Evaluation of microstructural features of a new polymeric organic stationary phase grafted on silica surface: A paradigm of characterization of HPLC-stationary phases by a combination of suspension-state ^{1}H NMR and solid-state ^{13}C-CP/MAS-NMR

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Abstract

Silica-supported poly(octadecylacrylate) (Sil-ODA$_n$), polymeric octadecylsilyl silica (polymeric ODS), and monomeric octadecylsilyl silica (monomeric ODS) were studied by a combination of suspension-state ^{1}H NMR and solid-state ^{13}C CP/MAS-NMR to probe the mechanisms underlying their functions as stationary phases for RP-HPLC. Sil-ODA$_n$, with a strong temperature dependent separation behaviour showed correspondent temperature dependent manifestations in both suspension-state ^{1}H NMR and solid-state ^{13}C CP/MAS-NMR experiments. With a gradual increase in temperature, intensity of proton signals (^{1}H NMR) of octadecyl moieties (mainly methylene groups) rose dramatically. This dramatic rise was at the same temperature of an endothermic peak detectable in its DSC thermogram indicating a relatively complete solid to liquid phase transition. In addition temperature dependencies of the ratio of trans to gauche conformed well to temperature dependencies of the separation factor between naphthacene and triphenylene (as a simple indicator of shape selectivity). Therefore NMR spectra of Sil-ODA$_n$ were used as a reference for ascertaining percentage of octadecyl moieties of liquid type mobility in the two other stationary phases. Using this method we determined percentage of liquid phase in polymeric ODS and monomeric ODS at various temperatures. We suggest a combination of suspension-state ^{1}H NMR and solid-state ^{13}C CP/MAS-NMR for structure-dynamic characterization of various kinds of hydrocarbon chains grafted onto the silica particles.
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1. Introduction

Much effort has been put into characterization of various kinds of grafted organic molecules on silica surface for use in reverse-phase high performance chromatography (RP-HPLC) [1–10] as separating potentials of grafted organic layers depend on their microstructural features. Identifying the structure–function relation of these silica-supported organic materials as well as other organic–inorganic hybrid materials enhances our knowledge of their underlying mechanisms of function, enabling scientists to make and use such products more effectively.

A number of physiochemical and computational methods from NMR spectroscopy to molecular dynamic simulation have been used along with several structure–retention relationship studies for this purpose. Raman spectroscopy has been used for determination of conformational and rotational order of stationary phases [11,12]. Fourier transform IR

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(FT-IR) and Fourier self-deconvolution (FSD) have been used for understanding the mechanism that leads to the elution order observed in the HPLC separation of enantiomers [13]. Potential of stationary phases for chiral descrimination can also be evaluated by NMR using two-dimensional NOESY technique [14,15].

Monomeric ODS and polymeric ODS are two popular stationary phases for RP-HPLC. The conformational nature of alkyl chains in monomeric ODS has been shown to be disordered in several researches using molecular dynamic simulation [16,17]. Extent of silane to silane bonding in both polymeric and monomeric ODS has been investigated using ^{29}Si-CP-MAS NMR spectroscopy [18]. ^{13}C CP-MAS NMR has shown that alkyl chain disorder increases with decreasing bonding density and increasing temperature [19,20]. Considerable efforts have been expended towards disclosing micro-architectural aspects of C18 stationary phases by determining the factors that affect separation of poly aromatic hydrocarbons (PAHs) by HPLC. Shape selectivity among C18 stationary phases generally increases with increasing bonding density regardless of the type of phase (monomeric or polymeric) or bonding chemistry. This suggests presence of size and shape specific slots amongst the self-assembled monolayer of alkyl chains in the stationary phase. However, a clear molecular picture of alkyl stationary phase architecture has remained elusive [21–24].

One method for identifying the architecture and functionality of ODS phases is evaluation of shape recognition of PAHs by using octadecyl bounded stationary phases in different ways [25].

Although polymeric ODS has the benefit of higher immobilization compared with monomeric ODS, but its potential to self-assemble is still perturbed because of short rigid covalent bounds of Si–O–Si either between the alkylsilane and silica surface or between two alkylsilane (cross-polymerization). In this paper we have introduced a new stationary phase by immobilization of poly(octadecylacrylate) onto porous silica particles (Sil-ODA$_n$) that differs from conventionally modified silicas because the distances between side chains are supposedly fixed and hence the variation in bonded phase ligand distances is overcome. On the other hand the octadecyl moieties are not directly bonded to the silica surface, so they can form a highly-ordered structure. In our previous studies [26–30] poly(octadecylacrylate), ODA$_n$ was grafted on porous silica particles and proved advantageous for use as stationary phase for geometrical detection that was strongly temperature dependent. This prominent temperature dependency in separation behaviour of grafted ODA$_n$ was coincident with an endothermic peak detectable by DSC. This unique behaviour of poly(octadecyacrylate) grafted on silica, Sil-ODA$_n$, can be regarded as a valuable opportunity to determine its relevant manifestations in NMR spectroscopy. Suspension-state ^{1}H NMR and solid-state ^{13}C-CP/MAS-NMR were performed at different temperatures to identify microstructure of Sil-ODA$_n$, polymeric ODS, and monomeric ODS. As will be discussed, Sil-ODA$_n$ can be used as a valuable reference for phase characterization of both polymeric and monomeric ODS. We hope this study will be found useful for providing more effective methods in characterization of organic–inorganic hybrid materials by NMR spectroscopy. Chemical composition of the stationary phases mentioned above are shown in Fig. 1.

