Poly(4-Vinylpyridine) as Novel Organic Phase for RP-HPLC. Unique Selectivity for Polycyclic Aromatic Hydrocarbons

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Poly(4-Vinylpyridine) as Novel Organic Phase for RP-HPLC. Unique Selectivity for Polycyclic Aromatic Hydrocarbons

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ABSTRACT

Subsidiary weak interaction such as π–π interaction shows us unique separation even in RP-HPLC. This communication shows the first example that poly(4-vinylpyridine) on silica provides extremely large selectivity with specificity for polycyclic aromatic hydrocarbons in methanol–water as a mobile phase. It also describes that the selectivity mode does not always obey those in conventional π-electron-containing stationary phases.

Key Words: Polyccyclic aromatic hydrocarbons; Poly(4-vinylpyridine); Novel organic phase; RP-HPLC.

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INTRODUCTION

Separation of polyaromatic hydrocarbons with ODS columns depends on the type of chemical modification of silica. Optimum separation has been often shown with polymeric ODS phases.[1] On the other hand, similar high selectivity has been realized with comb-shaped polymers with octadecyl side chains, especially at temperatures whose physical state is in a crystalline state on silica.[2–5] Both results indicate that the molecular orientation of octadecyl groups promotes molecular shape selectivity. However, direct interaction such as π–π interaction between organic stationary phase and solutes would increase selectivity. On this view point, there have been many types of π-electron-containing organic phases: for example, alkyl diphenyl,[6] coronene,[7] fullerene,[8,9] porphyrin,[10,11] poly(styrene),[12] poly(vinyl-naphtharene),[13] poly(acrylonitrile),[14] poly(methyl acrylate),[13,15] and poly(L-alanine).[16] In this communication, we introduce poly(4-vinylpyridine) as a novel π-electron-containing organic phase for HPLC. This shows extremely large selectivity for some of the polycyclic aromatic hydrocarbons.

EXPERIMENTAL

Poly(4-vinylpyridine), with a terminal reactive group at one side, was prepared with the telomerization method[3,17] of 4-vinylpyridine with 3-mercaptopropyl trimethoxysilane (Sch. 1). The average degree of polymerization (n) of the resultant polymers (VP$_n$, n is the average degree of polymerization) was estimated by ^{1}H-NMR spectroscopy, with using the protons of OCH$_3$ and a pyridine moiety. Two kinds of VP$_n$ with $n = 2$ or 22 were obtained by adjusting the initial molar ratio of the monomer. The resultant polymers were grafted onto silica by using the terminal trimethylsilyl group. YMC 120-S5 (diameter 5 μm, pore size 120 Å, specific surface area 300 cm^2 g^{-1}) was used as a porous silica. The amounts of immobilization were 24.9 and 27.1 wt% in VP$_2$ and VP$_{22}$, respectively.
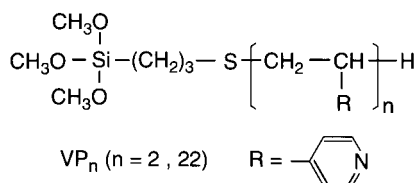
Scheme 1. Chemical structure of poly(4-vinylpyridine) with a terminal reactive group at one side.

The VP$_n$-grafted silica (Sil-VP$_n$) was packed into a stainless-steel column (4.6 mm I.D. $\times$ 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). Five milliliter of the sample dissolved in methanol was injected through a Reodyne Model 7125 injector. A chromatograph was obtained at flow-rate 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 times of samples and methanol, respectively. The separation factor (α) was given by the ratio of retention time. Poly(acrylo-nitrile)-grafted silica (Sil-AN$_n$, $n = 21$) was prepared according to a similar procedure[14] and then packed into a stainless-steel column (4.6 mm I.D. $\times$ 250 mm). ODS-5FJ (4.6 I.D. $\times$ 250 mm, Masis, Inc., Japan) and Wakosil 5CN (4.6 I.D. $\times$ 250 mm, Wako Pure Chemical Industries, Inc.) were used as reference columns. These are abbreviated as ODS and Sil-CN, respectively.

