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# Poly(4-vinylpyridine) as a reagent with silanol-masking effect for silica and its specific selectivity for PAHs and dinitropyrenes in a reversed phase

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## Abstract

This paper demonstrates that poly(4-vinylpyridine) is applicable as an effective masking reagent for silica to reduce undesirable side effects due to silanol groups. It also shows that this chemical modification brings about unique retention behaviors absolutely different from conventional ODS, which appear in molecular-shape selectivity for polycyclic aromatic hydrocarbons and in selectivity for position isomerism, especially for electron-withdrawing substitution compounds. Separation of 1,6- and 1,8-dinirtopyrenes as carcinogens is also described.
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## 1. Introduction

To analyze basic compounds with HPLC is very important because many pharmaceutical and bio-related materials often include amino groups. Especially, an RP-HPLC method using $C_{18}$-bonded silicas is very powerful and convenient for this purpose, and thus has been widely used around the world. However, serious problems have been noted; specifically peak-tailing and poor reproducibility through residual silanol groups on silica surfaces. To eliminate these undesirable properties, many end-capping reagents and methods have been developed. The most widely used reagents for this purpose include end-capping with trimethylchlorosilane [1,2] and hexamethyldisilazane [3] for silanol groups. These become more useful by coupling with high temperature treatment. Hydrosilation is also a very useful method for end-capping [4,5]. Alternatively, *n*-alkyl-bonded silica with embedded polar functional groups has been attracting

attention as an another approach to improve peak shape and reproducibility. It have been reported that these phases having amide or carbamate groups, showed a better peak shape for basic compounds compared with conventional *n*-alkyl-bonded phases [6,7]. This is often explained by the hydration layer on silica surface or the competitive interaction between its polar functional groups and silanols on silica [8].

In this paper, we wish to introduce a unique method to reduce undesirable silanol effect against basic compounds in HPLC. This method is based on the fact that a masking effect is brought about by poly(4-vinylpyridine) (Fig. 1) as a weak basic polymer which provides exclusion effect for basic compounds. In addition, we focus on polycyclic aromatic hydrocarbons (PAHs) and dinitroarenes because pharmaceuticals often possess aromatic groups and their geometrical and structural isomers often show extremely different action in the human body. Especially, 1,6- and 1,8-dinitropyrenes are targeted in this work because they are not only universally involved in diesel emissions but also recognized as one of the most hazardous human carcinogens [9,10]. Therefore, this paper also describes how poly(4-vinylpyridine)

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as an organic phase yields exceptionally high selectivity for PAHs, especially for electron-withdrawing substitution compounds.

## 2. Experimental

### 2.1. Preparation of $VP_n$ and grafting onto silica

Poly(4-vinylpyridine), $VP_n$, where $n$ is the average degree of polymerization, with a terminal reactive group at the one side was prepared by modification of the telomerization method [11,12] of 4-vinylpyridine with 3-mercaptopropyl trimethoxysilane. The typical synthetic procedure is as follows: ten ml of 4-vinylpyridine and 0.91 ml of 3-mercaptopropyl trimethoxysilane were mixed and then $N_2$ gas was bubbled for 20 min at room temperature. After addition of 0.1 g of AIBN, the mixture was stirred with bubbling $N_2$ gas at 60 °C for 6 h. The yellow solid obtained was collected and dissolved in 20 ml of chloroform. The solution was poured into 200 ml of hexane to precipitate as pale yellow powders. Similar precipitation was repeated three times and the collected powders were successively washed with *n*-hexane and dried in vacuo. Yield: 9.9 g, 91%. The average degree of polymerization was estimated by NMR spectroscopy to be 21.7. NMR data: $\delta_H$ (400 MHz; CDCl$_3$; Me$_4$Si) 0.7 (9 H, s, Si–OCH$_3$), 8.0–8.8 (4.34 H, m, 3- and 5-positions of pyridyl group).

The resultant $VP_n$ was grafted onto silica by using the terminal trimethoxysilyl group: 4.0 g of porous silica (YMC



Fig. 1. Schematic illustration of $VP_n$-grafted silica with the chemical structures of $VP_n$ and $St_n$ as polymeric organic phases.

