Enhancement of Diastereomer Selectivity Using Highly-Oriented Polymer Stationary Phase

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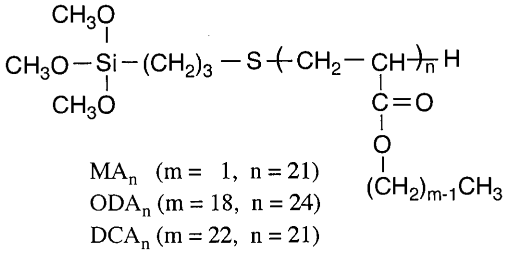
Enantiomer separation has been often realized with reversed phase liquid chromatography using diastereomerizing reagents with simply-hydrophobized silica. The present study demonstrates that diastereomer selectivity can be enhanced through highly-oriented organic phase produced by achiral poly(octadecyl acrylate)-grafted on silica and a carbonyl-π interaction due to the acrylate moiety is effective as the driving force for the selectivity enhancement.

Diasteromerization of enantiomeric isomers is very effective for chiral separation. When this technique is combined with reversed-phase liquid chromatography (RPLC) system, it yields very convenient analysis of enantiomer mixtures. Many diasteromerizing reagents have been developed.[1-7] These diasteromerizing reagents are characterized by the facts that chromophoric groups are included for sensitive detection and chiral separations are carried out by discriminating the hydrophobicity (or polarity) differences among the resulting diastereomers. Therefore, simply-hydrophobized silica such as octadecylated silica (ODS) has been often used for stationary phase of liquid chromatography. However, ODS usually shows low selectivity because the hydrophobicity difference among the diastereomers is small and ODS recognize mainly molecular hydrophobicity.

In this study, we focus on the fact that usual diasteremerizing reagents possess aromatic π-electrons which are useful as a π–π interaction source. In this communication, we report for the first time that poly(long-chain alkyl acrylate) with carbonyl-π electrons enhances diastereomer selectivity in RPLC mode and that it is brought only by high orientation of the polymer (schematically illustrated in Figure 2a).



$$CH_3O-\underset{OCH_3}{\overset{OCH_3}{Si}}-(CH_2)_3-S\!\left(CH_2-\underset{\underset{\underset{(CH_2)_{m-1}CH_3}{|}}{\overset{|}{\underset{O}{\overset{|}{C=O}}}}}{CH}\right)_n H$$

MA_n (m = 1, n = 21)
ODA_n (m = 18, n = 24)
DCA_n (m = 22, n = 21)

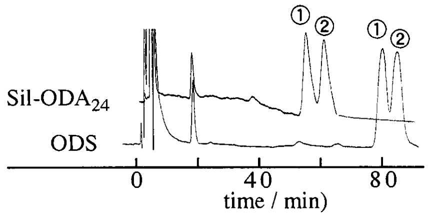


Figure 1. Typical chromatographs for the mixtures of the S-R (①) and S-S (②) isomers with Sil-ODA_{24} and ODS. Temperature, 20 °C. Mobile phase: methanol-water = 45 : 55.

(*S*)-(-)-(2,3-Naphthalenedicarboximidyl)propionyl fluoride (*Nip*-F)[4] is a commercially available diastereomerizing reagent for chiral separation against optically active amines. When the diastereomers of (*R*)- and (*S*)-phenylethylamines with (*S*)-*Nip*-F were analyzed by monomeric ODS[8] in methanol-water (45 : 55) at 20 °C, the separation factor (α) of 1.06 was obtained (Figure 1). This value is closed to that in the literature.[4] On the other hand, poly(octadecyl acryalate)-grafted silica[8] (Sil-ODA_{24}) provided a better α value (1.12) in the same condition than that of ODS (Figure 1). It was also observed that the α value was remarkably dependent on temperature: at 0 – 60 °C, α = 1.16 – 1.01 in Sil-ODA_{24} (Figure 2) and 1.09 – 1.01 in ODS. The detailed temperature dependence shows more significant information on understanding the enhancement mechanism. Figure 2 also indicates the selectivity enhancement compared with ODS. It is clear that distinct selectivity enhancement is observed only at temperature below 45 °C showing 1.8 – 2.2 times higher selectivity at 0 – 40 °C than those on ODS. To explain the distinct selectivity-jump at temperature around 45 °C in Figure 2, we detected that the silica-supported ODA_{24} had significant change in the physical state at the temperature. Differential scanning calorimetry (DSC) indicated that the immobilized ODA_{24} underwent a phase transition at temperature around 42 °C (T_c, peak-top temperature) in methanol-water (45 : 55). A polarization microscopic observation of ODA_{24} showed that a crystalline-to-isotropic phase transition was included at temperature around T_c. In addition, X-ray diffraction (4.16 Å with $2\theta = 21.3°$) in the silica-supported ODA_{24} provided similar patterns to non-supported ODA_{24} (4.15 Å with $2\theta = 21.4°$). These results indicate that the selectivity enhancement of Sil-ODA_{24} is realized at the highly-oriented structure of the immobilized phase.