2. Experimental

2.1. Sample preparation

2.1.1. Reagents

Silica particles 120-S5 (diameter 5 μm, pore size 120 Å, specific surface area 300 m^2 g^{-1}) were obtained from YMC, naphthacene and triphenylene, was purchased from Sigma–Aldrich (Germany). All other materials were from Sigma–Aldrich (Tokyo, Japan), or Fluka Chemica-Biochemica (Tokyo, Japan).

2.1.2. Synthesis of Sil-ODA$_{25}$ [poly(octadecylacrylate) grafted on porous silica particles]

Sil-ODA$_n$ was prepared by radical telomerization of octadecyl acrylate (ODA) with 3-mercaptopropyltri-methoxysilane (MPS) and following immobilization onto silica mostly according to our previously reported method [30]. Preparation procedure was as follows: ODA and MPS (30:1 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 an atmosphere of N$_2$ gas. The resulting white precipitates were gathered by filtration, washed successfully with methanol and acetone and dried in vacuo.

The structure and polymerization degree was determined by NMR spectroscopy. ^{1}H NMR shifts of ODA$_n$ were as follows: $\delta = 0.8$ ppm (SiCH$_2$), $\delta = 1.9$ ppm (CHCO), $\delta = 3.6$ ppm (SiOCH$_3$), $\delta = 4$ ppm (C(=O)OCH$_2$). The average degree of polymerization was determined to be 25 by the proton ratio at 3.6 and 4.0 ppm.

Then ODA$_{25}$ was readily immobilized onto porous silica by mixing in toluene at reflux temperature for 24 h. The product was gathered after filtration and several time washing with toluene and chloroform consecutively. After 72 h vacuum drying the amount of carbon and hydrogen were determined to be 18.3 and 3.2% by elemental analysis. Successive washing with chloroform as a good solvent for ODA$_{25}$ showed no significant change in elemental analysis results. Coverage of Sil-ODA$_{25}$ by octadecyl moieties was calculated to be 3 μmol m^{-2} and 726 μmol g^{-1}.

2.1.3. Synthesis of polymeric ODS

The same silica particles (YMC 120-S5) were mixed with octadecyltrichlorosilane at reflux temperature in toluene for 24 h. There was no effort for a controlled inclusion of water in the reaction. The product (a white powder) was gathered
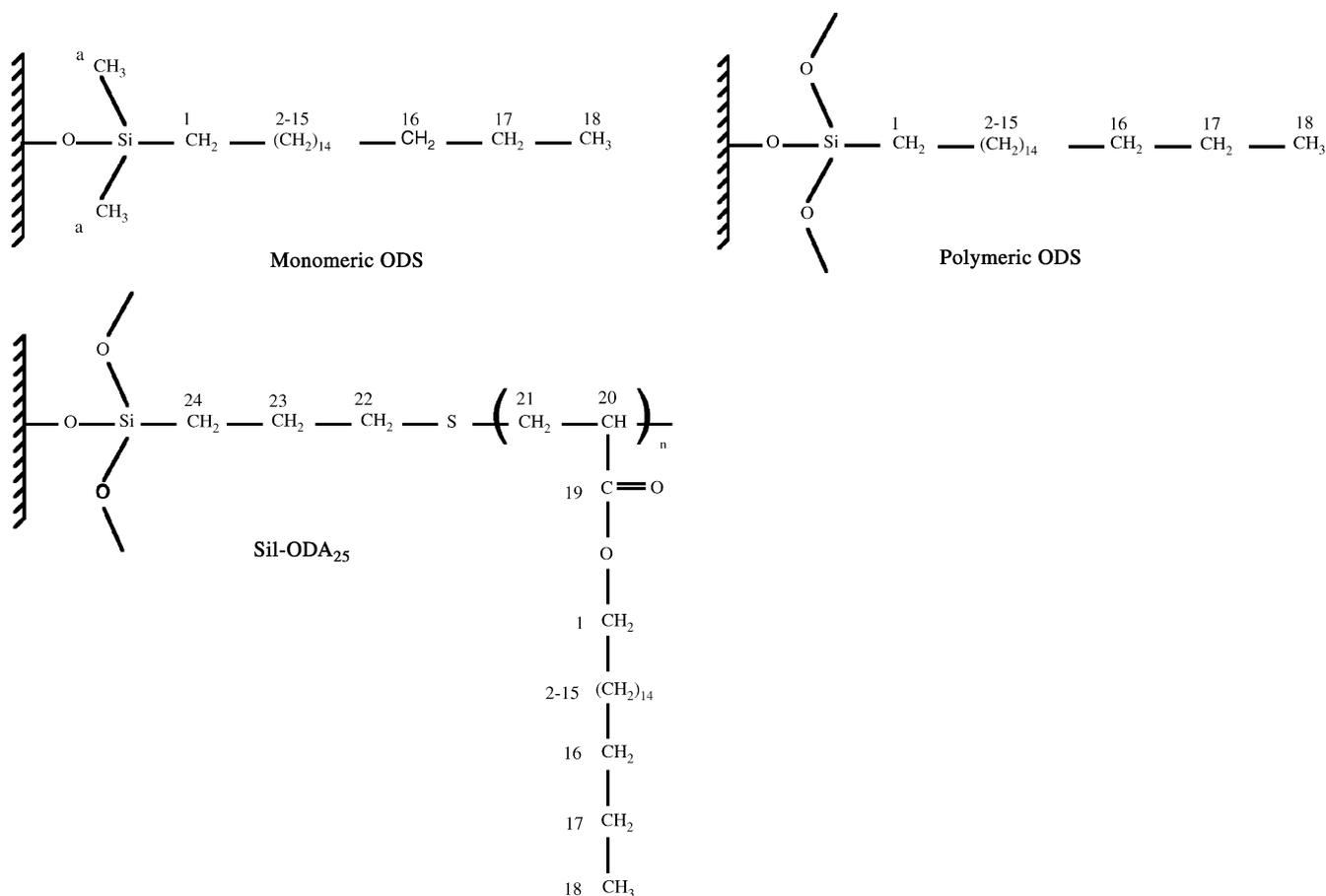
Fig. 1. Structure of the silica-supported stationary phases discussed in this paper: silica-supported polyoctadecylacrylate (Sil-ODA$_n$), polymeric octadecylsilyl silica (polymeric ODS), and monomeric octadecylsilyl silica (monomeric ODS).

