

Enhancement of Retentivity and Selectivity for PAHs in NP-HPLC by High-density Immobilization of Poly(4-vinylpyridine) as an Organic Phase on Silica

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Poly(4-vinylpyridine)-modified silica with high grafting density have been prepared by a grafting-from (g-from) approach through radical chain-transfer reactions. The widely used silane coupling agent 3-mercaptopropyltrimethoxysilane was used to prepare thiol-terminated silica. Chain-transfer reaction and polymerization of 4-vinylpyridine was carried out using α,α' -azobisisobutyronitrile as an initiator. Thiol-terminated silica and polymer-modified silica were both characterized qualitatively and quantitatively. The quantification of the organic phase has been done by thermogravimetric analysis and elemental analysis. Thus, the modified silica was used as a packing material and the retention behavior of polycyclic aromatic hydrocarbons (PAHs) was studied in normal-phase high-performance liquid chromatography. Results were compared with those of poly(4-vinylpyridine)-modified silica prepared by a grafting-to (g-to) approach. Commercially available aminopropyl-bonded silica and bare silica columns were also used as reference columns. The column of poly(4-vinylpyridine)-grafted silica prepared by the g-from method, having higher grafting density, provided the better retentivity and selectivity for PAHs compared to the other reference columns.

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Introduction

Poly(4-vinylpyridine)-modified silica (Sil-VP_n) has shown useful separation behaviors for polycyclic aromatic hydrocarbons (PAHs) in both normal-phase (NP)¹ and reversed-phase (RP)^{2,3} high-performance liquid chromatography (HPLC). The mixed mode of retention mechanism exhibited by a Sil-VP_n column is not achievable by more conventional normal-phase or reversed-phase columns;¹ thus, the Sil-VP_n has a potential for further development and study. On the other hand, PAHs are ubiquitous in the biosphere and because of their impact on health and environment much attention is needed for this class of compounds. Although RP-HPLC is quite popular, separation of PAHs having similar polarity is still a challenge if one uses this technique.⁴ On this viewpoint, NP-HPLC could be a better option for their separation.

We have reported the shape selectivity aspect of Sil-VP_n in NP-HPLC.¹ Shape selectivity characteristics for constrained solutes are reported to vary considerably among the stationary phases prepared by different synthetic approaches.⁴ For example Sander *et al.*⁵ observed the significant differences in the chromatographic properties of C₁₈ phases prepared by different ways. The variation in the morphology of the tethered alkyl chains has also been described based on the changes of the synthetic routes.⁶ Even the changes of polymerizing solvents have also resulted in dramatic changes in the retention behaviors of solute on the particular stationary phase.⁷

Different immobilization routes are reported for the

attachment of polymers on the surface, including (i) grafting-to (g-to), (ii) grafting-from (g-from) and (iii) surface copolymerization.⁸ The grafting-from synthetic approach offers the possibility to generate high densities of graft because of easy access of the reactive groups to the chain end of the growing polymer.⁸ The key is to introduce initiating points on the substrate surface; such introductions can be carried out through the assembly of self-assemble-initiators or the *in situ* modification of end group-functionalized surfaces by using self-assembling techniques.⁹ Among the g-from methods, radical chain-transfer reaction has also been used for the modification of solid surfaces.¹⁰

