Polymer Grafting to Silica Surface for Highly Selective RP-HPLC

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INTRODUCTION

Reversed-phase liquid chromatography (RP-HPLC) with, simply, octadecylated silica (ODS) and similar hydrophobized silicas, is the most popular method for analytical separation because of its wide applicability, with only slight modification of a mobile phase system. The separation mode is quite simple. It is usually understandable by the hydrophobic effect and partition coefficients of solutes, while highly-dense packing of organic phase on silica often brings selectivity increase based on a molecular slot effect.[1,2] However, if further specific selectivity is desired, even in RP-HPLC, its simple solution may be to immobilize a functional organic phase onto the silica surface, i.e., to develop new organic stationary phases. A typical example is seen in macrocyclic compound-immobilized silicas, such as those modified with porphyrin, cyclodextrin, and calixarene. In the case of porphyrin, its selectivity in HPLC is truly based on original functions derived from porphyrin.[3,4] Another solution can be expected by immobilization of a polymeric organic phase on silica. In this case, unexpected selectivity increase can be realized by a multiple-interaction mechanism, even if a polymer does not possess any macrocyclic structure. A typical example can be seen in poly(4-vinylpyridine)-grafted silicas.[5,6] The molecular-planarity selectivity towards polycyclic aromatic hydrocarbons (PAHs) compares to that in a porphyrin-immobilized silica in a reversed phase mode. In this article, we introduce polymer-grafting onto silica as an organic stationary phase for highly selective HPLC.

ADVANTAGE OF POLYMER GRAFTING

Polymer-grafting onto silica can be usually carried out by activation of silica surface and subsequent radical polymerization with a vinyl monomer. However, we recommend preparing a polymer with a reactive terminal group at one side, and then immobilizing it onto the silica surface, as schematically illustrated in Fig. 1. This method gives us many advantages.

1. A polymer with a terminal reactive group can be obtained by one-step telomerization. By choosing monomer, a function of the resultant polymer can be tunable.
2. Usual spectroscopy is applicable for determination of the chemical structure before immobilization onto silica. The stereoregularity is also estimated by NMR spectroscopy.
3. Telomerization usually leads narrow polydispersity of degree of polymerization. This promises homogeneity as an organic phase.
4. Polymerization degree can be controlled by the initial molar ratio of a vinyl monomer to a telogen. This is an essentially important feature because large polymers cannot penetrate into the pores of silica and, thus, this causes heterogeneity in surface modification.

The first successful result was seen in the grafting of poly(octadecyl acrylate) onto silica and its application for separation of PAHs in a reversed phase mode.[7] The polymer is obtained by telomerization of octadecyl acrylate, initiated with 3-mercaptopropyltrimethoxysilane (Fig. 1). The following immobilization is carried out by mixing with porous silica in a suitable solvent. The resultant polymer-grafted silica shows, not only extremely high separation of PAHs, but also specific temperature dependency on the selectivity, which is induced by an ordered-to-disordered transition of the grafted polymer.[7] The detail is described later.

GRAFTING OF DISORDERED POLYMERS

A simple application of our grafting method is seen for polymerization of styrene,[8] methyl acrylate,[9] acrylonitrile,[5] etc. (Fig. 1). Radical polymerization of these

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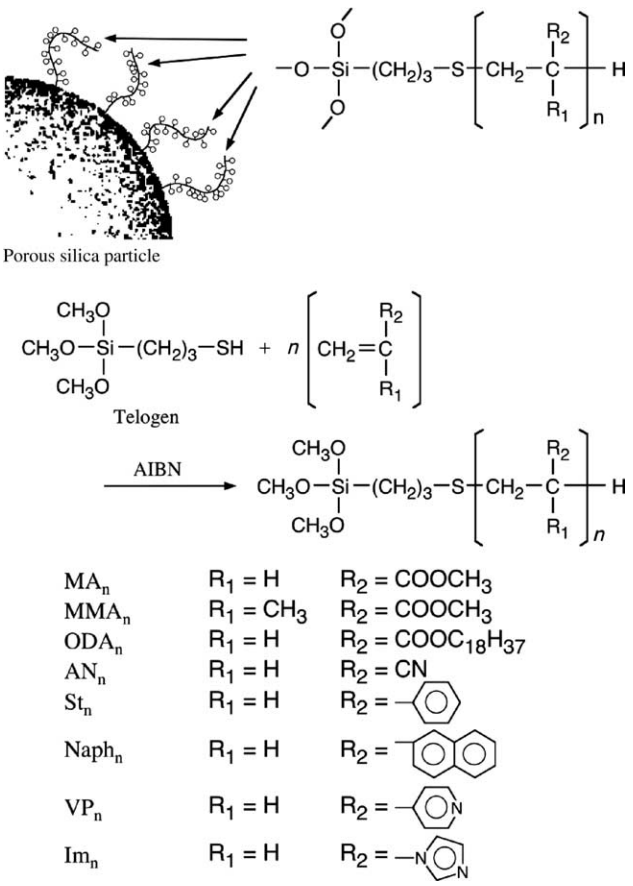

Fig. 1 Chemical structures of polymeric organic phases prepared by one-step telomerization. These polymers can be readily immobilized onto silica through the terminal methoxysilyl group.

