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Retention versatility of silica-supported comb-shaped crystalline and non-crystalline phases in high-performance liquid chromatography

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Abstract

Silica-supported comb-shaped polymer (Sil-ODA₁₈) provided a specific selectivity towards aromatic and non-aromatic solutes. Selectivity of Sil-ODA₁₈ and ODS (polymeric and monomeric) columns towards polycyclic aromatic hydrocarbons and geometric isomers have been discussed in respect of molecular shapes and sizes. The retention versatility of this phase arises from the orientation change on transition from the ordered crystalline to the less ordered non-crystalline state. Selectivity in the crystalline and non-crystalline regions of Sil-ODA₁₈ is similar to that of polymeric and monomeric ODS phases, respectively. Sil-ODA₁₈ column also exhibited the characteristic temperature dependency of liquid-crystalline phases, i.e., an increase in retention with increasing temperature on transition from crystalline to isotropic state. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Comb-shaped polymer; Shape selectivity; Phase transition; Crystalline phases; Non-crystalline phases

1. Introduction

Reversed-phase liquid chromatography (RPLC) is one of the most popular analytical separation methods. A survey of the market has shown that reversed-phase columns alone share approximately 60% of all commercial high-performance liquid chromatography (HPLC) columns [1]. ODS (octadecylsilyl silica gel) is the most widely used reversed-phase HPLC column. They are mainly two kinds; monomeric and polymeric. Monomeric ODS columns are generally used for routine analysis. On the other hand, poly-

meric ODS phases are essentially required for separation of complex mixtures. Polymeric phases exhibit unique selectivity towards polycyclic aromatic hydrocarbons (PAHs). Serious problems, however, arise with the performances of ODS columns. Selectivity varies with the phase type [2], substrate properties [3], phase density [4], temperature [5], and alkyl chain length of the bonded phase [6]. Partitioning is the dominant form of retention for small non-polar solutes in RPLC [7]. Partitioning increases with increasing surface coverage of silica by the grafted chains until it reaches a point at which the lateral packing constraints among neighboring chains give rise to chain ordering. Beyond the critical stationary phase density, further increase in phase density leads

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to decrease in partitioning into the grafted chains. Therefore, phase density is the most important factor for the selective retention. Proper selection of substrate properties is required to prepare ODS phases of desired phase densities [3]. These parameters are so interdependent that it needs a lot of efforts to prepare ODS phases of desired properties. Hence there are variations in performances among ODS columns. Unfortunately, an alternative to ODS could not be established yet.

On the other hand, liquid–crystalline stationary phases contribute to a great extent for gas chromatographic separations, particularly towards the structurally different substances, such as isomeric PAHs. Orderliness of liquid–crystalline phases is the origin of selectivity enhancement. Slender and planar molecules are able to fit with the ordered structure more effectively than bulky and non-planar molecules [8]. Slender and planar molecules are thus retained longer. Witkiewicz et al. quoted hundreds of references in the typical review article to describe the use of liquid crystals as stationary phases for gas chromatography (GC) [9]. Unfortunately, a very few works have been reported for HPLC compared to that of GC [10–15].

Liquid crystals coated on solid supports or capillary walls are used as stationary phases for GC, whereas only chemically bonded liquid crystals could be used for HPLC. Coated liquid crystals would result in rapid loss due to solubility in the mobile phase or to removal at high column pressure. Furthermore, liquid crystals may lose their properties after bonding to the solid supports. This is one of the problems of using liquid crystals in HPLC. Some materials when bonded to the polysiloxane backbone showed liquid–crystalline properties. Apfel et al. utilized these materials in GC [16]. Pesek and Jinno attempted to introduce such materials as stationary phases in HPLC [12,13]. They synthesized silane reagents of these materials and then bonded to silica. The bonding chemistry of these liquid crystals is similar to that of monomeric ODS phases. Like ODS phases, selectivity of these silica-supported liquid–crystalline phases varies with the phase density. It is assumed that slot-like structure is formed in such phases, and selectivity arises from penetration of solutes into the slots. The DSC (differential scanning calorimetry) measurement of these silica-bonded

phases, however, did not show any sharp phase transition. They observed only a positive heat-flow.

Talrose et al. studied a series of side-chain liquid–crystalline polymers by X-ray crystallography, and found these phases to be smectic and the rod-like substituents were preferentially aligned in one direction to form a comb-shaped structure [17]. If such comb-shaped polymers are introduced in HPLC, some advantages could be achieved over the conventional stationary phases. Some of them are described here. Selectivity variations between stationary phases arise from the differences in the degree of ordering. Comb-shaped structures of such kinds of polymers are only possible when some kind of orderliness exists (i.e., nematic, smectic, cholesteric, etc.). The distances between the side chains of comb-like polymers are usually constant. Therefore, selectivity variations due to the differences in phase densities could be overcome using such phases. The liquid–crystalline state of this kind of polymer is readily detectable by DSC measurement. Hence prediction or control of selectivity would be possible by correlating the orientation states (i.e., crystalline, liquid–crystalline or isotropic).

In order to introduce comb-shaped polymer in HPLC, we have prepared a side-chain polymer (ODA₁₈), poly(octadecyl acrylate), by a one-step telomerization of octadecyl acrylate with 3-mercaptopropyltrimethoxysilane. The polymer is readily immobilized onto porous silica gel through the reactive terminal group (Fig. 1) [18–22]. The DSC measurement showed a crystalline-to-isotropic phase transition. Therefore, ODA₁₈ forms highly oriented structures like liquid–crystalline state at temperatures below the phase transition temperature. The retention mode of Sil-ODA₁₈ is similar to RPLC (reversed-phase liquid chromatography) and it recognizes molecular hydrophobicity [21]. Sil-AN_n, silica-immobilized poly(acrylonitrile), provided higher separation factor towards geometric isomers, such as *cis*- and *trans*-stilbenes, than ODS. The π -electrons of CN groups of Sil-AN_n control the selectivity through π – π interaction [23]. It is also assumed that π – π interaction is the selectivity controlling factor in the crystalline regions of Sil-ODA_n towards *cis*- and *trans*-stilbenes [21]. We have further reported that: (i) relatively small-size molecules are able to accommodate into the non-crystalline regions more readily