RESULTS AND DISCUSSION

Retention Behavior for Alkylbenzenes

A series of alkylbenzenes is usable to evaluate the separation mode of packing materials in HPLC. The elution order of the chromatogram B in Fig. 1 is a typical example that ODS obeys a RPLC mode with reasonably large selectivity ($\alpha_{C18/C6}$, the k' ratio for dodecylbenzene to benzene $= 22.8$). However, Sil-VP$_{22}$ showed much less selectivity ($\alpha_{C18/C6} = 1.42$ in chromatogram A) although, the elution order is the same to that in ODS. This indicates that Sil-VP$_n$ seems to be less sensitive for the length of alkylchain of a solute, in spite of the fact that VP$_{22}$ has a hydrophobic main chain.

This abnormality is clearer by plotting the separation factor (α, the k' ratio for benzene) against the number of carbon atoms, as shown in Fig. 2. Interestingly, we have reported that poly(styrene)-grafted silica with the chemical structures similar to VP$_{22}$, shows much better selectivity for alkylbenzenes ($\alpha_{C18/C6} = 9.5$).[13] This indicates, that the nitrogen atom of pyridine moiety plays a specific role for lesser sensitivity to the hydrophobicity difference of solutes. To discuss this role, we refer to the retention behavior of poly(acrylonitrile)-grafted silica (Sil-AN$_n$, $n = 21$). This organic phase has nitrogen atoms. Sil-AN$_{21}$ showed $\alpha_{C18/C6} = 3.0$, while much smaller in Sil-VP$_{22}$ ($\alpha_{C18/C6} = 1.4$), as shown in Fig. 2. The exact mechanism to explain these specificities are not yet specified, but these small selectivities with Sil-VP$_{22}$ and Sil-AN$_{21}$ lead us to the following estimation: the nitrogen atom brings the strong polarization in pyridine or CN moieties to promote effective

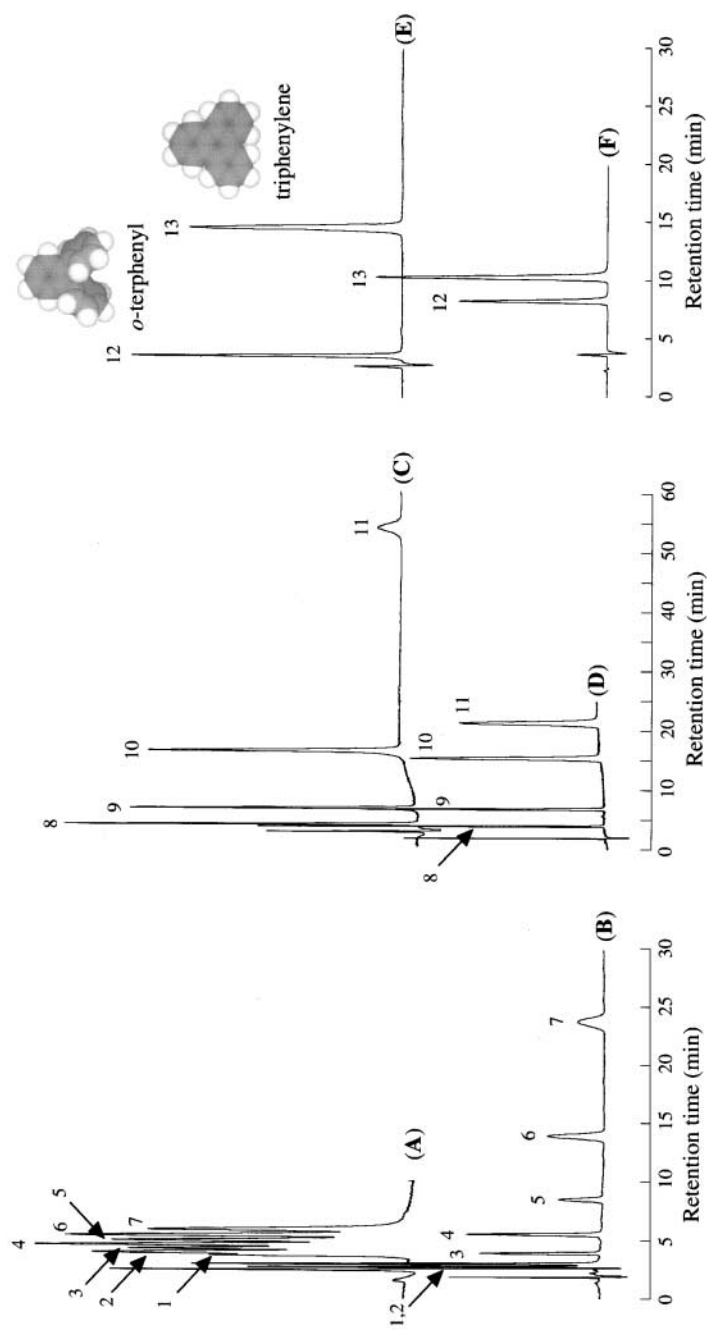***Figure 1.*** Typical chromatograms with Sil-VP$_{22}$ (A, C, and E) and ODS (B, D, and F) columns at 30°C. Mobile phase: (A), (B), (E), and (F), methanol–water (7:3); (C), and (D) methanol–water (7:3). Solutes: 1, toluene; 2, ethylbenzene; 3, butylbenzene; 4, hexylbenzene1 5, octylbenzene; 6, decylbenzene; 7, dodecylbenzene; 8, benzene; 9, naphthalene; 10, anthracene; 11, pyrene; 12, *o*-terphenyl; 13, triphenylene.
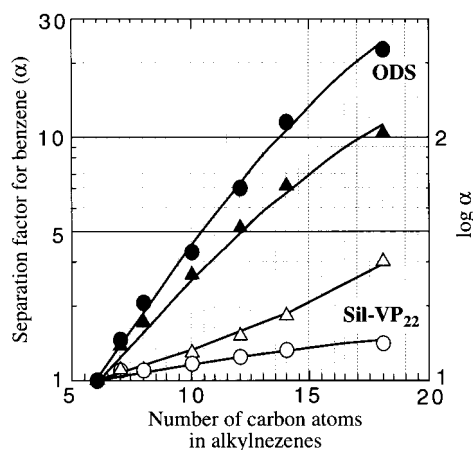
Figure 2. Relationship between the number of carbon atom and the separation factor and with Sil-VP$_{22}$ (○) , Sil-AN$_{21}$ (△), Sil-CN (▲), and ODS (●). Solutes: benzene, toluene, ethylbenzene, butylbenzene, hexylbenzene, octylbenzene, and dodecyl-benzene from the left in the figure. Mobile phases: Sil-VP$_{22}$, methanol–water $= 8 : 2$, 30°C; Sil-AN$_{21}$, methanol–water $= 6 : 4$, 35°C; Sil-CN, methanol–water $= 7 : 3$, 35°C; ODS, methanol–water $= 9 : 1$, 25°C.