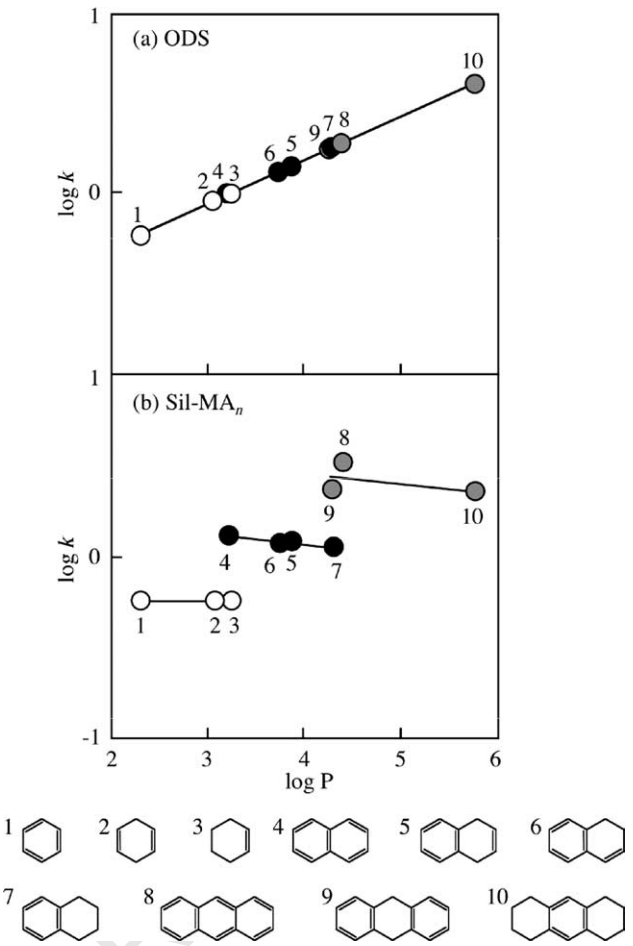

Fig. 2 Relationship between log k and log P with octadecylated silica (ODS) (a) and Sil-MA$_n$ (b). Mobile phases: (a) methanol–water (9:1); (b) methanol–water (7:3).

monomers provides their random polymers. However, the polymer-grafted silicas showed unique features as RP-HPLC packing materials. For example, when the polystyrene-grafted silica (Sil-PSt$_n$, where n is the average degree of polymerization) is applied for separation of PAHs, both the retention and separation factors are higher than commercially available phenyl-bonded silicas. In addition, the elution peaks are comparably symmetrical, although conventional porous poly(styrene-divinylbenzene) packing materials showed remarkable peak-tailing. These desirable properties are attributable to the fact that Sil-PSt$_n$ does not include a cross-linking structure in the bonded phase, but rather fluid to be a liquid.

Poly(methyl acrylate)-grafted silica (Sil-MA$_n$) showed unexpectedly unique selectivity in a reversed phase mode.[9] The unusual nature of Sil-MA$_n$ is emphasized by Fig. 2. Octadecylated silica shows a good linearity in the log k–log P plots (Fig. 2a), indicating that the elution order is understandable by molecular hydrophobicity of solutes. On the other hand, Sil-MA$_n$ provides no similar result. As shown in Fig. 2b, it seems to show that Sil-MA$_n$, instead, recognizes the molecular size (i.e., the number of the rings). This unusual result should be explained by including π–π interaction derived from a carbonyl group of an acrylate moiety. This interaction is estimated by the ab initio MO/MP2 calculations[10,11] (Fig. 3) to be stronger than a benzene π–benzene π interaction,[12] and is also experimentally supported by a substitution effect.[13] Thus, it can be specified as a carbonyl-π interaction.

Similar specificities in HPLC have been realized with poly(4-vinylpyridine)[5,6] and poly(acrylonitrile).[5] The similarity of these polymers can be characterized by the fact that their residual groups include locally polarized moieties with π-electrons. Here, their uniqueness is summarized through the results of poly(4-vinylpyridine)-grafted silica (Sil-VP$_n$): Sil-VP$_n$ is less sensitive for molecular hydrophobicity of solutes, as shown in a typical chromatogram of Fig. 4A. This is proof of a great difference from ordinary ODS. On the contrary, higher retention and selectivity (Fig. 4C) are observed for PAHs or π-electron-containing substances, especially sensitive for difference of the molecular planarities of solutes

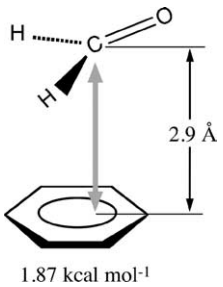

Fig. 3 Formaldehyde–benzene complex model by ab initio MO/MP2 calculations performed with the Gaussian 94 package.[11] The binding energy was calculated as a function of distance *R* between the carbon atom of formaldehyde and benzene plane, in which formaldehyde was removed perpendicularly to the benzene plane (plane-to-plane interaction) with the orientation fixed to that of the optimized geometry. A formaldehyde–benzene interaction (1.83 kcal mol^{-1}) is more effective than benzene–benzene[12] (0.49 kcal mol^{-1} in the parallel interaction) complex.

(Fig. 4E). In addition, Sil-VP_n shows specificity for ortho-isomers[6] better than for para-isomers, as shown in Fig. 5A. This selectivity is often seen in adsorption chromatography, but it should be noted that a mobile phase is aqueous or composed of polar solvents. These uniquenesses will compensate us for limited applicability in ordinary ODS and other π-electron-containing stationary phases in a reversed phase mode. The simple application is cited in Fig. 5B and C, while ODS shows almost no separation for these substances.

When *N*-isopropylacrylamide (NIPAM) is chosen as a vinyl monomer and the resultant polymer is grafted onto silica, its HPLC behavior is temperature-dependent because poly(NIPAM) has a typically lower critical solution temperature (LCST) around 32°C in an aqueous solution (Fig. 6).[15,16] Above its LCST, the organic phase behaves as a hydrophobic surface, which is due to dehydration of the grafted polymer chain. Recently, the separations of amino acid phenylthiohydantoins[17] and bisphenol A[18] are achieved by using this temperature-responsive chromatography with an aqueous solution as the mobile phase.

