

Crystalline polymer on silica. Geometrical selectivity for azobenzenes through highly-oriented structure

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Abstract

Poly(octadecyl acrylate), ODA_n, with a trimethoxysilyl group at one side of the terminal group was immobilized onto porous silica by using the reactive terminal group. The silica-supported ODA_n underwent crystalline-to-isotropic phase transition. Interaction between ODA_n and azobenzenes were evaluated by retention time in the liquid chromatography using the column packed with silica-supported ODA_n. The chromatograph showed complete separation against the geometrical isomers of azobenzenes in methanol–water. The temperature dependence of the separation factor indicated that the selectivity increased with the molecular orientation of ODA_n. Effect of carbonyl– π interaction due to the acrylate moiety on the selectivity was examined using seven kinds of *p*-substituted phenylazobenzenes with various Hammett's substituent constants. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Derivatives containing azobenzene and stilbene moieties undergo geometrical isomerization between *cis*- and *trans*-conformations. This transition is induced thermally or by irradiation of light and is accompanied by remarkable morphological change as well as physicochemical change. Therefore, many researchers have focused on using them as photo-sensitive functional groups: for example, crown ether bound to azobenzenes by Shinkai et al. [1,2], membrane-forming lipid containing azobenzene and stilbene moieties by Kunitake et al. [3,4], and residual azobenzene-containing polypeptides by Kinoshita et al. [5] and Sisido et al. [6]

In this paper, we describe separation of geometrical isomers of azobenzene derivatives using crystalline polymer-silica hybrids. This study can be characterized by the fact that the lipid membrane analogue-immobilized silica [7,8] (Fig. 1) was used for molecular recognition, and the geometrical selectivity was evaluated by the retention time in the liquid chromatography using the column packed with the silica. The lipid membrane-analogue (ODA_n) cannot form aqueous lipid bilayer structures because of its insolubility in water, but shows molecular orientation and phase transition

like lipid bilayer membranes in various polar organic solvents. We describe that the selectivity is absolutely sensitive to the molecular orientation of the analogue and carbonyl– π interaction due to the acrylate moiety is included in the molecular recognition mechanism using seven kinds of *p*-substituted phenylazobenzenes with various Hammett's substituent constants.

2. Experimental

2.1. Materials

Silica-supported poly(octadecyl acrylate) was prepared by radical telomerization of octadecyl acrylate (ODA) with 3-mercaptopropyltrimethoxysilane (MPS) and following immobilization onto silica according to our previously reported method [9]. Typical preparation procedure is as follows: ODA and MPS (20:1 in the molar ratio) were dissolved in ethanol. Azobisisobutyronitrile (20 mol.% for the mercapto group) was added to the ethanol solution at 80°C. The mixture was stirred for 6 h at 80°C under a N₂ gas atmosphere. The resulting white precipitates were gathered by filtration, washed successively with methanol and acetone and dried in vacuo. The structure and polymerization

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degree of the polymer were determined by NMR spectroscopy. ^1H NMR chemical shifts of ODA_n in CDCl_3 were as follows: $\delta = 0.8$ ppm (SiCH_2), $\delta = 1.9$ ppm (CHCO), $\delta = 3.6$ ppm (SiOCH_3), and $\delta = 4.0$ ppm (C(=O)OCH_2). The average degree of polymerization (n) was determined to be 23 by the proton ratio at 3.6 and 4.0 ppm.

ODA_{23} was readily immobilized onto porous silica by mixing in tetrachloromethane at reflux temperature for 72 h. YMC 120-S5 (diameter $5\ \mu\text{m}$, pore size $120\ \text{\AA}$, specific surface area $300\ \text{cm}^2\ \text{g}^{-1}$) was used as porous silica. Immobilization was confirmed by IR spectroscopy. The specific absorptions due to carbonyl and long-chain alkyl groups were detected around 1740 and $2900\text{--}3000\ \text{cm}^{-1}$, respectively. The amount of ODA_{23} immobilized was determined to be 19.9 wt% by elemental analysis. Successive washing with chloroform as a good solvent for ODA_{23} showed no significant change in the elemental analysis.