120-S5, diameter 5 μm, average pore size 12.6 nm, specific surface area 333 cm$^2$ g$^{-1}$) and 4.0 g of $VP_n$ were mixed in 40 ml of chloroform and then was stirred gently at 60 °C for 1 week. The silica was collected and washed successively with chloroform, an acetic acid–methanol mixture and methanol. The recovery was 4.95 g and the amount of immobilization was determined by elemental analysis to be 27.1 wt.%.

Poly(styrene)-grafted silica (Sil-St$_n$) was prepared according to the similar procedure [13]. The average degree of polymerization and the amount of immobilization on silica were 24.0 and 18.2 wt%, respectively.

### 2.2. Measurements

The $VP_n$-grafted silica (Sil-$VP_n$, n = 21) without any further end-capping procedure was packed into a stainless-steel column (4.6 mm i.d. × 250 mm) using a hexanol–chloroform (1:1) mixture and the liquid chromatographic property was examined using methanol-water as a mobile phase. The chromatograph included a JASCO 980 PU pump, a JASCO photodiode array detector (UVIDEC-100-IV) and a column heater (Sugai U-620 Type VP50). The solutes were dissolved in methanol with ultrasonication (45 W, 38 kHz) for 10 min and then filtered through a membrane filter (0.5 μm). Five microliter of the sample solution was injected through a Reodyne Model 7125 injector. Chromatograph was obtained at flowrate 1.0 ml min$^{-1}$. The retention factor ($k'$) was determined by $(t_r - t_0)/t_0$ where $t_r$ and $t_0$ are retention time of samples and methanol, respectively. The separation factor ($\alpha$) was given by the ratio of retention time.

Poly(styrene)-grafted silica (Sil-St$_n$) packed into a stainless-steel column (4.6 mm i.d. × 250 mm). ODS-5FJ (4.6 i.d. × 250 mm, Masis Inc., Japan) was used as a reference column.

Water–1-octanol partition coefficient (log $P$) was determined by retention factor with octadecylated silica, ODS (Inertsil ODS, 4.6 mm i.d. × 250 mm, GL Science Co. Ltd.): log $P = 3.759 + 4.207$ log $k$ ($r = 0.99997$) [14]. Log $P$s and dipoles of di-substituted benzenes were estimated by CAChe MOPAC with the AM1 option.

The structures of PAHs were estimated by HyperChem Ver 5.1 with molecular mechanics (until the energy changes were below 0.001 kcal mol$^{-1}$) and following semi-empirical AM1 method.

## 3. Results and discussion

### 3.1. Retention behaviors for basic compounds

A typical chromatographic behavior of poly(4-vinylpyridine)-grafted silica (Sil-$VP_n$) appeared in separation for a mixture of pyridine and phenol. As shown in Fig. 2, Sil-$VP_n$ showed a very small retention factor ($k = 0.18$) for pyridine with a good asymmetric factor ($A_s = 1.21$), while ODS-5FJ as a reference column showed a poor $A_s$ value
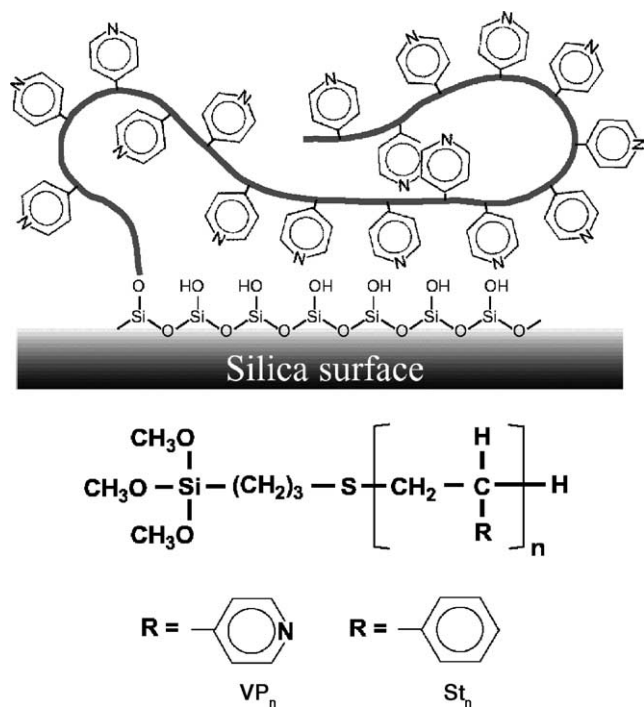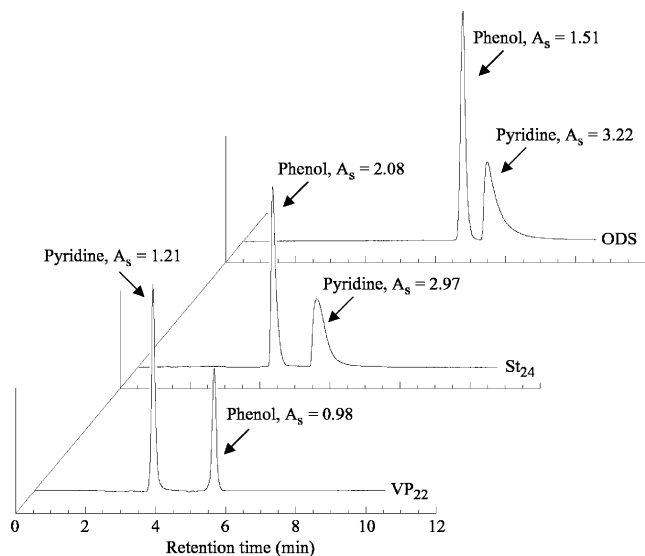
Fig. 2. Chromatograms for a mixture of pyridine and phenol with Sil-VP$_n$, Sil-St$_n$ and ODS (ODS-5FJ) at 30 °C. Mobile phase: methanol–water = 7:3.