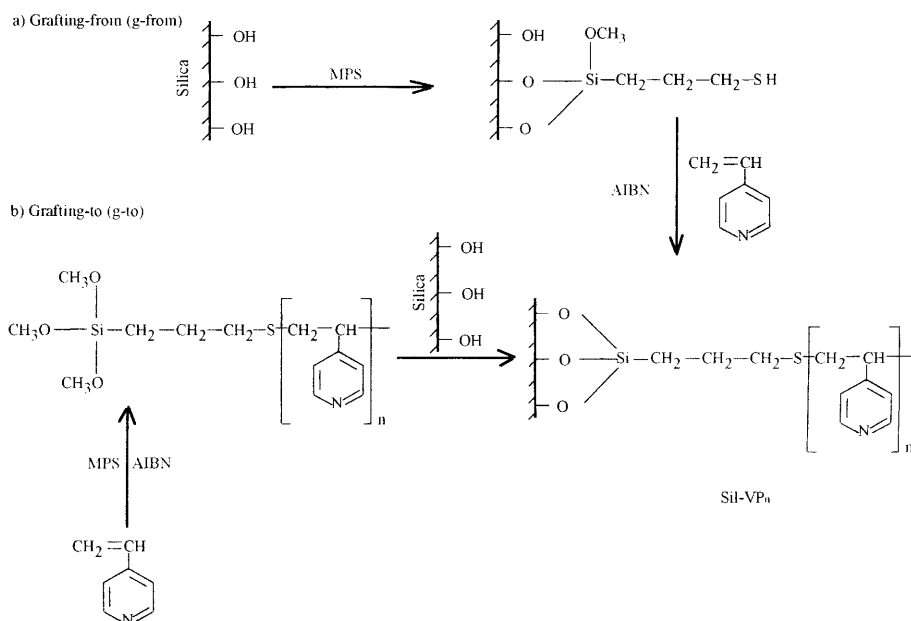
Herein, we report the modification of silica particles with poly(4-vinylpyridine) using g-from method [VP_n(from)] through a surface-initiated radical-chain transfer reaction. The modified silica particles are subsequently used to make HPLC columns and the chromatographic properties of PAHs are studied in NP-HPLC. The main objective of the present study is to compare how the synthetic routes affect the chromatographic properties of poly(4-vinylpyridine)-grafted silica (Sil-VP_n), especially for planar non-planar solutes.

Experimental

Materials

All polycyclic aromatic hydrocarbons (PAHs) elutes were commercially available and were used without further purification. 4-Vinylpyridine monomer was purchased from Sigma-Aldrich (Steinheim, Germany) and was used after distillation. 3-Mercaptopropyltrimethoxysilane (MPS) was purchased from Azmax Co. (Chiba, Japan). YMC silica gel

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Scheme 1 Routes of immobilization of poly(4-vinylpyridine) on silica surface: grafting-from (g-from) (a) and grafting-to (g-to) (b).

(120-S5; diameter, 5 μm ; pore size, 120 \AA ; specific surface area, $300 \pm 5 \text{ m}^2 \text{ g}^{-1}$) was used as porous silica particles.

Synthesis of stationary phases

Grafting-from (g-from) method (Scheme 1(a)). Five grams of YMC silica was suspended in 50.0 ml of toluene, 0.91 ml of MPS was then added to this suspension. The mixture was mechanically stirred under solvent reflux in an inert nitrogen atmosphere for 72 h. After cooling, the solid was filtered and then washed with toluene and acetone successively to remove un-grafted MPS. The obtained product, denominated Sil-SH, was dried in vacuum. In the next stage, 4.5 g of the modified silica was suspended in 40 ml of 4-vinylpyridine monomer and bubbled with nitrogen at room temperature for 10 min. Then 100 mg of α, α' -azobisisobutyronitrile (AIBN) was dissolved in 5 ml of 4-vinylpyridine monomer and the mixture was added to the suspension under nitrogen atmosphere. The suspension was mechanically stirred at 70°C for 6 h. The obtained product was washed several times with hot chloroform. The washing was repeated several times till the filtrate gave no precipitation with *n*-hexane to ensure complete removal of un-grafted poly(4-vinylpyridine). Finally, the material was washed with methanol, acetic acid and water, then dried in vacuum. The polymer grafted-silica [VP_n(from)] was characterized qualitatively by spectroscopic methods (spectra not shown) and quantitatively by elemental analysis (18.53% carbon and 2.82% nitrogen) and thermogravimetric analysis (TGA) [amount of immobilization 20.2 wt% (Fig. 1d)] and packed into a stainless-steel column (4.6 mm i.d. \times 250 mm).