by filtration after washing with chloroform and toluene for several times. After 72 h vacuum drying the amount of carbon and hydrogen were determined to be 23.3 and 4.3% according to elemental analysis. Successive washing with chloroform showed no significant change in elemental analysis results. Coverage of polymeric ODS by octadecyl moieties was calculated to be 4.9 μmol m^{-2} and 1078 μmol g^{-1}.

2.1.4. Synthesis of monomeric ODS

YMC 120-S5 particles were mixed with dimethyloctade-cylchlorosilane at reflux temperature in toluene for 24 h. The product was gathered after filtration and frequently washed with chloroform and toluene. After 72 h vacuum drying the amount of carbon and hydrogen was determined to be 13.8 and 2.7% based on elemental analysis. Successive washing with chloroform showed no significant change in elemental analysis results. Coverage of monomeric ODS by octadecyl moieties was calculated to be 2.5 μmol m^{-2} and 638 μmol g^{-1}.

2.2. NMR spectroscopy

All NMR spectra were measured by VARIAN UNITY[INOVA] AS400 at static magnetic field of 9.4 T using GHX Varian AS400 nanoprobe for suspension-state ^{1}H NMR and 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 terminology of VARIAN UNITY[INOVA]. We used spin rate of 2000–3500 Hz for suspension-state ^{1}H NMR and 4000–4500 Hz for solid-state ^{13}C-CP/MAS-NMR except polymeric ODS at 50 °C (spin rate of 2000–2500 Hz). The NMR under-peak areas were measures after correcting the base line and setting the total under-peak area to a value of 1000.

2.2.1. Suspension-state ^{1}H NMR

We made three suspensions adding 10 mg of each one of Sil-ODA$_{25}$, polymeric ODS, and monomeric ODS, to 100 μl methanol-d (CD$_3$OD) including 0.03% tetramethylsilane (TMS) and 0.05% hexamethyldisilane (HMS). Methanol-d was chosen because it had been the mobile phase during our past RP-HPLC observations. All samples were prepared at the same time using one single ampoule of CD$_3$OD. Hexamethyldisilane was added to work as a reference of signal intensity but protons of CD$_3$OD proved better for this purpose. Suspension-state ^{1}H NMR spectra were measured at temperatures: 20, 25, 30, 35, 40, 45 and 50 °C using GHX Varian AS400 nanoprobe. The parameters

used for measurement were delay time $d1 = 1.5$ s, pulse width pw $= 2.2$ μs, transient numbers $nt = 32$, spectral width sw $= 6000$ Hz. Water was suppressed using presaturation pulse sequence with saturation delay of 1.5 s and saturation power of 2 db. For assigning peaks, after determination of pulse width of 90° simple RELAY COSY (correlation spectroscopy) was done and the chemical shifts of the terminal methyl and methylene of octadecyl groups were determined. Shimming were adjusted for each temperature using a standard semi-automatic method. At the same time T_1-analysis and T_2 measurement via cpmgt$_2$ pulse sequence (Carr–Purcell Meiboom–Gill T_2) were done.

2.2.2. Solid-state ^{13}C-CP/MAS NMR

Hexamethyl benzene was used for adjusting the magic angle before each experiment. NMR frequency referencing was performed by adjusting methyl peak of HMB to 17.3 ppm. Solid-state ^{13}C-CP/MAS NMR spectra were measured at temperatures: 20, 25, 30, 35, 40, 45 and 50 °C using a line-broadening factor of 5 ($l_b = 5$). Hartmann–Hahn matching was satisfied via arrayed experiments on hexamethyl benzene. Other important parameters were as follows: spectral width, sw $= 50,000$ Hz; proton pulse width, pw90 $= 11.6$ μs; contact time for cross polarisation, cntct $= 5$ ms; delay before acquisition, $d1 = 2$ s except first few measurements in which it was 6 s; number of transients were 20,000 (for assigning relevant peaks) and 1000 (for variable temperature experiments). High power proton decoupling of 63 db with fine attenuation of dipolr $= 2500$ was used only during detection periods.

2.3. Chromatography

The chromatograph included a Gulliver 980 PU pump, a JASCO photodiode array detector (UVIDEC-100-IV) and a NESLAB column Jacket (Newington, USA). The polymeric ODS column (Shodex C18P 4E, 4.6 mm i.d. × 250, having 17.5% C) was purchased from Shodex (Tokyo, Japan). The monomeric ODS (Inersil ODS 3, 4.6 mm i.d. × 250, having 13.8% C) was purchased from G.L. Sciences (Tokyo, Japan). Sil-ODA$_n$ was packed into stainless-steel columns (4.6 mm i.d. × 250) using a hexanol–chloroform (1:1) mixture. Methanol–water (9:1) was used as mobile phase at a flow-rate of 1 ml min^{-1}. A 5 ml of the sample dissolved in methanol had been injected through Reodyne Model 7125 injector. The separation factor between naphthacene and triphenylene was used as an indicator of shape selectivity. The separation factor, $\alpha_{\text{naphthacene/triphenylene}}$, was defined as the ratio of the retention factor of naphthacene to the retention factor of triphenylene. The retention factors (k') were determined by the equation $(t_e - t_0)/t_0$, where t_e and t_0 are the retention times of the sample and void volume mark.