($A_s = 3.22$). This is direct proof that the VP$_n$ phase can work as a reagent with masking effect for silanol groups on silica. This masking effect is brought about by a unique mechanism because the elemental analysis of Sil-VP$_n$ indicates that 80% of silanol groups on silica still remain after grafting of VP$_n$. Therefore, the masking effect can be explained by the fact that VP$_n$ is covalently grafted at the terminal trimethoxysilyl group on a silica surface and that enhancement of masking effect can be realized by electrostatic interaction between the pyridine moieties and the remaining silanol groups on silica (Figs. 1 and 5). Supporting this assumption, when the pyridine moiety was replaced into a phenyl group, no effective masking was observed in Sil-St$_n$ ($A_s = 2.97$) as shown in Fig. 2. This indicates that a basic property derived from the nitrogen atom is essentially important for the masking effect. Calculation showed −0.142 at the nitrogen atom, which is effective for silanol proton interaction.

To confirm the applicability of VP$_n$ as a masking reagent, the retention behaviors were investigated using various basic compounds. As summarized in Table 1, the VP$_n$ phase showed satisfactory $A_s$ values (1.09–1.24) for bipyridine,

Table 1
Asymmetric factors for various basic compounds with Sil-VP$_n$ and Sil-St$_n$

| | Sil-VP$_n$ | | Sil-St$_n$ | |
|---|---|---|---|---|
| | $k$ | $A_s$ | $k$ | $A_s$ |
| Pyridine | 0.18 | 1.21 | 0.69 | 2.97 |
| 4,4′-Bipyridine | 0.22 | 1.21 | 1.01 | 2.99 |
| 2-Ethylpyridine | 0.16 | 1.22 | 1.06 | 2.48 |
| 4-Ethylpyridine | 0.17 | 1.24 | 1.38 | 2.44 |
| Aniline | 0.41 | 1.12 | 0.42 | 2.25 |
| Pyrimidine | 0.13 | 1.16 | 0.3 | 1.92 |
| Indole | 1.86 | 1.09 | 0.58 | 2.03 |
| Quinoline | 0.45 | 1.06 | 0.82 | 2.74 |

Mobile phase: methanol–water = 7:3, 30 °C.

