

rations; for example, the Sil-1 column showed a remarkably high separation factor with a specific temperature dependence for polyaromatics, recognizing molecular planarity [1] and molecular length [2] of sample solutes. No similar behaviour was observed with conventional reversed-phase liquid chromatographic packings such as octadecylated (ODS) silica gels. These attributes of Sil-1 are derived from the highly oriented structure of polymerized long-chain alkyl groups and their phase transition behaviour between the crystal and isotropic states (Fig. 1b).

In this paper, we report that Sil-1 shows geometrically selective retention as an HPLC column and its separation mechanism is also discussed.

2. Experimental

2.1. Materials

Sil-1 was prepared by telomerization of octadecyl acrylate with 3-mercaptoptrimethoxysilane and subsequent immobilization on silica gels as reported previously [1,2]. The average degree of polymerization of grafted poly(octadecyl acrylate) was estimated as 27 by NMR spectroscopy. Elemental analysis indicated that 20.6 mass-% of organic phase was immobilized on porous silica gels (YMC SIL-120-S5; diameter 5 μm ; pore size 120 \AA ; specific surface area 300 $\text{cm}^2 \text{g}^{-1}$).

2.2. Measurements

Sil-1 was packed into a stainless-steel column (250 mm \times 4.6 mm I.D.) and its liquid chromatographic properties were examined using methanol–water as eluent. The chromatograph included a JASCO Model 880 PU pump and a Shimadzu SPD-M6A UV–visible photodiode-array detector. A 5- μl volume of the sample dissolved in methanol was injected through a Reodyne Model 7125 injector. Chromatography was carried out at flow-rate of 0.5 ml min^{-1} . Differential scanning calorimetric (DSC) thermograms of polymer 1 or Sil-1 (5 mg) were

obtained using a heating rate of 2°C min^{-1} with a Seiko I & E SSC-580 with a DSC-10 differential scanning calorimeter.

3. Results and discussion

3.1. Retention behaviour of stilbene

The Sil-1 column gave a complete separation of a mixture of *trans*- and *cis*-stilbene at 25°C (Fig. 2). The theoretical plate number for the column and the separation factor (α , the ratio of the capacity factors of the *trans*- and *cis*-isomers) are 18 000 and 1.97, respectively. On the other hand, an ODS column (300 mm \times 4.6 mm I.D.) (GL Science) provided a very small separation factor ($\alpha = 1.07$ at 25°C). This large difference in α values indicates that Sil-1 has a specific retention mechanism, although Sil-1 includes a retention mode similar to that in reversed-phase liquid chromatography (RPLC) because the elution order is the same as that in ODS and the retention capacity increases with increase in mobile phase polarity.

The specificity of Sil-1 was emphasized by examining the temperature dependence of its retention behaviour. Fig. 3 shows the temperature dependences of the capacity factors (k') of stilbenes. With ODS, the k' values decreased with increase in temperature. Such negative slopes as in Fig. 3 are commonly observed with the usual RPLC packings [5,6]. These can be explained by an increase in the solubility of solutes from the stationary to the mobile phase

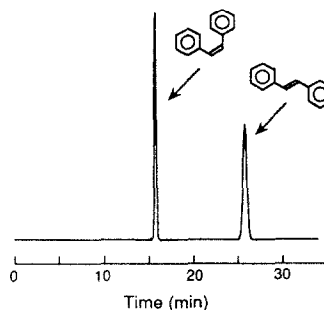


Fig. 2. Separation of a mixture of *trans*- and *cis*-stilbene with a Sil-1 column. Mobile phase: methanol–water (7:3).

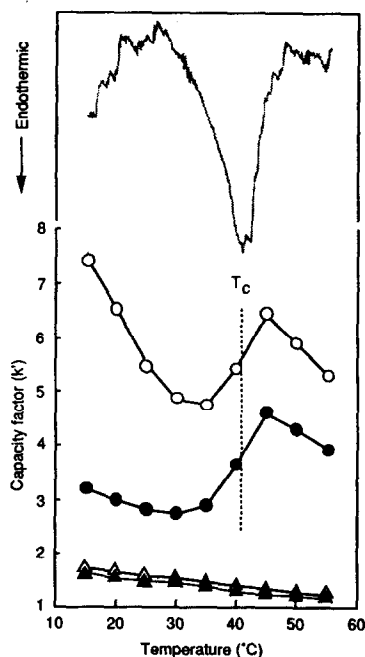


Fig. 3. Temperature dependences of the capacity factor of stilbene. DSC thermogram of Sil-1 in a methanol–water (7:3) dispersion shown as a dotted line (top). T_c corresponds to the peak-top temperature of the phase transition. Methanol–water mixtures were used as mobile phases (7:3 with Sil-1 and 9:1 with ODS). The k' value was determined by using the retention time of glycerol. \circ = *trans*-Stilbene with Sil-1; \bullet = *cis*-stilbene with Sil-1; \triangle = *trans*-stilbene with ODS; \blacktriangle = *cis*-stilbene with ODS.

with increase in temperature [5,6]. In contrast, Sil-1 provided critical bending points and positive slopes at temperatures between 35 and 45°C. Interestingly, this unusual temperature dependence includes a remarkable selectivity change. The α values are 2.33 (15°C), 1.97 (25°C), 1.64 (35°C), 1.55 (40°C), 1.40 (45°C), 1.38 (50°C) and 1.35 (55°C). With ODS, only small and constant α values (1.04–1.07 at 15–55°C) were observed.

