Chromatography

Original Paper

Molecular Shape Selectivity for Polycyclic Aromatic Compounds on a Poly(4-vinylpyridine) Stationary Phase in Liquid Chromatography

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Abstract
Poly(4-vinylpyridine) (P4VP) was introduced as a stationary phase in liquid chromatography (LC) and the retention behavior of polycyclic aromatic compounds (PACs) on the P4VP phase has been investigated by comparing with that observed on commercially-available octadecylsilica (ODS) and phenylbutylsilica (PBS) phases. On the P4VP phase, a good linear relationship between the logarithmic retention factor and the molecular size of planar PACs was obtained, however, a unique selectivity for some planar PACs was also observed. The P4VP phase demonstrated a specific retention trend, where planar PACs having "square-like" molecular shape were strongly retained. The above trend clearly suggested a unique selectivity of the P4VP phase for PACs. In addition, compared to the typical ODS and PBS phases, the P4VP phase showed a good molecular shape recognition capability for planar/non-planar PACs with a similar two-dimensional molecular size. The P4VP phase showed a very small retention for various alkylbenzenes, suggesting somewhat limited contribution of the hydrophobicity of the analytes to the retention. In the investigation for the selectivity to structural isomers, the P4VP phase exhibited a unique selectivity for isomers of dichlorobenzene and dibromobenzene when compared to the typical ODS and PBS phases. The trend can be interpreted as a dipole-dipole interaction between pyridyl groups in the P4VP ligands and the analyte.

Keywords: Stationary phase; Poly(4-vinylpyridine); Polycyclic aromatic compounds; Molecular shape selectivity; Liquid chromatography

1. Introduction
Liquid chromatography (LC) is one of the most powerful separation techniques for various types of complex mixtures and it has been employed in a wide range of scientific fields such as pharmaceutical, analytical and environmental chemistry [1-5]. In order to make sure an effective LC separation of real samples commonly encountered, a suitable setting of the separation conditions, especially, the choice of an appropriate stationary phase is quite important parameter [6-10]. Octadecylsilica (ODS) is widely employed as a stationary phase in LC because of the several advantageous features such as a good versatility, high separation performance, and significantly wide commercial availability with a reasonable cost [11-13]. However, there are still many types of complex mixtures, consisted of many closely-related components, that are quite difficult to be separated completely by single separation on typical ODS phases [14-21].

Recently, several types of synthetic polymers having heat resistance and chemical resistance were applied as a separation medium in LC and gas chromatography (GC) [22-28]. In contrast to conventional silicagel-based stationary phases, the stationary phase ligands could be introduced to the polymer backbone such as styrene/divinylbenzene-copolymer in polymer-based stationary phases, allowing the use of extremely severe separation conditions that are not adopted by typical ODS phases. Employment of fibrous synthetic polymers as an
Chromatography extraction medium in microscale sample preparation techniques has also been reported, including polymeric fiber-packed capillary and needle [29-41].

As an extension of the previous investigations, a poly(4-vinylpyridine) (P4VP) stationary phase [42-44] was introduced as an LC stationary phase in this work. Retention behavior of various polycyclic aromatic compounds (PACs) having different molecular shape and size was systematically investigated, where the molecular shape selectivity for these analytes was evaluated by comparing with that observed on the typical ODS and phenylbutylsilica (PBS) stationary phases. With a series of alkylbenzenes and structural isomers of PACs, additional investigations were also carried out to interpret the retention mechanism on the P4VP stationary phase.

2. Experimental

2.1. Reagents and solvents

All solvents were obtained from Kishida Chemical (Osaka, Japan), and the sample analytes including PACs were purchased either from Tokyo Chemical Industry (Tokyo, Japan) or Sigma-Aldrich (St. Louis, MO, USA). These chemicals were of analytical grade, and used without further purification. Water was purified by Milli-Q Water purification system (Merck Millipore, Darmstadt, Germany). Column temperature was controlled at 25ºC.

2.2. Poly(4-vinylpyridine) stationary phase

The P4VP column was supplied from DAICEL (Osaka, Japan), and the average particle size of this phase is 3 µm. Details of the P4VP phase can be found elsewhere [42]. The internal diameter of the packed column was 4.6 mm and the length was 150 mm. As shown in Fig. 1, the structure of the P4VP phase is consisted of polymeric pyridyl groups on a silicagel.