2.4. Differential scanning calorimetery

DSC experiments were performed using a heating rate of 1 °C min^{-1} with a Seiko I and E SSC-580 and a DSC-10 instrument. Using Seiko-AG-70 capsules one was filled with the sample and another empty one was used as the reference.

3. Results and discussions

3.1. Surface coverage

The molar amount of octadecyl moieties per 1 g silica (M) can be calculated as:

$$M(\mu\text{mol g}^{-1}) = 10^6 (P_C/100)/12n \qquad (1)$$

where P_C is the percentage of carbon element according to elemental analysis, and n is the corresponding number of carbon atoms per one octadecyl moiety which is 18 in both polymeric and monomeric ODS and 21 in case of Sil-ODA$_{25}$ (Fig. 1).

On the other hand weight percentage of the grafted phase (P_w) in each case can be calculated as:

$$P_w = 253 \times 10^{-4} M(n/18) \qquad (2)$$

as the molecular mass of a grafted octadecyl moiety is 253 Da.

Furthermore surface coverage (N) can be calculated as:

$$N(\mu\text{mol m}^{-2}) = M/\{S[(100 - P_w)/100]\}$$
$$= 10^6 P_C/[12nS(100 - P_w)] \qquad (3)$$

where S is surface area per 1 g of non-modified silica.

3.2. Suspension-state ^{1}H NMR spectroscopy

Although molecular mobility is usually probed by measurement of T_1 relaxation time, this was not the case for our study as NMR peaks of several methylene groups with different mobility (and hence different T_1 relaxation times) were superimposed having equal chemical shifts. Therefore we turned to a rather new but very simple approach; that is, determining percentage of octadecyl moieties with liquid type mobility in each case.

The form of motionally averaged Hamiltonian depends very strongly on the type and the time scale of molecular motion, and hence on the phase of matter [31]. In liquid or suspension-state NMR only those molecules or parts of molecules with very fast rotational motions are detectable. Motion must be in such a fast range that it can average out dipolar coupling and chemical shift anisotropy until related NMR peaks become narrow enough to be detected. Those molecules or molecule parts that have low mobility scales will give such a broad peak (because of dipolar coupling and chemical shift anisotropy contribution) that they cannot be detected.

The suspension-state ^{1}H NMR spectra were obtained for Sil-ODA$_{25}$, polymeric ODS, and monomeric ODS, at variable temperatures from 20 to 50 °C. Neither half-height width (line width) of methylene groups nor spin–spin relaxation time ($T_2 = 100$ ms) showed any significant change
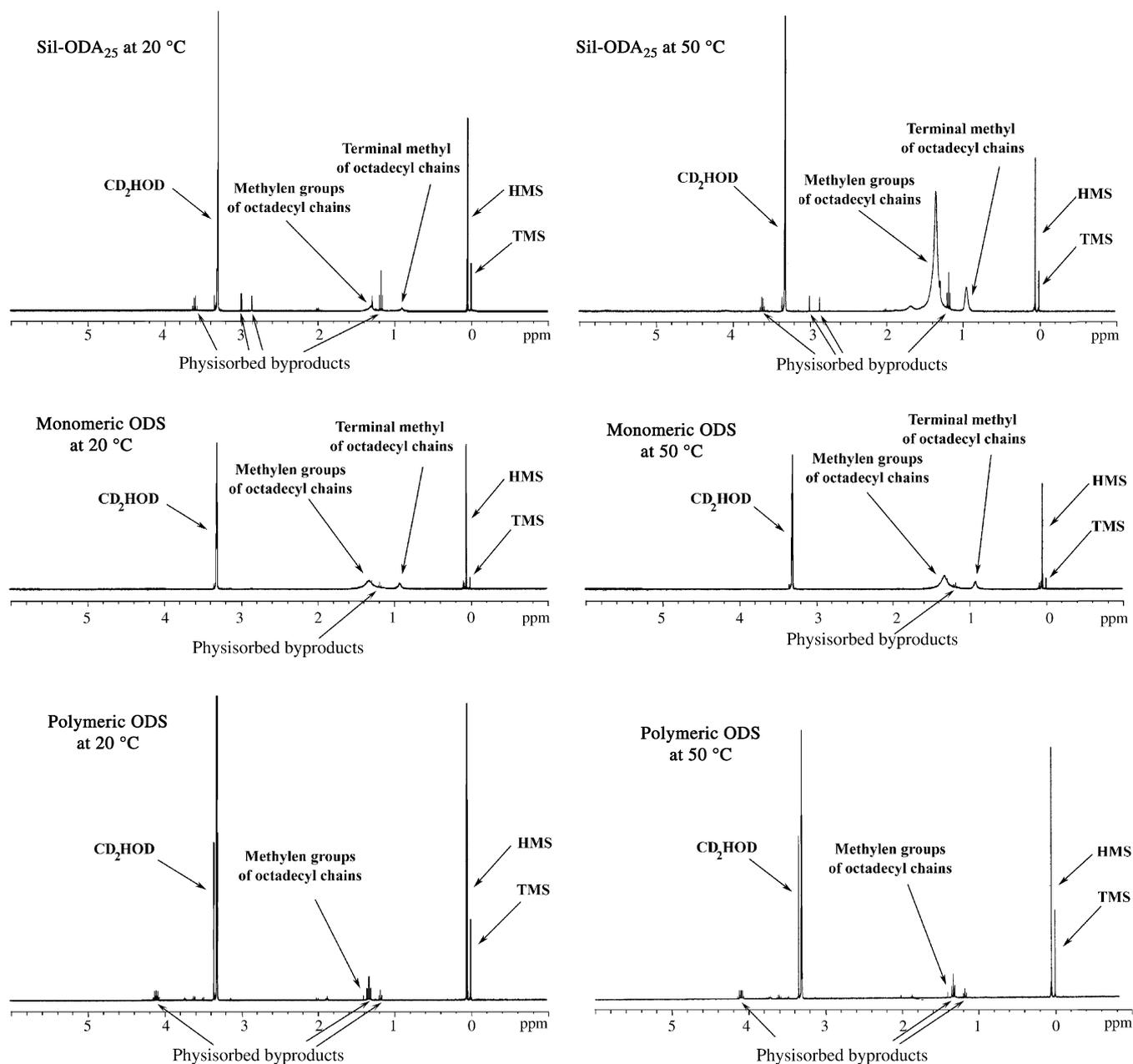
Fig. 2. Suspension-state ^{1}H NMR of Sil-ODA$_{25}$, monomeric ODS, and polymeric ODS: intensity of NMR peak of methylen groups in case of Sil-ODA$_{25}$ increases dramatically as temperature rises. However, monomeric ODS shows only a slight increase as temperature rises and polymeric ODS shows almost no increase.