Table 2
Comparison of assymetric factors with Sil-VP$_n$, ODSs and polar-embedded *n*-alkyl silicas

| | Pyridine | | Aniline | | Benzene | |
|---|---|---|---|---|---|---|
| | $k$ | $A_s$ | $k$ | $A_s$ | $k$ | $A_s$ |
| Sil-VP$_n$ | 0.18 | 1.21 | 0.41 | 1.12 | 0.49 | 1.15 |
| ODS-5FJ | 0.47 | 3.22 | 1.03 | 1.56 | 1.39 | 1.15 |
| Inertsil ODS[a] | 0.87 | 1.65 | 1.10 | 1.09 | 5.47 | 0.95 |
| Inertsil ODS-2[a] | 0.69 | 2.35 | 0.87 | 1.18 | 5.36 | 1.21 |
| Inertsil ODS-3[a] | 1.00 | 1.74 | 1.26 | 1.23 | 8.43 | 1.71 |
| Kromasil C18[a] | 1.13 | 4.70 | 1.13 | 1.34 | 7.00 | 1.03 |
| Kromasil C8[a] | 1.04 | 5.33 | 1.04 | 1.19 | 4.49 | 1.03 |
| Symmetry C8[a] | 1.04 | 2.90 | 1.50 | 1.18 | 7.91 | 1.03 |
| Supelco ABZ+[a] | 0.52 | 1.70 | 1.70 | 1.43 | 3.23 | 1.35 |
| Purospher[a] | 1.26 | 1.63 | 1.03 | 1.18 | 5.27 | 1.18 |
| Supelco Dis. Am[b] | 0.19 | 1.28 | 0.34 | 1.22 | 1.70 | 1.04 |
| Symm. Shield 18[b] | 0.45 | 3.04 | 0.83 | 1.18 | 4.30 | 1.18 |
| Symm. Shield 8[b] | 0.43 | 3.13 | 0.73 | 1.18 | 3.03 | 1.13 |

Mobile phase: methanol–water = 7:3.
[a] Referred from the data by McCalley [15].
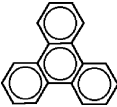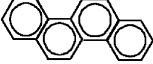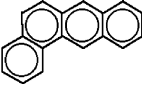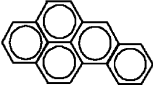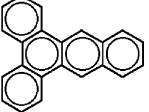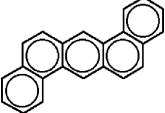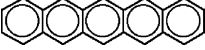[b] Referred from the data by McCalley [6].
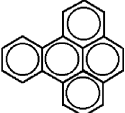
ethylpyridines, aniline, pyrimidine, indole and quinoline. However, benzylamine was remarkably retained. This is due to the much higher basicity of benzylamine as compared with that of VP$_n$. On the other hand, Sil-St$_n$ showed very poor $A_s$ values (1.92–2.99) for all basic compounds.

Table 2 compares the $A_s$ values for pyridine, aniline and benzene with Sil-VP$_n$, ODSs and polar-embedded *n*-alkyl silicas. Various ODSs are used as references because it is known that the $A_s$ values with ODSs are greatly dependent on the end-capping method and effectiveness of masking by the manufacturer. As shown in Table 2, it is clear that the masking effect of VP$_n$ is almost equal to those ODSs and polar-embedded *n*-alkyl silicas showing the highest masking effect [6,15]. For example, the VP$_n$ phase showed $A_s = 1.21$ for pyridine, which is a better value than that of the best ODS ($A_s = 1.63$) and polar-embedded *n*-alkyl silica ($A_s = 1.28$).

### 3.2. Lower sensitivity of VP$_n$ to molecular hydrophobicity

The feature of VP$_n$ is characterized not only by masking effect but also by lower sensitivity to molecular hydrophobicity and higher sensitivity to aromaticity of elutes. As shown in Fig. 3, Sil-VP$_n$ showed much smaller retention for dodecylbenzene ($C_{18}H_{30}$, $\log P = 8.52$) than for triphenylene ($C_{18}H_{12}$, $\log P = 5.28$) while ODS showed the opposite retention order. Similar unusuality of VP$_n$ can be found in the separation of structural isomers of PAHs. Typical examples are summarized in Table 3. When the retention behaviors are compared using PAHs with the same numbers of carbon atoms and π-electrons, the retention order in ODS obeyed $\log P$, which was a factor of molecular hydrophobicity, but Sil-VP$_n$ showed the opposite order. It seems that the VP$_n$ phase is insensitive to $\log P$ but rather recognizes the molecular shape of PAHs, preferring a disk-like molecule to a slender one.