GRAFTING OF ORDERED POLYMERS

Selectivity Increase Due to Ordering in Polymeric Organic Phase

It is difficult to control the stereoregularity of polymers in radical telomerization. However, the side chain ordering in the polymer can be realized if a long-chain alkyl compound is chosen as a vinyl monomer for telomerization. A preliminary example is reported with poly(octadecyl acrylate), ODA_n.[7] The resultant polymer can be easily grafted onto porous silica through a terminal

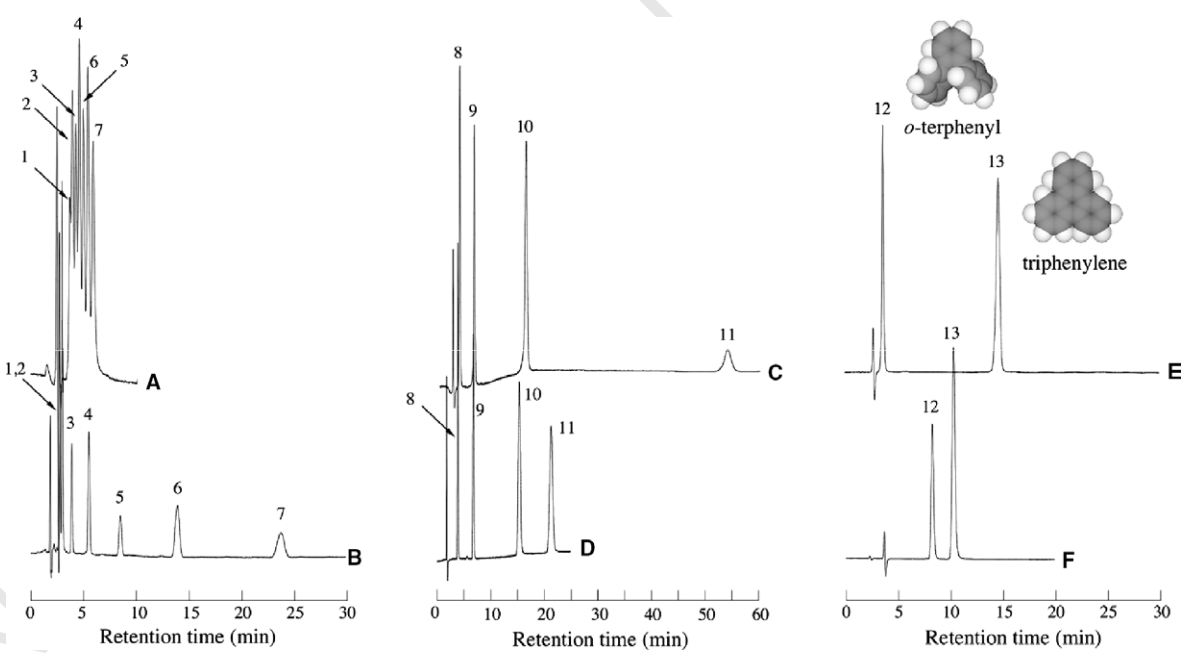

Fig. 4 Typical chromatograms with Sil-VP_n (A, C and E) and ODS (B, D and F) at 30°C.[5] Mobile phase: (A), (B), (E) and (F), methanol–water (7:3); (C) and (D), methanol–water (7:3). Solutes: 1, toluene; 2, ethylbenzene; 4, hexylbenzene: 5, octylbenzene; 6, decylbenzene; 7, dodecylbenzene; 8, benzene; 9, naphthalene; 10, anthracene; 11, pyrene; 12, *o*-terphenyl; 13, triphenylene.