with temperature. Fig. 2 shows ^{1}H NMR spectra of the three samples at 20 and 50 °C. We can see in Fig. 2 that intensity of NMR peaks representing terminal methyl and methylene groups of octadecyl moieties increases significantly in Sil-ODA$_{25}$. In case of transition from liquid crystalline phase to isotropic phase a fixed intensity but a declining line width (or an increasing T_2) was expected.

In the case of monomeric ODS, intensity of NMR peaks representing terminal methyl and methylene groups increased slightly, but for polymeric ODS, only the peak of methylene groups was detectable when a very high vertical scale was used for graphical presentation.

As all three samples were washed carefully with toluene and chloroform, the small sharp peaks detected in ^{1}H NMR spectra should be regarded as physisorbed by-products of grafting reactions. Interestingly the amount of physisorbed by-products in case of polymeric ODS or Sil-ODA$_{25}$ was higher than that of monomeric ODS, a fact that indicates presence of more closed spaces in the micro-architecture of polymeric ODS and Sil-ODA$_{25}$. As shown in Fig. 3, intensity of NMR signals from methylene groups of Sil-ODA$_{25}$ increase distinctly around an endothermic peak of DSC. Peak-top temperature was measured to be 38.4 °C for Sil-ODA$_{25}$ in presence of methanol. Intensities, to be comparable
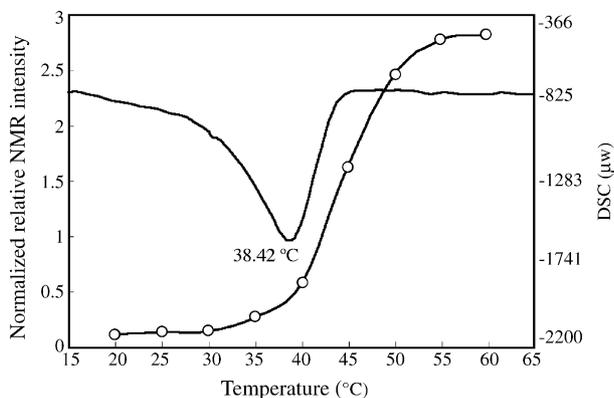
Fig. 3. Normalized relative ^{1}H NMR–NMR intensity of methylene groups of Sil-ODA$_{25}$ increase distinctly around 38 °C, which is just around the peak-top temperature of its DSC thermogram.

between different samples and at different temperatures, are weighted according to this formula (justification will be discussed in the following):

$$I(\mu mol^{-1}) = (I_m/I_D)/(4 \times 10^{-3}\ M)$$
$$= 250[I_m/(I_D \times M)] \qquad (4)$$

where I is the calculated normalized intensity (relative intensity of NMR signal per 1 μmol of octadecyl moieties) shown in Fig. 4, I_m the under-peak area of methylene peak, I_D the intensity of few protons belonging to methanol-d, and M is the amount of grafted octadecyl moiety per 1 g of modified silica.

The intensity of signals can be affected by time-to-time instrumental conditions, namely tuning of the observation channel. Considering the fact that instrumental conditions affect intensities of I_m and I_D equally and using one single ampoule of methanol-d for all measurements, the value of I_m/I_D can be assumed independent from instrumental conditions. At this stage the value of I_m/I_D represents all octadecyl moieties with liquid type mobility. Finally dividing this value by $4 \times 10^{-3}\ M$ will give us a normalized value for
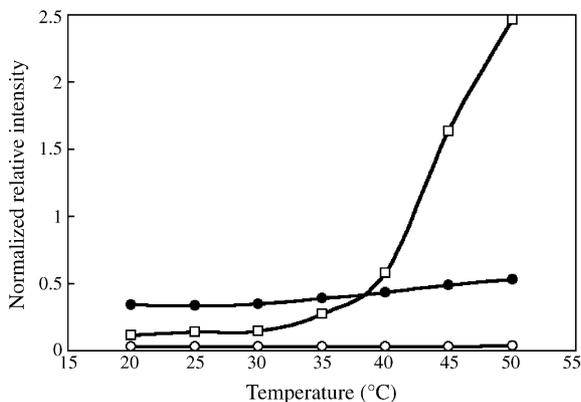
Fig. 4. Temperature dependencies of normalized relative ^{1}H NMR-intensities of methylene groups of Sil-ODA$_n$ (□), monomeric ODS (●), and polymeric ODS (○).

comparison between different experiments. M is the amount of grafted octadecyl moieties per 1 g of modified silica and 4×10^{-3} is included because NMR signal of only 4 mg of the modified silica was measured in each suspension-state ^{1}H NMR spectroscopy.