Grafting-to (g-to) method (Scheme 1(b)). Poly(4-vinylpyridine) with a terminal trimethoxysilyl group was prepared according to a previously reported telomerization method.¹⁻³ The polymer is grafted onto silica by mixing with chloroform at reflux temperature. The amount of grafting was quantified by elemental analysis (13.63% carbon and 2.14% nitrogen) and thermogravimetric analysis (TGA) [amount of immobilization 16.7 wt% (Fig. 1c)]. The prepared packing material [VP_n(to)]

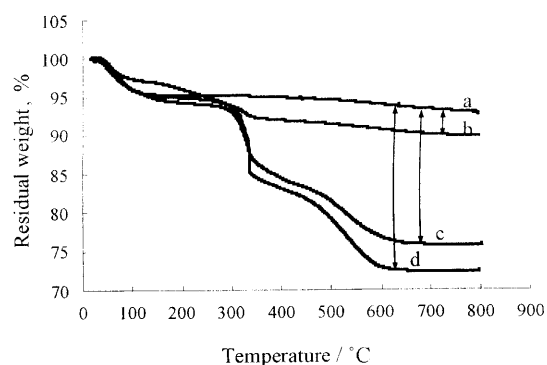


Fig. 1 Thermogravimetric data curve for bare silica (a), MPS-grafted silica (b), VP_n(to) (c) and VP_n(from) (d).

was then packed into a stainless-steel column (4.6 mm i.d. \times 250 mm). A bare silica column (Inertsil silica, 4.6 mm i.d. \times 250 mm, GL-Science, Inc., Tokyo, Japan) and an aminopropyl-bonded silica column (Lichrospher NH₂, 4.0 mm i.d. \times 250 mm, Kanto Chemicals, Co., Inc., Tokyo, Japan) were used as the reference columns.

Measurements

The analysis was conducted under an isocratic condition using a chromatograph with a pump (JASCO 980 PU), and a JASCO multiwavelength detector (MD-910). A mixture of *n*-hexane/2-propanol was used as the mobile phase. Five microliters of each sample were injected through an injector (Reodyne Model 7725). A flow rate of 1 ml min^{-1} was maintained in all the analyses. The column temperature was controlled by either a water/ethylene glycol jacket equipped with a Neslab RTE-111 circulator or by a column heater (Sugai U-620 Type VP30). The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where t_e and t_0 are the retention times of a sample and the deflection due to

injection of *n*-dodecane. The separation factor (α) was defined by the ratio of the retention factors.

Solid-state ^{29}Si NMR-CPMAS spectra were measured with a VARIAN UNITY^{NOVA} AS400. Thermogravimetric analysis (TGA) was performed using a TG/DTA 6300 (Seiko Instrument Inc., EXSTAR 6000, JST) with a heating rate of $10^\circ\text{C min}^{-1}$. The range of temperature extended from 30 to 800°C .

Results and Discussion

Calculation of the surface coverage

The surface coverage calculation has been done as per our previous report.¹¹ The molar amount of the grafted functional groups per 1 g of silica (M) can be calculated as:

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

Here P_C is the percentage of carbon obtained by the elemental analysis and n refers to the number of carbon atoms per one unit of the grafted functional groups. For MPS-grafted silica, n is equal to 3, whereas for $\text{VP}_n(\text{from})$ and $\text{VP}_n(\text{to})$ it is equal to 7. For the calculation of the molar amount of the functional groups per 1 g silica using nitrogen percentage, we have applied the following formula:

$$M (\mu\text{mol g}^{-1}) = 10^6(P_N/100)/14n$$

Here P_N is the percentage of nitrogen obtained by the elemental analysis and n refers to the number of nitrogen atoms per one unit of the grafted functional groups, which is 1 for both $\text{VP}_n(\text{from})$ and $\text{VP}_n(\text{to})$.

The weight percentage (P_W) of the grafted functional group in each case can be calculated by:

$$P_W = m \times 10^{-4} M$$

Here m is the molecular mass per one unit functional group, which is taken as 75 for MPS-grafted silica and as 105 for $\text{VP}_n(\text{from})$ and $\text{VP}_n(\text{to})$.

Similarly, surface coverage (N) in each case can be calculated by using the relation:

$$N (\mu\text{mol m}^{-2}) = M/\{S[(100 - P_W)/100]\}$$

Table 1 Element analysis results of the modified silica

	C, %	N, %	H, %	C/N
MPS-grafted silica	2.67	0	1.14	
$\text{VP}_n(\text{from})$	18.53	2.82	2.19	6.57
$\text{VP}_n(\text{to})$	13.63	2.14	1.77	6.37

