Conformational Effect of Silica-supported Poly(octadecyl acrylate) on Molecular-Shape Selectivity of Polycyclic Aromatic Hydrocarbons in RP-HPLC

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Poly(octadecyl acrylate) with a terminal reactive group was synthesized by radical telomerization in various solvents. The polymers were grafted onto porous silica for use in RP-HPLC, and the molecular recognition ability was investigated along with the selectivity for the structural isomers of polycyclic aromatic hydrocarbons. The mechanism of selectivity was also investigated with differential scanning calorimetry and NMR spectroscopic observations.

Introduction

Hydrophobized silicas, such as octadecylated silicas, are very useful as packing materials for reversed-phase high performance liquid chromatography, RP-HPLC. A simple modification of the mobile phase has expanded their applicability in analytical chemistry and separation technology. Similarly, a chemical modification of an organic phase on silica has provided versatility in the separation mode even in RP-HPLC. For these reasons, unique organic stationary phases have been created during the past decade.[1-16] Fullerene C_{60} bonded silica[3,4] showed higher selectivity for calixarene isomers, fullerenes C_{60} and C_{70}, and polycyclic aromatic hydrocarbons (PAHs). The stationary phases containing heavy atoms, such as sulfur, chlorine and bromine resulted in longer retentions of fullerenes.[2] Various porphyrin-silica stationary phases,[6-8] alkyl diphenyl-silicas[1] and (2,4,6-tri-*tert*-butylphenoxy)dimethyl-silicas[5] exhibited extraordinary shape selectivity in the separation of PAHs and fullerenes. We have reported that polymer-grafted silica exhibited selective separation in HPLC. Polymers with trimethoxysilyl terminal groups were synthesized using the telomerization method and chemically-grafted on silica particles. A number of synthetic polymers, such as poly(styrene),[10] poly(acrylonitrile),[11] poly(methylacrylate),[9,12] poly(vinylpyridine)[16] and poly(L-alanine),[14,15] were used to develop the polymer-silica hybrid materials as a stationary phase of HPLC. On the other hand, we have proposed that self-assembled systems, such as lipid aggregates, can provide a highly-ordered microenvironment leading to unique host-guest chemistry exceeding the functions of the original lipid.[17] Based on these points, we have focused on the ordered structures of poly(octadecyl acrylate), ODA_n and the ODA_n-grafted silica for applying to RP-HPLC.[18] Through these studies, we recognize that the weak subsidiary effects due to the carbonyl groups in the polymer work as main driving force for molecular-shape selectivity toward π-electron-containing compounds and the selectivity is specifically enhanced by ordering of the long-chain alkyl moieties.[19-22] The present study is aimed at controlling the ordered state of ODA_n (Fig. 1) by the polymerization condition, and evaluating its effect on the selectivity, with HPLC toward the structural isomers of terphenyls and four-ring polyaromatic hydrocarbons and with differential scanning calorimetry and NMR spectroscopy.

Experimental

Polymeric organic stationary phase
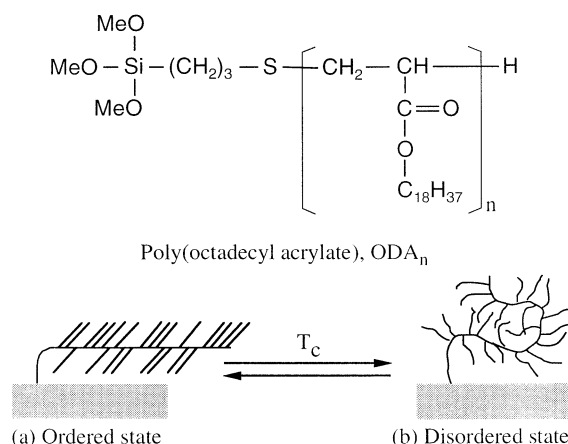 Three ODA_ns were prepared with different solvents according

Poly(octadecyl acrylate), ODA_n

(a) Ordered state   (b) Disordered state

Fig. 1 Chemical structure of ODA_n and proposed physical states of silica-supported ODA_n.

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to the previously reported method.[19,22]   The chemical structures were confirmed by ^{1}H-NMR spectroscopy. The average degree of polymerization was determined by proton ratio on the basis of the terminal methoxy groups. Immobilization of ODA$_n$ was also carried out by the previously reported method.[19,22]   The amount of immobilization was determined by elemental analysis.