Normalized intensity of ^{1}H NMR peak of methylene groups of Sil-ODA$_{25}$ begins to increase distinctly around 38 °C but no similar style of increase can be seen in case of monomeric ODS and polymeric ODS. According to suspension-state ^{1}H NMR and comparing normalized under-peak areas (assuming the same amount of octadecyl chains) between the three samples shown in Fig. 4, we can see that octadecyl moieties in case of Sil-ODA$_{25}$ show a dramatic increase of mobility towards liquid scale around 38 °C, just where it shows an endothermic peak in DSC thermogram.

We assumed 87.1% of octadecyl moieties of Sil-ODA$_{25}$ are in liquid state at 50 °C, as its normalized relative under-peak area increases 12.5% from 50 to 55 °C (from 2.46 to 2.78) but remains almost constant from 55 to 60 °C (from 2.78 to 2.82). Now if we assume normalized intensity of 2.82 indicative of 100% of octadecyl moieties with liquid type mobility we can develop a quantitative system for determining percentage of octadecyl moieties of liquid type mobility for each sample in each temperature. In other words when temperature is increased from 20 to 50 °C percentage of octadecyl/octadecyl-parts with liquid type mobility in Sil-ODA$_{25}$ increases from 3 to 87.1%, and in monomeric ODS from 12.3 to 18.5%. In case of polymeric ODS it is almost constantly around 1%.

3.3. Solid-state ^{13}C-CP/MAS NMR spectroscopy

Under the condition of magic angle spinning and dipolar coupling of protons, the chemical shift of methylene groups in ^{13}C-CP/MAS NMR Spectroscopy depends largely on the conformation of octadecyl chains [32,33]. For each central carbon atom in an octadecyl chain with the trans conformation, a chemical shift of about 33 ppm is expected. On the other hand, for conformations with rapid changes between gauche and trans, a chemical shift of about 30 ppm is expected [34].

In solid-state ^{13}C-CP/MAS NMR Spectroscopy cross polarization transfers magnetization from abundant proton nuclei to ^{13}C nuclei, a process that is critical for observation of this rare nucleus, but which can also create problems when comparing two under-peak areas. In other words intensity of one NMR peak depends not only on the amount of ^{13}C atoms with a particular chemical shift but also on the availability and average distance of their nearby protons. Although this problem needs a number of mathematical processes to be solved [35], we can still compare two ^{13}C-CP/MAS NMR peaks on which the cross polarization has equal effects, e.g. comparing under-peak areas between peaks of the gauche and the trans conformations. In other words, although we could not compare the ^{13}C NMR under-peak area of methylene groups, in which each ^{13}C nucleus is cross-polarized by two
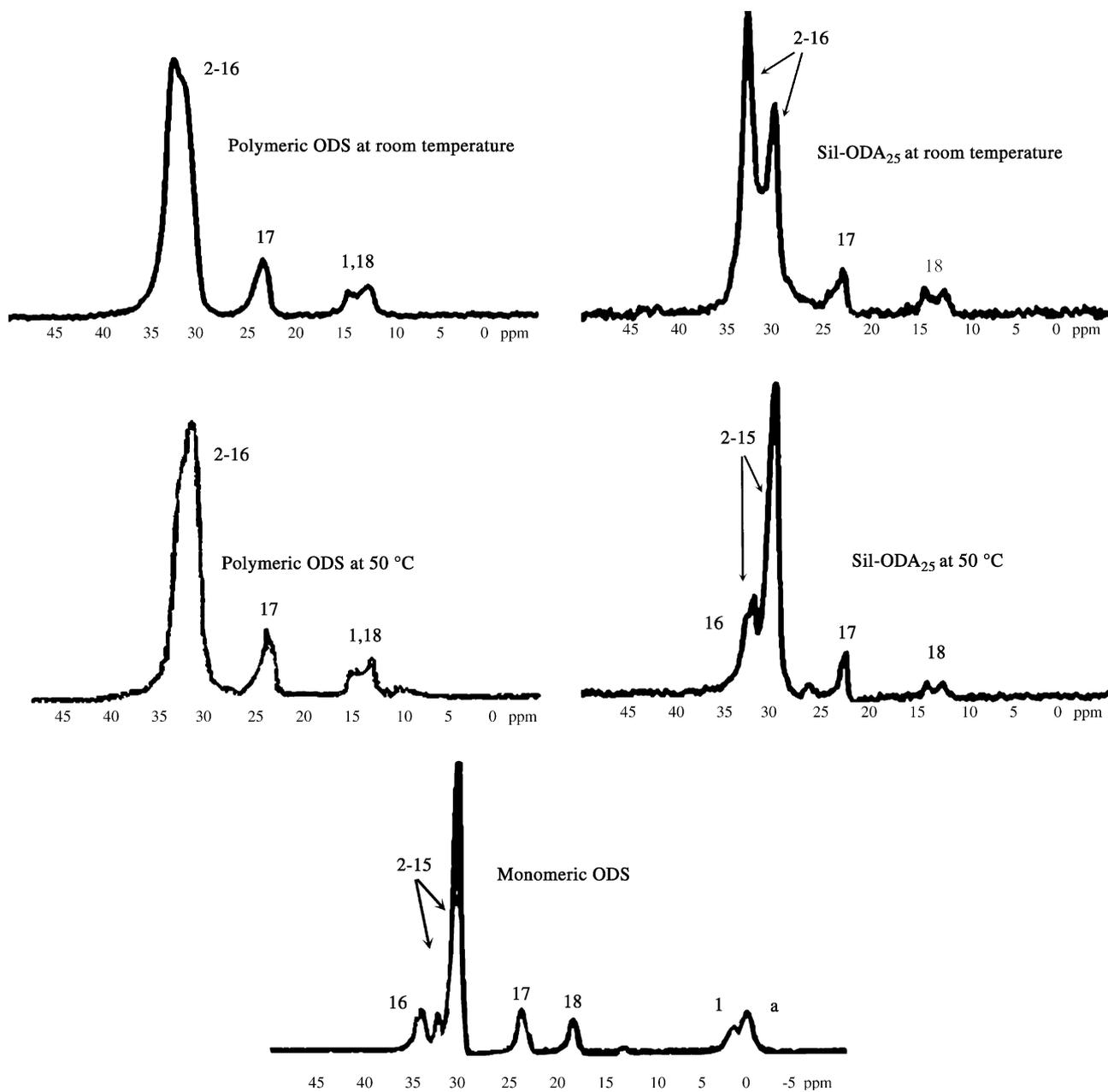
Fig. 5. Conformation of octadecyl moieties verified by solid-state ^{13}C-CP/MAS NMR Spectroscopy: octadecyl moieties with the trans conformation have a chemical shift of about 33 ppm but those with the gauche conformation have a chemical shift of about 30 ppm.