Table 3
Comparison of retention behaviors for PAHs with Sil-VP$_n$ and ODS at 30 °C

| | Elutes | | log $P$ | Sil-VP$_n$ | | ODS | |
|---|---|---|---|---|---|---|---|
| | | | | $k$ | $a$ | $k$ | $a$ |
| | Triphenylene | $C_{18}H_{12}$ | 5.28 | 23.64[a] | 1.42 | 2.76[b] | 0.79 |
| | Chrysene | $C_{18}H_{12}$ | 5.40 | 18.66[a] | 1.12 | 2.90[b] | 0.83 |
| | Benz[a]anthracene | $C_{18}H_{12}$ | 5.42 | 17.48[a] | 1.05 | 2.99[b] | 0.86 |
| | Napthacene | $C_{18}H_{12}$ | 5.71 | 16.70[a] | (1) | 3.48[b] | (1) |
| | Benzo[e]pyrene | $C_{20}H_{12}$ | 6.12 | 3.35[c] | 1.14 | 1.21[c] | 0.92 |
| | Benzo[a]pyrene | $C_{20}H_{12}$ | 6.30 | 2.93[c] | (1) | 1.32[c] | (1) |
| | 1,2:3,4-Dibenzanthracene | $C_{22}H_{14}$ | 6.47 | 4.24[c] | 1.41 | 0.91[c] | 0.68 |
| | l,2:5,6-Dibenzanthracene | $C_{22}H_{14}$ | 6.60 | 3.37[c] | 1.12 | 0.93[c] | 0.69 |
| | Pentacene | $C_{22}H_{14}$ | 7.15 | 3.00[c] | (1) | 1.33[c] | (1) |

[a] Mobile phases: methanol–water = 7:3.
[b] Mobile phases: methanol–water = 9:1.
[c] Mobile phases: methanol.

### 3.3. Retention behaviors for dinitrobenzenes and dinitropyrenes

It is estimated that the VP$_n$ phase can bring about weak electrostatic interactions such as dipole–dipole interaction with aromatic compounds. Therefore, the retention behaviors for various substituted benzenes were examined. Table 4 shows the results, indicating significant information on the specificity of the VP$_n$ phase: for example, (1) ODS and Sil-St$_n$ showed comparably higher retention factors for dimethyl- and dichlorobenzenes than the others. This is understandable by the fact that the molecular hydrophobicities (e.g., log $P$ = 2.80, 3.15 and 3.20 in *o*-, *m*- and *p*-dimethylbenzenes, respectively) are relatively higher than the others (e.g., log $P$ = 1.69, 1.49 and 1.48 in *o*-, *m*- and *p*-dinitrobenzenes, respectively). However, Sil-VP$_n$ showed the highest retention factor in *o*-dinitrobenzene.

(2) This difference was accompanied by that of the selectivity. Sil-VP$_n$ showed remarkable *o*-selectivity, especially for dicyano- and dinitro-compounds, while ODS and Sil-St$_n$ showed *m*- and/or *p*-selectivity. The selectivity for *o*- and *p*-isomers with Sil-VP$_n$ reached 2.31 and 2.81 in dinitro- and dicyano-compounds, respectively. The mechanism of this selectivity will be discussed in the next section.