Liquid chromatography

Silica-supported ODA$_n$ (Sil-ODA$_n$) was packed into a stainless-steel column (4.6 mm i.d. × 15 cm).   The chromatograph included a JASCO 980 PU pump and a JASCO MD-910 UV-visible photodiode array detector. A 5-μl volume of the sample was injected through a Reodyne Model 7125 injector. Chromatography was carried out with methanol or methanol–water mixtures as mobile phases at a flow rate of 1.0 ml min^{-1}. The retention factor (k) was determined by $(t_e – t_o)/t_o$, where t_e and t_o are retention time of the samples and methanol, respectively. The separation factor (α) was given by the ratio of the retention factors.

Other measurements

Thermograms by differential scanning calorimetry (DSC) of ODA$_n$ and their silica-supported derivatives were obtained at a heating rate of 1°C min^{-1} using a Seiko I & E SSC-580 with a DSC-10 instrument.

All NMR spectra were measured by VARIAN UNITY[INOVA] AS400 using a GHX Varian AS400 nanoprobe for suspension-state ^{1}H-NMR and solution-state ^{13}C-NMR, and a VARIAN 7-mm VT CP/MAS probe for solid-state ^{13}C-CP/MAS-NMR. All NMR parameters mentioned in this paper are compatible with the terminology of VARIAN UNITY[INOVA]. Hexamethyldisilazane was used as a reference for the peak intensity.

Results and Discussion

Phase transition behavior of ODA$_n$

ODA$_n$, where n is the average degree of polymerization, was obtained by the one-step telomerization of octadecyl acrylate with 3-mercaptopropyltrimethoxy silane. In the present study, three ODA$_n$s were obtained by telomerization in methanol, cyclohexane and benzene. The n values were determined by ^{1}H-NMR spectroscopy to be nearly close to n = 30, 35 and 35; they are abbreviated as ODA$_n$-M, -C and -B, respectively.

Differential scanning calorimetry (DSC) provided a distinct endothermic peak for all ODA$_n$s as shown in Fig. 2. The transition can be assured to include a solid-to-liquid phase transition. This was also confirmed by polarization microscopic observations.   Interestingly, the peak-top temperature (T_c) differs slightly depending on the polymerization solvent. This difference probably reflects the molecular orientation state among the long-chain alkyl groups because of no difference in their chemical structures. Therefore, this finding encourages us to investigate the effect of the side-chain orientation on the HPLC property.

These polymers were grafted with the terminal methoxy groups onto porous silicas, and then the resultant silicas (Sil-ODA$_n$) were evaluated as HPLC columns. The immobilization amounts were determined to be similar to each other by elemental analyses: 16.4, 18.5 and 17.5 wt% for ODA$_n$-M, -C and -B, respectively.
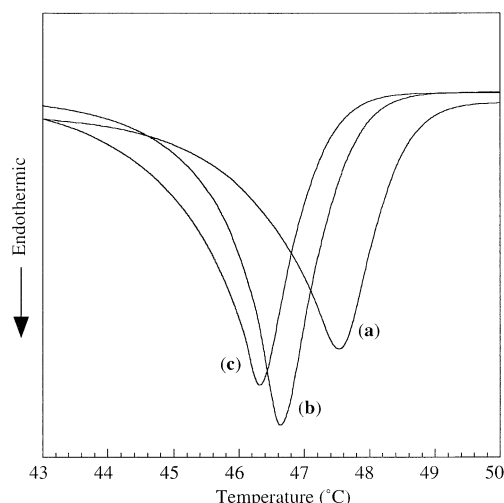

Fig. 2   DSC thermograms of ODA$_n$s prepared in methanol (a), cyclohexane (b) and benzene (c).

Temperature dependence of selectivity

Figure 3 shows typical chromatograms 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 small selectivity, such as α = 1.1 and 1.3 in monomeric and polymeric ODSs, respectively. On the other hand, complete separation (α = 3.0 – 3.3) was observed in all Sil-ODA$_n$s at 0°C while almost no separation was seen at 50°C. This can be explained as being one of the typical features of ODA$_n$, because ODA$_n$ forms ordered structures at temperatures below T_c (*e.g.* 0°C) and thus molecular planarity recognition appears at these temperatures. Our previous study indicated that a weak electrostatic interaction[23] between carbonyl-π from ODA and benzene-π from solutes is a main driving force for this recognition and enhanced molecular-shape selectivity through ordering of the carbonyl groups.[24]   On the contrary, ODA$_n$ became disordered at temperatures above T_c, forming an isotropic state. Subsequently, the separation ability was extremely reduced and became close to those of conventional ODSs.