protons, with that of terminal methyl groups in which each ^{13}C nucleus is cross-polarized by three protons, we could still compare gauche and trans ^{13}C NMR peaks because each ^{13}C nucleus from methylene groups in either gauche or trans conformations was cross-polarized almost equally by its two directly attached protons.

Fig. 5 shows solid-state ^{13}C-CP/MAS NMR spectra obtained from Sil-ODA$_{25}$, polymeric ODS, and monomeric ODS. Sil-ODA$_{25}$ shows two well-resolved peaks, trans ($\delta = 32.96$ ppm) and gauche conformations ($\delta = 29.98$ ppm). At room temperature polymeric ODS shows predominantly trans conformation ($\delta = 32.84$ ppm) with a high-field shoulder

indicating gauche conformation, but at 50 °C shows predominantly gauche ($\delta = 31.5$ ppm) with a low-field shoulder indicating trans.

The chemical shift of gauche conformation in case of polymeric ODS (31.5 ppm) is still considerably higher than that of Sil-ODA$_{25}$ (29.98 ppm). Note that the peak indicative of the gauche conformation represents the hydrocarbon chains with rapid conformational change between all-trans and gauche. Therefore, the fact that the chemical shift of the gauche peak of polymeric ODS has a 1.52 ppm higher value than that of Sil-ODA$_{25}$ implies that for any single octadecyl chain of polymeric ODS, the overall time of being in all-trans
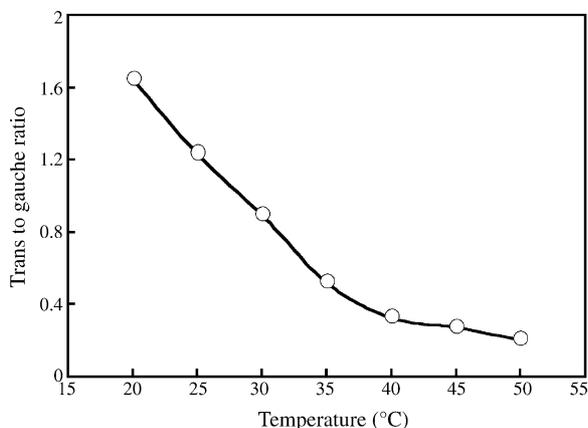
Fig. 6. Trans to gauche ratio in solid-state ^{13}C-CP/MAS NMR of Sil-ODA$_{25}$ decrease as temperature rise with an inclination between 35 and 40 °C just coincident with the sharp increase in the normalized relative ^{1}H NMR intensity of methylene groups shown in Fig. 3.

conformation is considerably higher than that of Sil-ODA$_{25}$. Monomeric ODS, however, shows almost only gauche conformation ($\delta = 30.4$ ppm). In case of Sil-ODA$_{25}$, because of its two well-resolved peaks a quantitative estimation of gauche and trans conformations was possible.

Interestingly, we see in Fig. 5 that there are two peaks rather than one around the chemical shift of $\delta = 14$ ppm in all samples except monomeric ODS. In case of polymeric ODS, this can be explained by assigning them to the carbon of terminal methyl group and the carbon directly (covalently) attached onto the silica surface (carbon atoms number 1 and 18 in Fig. 1) of polymeric ODS (as was assigned by some authors [35,36]).

But this cannot be the case for Sil-ODA$_{25}$ as the number of carbons directly (covalently) attached onto the silica surface is incomparable with the number of carbons belonging to terminal methyl groups (25 times less). So this duplication in the terminal methyl group peak might be interpreted as a chemical shift difference because of being located in two different microenvironments, for instance monolayer and bilayer membrane structures.

