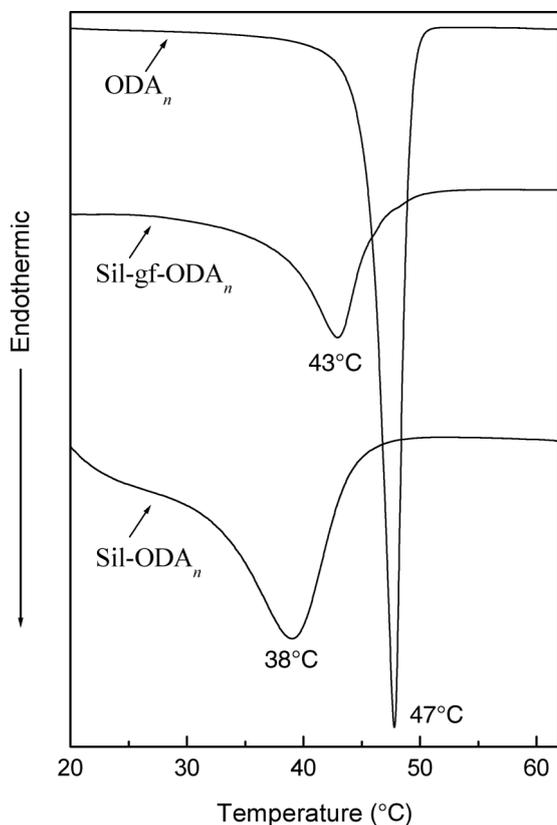


Fig. 5 Differential scanning calorimetry (DSC) thermograms of polymeric ODA (ODA_n), Sil-gf-ODA_n (grafting-from), and Sil-ODA_n (grafting-to).

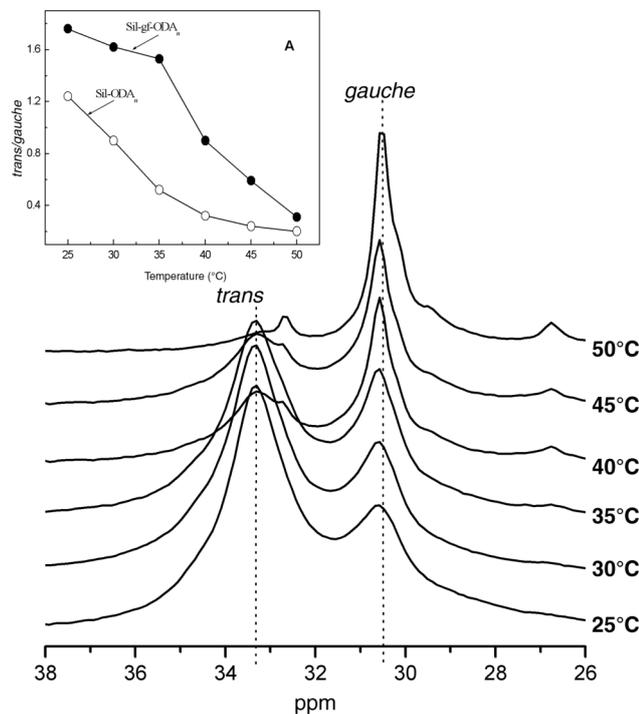


Fig. 6 Transformation of *trans* to *gauche* conformation of octadecyl moieties in solid-state ¹³C CP/MAS-NMR of Sil-gf-ODA_n with temperature. (A) Comparison of the ratio of *trans* to *gauche* conformation in solid-state ¹³C CP/MAS-NMR of Sil-gf-ODA_n and Sil-ODA_n.

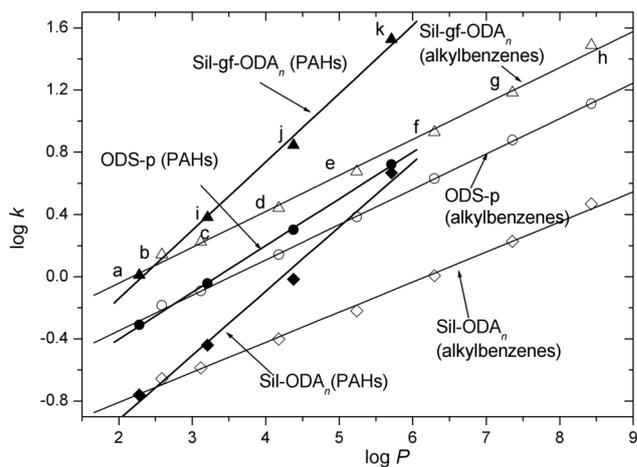


Fig. 7 $\log k$ vs. $\log P$ for ODS-p, Sil-ODAn, and Sil-gf-ODAn stationary phases. Eluates: a, benzene; b, toluene; c, ethylbenzene; d, butylbenzene; e, hexylbenzene; f, octylbenzene; g, decylbenzene; h, dodecylbenzene; i, naphthalene; j, anthracene; k, naphthacene. Mobile phase: methanol:water (90:10); Column temperature: 30°C; Flow rate: 1.00 ml/min.

approaches, as well as for ODS-p. The selectivity in ODAn is discussed focusing on molecular planarity and length in PAHs.