Poly(methyl acrylate) with a trimethoxysilyl group at one side of the terminal group (MA_n) was prepared and immobilized onto silica according to our previously reported method [10,11]. The average degree of polymerization was estimated to be 27 by ^1H NMR spectroscopy. Immobilization of the polymer onto silica was carried out according to that of silica-supported ODA_{23} . The amount of MA_{27} immobilized was determined to be 23.0 wt% by elemental analysis.

Phenylazobenzene was purchased and used after recrystallized from ethanol. *p*-Substituted phenylazobenzenes were synthesized by coupling reactions between nitrosobenzenes and corresponding *p*-substituted anilines [12–17]. The chemical structures were confirmed by NMR spectroscopy and elemental analyses. The melting points are as follows: mp $54.6^\circ\text{C}\text{--}55.3^\circ\text{C}$ ($-\text{OCH}_3$, Ref. [12] 55.5°C); mp $67.5^\circ\text{C}\text{--}68.6^\circ\text{C}$ ($-\text{CH}_3$); mp $89.0^\circ\text{C}\text{--}90.0^\circ\text{C}$ ($-\text{Br}$, Ref. [13] 89°C); mp $87.1^\circ\text{C}\text{--}87.9^\circ\text{C}$ ($-\text{Cl}$, Ref. [14] $88^\circ\text{C}\text{--}90^\circ\text{C}$); mp $67.9^\circ\text{C}\text{--}69.0^\circ\text{C}$ ($-\text{H}$, Ref. [15] 68°C); mp $108.2^\circ\text{C}\text{--}109.6^\circ\text{C}$ ($-\text{COCH}_3$, Ref. [16] 115°C); mp $118.8^\circ\text{C}\text{--}120.4^\circ\text{C}$ ($-\text{CN}$, Ref. [17] 120.5°C).

2.2. Photo-irradiation

Trans-to-cis isomerization of azobenzenes was carried out by irradiation of the $300\text{--}400\ \text{nm}$ light with a low pressure Hg lamp with a Toshiba UV-D35 filter at room temperature.

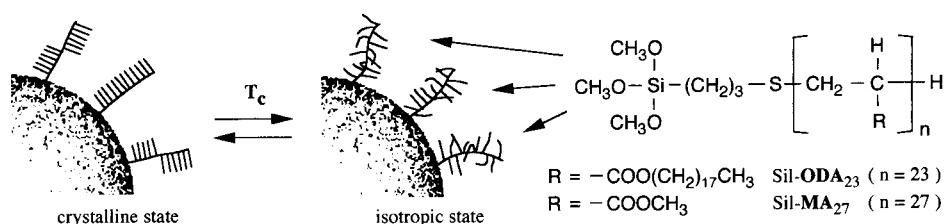


Fig. 1. Schematic illustration of grafting of lipid membrane analogue onto silica and chemical structures of poly(octadecyl acrylate) and poly(methyl acrylate) having a reactive terminal group at the one side of the main chain. ODA_{23} can undergo crystalline-to-isotropic phase transition on silica.

2.3. Measurements

Silica-supported ODA_{23} (Sil-ODA_{23}) and MA_{27} (Sil-MA_{27}) were packed into stainless-steel columns ($4.6\ \text{mm}$ i.d. $\times 250\ \text{mm}$) using a hexanol–chloroform (1:1) mixture and the liquid chromatographic property was examined using methanol–water or other polar organic solvent–water mixtures as mobile phases. The chromatograph included a JASCO 980 PU pump, a JASCO photodiode array detector (UVIDEC-100-IV) and a column heater (Sugai U-620 Type VP50). Ten microlitres of the sample dissolved in methanol was injected through a Reodyne Model 7125 injector. Chromatography was carried out at flow-rate 0.5 or $1.0\ \text{ml}\ \text{min}^{-1}$. The retention factor (k') was determined by $(t_r - t_0)/t_0$ where t_r and t_0 are retention time of samples and methanol, respectively. The separation factor (α) was given by the ratio of retention time.