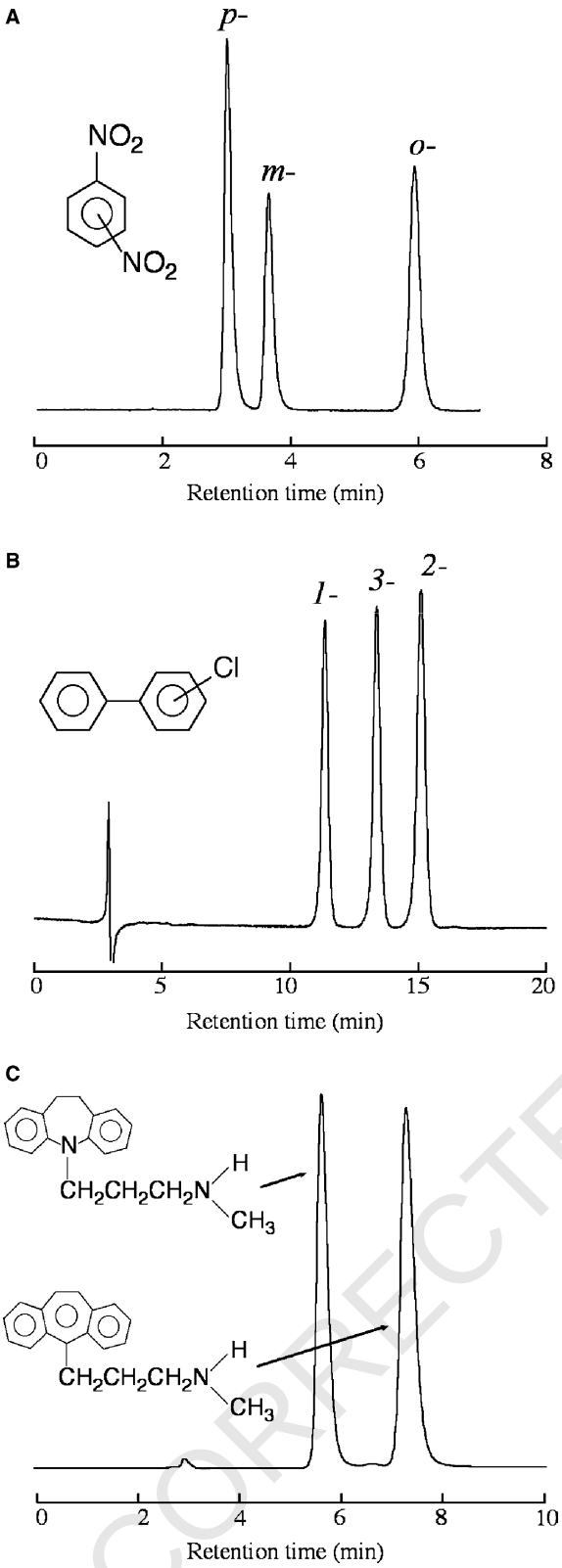

Fig. 5 Examples of chromatographic separation with Sil-VP_n at 30°C. Mobile phase: (A) and (B), methanol–water (6:4); (C), methanol–0.01 *M* KH_2PO_4 (7:3). Sample: (A), *o*-, *m*- and *p*-dinitrobenzenes; (B), 1-, 2- and 3-chlorobiphenyls; (C), desipramine and protriptyline.

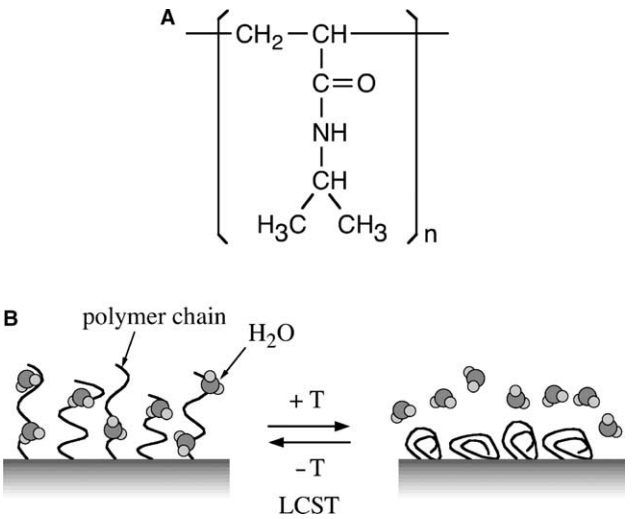

Fig. 6 (A) Chemical structure of poly(*N*-isopropylacrylamide) and (B) schematic illustration of temperature-responsive surface.

trimethoxysilyl group. Toluene and tetrachloromethane are good solvents for this procedure.

Poly(octadecyl acrylate) is characterized by differential scanning calorimetry (DSC)[19] and NMR spectroscopy.[20] The DSC thermogram shows a sharp endothermic peak (T_c) in both the heating and cooling processes. For example, the temperature of ODA_{27} ($n=27$) provides a peak around 49°C (T_{C_2}) with a shoulder (T_{C_1}) at 42°C–47°C in the heating process. Polarity microscopic observation indicated that T_{C_1} and T_{C_2} are assigned to crystalline-to-liquid crystalline and liquid crystalline-to-isotropic phase transitions, respectively. Similar phase transitions are also observed even after immobilization on silica. In a methanol–water (7:3) mixture as a mobile phase, a peak-top temperature (T_{C_2}) falls about 8°C, compared with the original T_{C_2}. This indicates that silica influences the orientation of bound ODA_{27}, but the bonded phase can maintain ordered structures and undergo crystalline-to-isotropic phase transition on silica, as illustrated in Fig. 7B.

The phase transition of ODA_n on silica can be also detected by a combination of suspension-state ^{1}H NMR and solid-state ^{13}C-CP/MAS-NMR spectroscopies.[20] For example, with a gradual increase in temperature, the intensity of proton signals (^{1}H NMR) of octadecyl moieties (mainly methylene groups) rises with a sharp inclination coincident with an endothermic peak in DSC thermogram, implying a relatively complete solid to liquid phase transition. In addition, the ratio of *trans*- to *gauche*-conformations can also be determined. This phase characterization method, in conjunction with conformation determination drawn from NMR spectroscopy, is important for a better understanding of the structure, dynamics, and separation behavior of organic layers grafted onto the