Table 2 Comparison of grafting density of the modified silica

	Surface coverage calculated by carbon percentage			Surface coverage calculated by nitrogen percentage		
	Amount of immobilization, wt%	Polymer density/ $\mu\text{mol g}^{-1}$	Polymer density/ $\mu\text{mol m}^{-2}$	Amount of immobilization, wt%	Polymer density/ $\mu\text{mol g}^{-1}$	Polymer density/ $\mu\text{mol m}^{-2}$
MPS-grafted silica	5.56	741.67	2.62			
$\text{VP}_n(\text{from})$	23.16	2205.95	9.57	21.15	2014.29	8.52
$\text{VP}_n(\text{to})$	17.04	1622.62	6.52	16.05	1528.57	6.07

Here S is surface area per 1 g of non-modified silica, which is equal to $300 \text{ m}^2 \text{ g}^{-1}$. Table 1 shows the results of elemental analysis, whereas Table 2 shows the results of surface coverage calculated by using carbon percentage and nitrogen percentage. The surface coverage calculated by using carbon percentage is comparatively higher than that obtained with nitrogen percentage. The difference could be due to the physisorbed organic solvents that were used while preparing and washing the polymer-grafted silica, while no nitrogen-containing solvents had been used during the synthetic and washing steps. The immobilization percentage obtained by TGA measurement (Fig. 1) is also in agreement with the immobilization percentage calculated by using nitrogen percentage. For example, the immobilization percentage of $\text{VP}_n(\text{to})$ calculated from TGA measurement is 16.7 wt% (Fig. 1c) and that calculated by using nitrogen percentage is 16.05 wt% (Table 2); the values are in agreement with each other. Similarly, the TGA-determined immobilization weight percentage of $\text{VP}_n(\text{from})$ is 20.2 wt% (Fig. 1d) and that calculated by using nitrogen percentage is 21.15 wt% (Table 2). Thus, whatever method was applied for the calculation of surface coverage, a higher percentage of organic phase was found on the silica surface of $\text{VP}_n(\text{from})$ compared to the percentage on that of $\text{VP}_n(\text{to})$.

Preformed polymers were grafted onto the silica surface during the preparation of $\text{VP}_n(\text{to})$. Thus the average polymerization degree was calculated from $^1\text{H-NMR}$ spectroscopy, results to be 21. However, in the case of $\text{VP}_n(\text{from})$, the polymerization was started from the surface of MPS-grafted silica, so the average polymerization degree of 3 was calculated, using elemental analysis results of MPS-grafted silica and poly(4-vinylpyridine)-grafted silica. While calculating the polymerization degree, it has been assumed that all SH-grafted silica reacted with 4-vinylpyridine monomers and that the distribution of the polymeric chain is homogeneous, which are very rare cases. Thus, significant error has been incurred in the calculation of the polymerization degree of $\text{VP}_n(\text{from})$, but still it can be predicted that the grafted polymers with $\text{VP}_n(\text{from})$ are short compared to $\text{VP}_n(\text{to})$, as the C/N ratio of $\text{VP}_n(\text{from})$ is also higher than that of $\text{VP}_n(\text{to})$ (Table 1). Thus, these results indicate that a higher percentage of the surface coverage is prevalent in $\text{VP}_n(\text{from})$ compared to that in $\text{VP}_n(\text{to})$.

Evaluation of $\text{VP}_n(\text{from})$ with HPLC measurements

The high retentivity and selectivity of PAHs with $\text{VP}_n(\text{to})$ in NP-HPLC have been discussed in our recent paper.¹ When *n*-hexane/2-propanol solvent system was used as a mobile phase, better resolution of PAHs was achieved compared to other conventional normal-phase and reversed-phase columns. In this paper, the same organic-phase modified silica was prepared by *g*-from method and the same set of PAHs was applied as solutes to examine the chromatographic efficiency of the present stationary phase. As shown in Table 3, increased retentivity and selectivity were achieved with $\text{VP}_n(\text{from})$ compared to the other

Table 3 Retention (k) and separation factors (α) for linear PAHs with various columns at 35°C

PAH	No. of double bonds	VP _n (from)		VP _n (to)		NH ₂		Silica	
		k^a	α	k^a	α	k^a	α	k^a	α
Benzene	3	0.34	1.94	0.20	1.95	0.09	1.44	0.42	1.69
Naphthalene	5	0.66		0.39		0.13		0.71	
Anthracene	7	1.55	2.37	0.82	2.12	0.18	1.38	1.15	1.62
Naphthacene	9	3.68		1.74		0.26		1.85	

a. Mobile phases: *n*-hexane/2-propanol (9:1) in VP_n(from), VP_n(to) and NH₂; *n*-hexane in silica.