DSC thermograms of ODA_{23} and Sil-ODA_{23} were obtained using a heating rate of $1^\circ\text{C}\ \text{min}^{-1}$ with a Seiko I and E SSC-580 and a DSC-10 instrument.

Trans-to-cis isomerization was monitored by ^1H NMR spectroscopy using a JEOL JNM-EX400.

Water–1-octanol partition coefficient ($\log P$) was determined by retention factor with octadecylated silica, $\log P = 3.134 + 4.203\log k'$ [18].

3. Results and discussion

3.1. Chromatographic detection of *trans-to-cis* isomerization

Fig. 2 shows the time course of the *trans-to-cis* isomerization in phenylazobenzene determined by ^1H NMR in CD_3OD . The content of the *cis*-isomer was determined by the proton ratio: $\delta = 7.81$ ppm (*d*) and 7.45 ppm (*m*) in the *trans*-isomer; $\delta = 7.17$ ppm (*t*), 7.07 ppm (*t*) and 6.74 ppm (*d*) in the *cis*-isomer. As shown in Fig. 2, the photo-irradiation induced *trans-to-cis* isomerization, but the maximum content of the *cis*-isomer was saturated at about 65 mol.%.

Fig. 3(a)–(c) show typical chromatograms of phenylazobenzene with Sil-ODA_{23} . Two kinds of peaks were observed at about 9 min and 23 min in their retention time (t_r). The photo-irradiation induced increase of the former peak

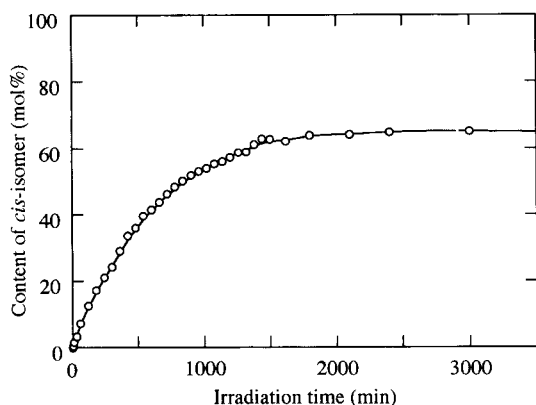


Fig. 2. Time course of *trans*-to-*cis* isomerization of phenylazobenzene by photo-irradiation at room temperature in methanol. The ratio of the *cis*-isomer was determined by ^1H NMR.

($t_r = 9$ min) with decrease of the latter peak ($t_r = 23$ min). These peaks can be ascribed to those of the *cis*- and *trans*-isomers by using a photodiode array detector. Therefore, the UV-visible spectra shown in Fig. 3(d) and (e) correspond to those of the *cis*- and *trans*-isomers without the impurity due to the geometrical isomers. Similar complete separation of the *cis*- and *trans*-isomers were observed in *p*-substituted phenylazobenzenes although their retention times are much dependent on the chemical structure of the substituent group.

3.2. Temperature dependencies on retention factor and separation factor

Fig. 4 includes the temperature dependencies on the retention factor (k') with Sil-ODA₂₃ and methanol–water (7:3) as a mobile phase. The samples are *p*-methoxyphenylazobenzene and *p*-cyanophenylazobenzene. These samples are selected as azobenzenes with typical electron-donating and -accepting substituents, respectively. The result provides significant information on the interaction mechanism between ODA₂₃ and azobenzenes. The k' values are much higher in the *trans*-isomers regardless of temperature and the substituent groups than in the *cis*-isomers. This can be explained by the facts that the *trans*-to-*cis* isomerization induces remarkable hydrophobicity change of azobenzene and the *trans*-isomers are more hydrophobic (less polar) than the *cis*-isomers. The hydrophobicities are evaluated by water–1-octanol partition coefficients¹ [18] ($\log P$): $\log P$ s are 0.75/3.032, 1.558/3.732 and 1.657/3.807 in *cis*-/