An additional interesting result was found at 35°C.   Only ODA$_n$ prepared in methanol (Sil-ODA$_n$-M) showed a peak separation (α = 1.26), which was not observed in the others (α, below 1.1).   Plots of the α value for *m*-terphenyl and *p*-terphenyl against the temperature (separation factor) make this difference clear as shown in Fig. 4. All Sil-ODA$_n$s provided remarkable selectivity changes at specific temperatures showing a distinct increase of the α value. However, those temperatures differ, for example at 40, 35 and 35°C in Sil-ODA$_n$-M, -C and -B, respectively. Figure 5 shows the van't Hoff plots (ln k *vs.* 1/T) for *m*-terphenyl on each stationary phase. The van't Hoff plots of all columns bent and their bending points were same to those of the α value plots in each column. Since non-linear van't Hoff plots were often explained due to conformational change of the organic phase,[25-27] it is clear that the differences in the bending points are related to those of T_c detected by DSC measurements (Fig. 2).

Similar temperature dependencies of the selectivity were observed in a series of four-ring aromatic hydrocarbons. As shown in Fig. 6, all Sil-ODA$_n$s show complete separation at 0°C but only Sil-ODA$_n$-M provides peak separation at 35°C. These
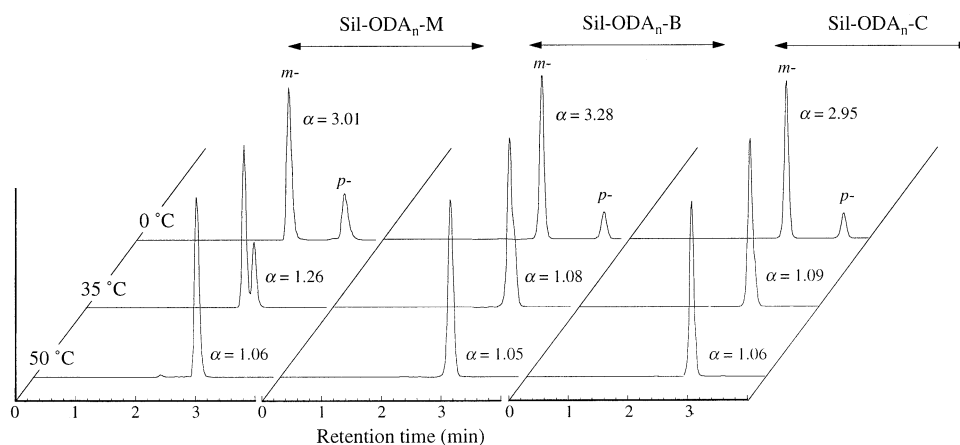
Fig. 3   Typical chromatograms of a mixture of *m*- and *p*-terphenyls with Sil-ODA$_n$ at 0, 35 and 50°C. Mobile phase: methanol.
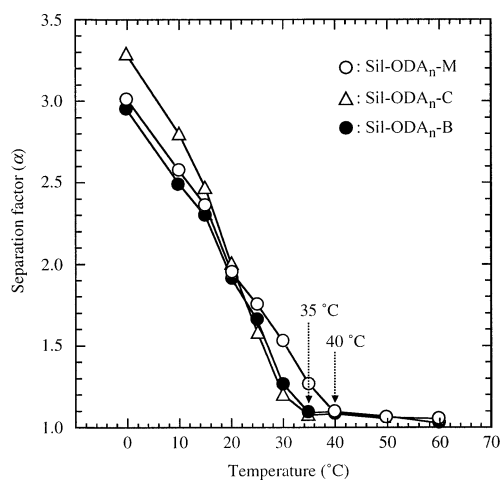

Fig. 4   Temperature dependencies of the separation factors between *p*- and *m*-terphenyls with Sil-ODA$_n$. Mobile phase: methanol.

Fig. 5   Typical van't Hoff plots for *m*-terphenyl with Sil-ODA$_n$.

results indicate that slight differences of the ordered structures of ODA$_n$ influence the selectivity.

Evaluation with NMR spectroscopy

The orientation of the side-chain alkyl groups of ODA$_n$ can be evaluated by the suspension-state ^{1}H-NMR of Sil-ODA$_n$ in methanol with a nanoprobe. The octadecyl methylene peak was very small and broadened at temperatures below T_c, but the intensity ratio of the methylene groups (C3 – C17, refer to Fig. 8) from ODA$_n$ and the methyl groups from reference (hexamethyldisilazane, HMDS) increased distinctly around T_c. The typical suspension state ^{1}H-NMR spectra of Sil-ODA$_n$-M and the temperature dependencies of those ratios are shown in Fig. 7. On the contrary, no similar increase could be seen in monomeric and polymeric ODSs. Therefore, this unusual increase of the intensity in ODA$_n$ can be explained by the fact that the mobility of the octadecyl groups increases with the ordered-to-disordered transition as shown in the DSC data.