Cis- and *trans*-stilbenes as geometrical isomers are good candidates for the evaluation of molecular-shape selectivity in HPLC. They have the same carbon number per molecule, and, thus, their molecular hydrophobicities are similar but the molecular planarities are absolutely different. The selectivity at 15°C is 1.33, 2.41, and 4.32 for ODS-p, Sil-ODAn, and Sil-gf-ODAn, respectively, clearly indicating higher selectivity by ODAn-grafted silica prepared by *grafting-from* method; however,

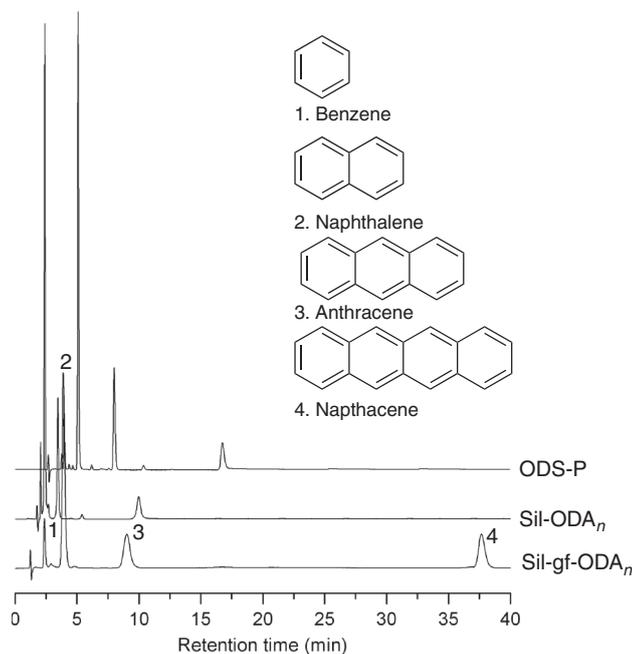


Fig. 8 Chromatograms for a mixture of benzene, naphthalene, anthracene, and naphthacene with Sil-gf-ODAn, Sil-ODAn, and ODS-p. Mobile phase: methanol:water (90:10); Column temperature: 30°C; Flow rate: 1.00 ml/min.

grafting-to approach also yielded a higher selectivity than that of ODS-p.

Another good example is the isomers of *o*-, *m*- and *p*-terphenyls. *o*-Terphenyl is bulky, and *p*-isomer is nearly planar. As summarized in Table 1, Sil-ODAn shows a better selectivity than that of ODS-p, and the selectivity is further enhanced in a high-density type, Sil-gf-ODAn, although its

Table 1 Retention and separation factors of PAHs for Sil-gf-ODAn, Sil-ODAn, and ODS-p stationary phases at 15°C.

Solute	C _n H _n	Sil-gf-ODAn		Sil-ODAn		ODS-p	
		<i>k</i>	α	<i>k</i>	α	<i>k</i>	α
Benzene	C ₆ H ₆	0.64		0.23		0.59	
Naphthalene	C ₁₀ H ₈	1.39	2.2	0.45	1.9	1.21	2.1
Anthracene	C ₁₄ H ₁₀	4.60	7.1	1.44	6.3	3.22	5.4
Triphenylene	C ₁₈ H ₁₂	9.67	1.5	3.60	1.4	6.78	1.2
Benz[a]anthracene	C ₁₈ H ₁₂	14.8	1.7	4.98	1.5	7.84	1.2
Chrysene	C ₁₈ H ₁₂	16.5	4.3	5.48	3.0	8.07	1.7
Naphthacene	C ₁₈ H ₁₂	41.4		10.7		11.4	
<i>O</i> -Terphenyl	C ₁₈ H ₁₄	1.19	4.0	0.72	1.9	2.46	1.7
<i>m</i> -Terphenyl	C ₁₈ H ₁₄	4.49	7.7	1.36	3.9	4.24	2.2
<i>p</i> -Terphenyl	C ₁₈ H ₁₄	8.82		2.82		5.53	

Mobile phase: methanol:water (90:10); Column temperature: 15°C; Flow rate: 1.0 ml/min.

selectivity decreases remarkably at higher temperatures such as 60°C to be similar to those of ODS-p. These results indicate that the ODA_n phase in a highly ordered state recognizes molecular planarity much better than ODS-p does; it should be noted that the organic phase in polymeric ODS has higher density than in conventional ODS.

To evaluate the planarity recognition capability of ODS phases, Tanaka et al.^[48] has introduced the separation for a special mixture composed of two homologous alkylbenzenes and non-planar and planar PAHs. As shown in Fig. 9, it is observed that all compounds are resolved better for Sil-gf-ODA_n than for ODS-p.

In addition, the ODA_n phase shows better selectivity for molecular slenderness (length). In Table 1, this is distinctly seen in the selectivity of a mixture of benz[a]anthracene, chrysene, and naphthacene, which are completely planar compounds but different in their molecular lengths. The selectivity is much higher in Sil-gf-ODA_n, ($\alpha_{\text{naphthacene/chrysene}} = 3.32$) than in ODS-p ($\alpha_{\text{naphthacene/chrysene}} = 1.60$).