On the basis of its specificity toward dinitro-substituted compounds, Sil-VP$_n$ was used for the separation of a mixture of 1,6- and 1,8-dinitropyrene. Both compounds have been detected in some carbon blacks and in particulate emissions from diesel engines, kerosene heaters and gas burners, and unfortunately they are recognized as human carcinogens and are one of the most hazardous compounds [9,10]. As shown in Fig. 4, Sil-VP$_n$ realized complete separation for them.
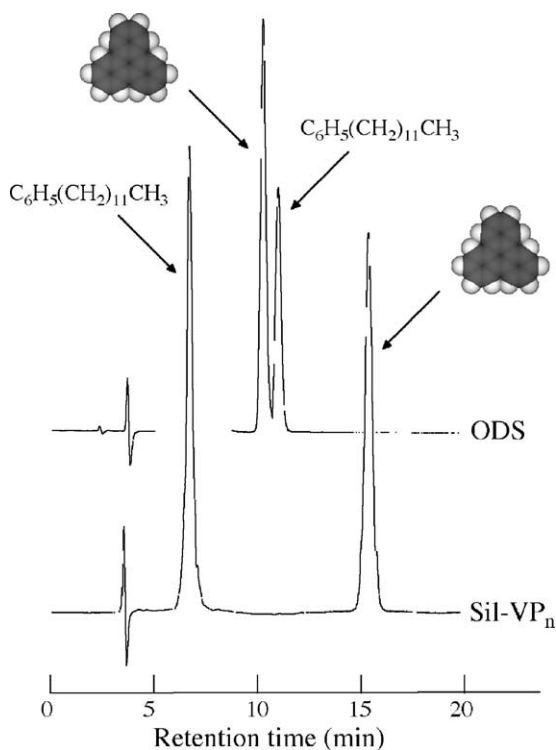
Fig. 3. Chromatograms for a mixture of dodecylbenzene and triphenylene with Sil-VP$_n$ and ODS (ODS-5FJ) at 30 °C. Mobile phase: methanol–water = 7:3 in Sil-VP$_n$; methanol–water = 9:1 in ODS.

Table 4
Separation factors for various position isomers of di-substituted benzenes with Sil-VP$_n$, Sil-St$_n$ and ODS

| Di-substituted benzene | | Sil-VP$_n$ | | Sil-St$_n$ | | ODS | |
|---|---|---|---|---|---|---|---|
| | | $k$ | $a$ | $k$ | $a$ | $k$ | $a$ |
| 1-CN | 2-CN | 0.59 | 2.81 | 0.22 | (1) | 0.14 | (1) |
| 1-CN | 3-CN | 0.25 | 1.19 | 0.22 | 1.00 | 0.15 | 1.07 |
| 1-CN | 4-CN | 0.21 | (1) | 0.18 | 0.83 | 0.15 | 1.07 |
| 1-NO$_2$ | 2-NO$_2$ | 1.20 | 2.31 | 0.32 | (1) | 0.22 | (1) |
| 1-NO$_2$ | 3-NO$_2$ | 0.72 | 1.38 | 0.39 | 1.23 | 0.36 | 1.64 |
| 1-NO$_2$ | 4-NO$_2$ | 0.52 | (1) | 0.32 | 1.00 | 0.34 | 1.55 |
| 1-C1 | 2-Cl | 0.53 | 1.33 | 0.54 | (1) | 1.34 | (1) |
| 1-C1 | 3-Cl | 0.43 | 1.08 | 0.57 | 1.06 | 1.69 | 1.26 |
| 1-C1 | 4-Cl | 0.40 | (1) | 0.54 | 1.00 | 1.46 | 1.09 |
| 1-CH$_3$ | 2-OH | 0.63 | 1.09 | 0.15 | (1) | 0.33 | (1) |
| 1-CH$_3$ | 3-OH | 0.61 | 1.05 | 0.14 | 0.88 | 030 | 0.91 |
| 1-CH$_3$ | 4-OH | 0.58 | (1) | 0.14 | 0.88 | 0.30 | 0.91 |
| 1-CH$_3$ | 2-CH$_3$ | 0.31 | 1.03 | 0.49 | (1) | 1.56 | (1) |
| 1-CH$_3$ | 3-CH$_3$ | 0.30 | 1.00 | 0.50 | 1.02 | 1.69 | 1.08 |
| 1-CH$_3$ | 4-CH$_3$ | 0.30 | (1) | 0.49 | 1.01 | 1.70 | 1.09 |
| 1-COCH$_3$ | 2-COCH$_3$ | 0.23 | 0.88 | 0.12 | (1) | 0.15 | (1) |
| 1-COCH$_3$ | 3-COCH$_3$ | 0.30 | 1.15 | 0.27 | 2.19 | 0.23 | 1.53 |
| 1-COCH$_3$ | 4-COCH$_3$ | 0.26 | (1) | 0.26 | 2.11 | 0.23 | 1.53 |
| 1-OCH$_3$ | 2-OCH$_3$ | 0.27 | 0.68 | 0.32 | (1) | 0.37 | (1) |
| 1-OCH$_3$ | 3-OCH$_3$ | 0.47 | 1.18 | 0.42 | 1.34 | 0.60 | 1.62 |
| 1-OCH$_3$ | 4-OCH$_3$ | 0.40 | (1) | 0.38 | 1.32 | 0.53 | 1.43 |