¹ Hydrophobized silica (C, 14.2%) was prepared by YMC GEL SIL-120-S5, dimethyloctadecylchlorosilane and trimethylchlorosilane and was packed into a stainless-steel column (4.6 mm i.d. \times 300 mm). The chromatography was carried out at a flow-rate of 0.5 ml min⁻¹ and with methanol–water (9:1). The standard samples are as follows: benzene ($\log P = 2.280$), naphthalene ($\log P = 3.208$), anthracene ($\log P = 4.378$), pyrene ($\log P = 5.030$), diphenyl ($\log P = 3.772$), toluene ($\log P = 2.588$), ethylbenzene ($\log P = 3.118$), butylbenzene ($\log P = 4.178$), hexylbenzene ($\log P = 5.238$), octylbenzene ($\log P = 6.297$), and decylbenzene ($\log P = 7.358$).

trans-*p*-cyanophenyl-, *p*-phenyl- and *p*-methoxyphenylazobenzenes, respectively. The large differences between the $\log P$ values of the isomers will give rise to those of the retention factors because ODA₂₃ works as a very hydrophobic organic phase in a polar medium such as methanol–water.

On the other hand, it is generally known that increase of temperature reduces a retention factor [19,20]. This is due to the increase of the solubility of the solutes from stationary to mobile phase with increase of temperature [19,20]. However, the distinct k' -jumps were observed at temperature around 35°C–45°C as shown in Fig. 4. This unusuality was accompanied by the distinct change of the separation factor (α) which was calculated by the ratio of the retention factors ($\alpha = k'_{\text{trans}}/k'_{\text{cis}}$). As shown in Fig. 5(a) and (b), the selectivity changes are observed at temperature around 35°C and much higher below the temperature than above. Neither similar k' -jump nor selectivity change were observed in simply-hydrophobized silica having an isotropic organic phase. These results strongly suggest that the retention mechanism cannot be understood only by hydrophobic effect and that the phase transition of ODA₂₃ would occur at temperature around 35°C–45°C. This assumption is supported by following results: firstly, DSC showed that the silica-supported ODA₂₃ provided an endothermic