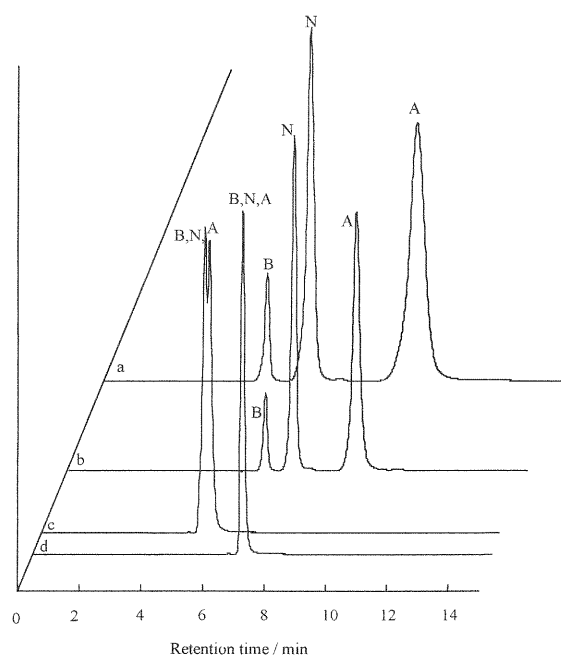


Fig. 2 Typical chromatograms for a mixture of PAHs with VP_n(from) (a), VP_n(to) (b), aminopropyl-bonded silica (c) and bare silica (d) at ambient temperature. Mobile phase, *n*-hexane/2-propanol (7:3); flow rate, 0.5 ml min⁻¹. B, Benzene; N, naphthalene; A, anthracene.

reference columns. Supporting the common trends of NP-HPLC adsorbents,¹² retention factors of PAHs had increased with the number of aromatic rings. On the contrary to the results for the common adsorbents of NP-HPLC, with VP_n phases the selectivity of the PAHs pair had increased based on the number of rings. Enhanced selectivity was observed for the PAHs having a higher number of rings though the difference of double bonds between the pairs is two in all the cases. Interestingly, with VP_n(from), higher selectivity was observed for all PAHs pairs except naphthalene/benzene pair than was achieved with VP_n(to) (Table 3). The increases of retention factor and separation factor with VP_n phases can be explained by π - π interactions between PAHs and pyridine rings of the polymer. Specificity of VP_n(from) can also be seen in the separation of PAHs using a mixture of *n*-hexane/2-propanol (7:3) as a mobile phase. Almost no resolution of PAHs was observed with aminopropyl-bonded column and bare silica column but very good resolution was obtained with VP_n phases and even better resolution had been observed with VP_n(from) (Fig. 2). The reproducibility of retention times of a solute with VP_n(from) phase had also been obtained in the range of $\pm 1\%$.

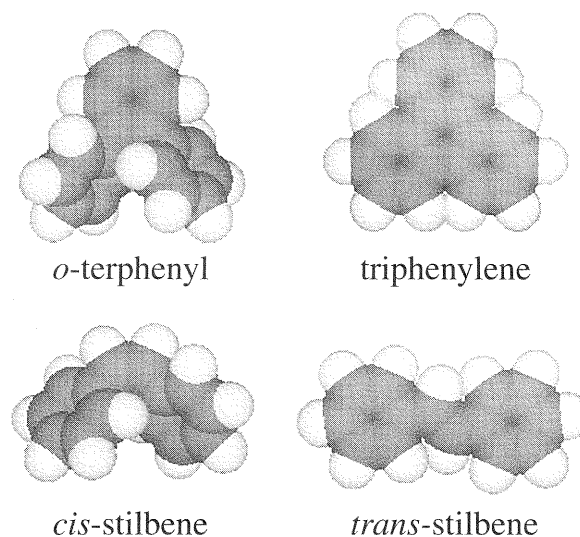


Fig. 3 CPK models of planar and non-planar PAHs.