To clarify the importance of the phase transition of the immobilized phase, the diastereomer selectivity was investigated with Sil-MA_{21} and Sil-DCA_{21} onto which poly(methyl acrylate) and poly(docosadecyl acrylate) were grafted. The former Sil-MA_{21} is always in an isotropic state at 0 – 60 °C. As a result, Sil-MA_{21} showed no diastereomer separation for the *Nip*-isomers (α = 1.0 at 0 – 60 °C). On the contrary, Sil-DCA_{21} with longer alkyl chains than Sil-ODA_{24} showed slightly higher selectivity (α = 1.18 and 1.06 at 0 and 45 °C) than Sil-ODA_{24} (α = 1.16 and 1.03 at 0 and 45 °C). DSC measurement showed that the immobilized DCA_{21} underwent a crystalline-to-isotropic phase transition at temperature around 58 °C (a peak-top temperature) in methanol-water (45 : 55). This higher T_c than that of Sil-ODA_{24} (42 °C) indicates that the DCA_{21} can maintain a highly-oriented structure even at higher temperature. Thease results strongly suggest that a

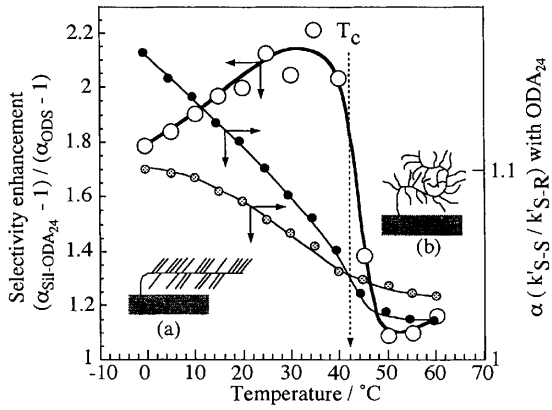
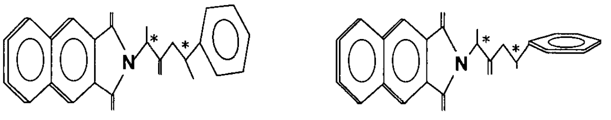


Figure 2. Temperature dependencies of the selectivities for the S-S- and S-R isomers through Sil-ODA$_{24}$. Mobile phases: methanol-water = 45 : 55 (○) and (●), actetonitrile-water = 33 : 67 (⊗). Figures (a) and (b) represent schematic illstration of crystalline and istropic states of grafted Sil-ODA$_{24}$.

crystalline state of the immobilized phase is essential to produce the selectivity enhancement.

We also investigated the effect of carbonyl groups because the structural difference between Sil-ODA$_{24}$ and ODS is characterized by them and a carbonyl π – benzene π interaction is effective for selectivity.[9,11] Here, if acetonitrile as a π-electron-containing medium was used instead of methanol, the α value decreased as shown in Figure 2. The value of 1.10 at 0 °C almost agreed with that in ODS at 0 °C (α = 1.09). Similar decrease of the selectivity was observed in acetone with π-electrons but not observed in 2-propanol. On the other hand, a DSC observation showed no significant difference in the phase transition behavior with acetonitrile. These results indicate that π-electron-containing media work as inhibitors for diastereomer selectivity regardless of the fact that the immobilized phase is in a crystalline state. These results show that the higher diastereomer separation can be realized both when the organic phase is in a highly-oriented state such as a crystalline state and a π-electron containing-solvent such as acetonitrile is not included in a mobile phase.

In our recent study, we have described that ODA$_n$ which is in a crystalline state showed molecular planarity recognition against polyaromatic hydrocarbons[9,10] and this ability would be realized through a carbonyl-π interaction.[9,11] However, there is no theoretical investigation. In this study, a carbonyl-π complex was evaluated against a formaldehyde-benzene complex model without solvent by the *ab initio* MO/MP2 calculations which were performed with Gaussian 94 package. The binding energy was calculated as a function of distance *R* between the carbon atom of formaldehyde and benzene plane, in which formaldehyde was moved perpendicularly to the benzene plane (plane-to-plane interaction) with the orientation fixed to that of the optimized geometry. According to our calculation result, a formaldehyde-benzene interaction (1.83 kcal mol^{-1}) is more effective compared with CH_4-benzene[12] (0.57 kcal mol^{-1}) and benzene-benzene[12] (0.49 and 1.78 kcal mol^{-1} in the parallel and perpendicular interactions, respectively) complexes. Therefore, we estimate that the diastereomer selectivity enhancement through Sil-ODA$_{24}$ and Sil-DCA$_{21}$ are brought with a π–π interaction due to carbonyl groups on a highly-oriented structure. As shown in Figure 3, the planar structure of the S-S isomer provides more suitable conformation for multiple interactions between the highly-oriented carbonyl groups and the aromatic π-electrons than that of the twisted *S-R* isomer.



S-S isomer S-R isomer

Figure 3. Structural difference between S-S and S-R isomers optimized by CAChe-Mopac Ver. 6.00 with the PM3 option.

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References and Notes

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8 ODA$_n$ was prepared with Fuji Silysia Super Micro Bead silica gel (100A-5D, diameter 5 μm, pore size 146 Å, specific surface area 330 cm^{2}g^{-1}) by the method reported previously.[9,10] The immobilized amount was 18.0 wt%. ODS was prepared with Fuji Silysia Super Micro Bead silica gel (100A-5D) by an ordinal procedure.[10]
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