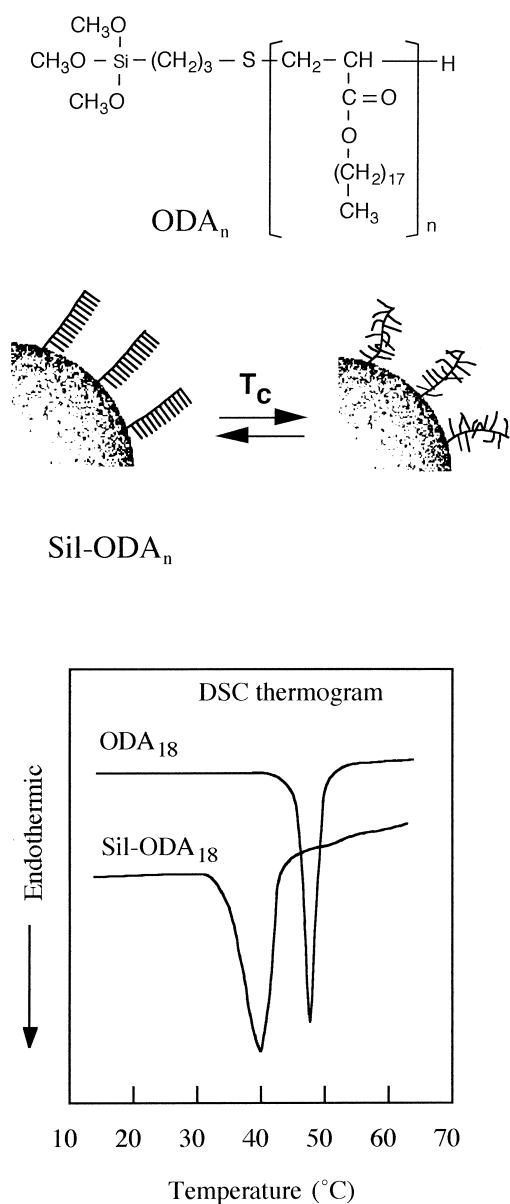


Fig. 1. Schematic illustration and DSC thermograms of silica-supported comb-shaped polymer ODA_{18} . The DSC was measured in methanol–water (80:20) dispersion in the heating process.

than large-size molecules; (ii) a k' -increase at around the phase transition temperature is related to the molecular planarity (sterically crowded PAHs are relatively non-planar, and hence they exhibited a k' -jump); (iii) a critical change in selectivity towards PAHs was observed between crystalline and isotropic

states [22]. One of the assumptions regarding the orientation of solute molecules with Sil-ODA_{18} at the crystalline state is ascribed to the fact that carbonyl groups exist on the same plane and solutes are aligned preferentially parallel to the ordered carbonyl groups, and hence the selectivity is largely controlled by π – π interaction. In such situations, the intensity of π – π interaction depends upon the length of solute molecules. The selectivity, however, cannot always be explained only by the π – π interaction. Further discussion is therefore needed for evaluation of the origin of selectivity for Sil-ODA_n .

2. Experimental section

2.1. Materials

Silica-supported polymer (Sil-ODA_{18}) was prepared, characterized and packed into a stainless steel column (150×4.6 mm) as reported previously [18–22]. YMC ($5 \mu\text{m}$, 120 \AA , $295 \text{ m}^2 \text{ g}^{-1}$) silica gel was used for immobilization of polymer. For comparison with Sil-ODA_{18} , a Vydac 201TP column was purchased from Hesperia (CA, USA), and Inertsil ODS from G.L. Sciences (Tokyo, Japan).

2.2. Chemicals

SRM 869 (benzo(a)pyrene (BaP), phenanthro(3,4-c)phenanthrene (PhPh) and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN)) was purchased from NIST (Gaithersburg, MD, USA) and the other test samples from Sigma–Aldrich (Tokyo, Japan) or Fluka Chemika-Biochemika (Tokyo, Japan). HPLC-grade methanol, acetonitrile and THF were obtained from Wako (Tokyo, Japan).

2.3. Measurements

Chromatography was performed using methanol or methanol–water or acetonitrile–water as the mobile phases at a flow-rate of 1 ml min^{-1} . The chromatography system included a Jasco type 880 PU pump and a type MD-910 UV–VIS photodiode array detector or a type 930 intelligent RI detector (Tokyo, Japan), a Rheodyne 7125 injection valve (Rheodyn,

Cotati, CA, USA) and a type U-620 column oven (Sugai, Tokyo, Japan), and a type C-R6A data processor (Shimadzu, Tokyo, Japan). Volumes of 5 μl of the samples dissolved in methanol or THF or acetonitrile were injected. The retention factor (k') was determined by the equation $(t_e - t_o)/t_o$, where t_e and t_o are the retention time of samples and void volume mark, respectively. The retention time of D_2O or a deflection mark due to injection was used as the void volume marker. The separation factor (α) is defined as the ratio of the retention factor of two solute molecules.

3. Results and discussion

3.1. Phase transition of ODA_{18}

The DSC thermograms for both ODA_{18} and Sil- ODA_{18} showed a crystalline-to-isotropic phase transition (Fig. 1). The peak-top temperature of Sil- ODA_{18} was 7°C lower than that of ODA_{18} . The ratio of isotropic phase to crystalline phase increases with increasing temperature between 30 and 46°C . Details have been discussed in the previous report [21].

3.2. A brief comparison between ODS and Sil- ODA_{18} phases

Monomeric ODS phases are usually prepared by the reaction of excess monofunctional silane (dimethylchlorooctadecylsilane) reagent with silica in an aprotic solvent and polymeric analogs using trifunctional silane (octadecyltrichlorosilane) in the presence of water [2,3]. Therefore, in monomeric phases bonds are only formed between the alkyl chain-substituted silane reagents and the silanol anchor groups on the silica gel base material, whereas in the case of the polymeric analogs bond formation not only occurs between the alkyl-substituted silane reagents and silanol groups of silicas, but also between the silane reagent molecules themselves. Thus, a highly cross-linked structure is formed in polymeric ODS phases. The third type of ODS phase is termed 'oligomeric'. The oligomeric phase is synthesized by the controlled sequential polymerization. Each reaction step is equivalent to a monomeric synthesis. The length of the polymeric

chain depends upon the number of reaction steps. The cross-linking is less favored for oligomeric phases. Several studies showed that polymeric ODS phases have greater shape-discriminating abilities compared to that of monomeric ODS phases [24–27]. The similarity of elution order of isomeric PAHs on liquid-crystalline phases in GC and on polymeric ODS phases in LC indicated that polymeric ODS phases might have a more ordered surface than monomeric phases [27]. Wise and Sander further studied the retention data of isomeric PAHs on polymeric (Vydac 201TP) and monomeric (Zorbax) ODS phases in LC, and liquid-crystalline (SB-SMECTIC) and methyl (SB-METHYL-100) polysiloxane phases in GC [28]. The GC columns were obtained from Lee Scientific (Salt Lake City, UT, USA). The comparison of LC and GC retention data indicated that the selectivity for isomeric PAHs on polymeric ODS and liquid-crystalline phases was very similar. Shah and Rogers compared monomeric and polymeric ODS phases by ^{13}C NMR and concluded that the polymeric phases must be more ordered [29]. The selectivity difference between monomeric and polymeric phases is thus a matter of degree of ordering rather than the fundamental difference in phase type. For comparison of the retention data on our newly developed column, we selected a polymeric Vydac 201TP and a monomeric Inertsil ODS column. Vydac 201TP is one of the commercially available densely loaded polymeric ODS columns.