From such results it can be predicted that VP_n(from) provides the specific interaction sites for PAHs, leading to better retentivity and selectivity compared to VP_n(to).

Molecular-shape selectivity

The chromatographic efficiency of VP_n(from) was further evaluated by planarity selectivity using triphenylene and *o*-terphenyl as solutes. Although *o*-terphenyl and triphenylene are not isomers, they have equal numbers of carbon atoms and π -electrons with absolutely different planarity (Fig. 3); thus the separation factor between this pair of compounds has been used to evaluate molecular-planarity selectivity.^{13,14} For the comparison of shape discrimination ability with VP_n(from) and with VP_n(to), the selectivity of this pair of compound was calculated at different temperatures. As shown in Fig. 4, higher separation factors were observed with VP_n(from) than with VP_n(to) at all temperatures. Further evidence of planarity selectivity can also be found in the separation of geometrical isomers of *trans*-*cis*-stilbenes (Figs. 3 - 5). When the graph (Fig. 4) was plotted between the selectivity of this pair and temperature, a higher separation factor was obtained in VP_n(from) compared to that in VP_n(to). These findings indicate that higher shape discrimination ability for planar/non-planar PAHs was prevalent with VP_n(from). The graphs in Fig. 4 and the chromatograms in Fig. 5 show that the better resolution of planar/non-planar solutes can be obtained at lower temperature.

Even for the isomers of four-ring PAHs, higher retentivity and selectivity (Table 4) was observed with VP_n(from) compared to other reference columns. The increases of retentivity and

selectivity for the structural isomers of four-ring PAHs having the same number of π -electrons cannot be understood if we consider only π - π interaction. Thus, multiple interactions including π - π interaction enhance the retentivity and selectivity of PAHs.

Discussion on the high retentivity and selectivity of PAHs with $VP_n(\text{from})$

We have reported that the interaction between pyridine rings and PAHs is the main driving force for solute retention with VP_n phase.¹⁻³ On the other hand, pyridine rings are also involved in the formation of hydrogen bonds between the pyridine nitrogens and the surface hydroxyl protons of Sil- VP_n .^{1,3,15} As discussed in the introductory section, g-from approach is an appropriate method for growing high density of the graft on the surface of silica.⁸ Supporting this assumption, we observed a higher percentage of weight loss in TGA in case of $VP_n(\text{from})$ (Fig. 1d) compared to $VP_n(\text{to})$ (Fig. 1c); elemental analysis results also

showed the higher percentage of carbon and nitrogen along with higher C/N ratio in $VP_n(\text{from})$ (Table 1) compared to $VP_n(\text{to})$. The calculated surface coverage is also higher with $VP_n(\text{from})$ compared to $VP_n(\text{to})$ (Table 2 and Fig. 1). Another proof of the higher surface coverage can also be obtained from ^{29}Si NMR-CPMAS spectra. The more intense peak of T^2 species in $VP_n(\text{from})$ (Fig. 6a) compared to $VP_n(\text{to})$ (Fig. 6b) in solid-state ^{29}Si NMR-CPMAS is attributed to the higher amount of silicon atom of type $T^2[\text{Si}(2\text{OSi}, \text{R}, \text{OR})]$.⁸ The absence of $T^1[\text{Si}(\text{OSi}, 2\text{OH}, \text{R})]$ species from ^{29}Si NMR-CPMAS spectra has been reported as an indication of a high degree of crosslinking of the trifunctional species on the silica surface.¹⁶ These results show that $VP_n(\text{from})$ consists of a larger amount of short, tethered and crosslinked polymer brushes on the silica surface. So, it can be estimated that, due to high grafting density, in $VP_n(\text{from})$ the space between the immobilized polymers is reduced and only a