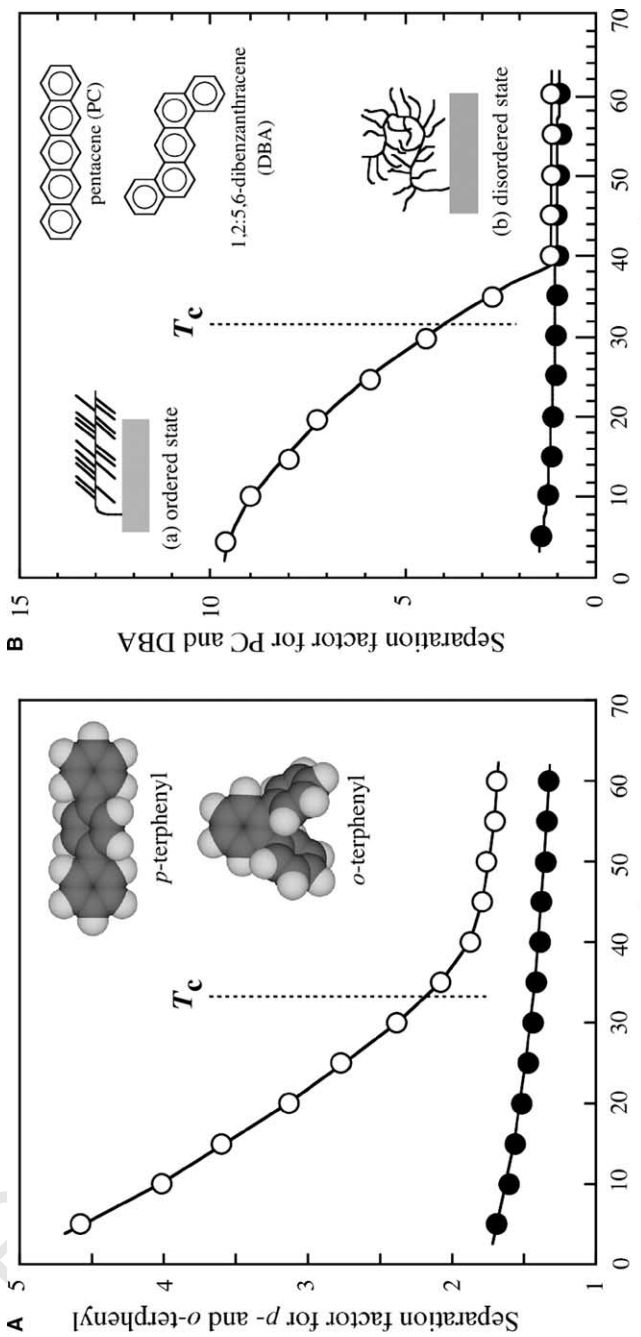

Fig. 7 Temperature dependencies on the separation factor with Sil-ODA$_n$ (open circles) and ODS (solid circles). Mobile phase: methanol (a); ethanol (b).

silica surface. Interestingly, this method clarified that most of the octadecyl chains in the case of monomeric ODS, despite having a gauche conformation, were still in a solid phase and only 12.3%–18.5% of them have enough mobility to be considered as being in a liquid phase.[20]

Sil-ODA$_n$ shows ODS-related retention orders for usual hydrophobic solutes in a reversed phase mode, but the unique performance of Sil-ODA$_n$ is emphasized by temperature dependency. Sil-ODA$_n$ shows distinct bending in the plots of temperature vs k in HPLC. T_c of Sil-ODA$_n$ observed completely agrees with the temperature of the bending point. This is accompanied by the remarkable selectivity change. A typical example is shown in Fig. 7. Both increases of the retention and separation factors always appear at temperature below T_c, where the physical state is in an ordered form. On the other hand, higher selectivity is clearly observed for PAHs vs non-aromatic hydrocarbons. To clarify the separation mechanism of Sil-ODA$_n$, we have investigated the selectivity with geometrical isomers from various substituted azobenzene compounds.[13] 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 by a carbonyl π moiety in ODA$_n$. As supporting this assumption, a carbonyl group-containing polymeric organic phase, Sil-MA$_n$ with neither a long-chain alkyl group nor any ordered structure shows no bending behavior in the temperature-selectivity plots, but also the separation factor is higher when compared with ODS.[13] In addition, the separation factor decreases with addition of acetone to a mobile phase, which can work as an inhibitor for a carbonyl π–benzene π interaction. No similar decrease is observed by 2-propanol. Theoretical study of a carbonyl π–benzene π interaction with a model system has also been done with ab inito calculation (Fig. 3).[10–12] Therefore, it is concluded that solute molecules are not incorporated into a crystalline (ordered) phase, but rather adsorbed onto the aligned carbonyl groups. On the other hand, the isotropic (disordered) bonded does not have such specificity. The solutes can partition into the bonded phase and, thus, the separation mode is similar to that of ODS.

Effect of Stereoregularity of Polymeric Organic Phase on Selectivity Increase

As briefly mentioned above, a stereoregularity of polymers is hardly controlled in a radical telomerization, although it would be an important factor to increase the selectivity. However, it is certain that stereoregularity can be influenced by solvent and temperature in a telomerization process. In support of this, when the telomerization is prepared in methanol, benzene, or cyclohexane, and then the resultant polymers, abbreviated as ODA$_n$-M, ODA$_n$-B and ODA$_n$-C respectively, are applied as organic stationary phases, significant selectivity difference is detected among them.[21] A typical example can be shown by the separation of a mixture of *m*- and *p*-terphenyls. These structural isomers are useful for evaluating the molecular shape selectivity because the molecular shape differs in planarity and length, but the hydrophobicity is similar. Therefore, a conventional RP-HPLC shows very low selectivity, e.g., $\alpha = 1.1$ and 1.3 in monomeric and polymeric ODSs, respectively, while complete separations ($\alpha = 3.0$–3.3) are observed in all Sil-ODA$_n$ at 0°C. Here, if the separation factor is compared with the results at 35°C, the solvent effect in the telomerization makes it clear. This difference can be explained by the fact that the phase transition temperature of ODA$_n$-M is a little higher (40°C in a peak-top temperature) than the others (35°C). This difference reflects the molecular orientation among the long-chain alkyl groups. The orientation of the side chain alkyl groups of ODA$_n$ can be also evaluated by the suspension-state ^{1}H NMR in methanol with the nanoprobe.[20] The octadecyl methylene peak is very small and broadened at 0°C, but the normalized intensity begins to increase distinctly around 30°C. This temperature is close to the phase transition temperature. This unusual increase of the intensity in ODA$_n$ can be explained by the fact that the mobility of octadecyl groups increases with the ordered-to-disordered transition, as shown in the DSC data. On the other hand, when ^{13}C NMR spectroscopy of ODA$_n$ is carried out in chloroform-d at room temperature, all ODA$_n$ provides nine distinct peaks in the range of 10–70 ppm. All peaks are assigned reasonably by considering electronegative forces of their neighboring moieties. On the basis of the assignment, it is estimated that ODA$_n$-C and ODA$_n$-B show relatively high polydispersity in the tacticity compared to ODA$_n$-M. This estimate agrees with the fact that the phase transition temperature of ODA$_n$-M is a little higher than the others. This study cannot confirm exact conformation of the carbonyl groups in ODA$_n$ but shows that the microenvironmental difference in polymer influences the resultant selectivity. This finding is very valuable because it indicates that conformational control of the polymer main chain would lead to high selectivity in HPLC.