In contrast, octadecyl acrylate is first polymerized and then immobilized at one end of the reactive polymer chain onto silica gel to yield Sil- ODA_n . The distances between the side chains, i.e., octadecyl acryl groups, are fixed. On the other hand, like monomeric ODS phases the distances of the polymer main chains vary with the surface coverage. The side chains of the polymer are structurally similar to octadecyl groups of ODS except carbonyl groups. Such kinds of side-chain polymers preferentially align in one direction to form a comb-like structure. The immobilization of ODA_n onto silica gel was in fact carried out at isotropic temperature for 24 h to attend the maximum surface density (16.37% C or 21.05 wt%). Therefore, when the polymer becomes aligned at the crystalline state, the spaces between the polymer main chains increase, and the access of

solutes becomes easier. Penetration between the acrylate moieties is restricted to the size of solutes as the spaces are defined by $-\text{CH}_2-$. The comparison of bonding chemistry between SiI-ODA_{18} and ODS phases are shown in Fig. 2.

3.3. Assessment of column selectivity

Problems arise with commercially available both polymeric and monomeric ODS phases in knowing what is offered by the individual manufacturers,

because they often do not want to reveal the bonding chemistry. Sander and Wise devised a simple empirical test to gauge the relative nature of ODS columns [2–6,24,30]. They used a SRM 869 test mixture for assessment of ODS columns. SRM 869 contains benzo(a)pyrene (BaP), phenanthro(3,4-c)phenanthrene (PhPh) and 1,2:3,4:5,6:7,8-tetrabenzonaphthalene (TBN). They suggested that the elution order of this test mixture could be used to screen unknown phases, and for column selectivity towards more complex PAHs mixture. The elution order on

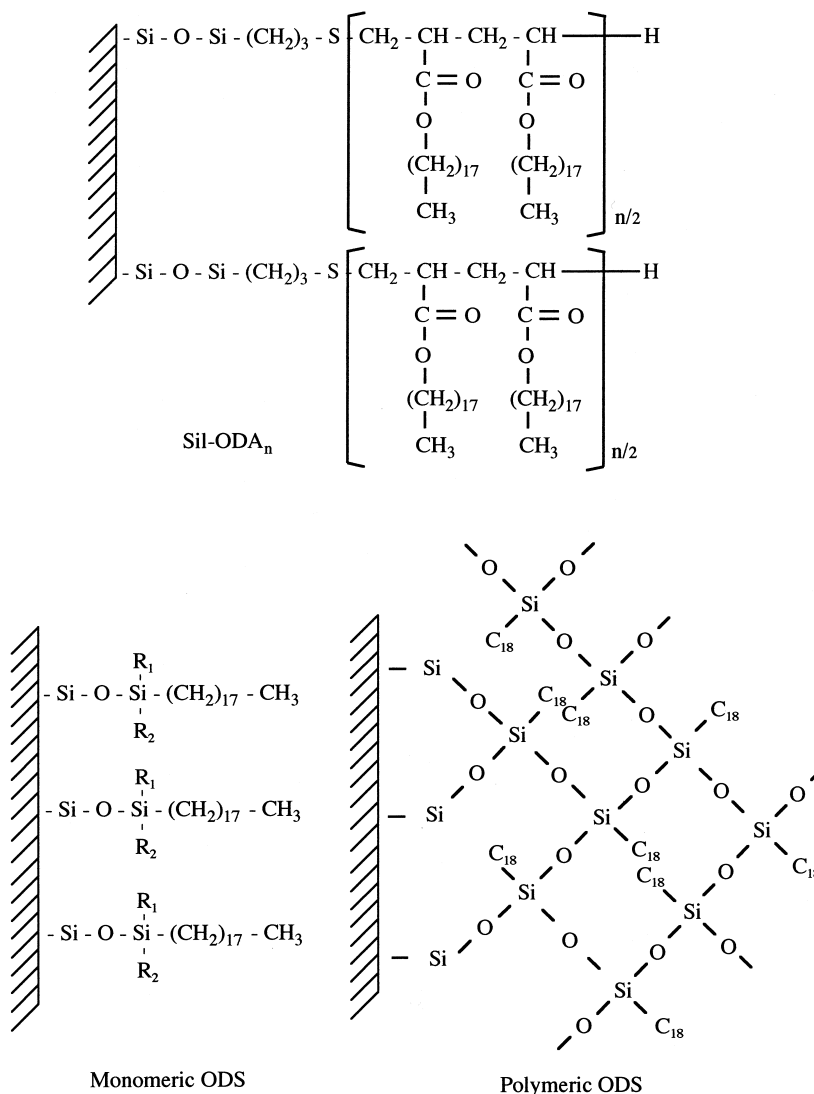


Fig. 2. Comparison of the bonded-phase linkage between SiI-ODA_{18} and ODS.

monomeric phases is usually $\text{BaP} \leq \text{PhPh} < \text{TBN}$, and on polymeric phases, $\text{PhPh} < \text{TBN} \leq \text{BaP}$. To characterize the phase selectivity, the k' ratios of TBN and BaP ($\alpha_{\text{TBN}/\text{BaP}}$) are used. The value of $\alpha_{\text{TBN}/\text{BaP}} \leq 1$ is assigned for polymeric phases, and $\alpha_{\text{TBN}/\text{BaP}} \geq 1.7$ for monomeric phases. Furthermore, densely loaded monomeric phases or lightly loaded polymeric phases were defined as 'intermediate' ODS phases. Columns show the elution order of $\text{PhPh} < \text{BaP} < \text{TBN}$, and $1 < \alpha_{\text{TBN}/\text{BaP}} < 1.7$ are termed 'intermediate'.

The elution order of SRM 869 on Sil-ODA₁₈ at the crystalline temperature is $\text{PhPh} < \text{TBN} \leq \text{BaP}$, while at the non-crystalline temperature, $\text{PhPh} < \text{BaP} < \text{TBN}$. The selectivity factor ($\alpha_{\text{TBN}/\text{BaP}}$) in the crystalline regions is below 1, and $\alpha_{\text{TBN}/\text{BaP}}$ is greater or less than 1.7 in the non-crystalline regions depending on the temperature and the solvent systems. The comparison of $\alpha_{\text{TBN}/\text{BaP}}$ versus temperature between Sil-ODA₁₈ and ODS columns in different solvent systems is shown in Fig. 3. The data indicate that the selectivity in the crystalline and non-crystalline regions of Sil-ODA₁₈ is similar to that of polymeric and monomeric or intermediate ODS phases, respectively.