^{13}C-NMR spectroscopy of ODA$_n$ was carried out in chloroform-*d* at room temperature. All ODA$_n$s provided nine distinct peaks in the range of 10 – 70 ppm (Fig. 8). The peaks at 14 ppm and 29.7 ppm can be assigned to be C18 as terminal methyl groups and C4 – C14 as most of the methylene groups, respectively. Some peaks at 64.6 ppm (C1) and 41.3 ppm
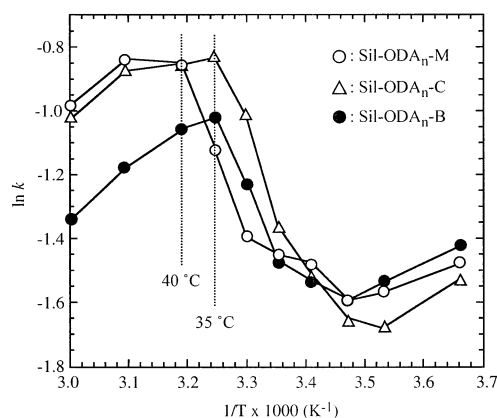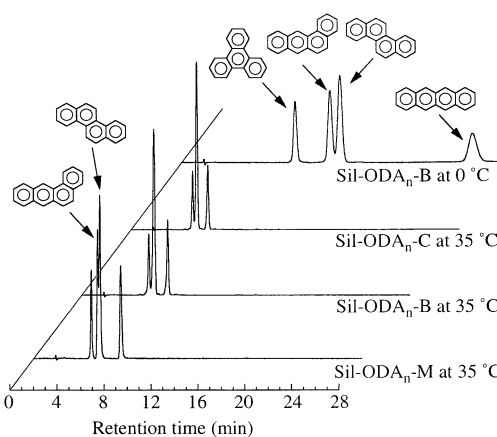

Fig. 6   Chromatograms of a mixture of four-ring aromatic hydrocarbons with Sil-ODA$_n$ at 0 and 35°C.   Mobile phase: methanol–water = 9:1.

(C20), assigned by previous works,[28] were also used to determine the chemical shifts of C2, C3, and C15 – C19. Additional supporting data were obtained by the 2D-INADEQUATE test.[29,30] The INADEQUATE correlation peaks were determined at 3660 Hz (14 ppm and 22.6 ppm), 5430 Hz (25.8 ppm and 28.5 ppm), 5450 Hz (22.6 ppm and 31.9 ppm),
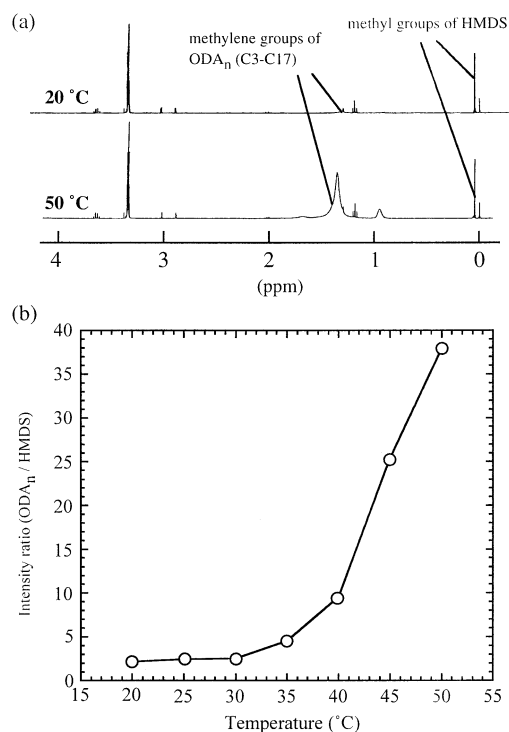
Fig. 7 (a) Suspension-state ^{1}H-NMR-spectra of Sil-ODA$_n$-M and (b) temperature dependency of the intensity ratio of methylene groups (C3 – C17) from ODA$_n$ and the methyl groups from standard (hexamethyldisilazane).