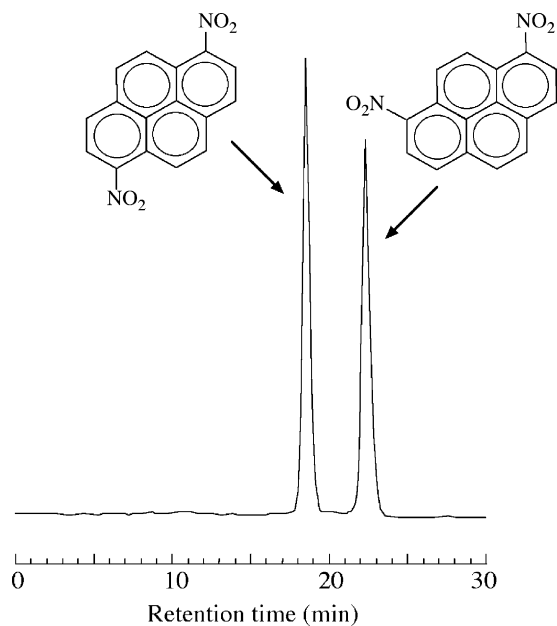Mobile phase: methanol–water = 8:2 at 35 °C.



Fig. 4. Chromatograms for a mixture of 1,6- and 1,8-dinitropyrenes with Sil-VP$_n$. Mobile phase: methanol–water = 8:2 at 30 °C. Flow rate: 0.5 ml min$^{-1}$. Detection: 400 nm.

### 3.4. Discussion on selectivity

In order to explain retention behaviors with Sil-VP$_n$, we propose the following assumptions: (1) the masking effect for silanol groups on silica was observed in Sil-VP$_n$ while not in Sil-St$_n$. This indicates that a nitrogen atom in a pyridine ring is essentially important for the masking effect. Since the calculation showed that the point charge of a nitrogen atom in a pyridine ring was estimated to be −0.142, it is considered that nitrogen atoms in VP$_n$ interact with silanol groups through hydrogen bonding as shown in Fig. 5. As a result, the silanol groups are masked with VP$_n$ and then undesirable adsorption for basic elutes can be suppressed. (2) Sil-VP$_n$ showed lower sensitivity to molecular hydrophobicity, but higher sensitivity to molecular aromaticity. These facts enable us to estimate
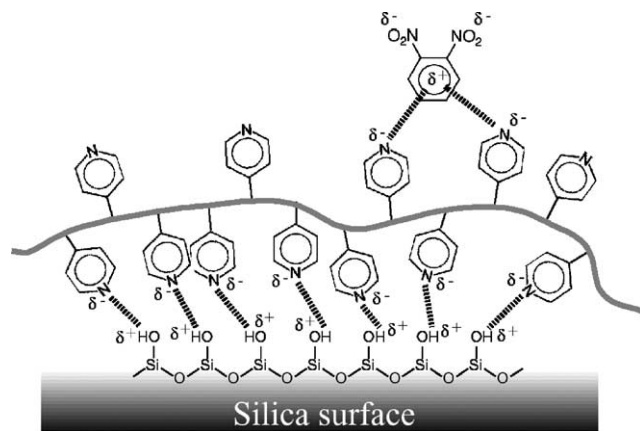