3.4. Selectivity and interaction of PAHs with Sil-ODA₁₈ and ODS phases

The theory and the experiments pertinent to the retention mechanism of molecular retention for small molecules in RPLC reviewed by Dorsey and Dill agree to the fact that solute molecules partition into, rather than adsorbing onto, the stationary phase [31]. Solute molecules are approximately embedded within the grafted chains in the partition process. They will distribute where the chain order is the smallest (i.e., entropic repulsion is minimum). Thus they will preferentially distribute near the chain ends than the anchored ends. The driving forces that control partitioning of solutes into the interphase arise from the chain organization imposed by the interfacial constraints, and the other from the neighboring interactions of solute molecules just like bulk-phase (oil/water) partitioning model. The principal driving force for the latter is the relative chemical affinity for mobile- or stationary-phase molecules. Partitioning of solutes into the interphase is strongly dependent upon the surface density of the grafted chains. Ordering of grafted chains arises from the boundary condition imposed by the interface and lateral inter-

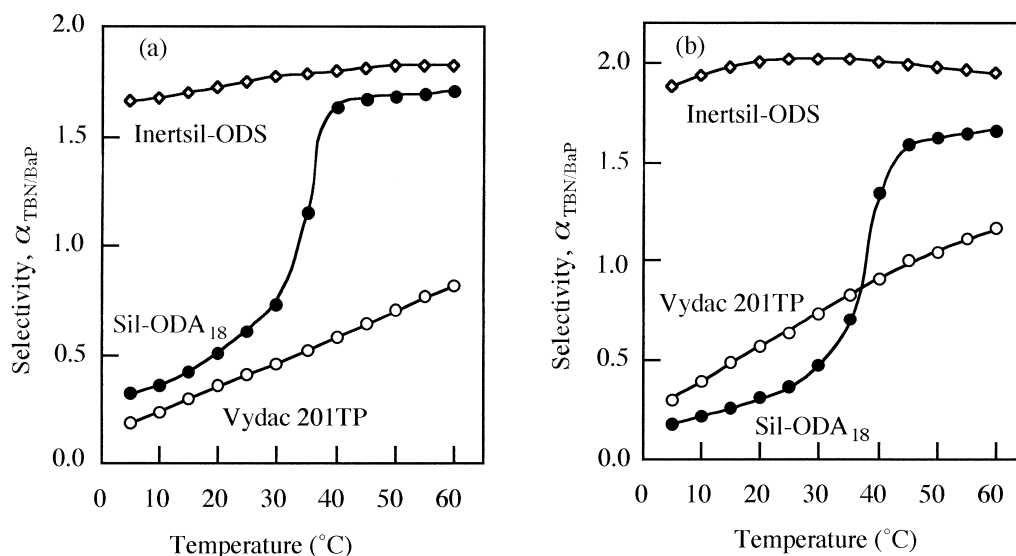


Fig. 3. Phase selectivity ($\alpha_{\text{TBN}/\text{BaP}}$) plotted as a function of temperature. Column, Sil-ODA₁₈ and ODS; mobile phase, methanol (a), and acetonitrile–water (85:15) (b); flow-rate, 1 ml min⁻¹.

actions among neighboring chains. Partitioning of solutes increases with increasing surface coverage of silica by hydrocarbon chains until it reaches a point at which the lateral packing constraints among neighboring chains give rise to chain ordering. Beyond this density, further increases in surface density result in entropic expulsion of solutes (i.e., at maximum density, no solute will partition into the phase). In other words, partitioning of solutes into the interphase decreases with increasing phase organization.

The selectivity for PAHs on Sil-ODA₁₈, Vydac 201TP (polymeric) and Inertsil-ODS (monomeric) columns has been discussed here. The purpose of this study is characterization of Sil-ODA₁₈ rather than evaluation of its efficiency for the separation of PAHs. The structures of the test samples are shown in Fig. 4. The retention of PAHs with equal numbers of aromatic carbons decreases with increasing mean molecular size, but the retention increases with increasing the number of aromatic carbons [32]. Thus dibenz(*a,c*)anthracene (C₂₂H₁₄) eluted after benzo(*a*)pyrene (C₂₀H₁₂) on Sil-ODA₁₈ ($\alpha_{\text{TBN/BaP}}=0.45$) and Inertsil-ODS ($\alpha_{\text{TBN/BaP}}=1.85$). On the other hand, a reverse elution was observed on Vydac 201TP ($\alpha_{\text{TBN/BaP}}=0.48$) (Fig. 5). This kind of discrepancy was also observed with Sil-ODA₁₈. For example, naphthacene* (C₁₈H₁₂) eluted after benzo(*e*)pyrene (C₂₀H₁₂) on Sil-ODA₁₈. In fact, naphthacene (C₁₈H₁₂) eluted after perylene (C₂₀H₁₂). Likewise, pentacene* (C₂₂H₁₄) eluted after naphtho(2,3-*a*)pyrene (C₂₄H₁₄) [22].

Both enthalpically and entropically, block-like PAHs would prefer to align themselves with the ordered stationary phases [33]. Janini and co-workers observed that the retention of isomeric PAHs measured on liquid–crystalline phases for gas chromatographic separation increased with increasing the length to the width of solute molecules [34–36]. The effect of length/breadth (*L/B*) ratio on retention was explained by Wise and Sander for ODS phases in terms of slot-model [4]. Long-narrow molecules penetrate and are able to fit with the slots more readily than square-shaped molecules, and consequently they have increased retention. In general, the retention of isomeric PAHs in RPLC increases with increasing *L/B* ratio [27]. The *L/B* ratio is defined as the ratio of length to breadth dimensions of a box

drawn about the molecule such that the maximum *L/B* value is produced. We have observed that the retention of isomeric PAHs (i.e., triphenylene, benzo(*a*)anthracene, chrysene, naphthacene, or benzo(*e*)pyrene, perylene, benzo(*a*)pyrene, or dibenz(*a,c*)anthracene, dibenz(*a,h*)anthracene, pentacene) on Sil-ODA₁₈ increases with increasing *L/B* ratio [22].

The elution of benzo(*ghi*)perylene* and dibenz(*a,h*)anthracene on ODS depends upon the phase type [30]. Benzo(*ghi*)perylene elutes after dibenz(*a,h*)anthracene on monomeric or intermediate ODS phases. The elution order of benzo(*ghi*)perylene and dibenz(*a,h*)anthracene, however, varies between polymeric phases. For example, benzo(*ghi*)perylene eluted prior to dibenz(*a,h*)anthracene on an ODS phase ($\alpha_{\text{TBN/BaP}}=0.38$), whereas it eluted after dibenz(*a,h*)anthracene on another ODS phase showing $\alpha_{\text{TBN/BaP}}=0.65$ [30]. We found that benzo(*ghi*)perylene eluted before dibenz(*a,h*)anthracene on Sil-ODA₁₈ in the crystalline region ($\alpha_{\text{TBN/BaP}}=0.45$) and on Vydac 201TP ($\alpha_{\text{TBN/BaP}}=0.48$), whereas it eluted after dibenz(*a,h*)anthracene on Sil-ODA₁₈ in the non-crystalline regions and on Inertsil-ODS ($\alpha_{\text{TBN/BaP}}=1.85$) when methanol–water (90:10) was used as an eluent (Fig. 5). The selectivity changes on transition from crystalline to non-crystalline state further showed that the elution order of PAHs on Sil-ODA₁₈ at 37°C was identical with that on Inertsil-ODS at 20°C. The change in selectivity between crystalline and non-crystalline states is in agreement with the retention theory predicted by Jinno et al. [37]. According to the theory, a critical change in selectivity toward PAHs would occur near the phase transition due to the change in solid-like to liquid-like behavior of the phase. The change in selectivity between crystalline and non-crystalline states is also in agreement with the partition theory. Details have been discussed in the relevant sections.