3.2. Retention mechanism of Sil-1

The temperature shown as a dotted line in Fig. 3 indicates the phase transition behaviour (T_c , peak-top temperature) of the immobilized comb-shaped polymer 1 on silica gels, which was directly observed by DSC measurements in the

methanol–water (7:3) dispersion state. Polarization microscopic observation indicated that this transition was from the crystalline to the isotropic state of the immobilized polymer 1. This temperature lies in the temperature range at which the k' values increase in Fig. 3. Therefore, the unusual increase in retention is related to the phase transition of immobilized polymer 1 and the separation factor was much higher at crystal-state temperatures ($\alpha = 2.33$ at 15°C) than at isotropic-state temperatures ($\alpha = 1.35$ at 55°C). This indicates that the oriented structure of immobilized phase provides high selectivity for geometric isomers.

On the other hand, the positive slope of the k' versus temperature plots at 30–45°C does not show a change in the retention mechanism. For this unusual positive slope, we have following explanation: immobilized 1 forms a mixed phase containing both crystalline and isotropic phases and the ratio of isotropic 1 to crystalline 1 increases with increase in temperature at 30–45°C. Therefore, the capacity factor (k') also increases with increase in this ratio, because k' is larger in the isotropic than in the crystalline state.

In order to understand this unusual specificity, we have discussed previously that highly oriented structures of an immobilized phase can recognize the molecular planarity of solutes [1–3]. For example, a planar compound, triphenylene, is more strongly incorporated in a crystal-state medium than a corresponding non-planar compound, *o*-terphenyl [$\alpha = 4.0$ (25°C) and 1.5 (25°C) in Sil-1 and ODS, respectively] [1,3]. In support of this, the stilbene isomers have very different molecular shapes. According to this assumption, it is considered that the steric bulkiness of *cis*-stilbene prevents it from being incorporated into a crystal medium.

However, in this study we encountered retention phenomena that cannot be explained only by this assumption. For example, Sil-1 showed geometrically selective retention for 1,2-bis(phenylsulphonyl)ethylene isomers ($\alpha = 1.60$ at 25°C; Fig. 4), but showed almost no selectivity for geometrical isomers of 1,4-dichloro-2-butene (Fig. 4), 1,2-dimethylcyclohexane and deca-

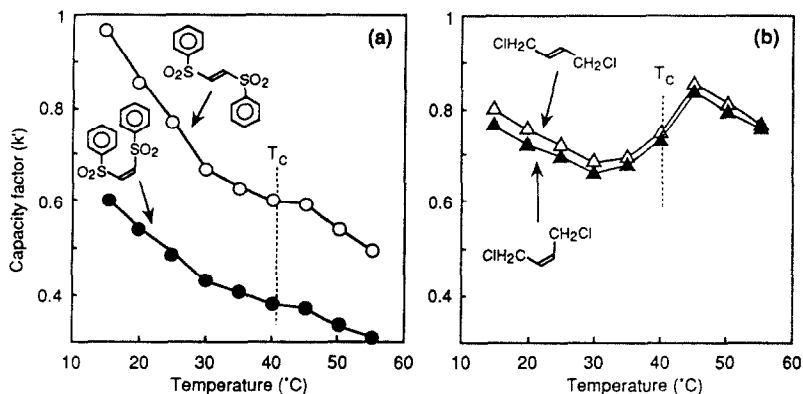


Fig. 4. Temperature dependences of the capacity factor of geometrical isomers of (a) 1,2-bis(sulphonyl)ethylene and (b) 1,2-dichloro-2-butene. Mobile phase: methanol–water (7:3).

hydronaphthalene ($\alpha = 1.04$, 1.05 and 1.08 at 25°C, respectively). However, the k' versus temperature plots showed a distinct jump at temperatures around the phase transition temperature in all samples. This indicates that the difference in bulkiness between *cis*- and *trans*-isomers is not always reflected in geometric selectivity.

On the basis of these results, our previously proposed mechanism for molecular recognition should be modified and developed as follows. (1) Immobilized polymer **1** forms a highly oriented structure at its crystal-state temperatures. A planar compound such as *trans*-stilbene can be easily incorporated in this oriented polymer. This mechanism is analogous to the fact that cholesterol as a rigid and planar compound is readily incorporated into lipid bilayer membranes which form a highly oriented structure. (2) Immobilized polymer **1** interacts with a double bond of the solutes, recognizing its π -electron density. Perhaps this interaction is derived from carbonyl π -electrons of acrylate moieties, which can work as electron-accepting groups. This assumption is supported by following facts: (i) it was confirmed that cross-linked poly(methyl acrylate) polymer (MA) particles as a reference showed higher selectivity for various isomeric aromatics compared with ODS, although these MA particles provided comparably small k' values because of the absence of a long-chain alkyl group; (ii) from calculation of

the energy level of the HOMO (highest occupied molecular orbital) in stilbene, the *trans*-isomer is more electron donating (-8.63 eV) than the *cis*-isomer (-9.06 eV) [calculation of the energy level of the HOMO was carried out with Materia (MOPAC 6.00 with PM3 option) of Teijin System Technology (Japan)]. A similar interaction between benzene and formic acid was calculated by Bredás and Street [7]. On the other hand, geometrical isomerism for 1,4-dichloro-2-butene having non-conjugating substituent groups provides no significant difference in the energy level of the HOMO. In this case, Sil-1 shows a small selectivity for retention of these isomers.

Acknowledgements

We are grateful to Mr. N. Nakamura for his capable assistance. We also thank Mr. Goda and Mr. Ohe of Sumitomo Seika Chemicals for the use of Materia. This study was supported in part by a Grant-in-Aid for Scientific Research from Fukuoka Science and Technology Foundation (Japan).

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