To discuss the effect of structural ordering of the polymer on selectivity, we have been focusing on secondary structures of poly(α-amino acid)s, i.e., polypeptides.[22,23] They provide rigid and exact conformations, such as α-helix and β-structure, spontaneously if polymerization is done with a purely chiral amino acid. The poly(L-alanine), poly(L-leucine), and poly(L-phenylalanine) with a terminal reactive trimethoxysilyl group can be prepared by the corresponding *N*-carboxyanhydrides and 3-aminopropyltrimethoxysilane as an initiator for polymerization. However, in this case, a serious problem is often accompanies the procedure. This is due to lower solubility of the resultant polypeptides and, particularly, becomes formidable with an increase of the

polymerization degree. Therefore, grafting of polypeptides is usually carried out by immobilization of 3-aminopropyltrimethoxysilane onto porous silica and subsequent polymerization with *N*-carboxyanhydride initiated with the activated silica. The secondary structures of polypeptides on silica can usually be estimated by the absorption bands ascribed to an amide I and II in IR spectroscopy, because the strong absorption due to silica overlaps with that of an amide V. IR spectroscopy indicated that the main secondary structures of poly(L-alanine) and poly(L-leucine) on silica were the β-structure and the α-helix, respectively.[22]

When poly(L-alanine)-grafted silica is applied for separation of PAHs in a reversed phase mode, we can encounter various specific selectivities. For example, $\alpha =$ 10.4 was obtained for a mixture of *p*- and *o*-terphenyls, while $\alpha = 1.5$ in ODS. Both *p*- and *o*-terphenyls possess the same numbers in carbon atoms and π-electrons, but the molecular planarity is entirely different. As indicated in the Corey-Pauling-Koltun (CPK) models of Fig. 7A, *p*-terphenyl is a little twisted (almost planar), but more slender (linear) than *o*-terphenyl. No similar enhancement of the selectivity is observed in poly(L-leucine)- and poly(L-phenylalanine)-grafted silica. These polypeptides show, rather, similarity to ODS: e.g., $\alpha = 1.7$ in poly(L-leucine)-grafted silica.

To explain the high selectivity of the poly(L-alanine) phase, we apply a multiple carbonyl π-to-benzene π interaction mechanism on highly-ordered structures. As shown in the schematic illustrations of Fig. 8, the carbonyl groups in the poly(L-alanine) main chain as a π-electron source are well-oriented because the peptide main chain is in a rigid β-form structure on silica. On the basis of these facts, we explain the multiple π–π interaction mechanism:

1. Polycyclic aromatic hydrocarbons can interact with the carbonyl groups. The methyl group of the poly(L-alanine) side chain does not prevent electrostatic interaction (Fig. 8a).
2. On the contrary, poly(L-leucine) does not offer a chance to provide this interaction because the residual isobutyl groups are too bulky to approach each other (Fig. 8b). Also, it should be noted that poly(L-leucine) provides only α-helices, even on silica and, thus, their carbonyl groups are absolutely covered with the bulky residual groups. As a result, the poly(L-leucine) phase showed only hydrophobicity recognition similar to ODS. The selectivity is close to that in ODS ($\alpha = 1.5$).
3. The carbonyl groups in poly(L-alanine) should be aligned unidimensionally along the β-structure form. This conformation promotes the multiple carbonyl-π interaction, which works more effectively with longer (slender and linear) PAHs than with shorter ones. Fig. 8a and b show that a longer and planar PAH, such as pentacene, yields higher contact points with poly(L-alanine) than a disk-like PAH such as coronene.

Multi-Anchoring Effect on Selectivity Increase

Porphyrin-derivatized bonded phases show unique shape selectivity, retaining planar PAHs.[3,4] Similar molecular-planarity selectivity is also observed in cholesteryl-10-undecenoate and 4,4′-dipentyldiphenyl bonded phases. These phases contain rigid structures and, thus, the limited mobility in their organic phases contributes to the molecular-shape selectivity. A similar effect on selectivity increase can be seen in the comparison of the retention behaviors between polymeric and monomeric ODSs.[2] Therefore, to increase the selectivity, decreasing molecular mobility of ODA_n having plural reactive groups in the side chain has been synthesized and immobilized onto silica. This polymeric phase can be obtained by co-telomerization with γ-methacryloxypropyltrimethoxysilane. As expected, this polymer phase-immobilized silica showed better selectivity for PAHs: e.g., 1.4 times higher selectivity for

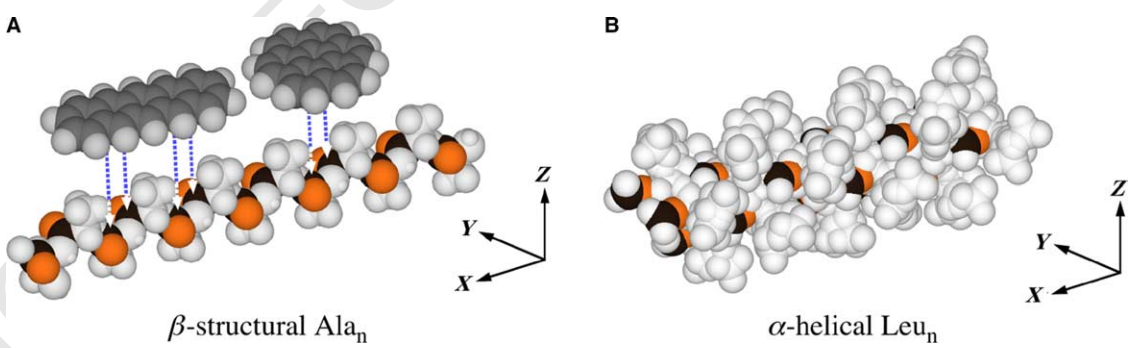

Fig. 8 CPK models of (A) β-structural Ala_n and (B) α-helical Leu_n derived from PEPCON. The black atoms present carbonyl carbons. A linear and planar solute such as pentacene provides more effective interaction area with the carbonyl groups one-dimensionally-aligned on the rigid main chain than a disk-like solute such as coronene (A). On the other hand, the carbonyl groups of Leu_n are covered with their bulky residual groups (B).[22]