Selectivity of Sil-ODA₁₈ towards SRM 869 suggests that $\alpha_{\text{TBN/BaP}}$ would be a good indicator for correlating the separation of PAHs on Sil-ODA₁₈ and Vydac 201TP phases. Some discrepancies were, however, observed during the course of phase characterization. For example, a complete separation for benz(*a*)anthracene and chrysene ($\alpha=1.22$) was obtained on Vydac 201TP ($\alpha_{\text{TBN/BaP}}=0.48$), whereas $\alpha=1.08$ was achieved on Sil-ODA₁₈ ($\alpha_{\text{TBN/BaP}}=$

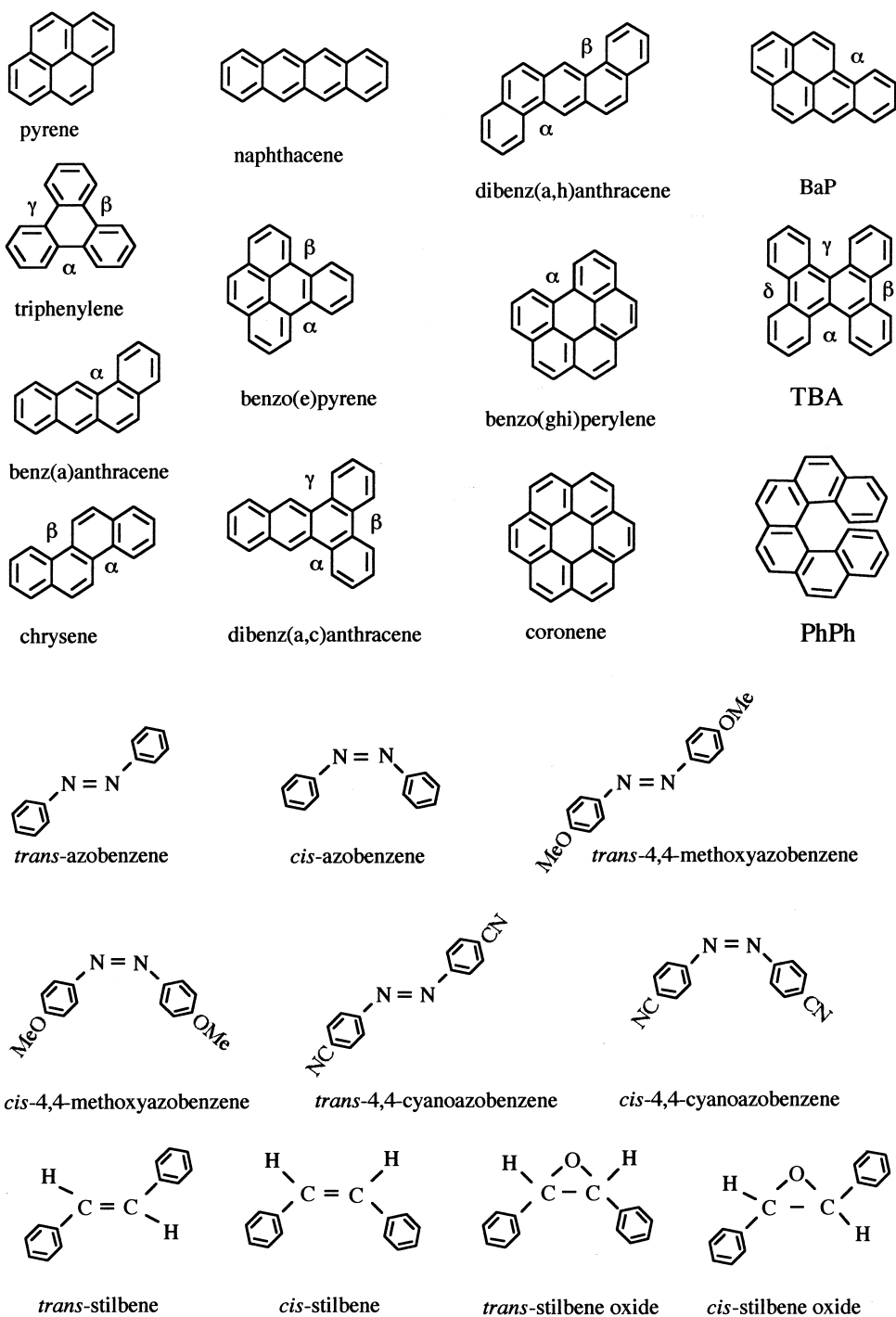


Fig. 4. Structures of PAHs and geometric isomers. Crowded regions (bay-regions) of PAHs are represented by α , β , γ , δ .