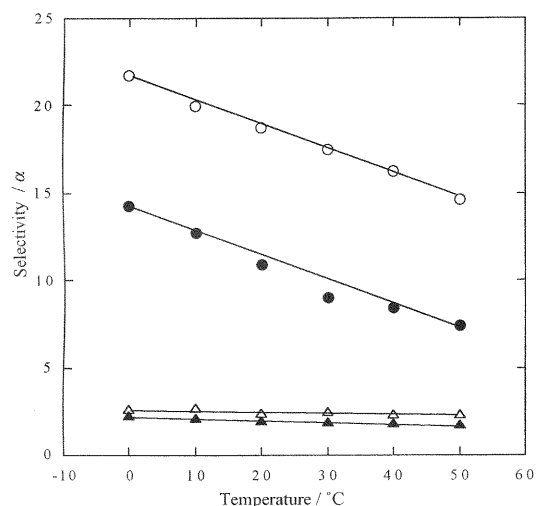


Fig. 4 Comparison of selectivity of planar/non-planar PAHs with $VP_n(\text{from})$ (O, Δ) and $VP_n(\text{to})$ (\bullet , \blacktriangle) at different temperatures. (O, \bullet) triphenylene/*o*-terphenyl; (Δ , \blacktriangle) *trans*-/*cis*-stilbenes.

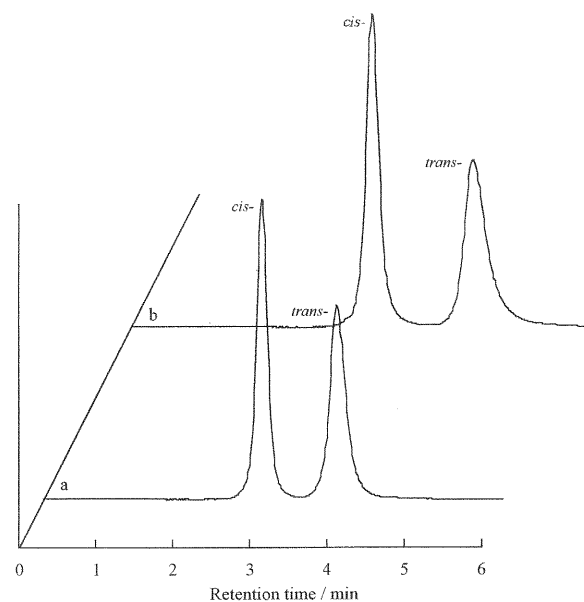
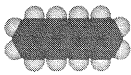





Fig. 5 Typical chromatograms for a mixture of *cis*-/*trans*-stilbenes with $VP_n(\text{from})$ at 40°C (a) and 20°C (b). Mobile phase, *n*-hexane/2-propanol (9:1); flow rate, 1 ml min⁻¹.

Table 4 Retention factor (k) and separation factor (α) for four-ring PAHs with $VP_n(\text{from})$, $VP_n(\text{to})$, NH_2 and silica at 35°C

PAH		$VP_n(\text{from})$		$VP_n(\text{to})$		NH_2		Silica	
		k	α	k	α	k	α	k	α
Naphthacene		3.680	1.51	1.74	1.46	0.26	1.15	0.12	1.08
Benz[<i>a</i>]anthracene		4.09		1.89		0.27		0.12	
Chrysene		4.64		2.12		0.28		0.12	
Triphenylene		5.540		2.54		0.30		0.13	

Mobile phases: *n*-hexane/2-propanol (9:1) in $VP_n(\text{from})$, $VP_n(\text{to})$, NH_2 and silica.

few pyridine rings of poly(4-vinylpyridine) are involved in the formation of hydrogen bonds with residual silanol groups of silica compared to VP_n(to) as shown in Fig. 7. Therefore more

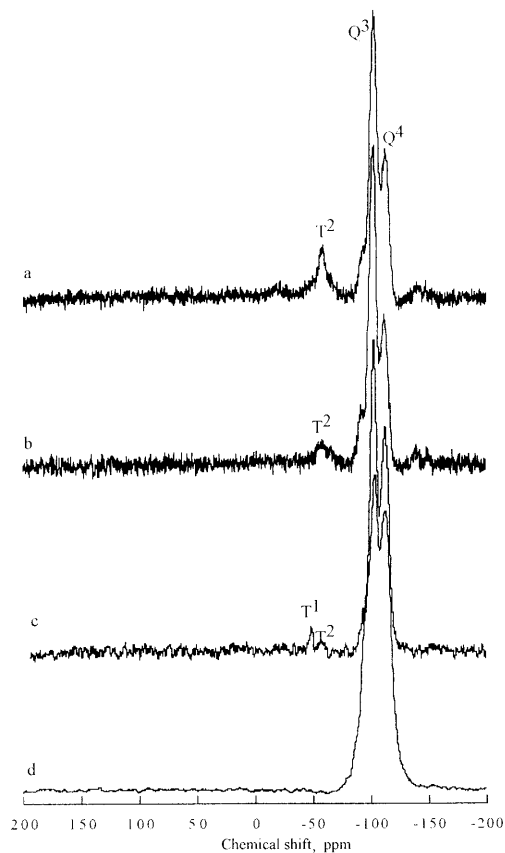


Fig. 6 Solid-state ^{29}Si NMR-CPMAS spectra of VP_n(from) (a), VP_n(to) (b), MPS-grafted silica (c) and bare silica (d).