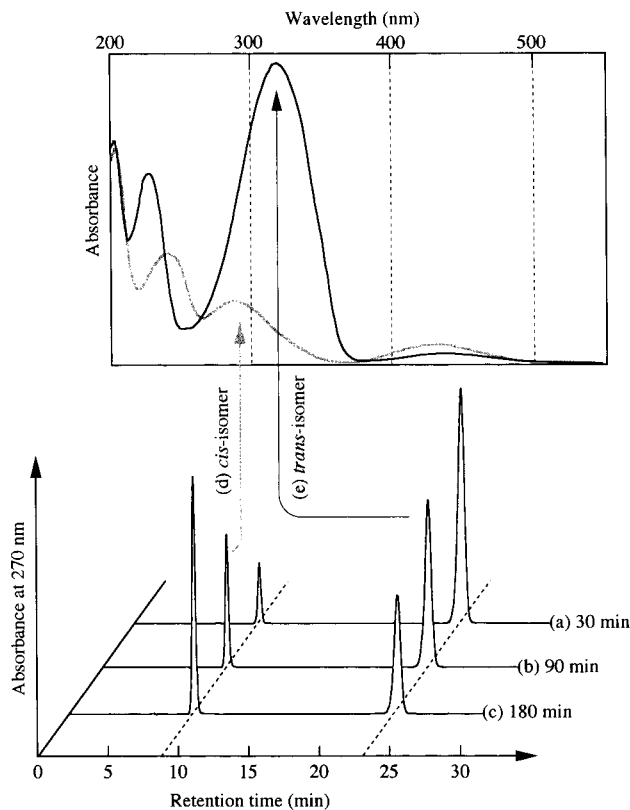


Fig. 3. Typical chromatograms of phenylazobenzene after photo-irradiation for: (a) 30 min; (b) 90 min; and (c) 180 min with Sil-ODA₂₃. Mobile phase, methanol–water (7:3) at 25°C. Flow-rate, 0.5 ml min⁻¹. The UV-visible spectra (d) and (e) are derived from the *cis*- and *trans*-phenylazobenzene, respectively.

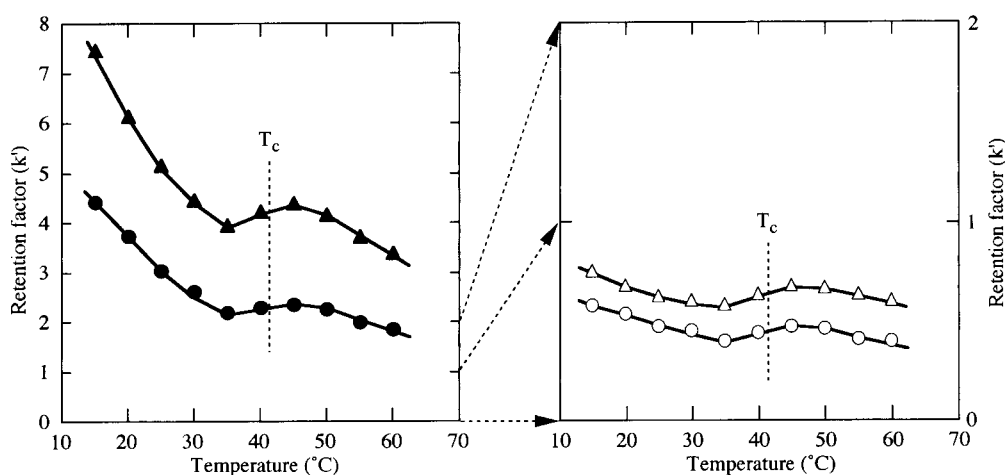


Fig. 4. Temperature dependencies on the retention factor (k') with Sil-ODA₂₃. Mobile phase, methanol–water (7:3). Flow-rate, 1.0 ml min⁻¹. (○) *cis-p*-cyanophenylazobenzene, (●) *trans-p*-cyanophenylazobenzene, (△) *cis-p*-methoxyphenylazobenzene, and (▲) *trans-p*-methoxyphenylazobenzene.

transition² from crystalline to isotropic states at temperature around 41°C (T_c) in methanol–water (7:3). The T_c of ODA₂₃ is closely related to the temperatures showing the k' -jump (Fig. 4) and the selectivity change (Fig. 5). In addition, it should be noted that the separation factor observed at the crystalline temperature is much higher than that obtained by extrapolating the separation factor at the isotropic phase transition temperature. Therefore, we estimate that this gap is derived from the molecular orientation effect without hydrophobic effect.

Secondly, poly(methyl acrylate)-immobilized silica (Sil-MA₂₇) also showed complete separation for the geometrical isomers of azobenzenes in methanol–water (7:3) as a mobile phase. However, no k' -jump was observed and the selectivity was very low (Fig. 5) at temperature

between 15°C and 60°C: for example, the selectivities for *p*-methoxyphenylazobenzene were 3.0–2.3 in Sil-MA₂₇ and 10.0–5.7 in Sil-ODA₂₃ at 15°C–60°C. Sil-MA₂₇ does not show a crystalline-to-isotropic phase transition because of no long-chain alkyl group.