Fig. 5. Proposed mechanisms of masking effect with VP$_n$ for silanol groups on silica surface and dipole–dipole interaction with o-dinitrobenzene.
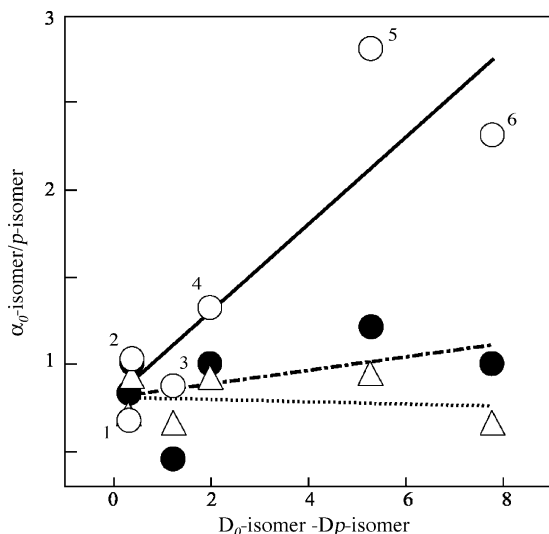
Fig. 6. Relationship between the selectivity for di-substituted benzenes and their dipoles, with Sil-VP$_n$ (open circles), Sil-St$_n$ (solid circles) and ODS (open triangles). Mobile phase: methanol–water = 8:2 at 35 °C. Elutes: 1, -OCH$_3$; 2, -CH$_3$; 3, -COCH$_3$; 4, -Cl; 5, -CN; 6, -NO$_2$.

that VP$_n$ can interact with not only the silanol groups but also $\pi$-electron-containing compounds: As shown in Fig. 5, some pyridine rings face toward silica surface because of hydrogen bonding with silanol groups, and thus the mobility of polymer chains must be reduced. On the other hand, some pyridine rings expose to the mobile phase, and thus provide an adsorption site. (3) Sil-VP$_n$ showed the specific $o$-isomer selectivity while ODS and Sil-St$_n$ did not show such selectivity. This result indicates that the selectivity due to VP$_n$ cannot be explained by $\pi$–$\pi$ interaction, including charge transfer interaction. In addition, this selectivity due to VP$_n$ is found in dinitro- and dicyano-substitutents which can be characterized as strong electron-withdrawing groups but not found in dimethyl and dimethoxy-substitutents which are electron-donating groups. The calculation showed that the difference of dipoles due to position isomerism is remarkably higher in electron-withdrawing groups than in electron-donating groups. For example, the dipoles of $o$- and $p$-dinitrobenzenes are 7.80 and 0.02, and thus the difference corresponds to 7.78, but it is only 0.4 between $o$- and $p$-dimethylbenzenes. These facts imply that the difference of dipoles is important for $o$-isomer selectivity. Fig. 6 shows the relationships between the selectivity ($\alpha_{o\text{-isomer}/p\text{-isomer}}$) and the difference of dipoles ($D_{o\text{-isomer}} - D_{p\text{-isomer}}$). It seems that the selectivity with Sil-VP$_n$ have relevance to the difference of dipoles while the selectivities with ODS and Sil-St$_n$ were independent on the difference of dipoles. Based on these results, we estimated that the high selectivity of VP$_n$ for position isomers of dinitro- and dicyanobenzenes can be brought about through electrostatic interaction such as a dipole–dipole interaction (Fig. 5). Supporting this assumption, it was reported that $p$-nitrophenyl-bonded silica showed selective retentions for $o$-isomer in dinitro- and chlorobenzenes [16]. Through the comparison with conventional pyrenyl-bonded silica, it

was explained that $p$-nitrophenyl-bonded silica is more favorable with the more dipolar isomers through dipole–dipole interaction [17]. (4) Table 3 indicates that the VP$_n$ phase recognizes the molecular shape of PAHs. It is obvious that disk-like PAHs (with a low LB ratio) are more retained by VP$_n$ than linear PAHs (with a high LB ratio). The molecular-shape selectivity has been generally realized by molecular ordering of organic phase on silica. Typical example can be seen in high dense ODS with more than 25 wt.% of octadecyl group [18,19]. Similarly, organic phases with well-organized carbonyl groups showed molecular-shape selectivity, retaining linear PAHs than disk-like PAHs [12,20,21]. Therefore, we estimated that random coiled VP$_n$ does not provide any ordered sites such as molecular slits but rather provides less-ordered sites to lead to multiple $\pi$–$\pi$ interaction, preferably to disk-like PAHs.

In conclusion, we have introduced the versatility of VP$_n$ as a polymeric organic phase for silica. Its features can be summarized as (1) masking effect for silanol groups, (2) lower sensitivity for molecular hydrophobicity of elutes, (3) specific selectivities for $o$-isomers and (4) molecular-shape selectivity for PAHs. As a result, the VP$_n$ phase realizes a separation mode far from conventional RP-HPLC columns, and thus its applicability may be expanded.

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