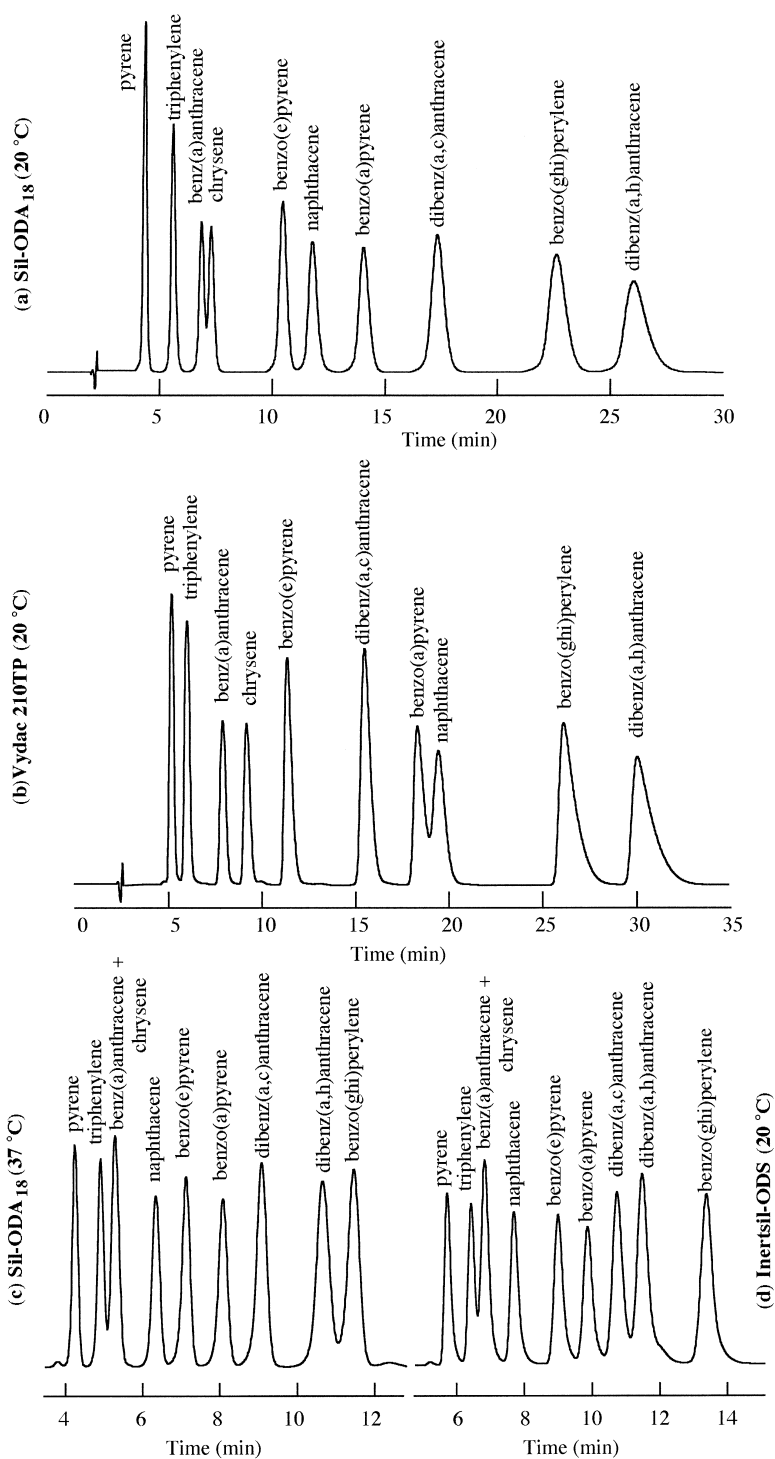


Fig. 5. Selectivity of PAHs on Sil-ODA₁₈ and ODS columns. Mobile phase, methanol–water (90:10); flow-rate, 1 ml min⁻¹.

0.45). Even a complete separation on Sil-ODA₁₈ could not be obtained at 10°C when $\alpha_{\text{TBN/BaP}}=0.32$. Such discrepancy arises from the differences in phase chemistry between Sil-ODA₁₈ and Vydac 201TP. The penetration (partition) of solutes into polymeric ODS phases is determined by the phase density. Relatively slender solutes penetrate deep into the phase more readily than the square-shaped molecules. Moreover, the extent of interactions is related to the phase thickness. Interactions increase with increasing phase thickness. Selectivity with polymeric phases is determined by the relative partitioning of solutes into the phase. In contrast, partitioning of solutes into the ordered (stretched) side chains of Sil-ODA₁₈ at the crystalline state would be entropically less favorable due to highly ordered system. Solute likely interact with Sil-ODA₁₈ at the crystalline state by adsorption rather than partition. Partition gradually increases on transition from the ordered crystalline to the less ordered non-crystalline state. Thus selectivity at the crystalline state is probably controlled by adsorption, and at the non-crystalline state, by partition. Selectivity at the crystalline state further depends upon how effectively solutes are able to accommodate with the ordered side chains of fixed spaces. Therefore, interactions depend upon solute incorporation as well as the number of side chains that would get in close contact. We assume that both chrysene and benz(*a*)anthracene were associated mainly with a single side chain. Interactions with the adjacent side chains would be less distinctive for this pair of solutes. When planar solutes incorporate with the side chains of Sil-ODA₁₈ at the crystalline state, interactions with the side chains of the neighboring polymer main chains would also be less contributive for such a structure with low surface coverage (C%=16.37). In such a situation, the selectivity for chrysene and benz(*a*)anthracene would likely be controlled by the molecular slenderness. Because of slenderness chrysene ($L/B=1.72$) would have a greater contact area than benz(*a*)anthracene ($L/B=1.58$). In contrast, chrysene penetrated into a larger number of slots of Vydac 201TP than benz(*a*)anthracene. The interactions of chrysene with Vydac 201TP relative to benz(*a*)anthracene thus could be assumed to be much greater than the situation with Sil-ODA₁₈, and hence

Vydac 201TP provided a better separation factor than Sil-ODA₁₈.

Certain PAH molecules have non-planar conformations that result from steric hindrance of approximate hydrogen and/or carbon atoms [38,39]. Such strain creates a twist in the molecule which is characterized by the dihedral angle between affected atoms. The 'dihedral angle of distortion' was described by Garrigues et al. for methyl-substituted PAHs [38]. The distortion angle is defined as the dihedral angle between the aromatic rings opposite to each other (i.e., non-adjacent rings) in the bay region. Tetrabenzonaphthalene (TBN) occupies four sterically crowded regions which make the molecule non-planar. The effect of the relative non-planarity of solutes in retention on Sil-ODA₁₈ is also detectable in the k' -temperature plot [22]. For example, TBN is less planar than BaP, and consequently exhibited a distinct k' -jump at around the phase transition temperature. In contrast, BaP showed a decrease in retention with increasing temperature. Because of planarity and slenderness BaP would be able to fit with the side chains at the crystalline state more effectively than TBN, and consequently BaP would have a greater contact area as well as a close interaction. The effects of molecular planarity and slenderness in penetrating into the densely loaded polymeric ODS phase are also significantly important. Planar solutes penetrate more readily into the slots than non-planar molecules. Furthermore, retention at the crystalline state of Sil-ODA₁₈ depends upon the number of side chains that would get in close contact with solutes. With increasing breadth, B (i.e., decreasing L/B), the possibility of interaction of solutes with the adjacent side chains increases, whereas the interaction decreases with increasing B in case of polymeric ODS because solutes are prevented entering into the slots. Thus $\alpha_{\text{TBN/BaP}}$ values cannot always correlate the selectivity between Sil-ODA₁₈ and polymeric ODS columns.

The effect of L/B ratio on retention is also understood from the elution order of BaP ($L/B=1.50$) and dibenz(*a,c*)anthracene ($L/B=1.24$). They eluted on Vydac 201TP with increasing L/B ratio, whereas a reverse elution was observed on Sil-ODA₁₈. The small L/B ratio likely favors dibenz(*a,c*)anthracene to retain longer on Sil-ODA₁₈ be-

cause of the adjacent-chain effects. In contrast, dibenz(*a,c*)anthracene are prevented entering into the polymeric ODS phases because of its small L/B ratio. Thus the possibility of interactions are much smaller than BaP.

Naphthacene would readily be accommodated with the ordered chains of Sil-ODA₁₈ at the crystalline state than benzo(*e*)pyrene because of planarity and slenderness. We assume that all four rings of naphthacene would come into close-contact with the side chain, whereas three rings of benzo(*e*)pyrene, and hence naphthacene, is longer retained. Similarly, dibenz(*a,h*)anthracene ($L/B=1.79$) eluted after benzo(*ghi*)perylene ($L/B=1.12$) because of slenderness

The results indicate that the selectivity for PAHs on Sil-ODA₁₈ at crystalline temperature is mainly controlled by the incorporation of solute molecules on the ordered side chains.