These results indicate that the crystalline (highly-oriented) structure of ODA₂₃ increases geometrical selectivity. Here, it should be noted that *trans*-to-*cis* isomerization of azobenzene is accompanied by drastic morphological change as well as hydrophobicity change. For example, as shown in Fig. 6, the isomerization induces the change of the molecular planarity. The minimum thicknesses are 3.02 and 5.62 Å in the *trans*- and *cis*-isomers, respectively.³ Perhaps, the crystalline ODA_n prefers a thin and planar isomer to a bulky isomer. Such a phenomenon is similar to the fact that a rigid and planar cholesterol can be easily incorporated into lipid bilayer membranes with highly-oriented structure. We have been briefly reported that Sil-ODA_n can recognize molecular bulkiness: for example, Sil-ODA_n provided remarkable k' -jump at temperature below and above T_c against adamantane (6.53 Å in the minimum thickness, Fig. 6) as a bulky hydrocarbon [9]. Molecular-bulkiness recognition by crystalline ODA_n are further discussed elsewhere [21].

3.3. Contribution of carbonyl- π interaction

ODA₂₃ has a lot of carbonyl groups per molecule. Such π -electrons can work as a driving force for selectivity

Table 1
Effect of solvent on the separation factor between *cis*- and *trans*-isomers of *p*-substituted (-R) phenylazobenzenes with Sil-ODA₂₃

-R	Solvent	(%) ^a	k'_{cis}	k'_{trans}	k'_{trans}/k'_{cis}
-H	Methanol	(70)	0.58	3.35	5.81
	Ethanol	(55)	0.66	3.70	5.63
	2-Propanol	(45)	0.73	3.74	5.10
	Acetone	(55)	1.08	3.96	3.67
-CN	Acetonitrile	(55)	0.99	3.64	3.68
	Methanol	(70)	0.50	3.16	6.35
	Ethanol	(55)	0.52	2.86	5.45
	2-Propanol	(45)	0.58	2.62	4.51
-OCH ₃	Acetone	(55)	1.00	3.75	3.75
	Acetonitrile	(55)	0.87	3.54	4.06
	Methanol	(70)	0.66	5.36	8.16
	Ethanol	(55)	0.64	4.93	7.68
-OCH ₃	2-Propanol	(45)	0.65	4.44	6.80
	Acetone	(55)	1.04	4.65	4.45
	Acetonitrile	(55)	0.97	4.56	4.70

Mobile phase: water–organic solvent mixtures at 25°C.

^aPresents the vol.% of organic solvents.

² DSC and polarization microscopic observation indicated that ODA₂₃ was in a crystalline state below 42°C and an isotropic state above 50°C. The peak-top temperature was 48°C. A liquid crystalline state existed at temperature between 42°C and 47°C. Similar phase transitions were also observed in methanol–water (7:3), accompanied by a slight decrease in the peak-top temperature by 2°C–4°C. Immobilization of ODA₂₃ onto silica further lowered the phase transition temperature. The peak-top temperature was 41°C in methanol–water (7:3).

³ The molecular shape of azobenzene was estimated by Sony-Tektronix MOPAC (Ver. 6.0 with PM3 option).