As shown in Fig. 5, at 20 °C Sil-ODA$_{25}$ has a dominance of trans conformation, polymeric ODS shows both conformations, and monomeric ODS shows only a very small (if any) amount of trans conformation. Some authors [36] compared microstructures of grafted C$_{18}$ and C$_{22}$ bifunctional silane ligands with C$_{30}$ and C$_{34}$ trifunctional ones where gauche conformation was determined for C$_{18}$ and C$_{22}$ but trans for C$_{30}$ and C$_{34}$. Although they implied that it is the chain length that affects conformation, this study by determining trans conformation for C$_{18}$ trifunctional ligands shows that conformation of the grafted alkylsilanes is more affected by features of the head than by length of the tail.

As shown in Fig. 6, the ratio of trans to gauche conformation decreases as temperature increases. The trend of decrease of the ratio of trans to gauche showed an inclination around 35–40 °C just like Fig. 3. Polymeric ODS seems reluctant
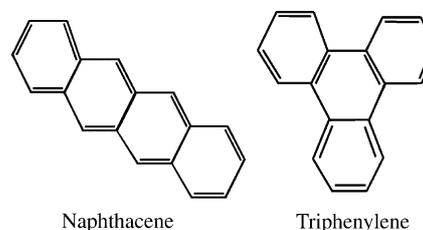
Fig. 7. Naphthacene and triphenylene have similar molecular weight but different shapes therefore the separation factor, $\alpha_{naphthacene/triphenylene}$, can show the ability of each stationary phase to detect molecular shapes.

to lose trans conformation (a significant low-field shoulder still remain at 50 °C), and monomeric ODS doesn't show significant change in its gauche conformation. A quantitative estimation of the gauche and trans conformations was not possible in case of polymeric ODS as the two related peaks were not resolved enough.

3.4. Chromatography

We used the separation factor, $\alpha_{naphthacene/triphenylene}$ as a quantitative index of ability to detect linearity of compounds. As shown in Fig. 7, naphthacene and triphenylene have equal molecular weights but naphthacene is apparently more linear than triphenylene, so their mechanism of separation cannot be based on possessing different hydrophobicities but must mainly be based on possessing of different geometries. Hence the separation factor between them, $\alpha_{naphthacene/triphenylene}$, can be regarded as a quantitative index of shape selectivity of the stationary phase. Fig. 8 shows temperature dependencies of $\alpha_{naphthacene/triphenylene}$ in the three stationary phases. It is evident that the shape selectivity of Sil-ODA$_n$ was highest at low temperatures but fell as temperature increased from 20 to 50 °C. Polymeric ODS showed a moderate but rather stable
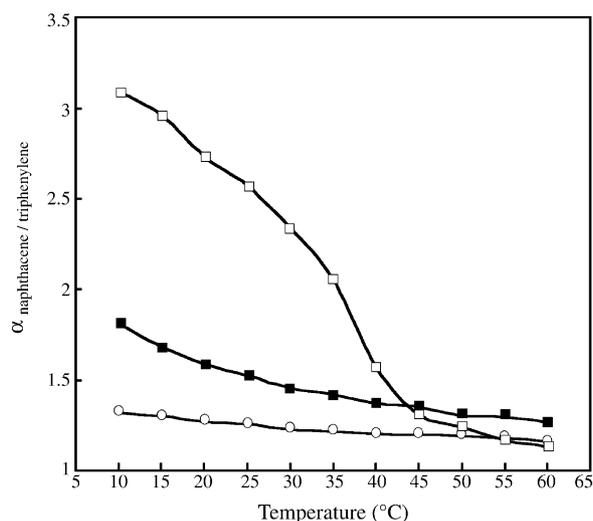
Fig. 8. Temperature dependency of $\alpha_{naphthacene/triphenylene}$ in, Sil-ODA$_n$ ($\square$), polymeric ODS ($\blacksquare$), and monomeric ODS ($\bigcirc$): shape selectivity of Sil-ODA$_n$ is highest at low temperatures but decreases dramatically as temperature increases from 20 to 50 °C.

Table 1
Summarised characterization of Sil-DA$_{25}$, monomeric ODS, and polymeric ODS

Stationary phase	Dominant phase	Conformation	Ability to detect linearity
Sil-ODA$_{25}$ (at low temperature)	Solid	Mostly trans	High
Sil-ODA$_{25}$ (at high temperature)	Liquid	Gauche	Low
ODS (monomeric)	Solid	Gauche	Low
ODS (polymeric)	Solid	Partially trans	Medium

shape selectivity and monomeric ODS showed the lowest shape selectivity. It is quite justifiable to compare our NMR findings with chromatography data as the carbon percentage (and hence surface coverage) of synthesized monomeric ODS and the monomeric C$_{18}$ column were exactly the same (13.8%). In case of polymeric ODS although surface coverage in polymeric C$_{18}$ column was significantly lower than the polymeric ODS that was subjected to NMR experiments (17.5% compared to 23.3%) the polymeric C$_{18}$ column still showed a good shape selectivity.

4. Conclusion

Comparative methods based on suspension-state ^{1}H NMR can be used for categorizing mobility type (liquid scale or solid scale) of grafted alkyl chains on silica or any other supporting particles. This phase characterization method in conjunction with conformation determination drawn from solid-state ^{13}C-CP/MAS-NMR is important for better understanding of structure, dynamic, and separation behaviour of organic layers grafted on the silica surface. Final characterizations of the polymeric ODS and monomeric ODS together with Sil-ODA$_n$, itself, are summarised in Table 1.

Trans conformation indicates ordered packing of octadecyl moieties that can easily explain both the better shape selectivity of polymeric ODS (compared with monomeric ODS) and the strong temperature dependency in shape selectivity of Sil-ODA$_{25}$.

Interestingly most of the octadecyl chains in case of monomeric ODS, despite having gauche conformation, are still in solid phase and only 12.3–18.5% of them have enough mobility to be considered as being in liquid phase.

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