The above findings suggest that Sil-ODA₁₈ would be able to make significant contributions to the separation of PAHs, like polymeric ODS phases. In addition, since the retention mechanism is different between the two types of stationary phases, Sil-ODA₁₈ thus offers the possibility to solve the failures of polymeric ODS phases. Furthermore, selectivity differences for ODS columns from the same manufacturer but from different production lots were even observed [40–42]. Selectivity greatly varies with the phase density [4]. Particularly for polymeric ODS phases, cross-linking and branching of silane linkage determine the density of polymeric phases, and the extent of cross-linking depends on the particle sizes and pore diameters of silica gel and the amount of water present in the reaction system as well [2,3]. The reactive silane reagent molecules may anchor during polymerization at a point far from the silica surface. The degree of polymerization is almost never known for such kind of polymerization. The surface density numbers are estimated as a rough indication of true chain density. Sander and Wise stated that ‘the use of surface coverage values to calculate interchain distances is probably not justified for polymeric phases’ [2]. The preparation of polymeric ODS phases hence does not always ensure the performances of the final product. In contrast, the synthesis of Sil-ODA₁₈ is highly reproducible. The ODA_{*n*} is prepared by a one-step

reaction, and the degree of polymerization is readily controlled by the adjustment of the initial molar ratio of the reactants. We prepared polymeric chains of various lengths ($n=18-33$). The selectivity changes with increasing the length of the polymer chain of Sil-ODA_{*n*} were not observed so far. The number of theoretical plates of this column for benzo(*a*)pyrene was 40 000/m in an 85% acetonitrile–water mobile phase system. The performances of this stationary phase were checked using silica gels of different particle sizes, pore diameters and surface areas. Silica gel of 5 μm particle size, 100–120 Å pore diameter and 300–400 m²/g surface area provided better performances. Furthermore, the selectivity and the separation factor for the test solute molecules on different types of silica-supported ODA₁₈ were found to be almost identical although they had different surface coverages. This also suggests that separation of solute molecules is likely not much dependent upon the phase density. The inlet pressure of this column (150×4.6 mm) was around 30 kg cm⁻¹ at 25°C when methanol was used as an eluent at a flow-rate of 1 ml min⁻¹.

In addition to the above, phase transition of Sil-ODA₁₈ is readily detectable by DSC, and hence the orientation order. Selectivity is thus readily predicted or controlled by correlating the orientation order. On the hand, the existence of liquid–crystalline state (i.e., degree of phase organization) is assumed from practice. Phase transition of ODS phases is often demonstrated by Vant’s Hoff plots. Cole and Dorsey have reported that although the non-linearity in Vant’s Hoff plots may be an indication of a change in retention mechanism, simple morphological changes in the bonded phases do not necessarily change the intrinsic mechanism [43,44]. Such kind of curvature may arise from other thermodynamic process than the stationary phase changes only. Furthermore, the phase transition of ODS phases observed by different investigators was found to vary with the phase density, phase type, alkyl chain length and the choice of solute as well [45]. Thus the prediction of selectivity with ODS would not always be as simple as with Sil-ODA₁₈.

Selectivity variations with polymeric ODS phases arise from the differences in the relative partitioning of solutes into the stationary phases. Because of

entropic repulsion partitioning of solutes into densely loaded polymeric ODS phases is smaller than into lightly loaded phases. This entropic repulsion would be more pronounced towards square-shaped solutes than slender solutes. Therefore, partitioning depends upon the phase density as well as the shapes of solutes. Densely loaded polymeric phases thus provide more selective retention towards isomeric solutes. In contrast, the results indicate that adsorption, rather than partitioning, is the dominating mechanism at the crystalline state of Sil-ODA₁₈. Selectivity at the crystalline state arises from the effective incorporation of solutes on the ordered side chains of fixed spaces and hence variation in selectivity due to differences in phase densities does not take place. Selectivity with such thermotropic stationary phases usually varies with the change in the orientation order.

3.5. Selectivity of Sil-ODA₁₈ and ODS columns towards geometric isomers

The separation factor ($\alpha = k'_{\text{trans}}/k'_{\text{cis}}$) for isomeric azobenzenes and stilbenes was measured on Sil-ODA₁₈ and ODS phases at 20°C using methanol–water (70:30) as an eluent. The value of α for *cis*- and *trans*-azobenzenes on Sil-ODA₁₈ was 6.95. When electron donating –OCH₃ groups were introduced on the *para* positions of *cis*- and *trans*-azobenzenes, i.e., *cis*- and *trans*-4,4-methoxyazobenzenes, α increased to 10.07. We have reported that the retention of geometric isomers on Sil-ODA₁₈ is enhanced by the carbonyl– π interaction, and this is more pronounced towards *trans*-isomers [21]. The calculation of energy level of HOMO (highest occupied molecular orbital) showed that *trans*-isomer is more electron-donating. It is therefore reasonable to consider that the higher separation factor towards isomeric *cis*- and *trans*-4,4-methoxyazobenzenes than the unsubstituted azobenzenes arises from the induction of π – π interaction. The separation factor, however, also increased when electron withdrawing –CN groups were introduced, i.e., *cis*- and *trans*-4,4-cyanoazobenzenes ($\alpha = 8.39$). The values of α increased on Sil-ODA₁₈ in the order of 4,4-methoxyazobenzenes > 4,4-cyanoazobenzenes > azobenzenes. The separation factor of these isomers on Vydac 201TP also followed the same order (i.e.,

$\alpha = 14.66, 13.28, 10.15$, respectively). On the other hand, the values of α were almost the same on Inertsil-ODS (i.e., 6.82, 7.11, 6.84, respectively). The selectivity for geometric isomers on Vydac 201TP arises from penetration of solutes into the slot-like structure. Because of planarity, *trans*-isomers are able to penetrate more readily into the slots than *cis*-isomers. Partitioning of solutes into such polymeric structure is controlled by the phase organization. In contrast, such kind of slot-like structures usually do not form in monomeric ODS phases at this temperature. The values of separation factor indicate that partitioning driving forces of these pairs into monomeric ODS are less distinctive. On the other hand, separation of these isomers on Sil-ODA₁₈ depends upon the substituent groups. The separation factor on Sil-ODA₁₈ increased in the same order as observed on Vydac 201TP. This suggests that the selectivity for geometric isomers on Sil-ODA₁₈ is likely controlled by the slot-like structure.

Further studies showed that the separation factor for isomeric *cis*- and *trans*-stilbenes on Sil-ODA₁₈ ($\alpha = 2.25$) was higher than that on Vydac 201TP ($\alpha = 1.82$). On the other hand, a partial separation was obtained on Inertsil-ODS ($\alpha = 1.14$). Should we then claim that this increased selectivity with Sil-ODA₁₈ arises from the induction of π – π interaction? For evaluation of the contribution of π – π interaction, we selected non-conjugated *cis*- and *trans*-stilbene oxides. If the carbonyl groups of Sil-ODA₁₈ truly control the selectivity of geometric isomers through π – π interaction, the separation factor for stilbene oxides should be significantly smaller than stilbenes. The separation factors for stilbenes and stilbene oxides are, however, almost the same, i.e., 2.25 and 2.21, respectively. The α values on Vydac 201TP and Inertsil-ODS are 2.06 and 1.79, respectively. Furthermore, for both conjugated stilbenes and non-conjugated stilbene oxides, the values of α on Sil-ODA₁₈ are higher than that on Vydac 201TP. Thus, again agreeing to the fact that the shape discrimination on Sil-ODA₁₈ towards geometric isomers originates from the incorporation of solutes with the ordered side chains.

The separation factor for *cis*- and *trans*-stilbenes on Sil-MA₂₁, poly(methyl acrylate), ($\alpha = 1.61$) was significantly smaller than that on Sil-ODA₁₈ at the crystalline state ($\alpha = 2.25$), but much higher than that

on Inertsil-ODS ($\alpha=1.14$). The value of α on Sil-ODA₁₈, however, decreased to 1.35 at the isotropic state (55°C). The orientation order of Sil-MA₂₁ is not maintained because of the lack of long alkyl side chains [46]. The orientation may be considered similar to that of monomeric ODS phases. The increased retention factor on Sil-MA₂₁ has been described in terms of π - π interaction [46]. The long-chain alkyl groups of Sil-ODA₁₈ at the isotropic state probably prevents solutes coming into close contact with the carbonyl groups. Hence π - π interaction would likely be less contributive at the isotropic state.