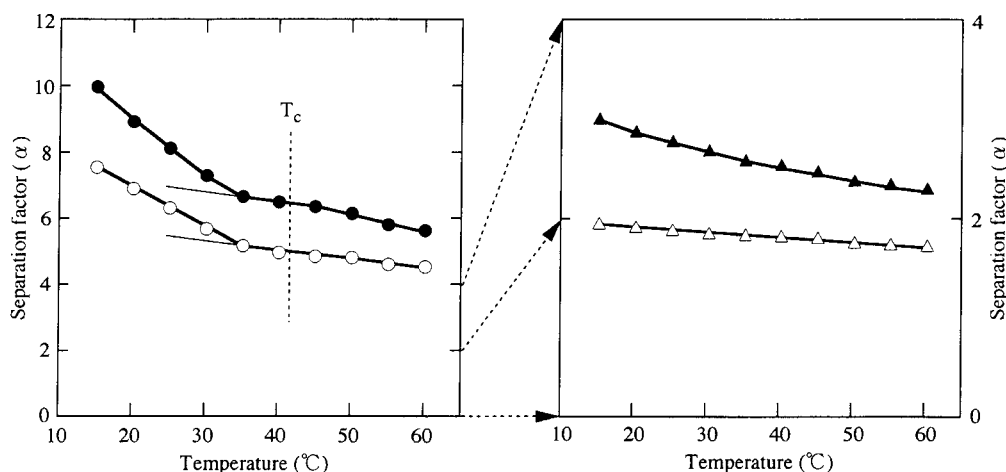


Fig. 5. Temperature dependencies on the separation factor (α) between *trans*- and *cis*-isomer with: (a) Sil-ODA₂₃; and (b) Sil-MA₂₇. Mobile phase, methanol–water (7:3). Flow-rate, 1.0 ml min⁻¹ in Sil-ODA₂₃ and 0.5 ml min⁻¹ in Sil-MA₂₇. (○, △) *p*-cyanophenylazobenzene, and (●, ▲) *p*-methoxyphenylazobenzene.

[22–25]. Therefore, we estimate that carbonyl– π interaction will be included in the selective retention with Sil-ODA₂₃. Both electrostatic and charge transfer interactions are mainly included in it. As shown in Fig. 5, the geometrical selectivity is higher in the *p*-methoxy derivative ($\alpha = 10.0$ at 15.0°C) than in the *p*-cyano derivative ($\alpha = 7.6$ at 15.0°C). These substituent groups are typical electron-donating and -accepting groups, respectively. In order to clarify the importance of the substituent group for the selectivity, seven kinds of *p*-substituted phenylazobenzenes (-0.27 – 0.66 in the Hammett's substituent constant, σ) were selected as samples and their retention factors were examined. As a result, the α – σ plots in Sil-MA₂₇ provide a good linearity (a correlation coefficient, $r = 0.98$) with a reaction constant ρ , -0.85 [Fig. 7(b)]. This negative ρ value indicates that the geometrical selectivity is dependent on an electron-donating property of the substituent group. Perhaps an electron-accepting property is derived from carbonyl groups of the acrylate moieties. On the other hand, an extremely small correlation coefficient ($r = 0.29$ at 15°C) was obtained in the α – σ plots with Sil-ODA₂₃ [Fig. 7(a)]. This plot rather seems to show that Sil-ODA₂₃ does not provide carbonyl– π interaction. However, a remarkable increase of the correlation coefficient from 0.29 to 0.64 was observed with an increase in temperature from 15°C to 60°C [Fig. 7(a)]. ODA₂₃ is in an isotropic state at 60°C ($T_c = 41^\circ\text{C}$). This indicates that carbonyl– π interaction becomes effective under the isotropic state condition.

Effect of carbonyl– π interaction can be also evaluated by using π -electron-containing solvents instead of methanol as mobile phases. Table 1 summarizes the geometrical selectivity in various mobile phases. The ratios of organic solvents to water were adjusted so that the hydrophobicity difference between mobile phases would be small, because methanol is the most hydrophilic solvent and thus the decrease of the hydrophilicity causes a remarkable k'

decrease. As shown in Table 1, the smallest separation factor were observed by using acetone and acetonitrile regardless of the substituent group. The big differences between the separation factors in 2-propanol and acetone have a particularly significant meaning. These results strongly support the point that the geometrical selectivity is reduced by π – π interaction between azobenzenes and mobile phases.

In conclusion, we have described the immobilization of ODA₂₃ as a lipid membrane analogue onto silica and that the ODA₂₃ shows high geometrical selectivity for azobenzenes. The recognition ability is especially higher at the crystalline temperatures of ODA₂₃ than at the isotropic temperature.