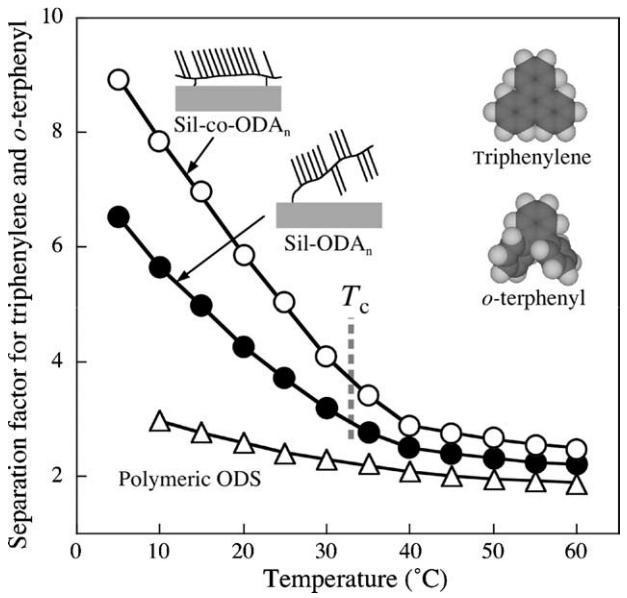

Fig. 9 Temperature dependencies of the separation factors between triphenylene and *o*-terphenyl with multi-anchored ODA$_n$ (open circles), Sil-ODA$_n$ (solid circles) and polymeric ODS (open triangles). Mobile phase: methanol–water = 9:1.

the separation of triphenylene and *o*-terphenyl than Sil-ODA$_n$ (Fig. 9). This is a typical example that the immobilization method of polymer on support materials influences the resultant molecular-shape selectivity and, thus, this finding encourages us to investigate the multi-anchoring effect for various polymeric phases.

Application for Chiral Discrimination

It is well known that, for biologically active substances, one enantiomer shows different biological activity from the other. For example, the studies by Mori et al. on the relationship between optical purity and biological activity of insect pheromones have revealed that the biological activities were dramatically changed by their optical purities. Therefore, it is important to determine the absolute configuration and accurate optical purity of biologically active compounds.

For this purpose, a diastereomer method has been widely used for determination of the absolute configuration and optical purity and, thus, many diastereomerizing reagents have been developed for racemic amines, alcohols, and amino acids.[10,14] When this method is combined with RP-HPLC, it yields very convenient and quick analyses of enantiomer mixtures. Diastereomerizing reagents can be generally characterized by the fact that chromophoric groups are included for sensitive detection and chiral separation is realized by discriminating the hydrophobicity (or polarity) difference between the resulting diastereomers. Therefore, π–π interaction-supported RP-HPLC with ODA$_n$ would show much better discrimination for the resultant diastereomers, compared with ordinary ODS.

Fig. 10A shows a typical example for *N*-acyl amino acid diastereomerized with *N*-dansyl amino acid.[24] Diethyl phosphorocyanidate (DEPC) is adopted as a condensation reagent instead of conventional dicyclohexylcarbodiimide (DCC), to avoid racemization during the reaction. Fig. 10A shows the time course of chromatograms for the methyl ester of DL-phenylalanine diastereomerized by *N*-dansyl-L-proline with Sil-ODA$_n$. The chromatogram, a minute after addition of DEPC to the mixture of DL-phenylalanine and *N*-dansyl-L-proline, provided two new peaks with λ_{max} of 350 nm at 6.8 min and 8.5 min which are attributable to the absorption based on a dansyl group. It is also assigned that the first peak, at 6.8 min, has the same retention time as that obtained by the diastereomer of L-phenylalanine methyl ester with *N*-dansyl-L-proline. The selectivity in Sil-ODA$_n$ is temperature-dependent and much higher at temperature below T_c ($\alpha = 1.45$, $-10°C$) than that in ODS ($\alpha = 1.16$, $-10°C$).

Another good example is shown in Fig. 10B.[25] Ohrui et al. have developed the chiral labeling reagents, 2-(2,3-anthracenedicarboximide)cyclohexane carboxylic acid and 2-(2,3-anthracenedicarboximide)cyclohexanol to discriminate the diastereomers having chiral centers separated by more than four bonds. The use of these reagents made it possible to separate the branched fatty acids or alcohols having a branched methyl group by reversed phase HPLC with ODS. However, it was necessary to apply low column-temperature conditions (around $-40°C$) for their separation because the mobility of octadecyl groups in ODS should be remarkably reduced. To solve this problem, Sil-ODA$_n$ has been applied instead of conventional ODS because the ODA$_n$ phase is rigid and ordered in the side chain at temperatures below T_c.

Fig. 10B shows chromatograms for the diastereomers, *S,S,R*-**1** and *R,R,R*-**1** by HPLC with Sil-ODA$_n$ and ODS at 0°C.[25] No separation is observed in ODS, where it was necessary to apply lower column-temperature conditions for a substantial separation. Even each injection showed almost no separation at 0°C ($k_{R,R,R}$ and $k_{S,S,R} = 15.2$ with ODS). On the contrary, complete separation is observed in Sil-ODA$_n$ ($k_{R,R,R} = 21.0$, $k_{S,S,R} = 17.3$, $\alpha = 1.21$). There is a series of the Ohrui's reagents; then similar good results are always obtained with Sil-ODA$_n$, compared with ODS. This is due to the fact that the separation is supported with π–π interaction as well as a hydorophobic effect.