As mentioned above, the ODA_n phase has a temperature-dependent ordered-to-disordered phase transition. Fig. 10 shows the selectivity for a planar triphenylene and a non-planar *o*-terphenyl in Sil-gf-ODA_n, Sil-ODA_n, and ODS-p. It shows that the effect of temperature is very low for ODS-p, while Sil-gf-ODA_n and Sil-ODA_n both showed remarkable temperature dependence with distinctly higher selectivity in Sil-gf-ODA_n than in Sil-ODA_n below the phase

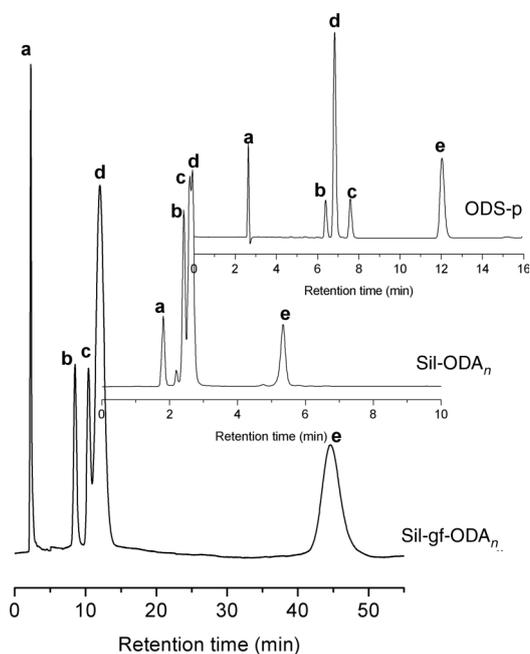


Fig. 9 Chromatograms for the Tanaka test mixture with Sil-gf-ODA_n, Sil-ODA_n, and ODS-p. Eluates: a, uracil; b, butylbenzene; c, pentylbenzene; d, *o*-terphenyl; e, triphenylene. Mobile phase: methanol:water (90:10); Column temperature: 30°C; Flow rate: 1.0 ml/min.

transition temperature, which is attributed to the ordered/disordered state of the ODA_n moiety.

PEPTIDE LIPID-GRAFTED TYPES

In recent years, there has been renewed attention in synthetic polypeptides because of their potential application as biodegradable and biomedical polymers, as well as their ability to form highly ordered hierarchical structures through non-covalent forces such as hydrogen bonding. Incorporation of a high degree of amino acid functionality and chirality in the polymer chains can enhance the potential to form secondary structures (α -helix and β -sheet) and higher ordered structures. On the other hand, it is known that special kinds of peptide-derived lipids can form highly ordered structures such as lipid bilayer membranes.^[21] In these cases, mostly hydrogen bonding interactions with peptide (amide) bonds play an important role in molecular assembling. One of the successful examples is an L-glutamic acid-derived lipid which can produce not only nanofibrillar aggregates such as helical and tubular structures but also show supramolecular functions on the base of chirally-ordered structures.^[21] Therefore, the application of these self-assembling system is of great interest to scientists dealing with the preparation of new packing materials for HPLC.

With this background, we have done direct immobilization of a dialkyl L-glutamide-derived lipid (Glu-1) onto porous silica (Fig. 11).^[33] Extremely enhanced selectivity was obtained for shape-constrained solutes, e.g., PAHs, aromatic positional isomers, and nucleic acid constituents. This finding encouraged us to develop polymeric peptide lipid type stationary phases.

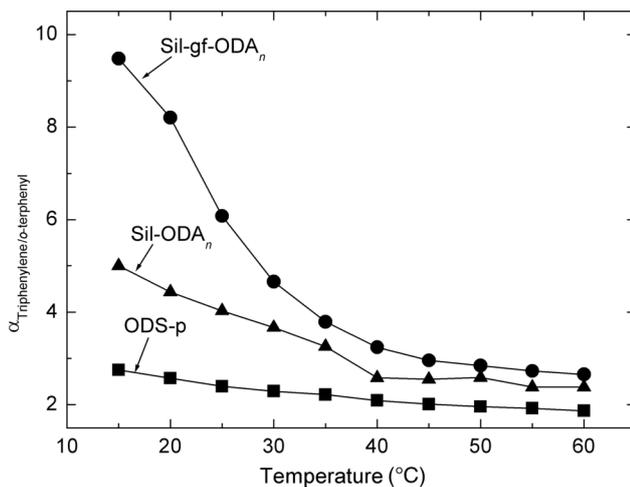


Fig. 10 Temperature dependencies of the separation factors between triphenylene and *o*-terphenyl with Sil-gf-ODA_n, Sil-ODA_n, and ODS-p. Mobile phase: methanol:water (90:10); Flow rate: 1.0 ml/min.

be remarkably enhanced by molecular ordering, and this concept leads us to highly selective HPLC.

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