pyridine rings are available for the interaction with a solute, leading to better retentivity and selectivity. Zhao and Brittain in their review article¹⁷ have also suggested that the tethered dense polymer chains are crowded and are forced to stretch away from the surface so as to avoid overlapping and that such behaviors result in the novel properties of the polymer brushes. In addition to the availability of more pyridine rings for interaction, the high grafting density of the organic phase may also promote the ordering of the polymer chains in VP_n(from) more than in VP_n(to) as is reported by Sentell and Dorsey¹⁸ because molecular planarity selectivity has often been described with the ordered organic phase.¹⁹ Supporting this assumption, highly ordered poly(octadecylacrylate) as a comb-shaped polymer having isotropic-to-crystalline phase transition provided the remarkably higher selectivity only at crystalline temperatures.²⁰ Though other proofs for this assumption is not in our hand, it is sure that the better shape discrimination ability of the VP_n(from) phase is due to the higher grafting density of the organic phase, which brings about the multiple interaction effects including polymeric and orientation effects of the polymer. Further investigations on the polymeric and orientation effects of the polymer are in progress.

Conclusions

Poly(4-vinylpyridine)-grafted silica has been prepared by a grafting-from approach through a radical chain-transfer reactions. Thus, the modified silica particles have been recognized to have higher immobilization percentage and have showed enhanced selectivity and retentivity compared to the poly(4-vinylpyridine)-grafted silica prepared by grafting-to method. The increased retentivity of VP_n(from) along with enhanced selectivity for planar PAHs would be an additional advantage in the field of separation science.

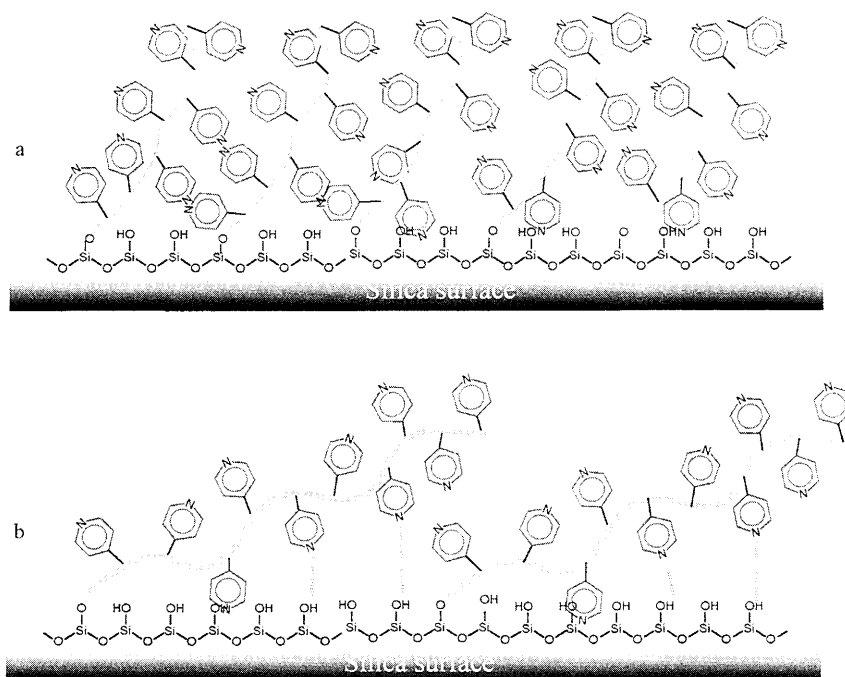


Fig. 7 Proposed models of immobilization of polymers in VP_n(from) (a) and VP_n(to) (b).

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