CONCLUSIONS

Lipid bilayer membrane systems, having gel (solvated crystalline state)-to-liquid crystalline phase transitions are attractive as specific organic media for separation

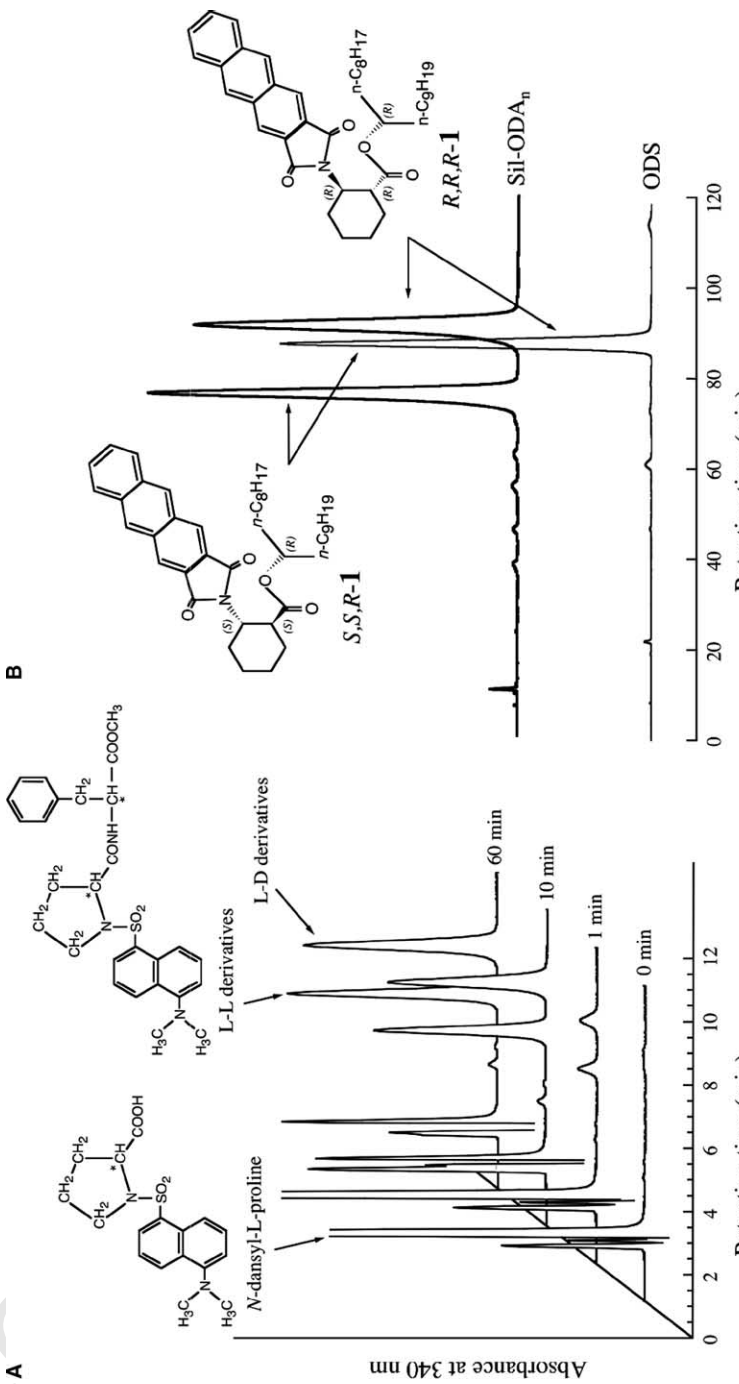

Fig. 10 (A) Time course of diastereomerization of DL-phenylalanine with *N*-dansyl-L-proline using DEPC[24] and (B) chromatograms of the mixture of *S,S,R*-**1** and *R,R,R*-**1** with Sil-ODA$_n$ and ODS.[25] Mobile phases: (a), methanol–water (7:3) at 25°C; (b), methanol at 0°C.

chemistry. The first approach in HPLC was direct immobilization of a phosphatidylcholine lipid onto silica. This modified silica shows interesting selectivity against amino acids, but the separation mode is too complicated, due to the zwitterionic property of the immobilized molecule. In addition, no lipid membrane function is realized on the silica because of the direct immobilization with covalent bonding, which prohibits lateral diffusion of lipids from forming highly-ordered structures that lead to supramolecular functions of lipid membrane systems.

Sil-ODA$_n$ has been developed to address this question.[7] The ODA$_n$ phase does not make lipid membrane structures in an aqueous system, but it possesses similar functions, such as side-chain ordering and phase transition behavior between ordered and disordered states. However, the resultant selectivity in HPLC often excelled, better than expected. This is due to a sort of a side effect and, then, it may be called a polymeric effect, which promotes multiple interactions to increase the selectivity. Molecular ordering of functional groups particularly enhances it.

This article has revealed that a grafting method is useful to direct a polymeric effect in HPLC. The advantage of this method is quite clear. Firstly, grafted polymers are not appreciably influenced by carrier particles. This feature is very important to maintain the original functions of polymers. For example, ODA$_n$ can undergo a phase transition, even after immobilization on silica. The other advantage is based on the fact that the functions of polymers are absolutely tunable by judicious selection of the monomer. Copolymerization would expand their versatility remarkably. Also, potential applicability of a polymer grafting method for surface modification must be limited to use in HPLC.

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