Table 1   Peak width at the half height ($W_{1/2}$) in C4 – C14 methylenes by ^{13}C-NMR spectroscopy

	Polymerization solvent	$W_{1/2}$/Hz
ODA$_n$-M	Methanol	12.7
ODA$_n$-C	Cyclohexane	19.4
ODA$_n$-B	Benzene	20.7

However, our silica-supported ODA$_n$ used in this study has only 16.4 – 18.5 wt% in the organic phase, and thus the content of an octadecyl group is even smaller (15 wt%), but the α value is much higher than that of conventional ODS. This is not only because ODA$_n$ has carbonyl groups as a π-electron source for an electrostatic interaction but also because it can form ordered structures at temperatures below T_c to promote multiple π-π interactions. Therefore, microenvironmental effect around the carbonyl groups cannot be negligible, but should be significant for our discussion. The present study cannot confirm an exact conformation of the carbonyl groups, but shows that the microenvironmental difference in the 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.

Acknowledgements

5550 Hz (25.8 ppm and 29.7 ppm), and 6120 Hz (29.3 ppm and 31.9 ppm). Therefore, the peaks of C17 can be determined to be at 22.6 ppm. Also C16 at 31.9 ppm, C15 at 29.3 ppm, C2 at 28.5 ppm, and C3 at 25.8 ppm. All assignments are reasonable when considering electronegative force of their neighbouring moieties except for C16, which is rather embarrassing. This is peculiar because it is higher than that of both neighbours. However, other researchers have also assigned the ^{13}C-NMR results of the carbon element located at the same position in similar compounds (octadecyl silane) to be higher than that of their neighbouring elements.[31,32]

   Based on the assignment, the peak width of the half height ($W_{1/2}$) in Fig. 8 was compared to obtain conformational information on the main chain of ODA$_n$. It is clear, as shown in Table 1, that the $W_{1/2}$ value of ODA$_n$-M is smaller than the others. Since the polymers were dissolved as isotropic states, the broadening of the signal is not due to molecular ordering, but rather polydispersity in the conformation of the main chain. Although the exact tacticity was not specified in this study, it is estimated that ODA$_n$-C and ODA$_n$-B show relatively high polydispersity in the tacticity than ODA$_n$-M. This estimation agrees with the fact that the phase transition temperature of ODA$_n$-M is slightly higher than the others.

Conclusions

We have clarified that the molecular-shape selectivity for PAHs with ODA$_n$ is influenced by the polymerization condition. According to Sander et al., the molecular-shape selectivity can be explained by the immobilization density of an octadecyl group on silica, and thus distinct enhanced selectivity can be realized by high-density ODS (more than 25 wt%).[33,34]

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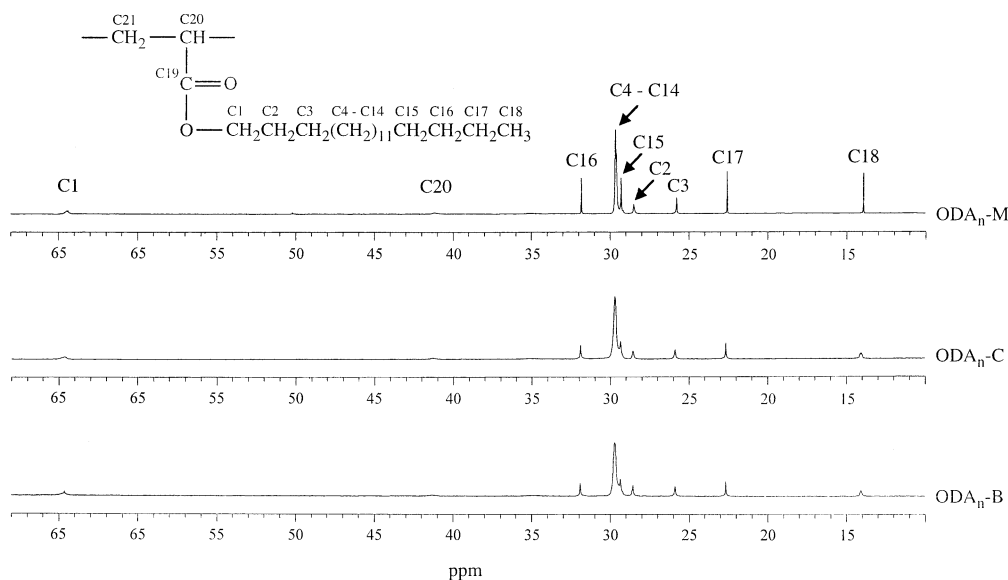
Fig. 8   Assignment of ^{13}C-NMR spectra of ODA$_n$. Solvent: chloroform-d.

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