interaction with the residual silanol groups on silica because of no endcapping treatment in the preparation process. VP$_{22}$ and AN$_{21}$ should be tightly constricted on silica by multiple interactions between the locally polarized nitrogen atom and the silanol group to reduce the mobility. This interaction causes the reduction of the partition of solutes into the organic phase. As supporting this estimation, simply-cyanopropylated silica (Sil-CN) showed much better selectivity ($\alpha_{C18/C6} = 10.2$), as shown in Fig. 2, than Sil-AN$_{21}$ ($\alpha_{C18/C6} = 3.0$), but rather behaved similarly to ODS. Probably, the flexible propyl spacer works as a hydrophobic phase in the case of Sil-CN.

Retention Behavior for Polycyclic Aromatic Hydrocarbons

Specific separation with Sil-VP$_{22}$ can be shown by evaluating the separation factor for polycyclic aromatic hydrocarbons. Typical chromatograms are presented in (C) and (E) in Fig. 1. Sil-VP$_{22}$ shows extremely high selectivity: $\alpha_{C18/C6}$ (the k' ratio of pyrene for benzene) reached 21.2, while the values are 11.2, 5.4, and 4.9 in Sil-AN$_{21}$, Sil-CN, and ODS, respectively (Fig. 3). The selectivity is higher in the order of Sil-VP$_{22}$ > Sil-AN$_{21}$ > Sil-CN > ODS, which is in reverse order in the separation for alkylbenzenes.
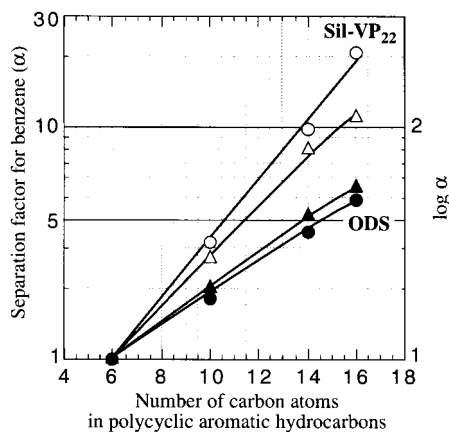
Figure 3. Relationship between the number of carbon atom and the separation factor and with Sil-VP$_{22}$ (O) , Sil-AN$_{21}$ ($\triangle$), Sil-CN ($\blacktriangle$), and ODS ($\bullet$). Solutes: benzene, naphthalene, anthracene and pyrene from the left of the figure. Mobile phases: Sil-VP$_{22}$, methanol–water $= 8:2$, 30°C; Sil-AN$_{21}$, methanol–water $= 6:4$, 35°C; Sil-CN, methanol–water $= 7:3$, 35°C; ODS, methanol–water $= 9:1$, 25°C.