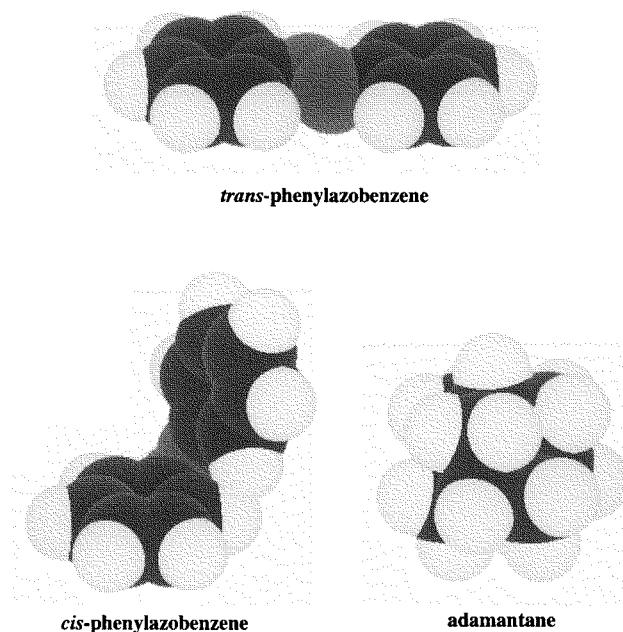


Fig. 6. Possible structures of phenylazobenzene and adamantane estimated by MOPAC Ver. 6.00 calculation with PM3 option.

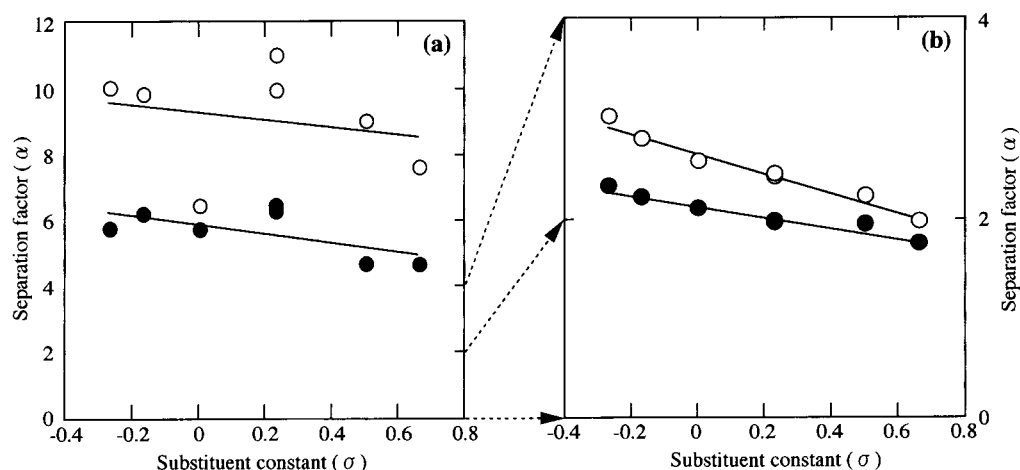


Fig. 7. Plots of the separation factor (α) between *trans*- and *cis*-isomer and the Hammett's substituent constant (σ) of *p*-substituted phenylazobenzenes with: (a) Sil-ODA₂₃; and (b) Sil-MA₂₇ at 15°C (○) and 60°C (●). Hammett's substituent constant (σ): -0.27 (-OCH₃); -0.17 (-CH₃); 0.0 (-H); 0.23 (-Cl); 0.23 (-Br); 0.50 (-COCH₃); and 0.66 (-CN). Mobile phase, methanol–water (7:3). Flow-rate, 1.0 ml min⁻¹ in Sil-ODA₂₃ and 0.5 ml min⁻¹ in Sil-MA₂₇.

This is because the molecular-planarity is recognizable by the highly-oriented structures. In this study, it was also clarified that carbonyl- π interaction was included as an additive effect for increasing the selectivity.

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