The results further suggest the possibility of alignment of geometric isomers or PAHs at the crystalline temperature with the ordered side chains of Sil-ODA₁₈ than with the ordered carbonyl groups. The alignment of such solute molecules with the ordered alkyl chains would not be unreasonable as π - π interaction is much weaker than the partitioning driving force. Thus π - π interaction could be assumed as an additive rather than the predominating factor for the selectivity enhancement of solutes on Sil-ODA₁₈ at the crystalline state.

3.6. Retention behaviors of solutes with Sil-ODA₁₈

Retention of solutes on liquid-crystalline phases in GC usually decreases with increasing temperature in the solid liquid-crystalline region. A minimum appears in the curve followed by an increase in retention at around the transition point of mesophase, and then again decreases when it reaches the isotropic state [9]. This kind of behavior, however, was not observed with all liquid-crystalline phases. Fortunately, our Sil-ODA₁₈ column exhibited a similar pattern of temperature dependency. Fig. 6 shows the characteristic temperature dependency of solute molecules, such as cycloalkanes, alkanes, rigid polycyclic molecules and PAHs, on Sil-ODA₁₈. On the conventional reversed-phase liquid chromatography, like ODS, the retention of solutes usually decreases with increasing temperature. We also observed that the elution order of dodecane, cyclododecane, pyrene, triphenylene and adamantane on Sil-ODA₁₈ at the isotropic state is identical with that on ODS phases.

The cross-over between cyclododecane and PAHs

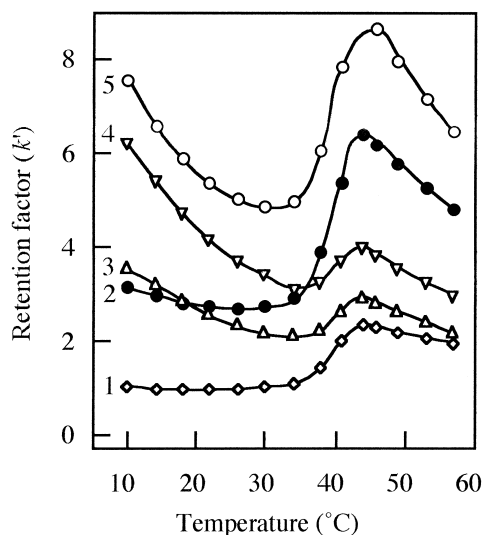


Fig. 6. Typical temperature dependency of aromatic and non-aromatic hydrocarbons on Sil-ODA₁₈. Solutes: adamantane (1), cyclododecane (2), pyrene (3), triphenylene (4), dodecane (5). Mobile phase, methanol-water (80:20); flow-rate: 1 ml min⁻¹.

provides important information regarding interaction of solutes with the crystalline and the non-crystalline phases of Sil-ODA₁₈. We assume that because of planarity pyrene and triphenylene would have greater contact areas and close interactions at the crystalline state than cyclododecane. Yan and Martire have derived a molecular theory to describe the selectivity enhancement for block-like solutes in anisotropic stationary phases [47]. The theory predicts that solute-stationary phase contact area with the isotropic state is much larger than that with anisotropic state. If molecules are viewed three dimensionally, the volumes of non-planar cycloalkanes will be larger than the corresponding planar PAHs. The k' -jump from 35 to 45°C for cyclohexane and *trans*-decalin was found to be 5 and 8 times higher than benzene and naphthalene, respectively [22]. We have also observed that the k' -jump from 30 to 45°C for cycloalkanes is much higher than the corresponding alkanes [48]. We assume that the k' -increase with increasing temperature is related to the increase in solute-stationary phase contact-volume, and which is more pronounced towards non-planar cyclododecane than the relatively planar pyrene and triphenylene. Such a shape-related solute-stationary phase interaction in the crystalline and non-crystalline

regions ultimately resulted in a cross-over between these solutes.

The effect of molecular planarity for retention on Sil-ODA₁₈ is also understood from the selectivity of similarly shaped planar coronene and non-planar phenanthro(3,4-*c*)phenanthrene (PhPh). The separation factor ($k'_{\text{coronene}}/k'_{\text{PhPh}}$) was about 12 at the crystalline temperature (25°C), which drastically decreased to 3 at isotropic temperature (55°C). Because of planarity coronenes were effectively incorporated with the side chains at crystalline temperature than PhPh. On the other hand, PhPh were retained longer at isotropic temperature (55°C) than at crystalline temperature (25°C) because of the increased contact volume. At 25 and 55°C, the values of k' for coroner were 17.86 and 5.80, and for PhPh, 1.48 and 1.89, respectively.

The increase in k' on transition from crystalline to non-crystalline region is in agreement with the retention model of partition theory. The k' increase with increasing temperature indicates the dependency of enthalpies and entropies of transfer (and hence the chemical potential) of solutes from mobile phase to stationary phase. Because of the entropic repulsion solute molecules would not be able to partition into the ordered crystalline state. They would likely interact with the stationary phase by adsorption. Partitioning of solutes, however, gradually increased on transition from the ordered crystalline to the less ordered non-crystalline state and resulted in a k' increase.

4. Conclusions

The ordering of stationary phases is considered to be the origin of shape selectivity in RPLC. The silica-supported comb-shaped polymer (Sil-ODA₁₈) provides three kinds of phase organization (i.e., crystalline, liquid–crystalline and isotropic). Adsorption is the dominating mechanism for the selective retention at the crystalline state, whereas partitioning is the dominating mechanism at the non-crystalline state. The selectivity for PAHs on Sil-ODA₁₈ at the crystalline and non-crystalline states is similar to that on polymeric and monomeric ODS phases, respectively. Thus a single column may solve separation problems of both types of ODS phases. A change in

selectivity near the phase transition temperature further offers the possibility of separation of critical solute mixtures (i.e., solutes difficult to resolve by other columns) by selecting the proper temperatures. Furthermore, selectivity can readily be predicted or controlled correlating the DSC thermogram (i.e., the orientation states).

The long-chain alkyl groups (side chains) of Sil-ODA₁₈ play the major role in solute retention. The selectivity at the crystalline states arises from the effective alignment of solutes on the ordered side chains. The distances between the side chains is constant for such structures. The use of Sil-ODA₁₈ thus eliminates the selectivity variations due to differences in phase densities between columns of the same type (i.e., ODS). Partitioning of solutes into such a structure is related to the orientation state of the phase. Partitioning increases with decreasing phase organization of Sil-ODA₁₈, and hence the retention increases on transition from ordered crystalline state to the less ordered non-crystalline state.

Thus, our newly developed column offers not only solutions for analysts to solve separation problems in HPLC, but also a new line of understanding of the retention mechanism in RPLC.

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