Table 1 emphasizes uniqueness of Sil-VP$_{22}$. The separation factor between triphenylene and *o*-terphenyl reached 11.48 in Sil-VP$_{22}$. This value is extremely high because of comparison with the values of ODS ($\alpha = 1.5$), Sil-AN$_{21}$ ($\alpha = 6.5$), and Sil-CN ($\alpha = 1.9$). It has been reported that the C$_{60}$-fullerene-bonded silica showed good selectivity ($\alpha = 3.6$) for this separation, and then, the selectivity was explained by the molecular planarity recognition through π–π interaction.[9] This shows how large selectivity is obtained with Sil-VP$_{22}$. On the other hand, the effective selectivity was also observed for the analogues of four-ring compounds such as naphthacene (Table 2), and also it should be noted, that the elution order did not agree with those in Sil-AN$_{21}$, Sil-CN, and ODS (Sch. 2). Typical chromatograms are shown in Fig. 4.

The elution order difference cannot be explained only by π–π interaction with a solute, but should be rather discussed with conformational effect of the polymer chains.[18] We have discussed, through highly-oriented polymers as bonded phases, that multiple interaction is more effective for linear solutes with the large aspect ratio such as naphthacene and pentacene [see Fig. 5(A)] than disk-like solutes with the small aspect ratio such as pyrene and coronene when π–π interaction sources such as carbonyl groups are one-dimentionally ordered on silica.[5,19] It has been also reported that the *ab initio* MO/MP2 calculations, which were performed with Gaussian 94 package, showed that
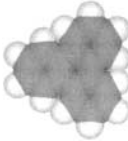
Table 1.   Comparison of the separation factor for polycyclic aromatic hydrocarbons.

	Sil-VP$_{22}$[a]		Sil-AN$_{21}$[b]		Sil-CN[c]		ODS[d]	
	k'	α	k'	α	k'	α	k'	α
C$_{18}$H$_{12}$ Triphenylene	7.13	11.48	5.22	6.50	2.92	1.86	2.76	1.45
C$_{18}$H$_{14}$ o-Terphenyl	0.62	1.00*	0.80	1.00*	1.58	1.00*	1.90	1.00*

Note: The separation factor (α) was evaluated by using the compounds marked with *.
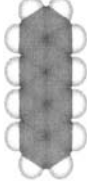[a]Methanol–water = 8 : 2, 30°C.
[b]Methanol–water = 6 : 4, 35°C.
[c]Methanol–water = 7 : 3, 35°C.
[d]Methanol–water = 9 : 1, 25°C.

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Table 2. Comparison of the separation factor for polycyclic aromatic hydrocarbons.

		Sil-VP$_{22}$[a]		Sil-AN$_{21}$[b]		Sil-CN[c]		ODS[d]	
		k'	α	k'	α	k'	α	k'	α
Naphthacene	$C_{18}H_{12}$	5.02	1.00*	7.96	1.00* ⟨1.00⟩	3.57	1.00* ⟨1.00⟩	3.48	1.00* ⟨1.00⟩
Benz[a]anthracene	$C_{18}H_{12}$	5.42	1.08	6.71	0.84 ⟨1.19⟩	3.41	0.96 ⟨1.04⟩	2.99	0.86 ⟨1.16⟩
Chrysene	$C_{18}H_{12}$	5.78	1.15	6.48	0.81 ⟨1.23⟩	3.04	0.85 ⟨1.18⟩	2.90	0.83 ⟨1.20⟩
Triphenylene	$C_{18}H_{12}$	7.13	1.42	5.22	0.66 ⟨1.52⟩	2.92	0.82 ⟨1.22⟩	2.76	0.79 ⟨1.27⟩

Note: The separation factor (α) was evaluated by using the compounds marked with *.
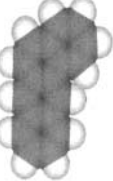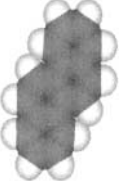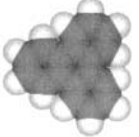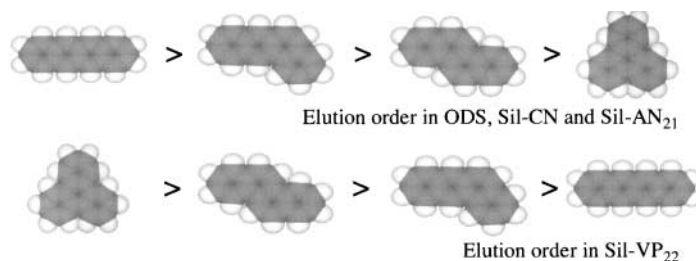[a]Methanol–water = 8 : 2, 30°C; [b]Methanol–water = 6 : 4, 35°C; [c]Methanol–water = 7 : 3, 35°C; [d]Methanol–water = 9 : 1, 25°C.

Elution order in ODS, Sil-CN and Sil-AN$_{21}$

Elution order in Sil-VP$_{22}$

Scheme 2. Elution order of four-ring aromatic hydrocarbons with chemically-modified silicas.

carbonyl π-benzene π interaction was significantly large[20] compared to CH$_4$-benzene π and benzene π–benzene π interactions.[21] Therefore, we assume that the VP$_{22}$, as a bonded phase, be disordered on silica because of no driving force for ordering but tightly interact with the residual silanol group. Any direct observation is not yet obtained for this assumption, but it is sure that the
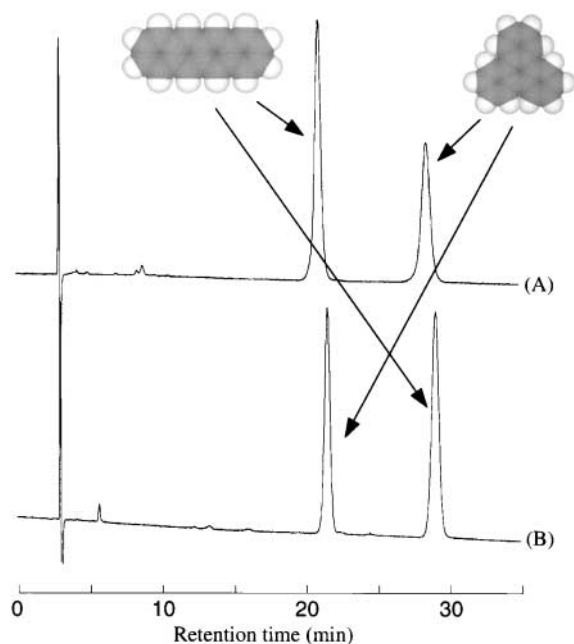

Figure 4. Chromatograms for the mixture of naphthacene and triphenylene with Sil-VP$_{22}$ (A) and ODS (B). Mobile phase: methanol–water $= 8:2$, 30°C.

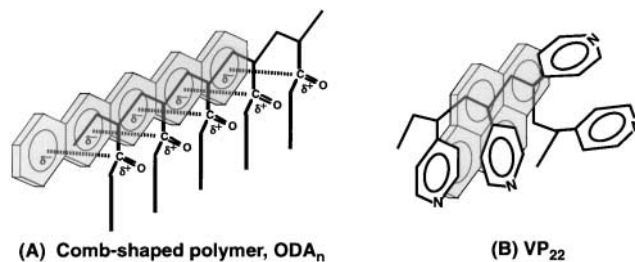**(A) Comb-shaped polymer, ODAₙ**   **(B) VP₂₂**

Figure 5. Schematic illustration of enhanced selectivity for polycyclic aromatic hydrocarbons with Sil-ODA$_n$ and Sil-VP$_n$. (A) Poly(octadecyl acrylate) (ODA$_n$) as a bonded phase is in a crystalline state at temperature below T_c. The selectivity is higher for an eluate with the large aspect ratio.[5,19] (B) VP$_n$ as a bonded phase does not form any ordered structure. The selectivity is higher for an eluate with the small aspect ratio.
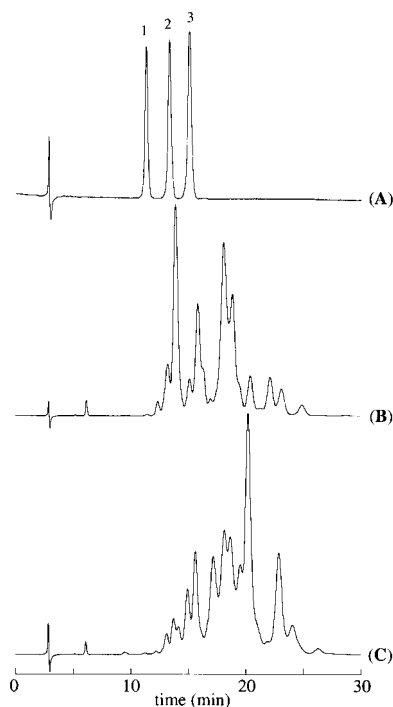
Figure 6. Examples of chromatographic separation with Sil-VP$_{22}$. Temperature Mobile phase: methanol–water $= 60:40$, $30°C$. Detection: 254 nm. Samples A: 1, *o*-chlorobiphenyl; 2, *p*-chlorobiphenyl; 3, *m*-chlorobiphenyl. Sample B: mixture of polychlorobiphenyl (KC-400). Sample C: mixture of polychlorobiphenyl (KC-500).
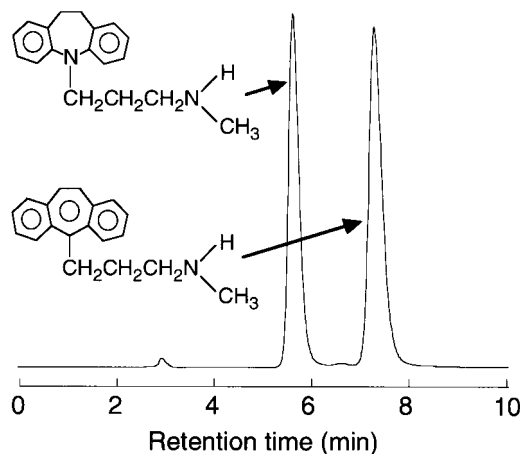
Figure 7. Separation of the mixture of desipramine and protriptyline as antidepressants with Sil-VP$_{22}$. Mobile phase: methanol-0.01 M–KH$_2$PO$_4$ = 70 : 30, 30°C. Detection: 254 nm.

disordered structure of VP$_{22}$ must prefer to contact with disk-like solutes with the small aspect ratio such as pyrene and triphenylene [see Fig. 5(B)], rather than linear solutes such as naphthacene. As supporting these assumptions, it was confirmed that the selectivity for the series of four-ring compounds reduced by ca. 40% when the polymerization degree decreased from 22 to 2, but no change was observed in the elution order. This indicates that the specificity of VP$_{22}$ includes polymeric effects.

In conclusion, we can summarize the uniqueness of Sil-VP$_{22}$ as a novel organic stationary phase for HPLC as follows: (1) Sil-VP$_{22}$ is less sensitive for molecular hydrophobicity of solutes. This is a proof of great difference from ordinal ODS. (2) High selectivity is observed for polyaromatic hydrocarbons or π-electron-containing substances, especially sensitive for difference of molecular planarity. However, the retention order is not always explained by π–π interaction mechanisms between stationary phase and solutes. This will compensate us for limited applicability in ordinal ODS and other π-electron-containing stationary phases in RPLC mode. The simple application is cited in Figs. 6 and 7. (3) It should be also noted, that VP$_{22}$ is readily prepared by one-step polymerization and then simple immobilization procedure. (4) The VP$_{22}$ bond shows unique results for anionic or cationic elutes. This is derived from a basic property of the vinylpyridine moiety. The detail is under investigation.

ACKNOWLEDGMENT

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