![Silicagel](image)

**Fig. 1.** Chemical structure of the P4VP stationary phase.

2.3. Chromatographic measurements

LC system was consisted of a PU-1585 pump (Jasco, Tokyo, Japan) and a model MD-910 photodiode array detector (Jasco), and a model 7725 injector (Rheodyne, Cotati, CA, USA). Data analysis software used was Borwin PDA (Jasco) running on a personal computer. As the mobile phase, mixture of methanol/water, acetonitrile (ACN)/water and ACN/20 mM phosphate buffer (pH 7.5) were employed unless otherwise specified, and the flow-rate was set at either 1.0 or 0.50 mL/min. For all the experiments, an injection volume of 20 µL was used. UV detection wavelength was determined in the preliminary experiments for all the analytes in order to make sure the effective detection of each analyte. The relative standard deviations (RSDs) were less than 3% for all the retention time measurements in this work.

3. Results and discussion

3.1. Molecular shape selectivity for PACs on the P4VP stationary phase

LC measurements of PACs were carried out using the P4VP stationary phase, and compared with commercially available stationary phases. For comparison, following three stationary phases were employed: a Develosil ODS-UG-5 (monomeric ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical, Seto, Japan), a Develosil ODS-A-5 (polymeric ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical) and a YMC-Triart Phenyl (PBS phase; 4.6 mm i.d., 150 mm length; YMC, Kyoto, Japan).

For a systematic analysis of retention behavior of PACs, \( F \) number, which represents a molecular size, was introduced in this work. \( F \) number was proposed by Hurtubisse et al. [45,46] and defined as follows:

\[
F = \text{(number of double bonds)} + \text{(number of primary and secondary carbons)} - 0.5 \times \text{(number of non-aromatic rings)},
\]

Regarding the relationship between the \( F \) number of PACs and the corresponding logarithmic retention factor, a similar trend was also observed on the PBS phase in our previous work [48]. As shown in Fig. 2, the P4VP stationary phase also exhibits a high linear correlation between the logarithmic retention factor and the \( F \) number for planar PACs. For non-planar PACs, however, the retention trend on the P4VP was similar to on the polymeric ODS phases which showed a significantly small retentivity to non-planar PACs.

The P4VP stationary phase indicated a unique retention trend for some PACs when focusing the length-to-breadth \((L/B)\) ratio proposed by Wise et al. and Kaliszan et al. [49-51]. \( L/B \) ratio is defined as the maximized length to breadth ratio of the two-dimensional molecules. In other words, the parameter can quantitatively classify the molecules having "rod-like" shape from the one having "square-like" shape, where a large \( L/B \) means more "rod-like" molecular shape if the number of aromatic rings, i.e. \( F \) number, is the same.
The results suggest that the P4VP stationary phase has a good selectivity for recognizing planar and non-planar PACs with a similar molecular size. In the P4VP phase, the retention of non-planar analytes was quite smaller than that observed on other three phases, while planar analytes were retained longer as similar to the retention trend on the conventional polymeric ODS stationary phase. In order to interpret the above trend, it has been proposed that the polymeric ODS phase has a "slot-like" structure on the silica support, where the structure enables an effective interaction with analyte molecules having large L/B values [47,52,53]. As found from previous investigations, in the case of monomeric ODS phase, the elution order of these four-rings PACs was the same as the polymeric ODS, although the molecular shape selectivity of monomeric ODS was somewhat smaller than polymeric one due to a relatively low ligand density of octadecyl functional groups on the silica support [47].

On the P4VP stationary phase, however, "square-like" molecules with a small L/B ratio were retained longer than "rod-like" molecules. The trend is clearly different from that observed on typical ODS phases. From the above results, it can be assumed a relatively shallow "slot-like" structure on the surface of the P4VP phase because of an effective inter- and intra-molecular interactions between the pyridyl groups induced by the π-π interaction. Since a large number of pyridyl functionalities are existed in the P4VP ligand, these pyridyl groups could be interacted easily. The interaction between the pyridyl groups existed in adjacent ligands could also be possible on the basis of the flexible ligand backbone structure of the P4VP phase.

For the investigation of the molecular planarity recognition capability of the P4VP phase, triphenylene with a planar shape and o-terphenyl with a non-planar shape, proposed by Tanaka et al. and Jinno et al., were introduced as the sample probes [54,55]. The solute pair is usually employed for the evaluation of molecular planarity recognition capability because of the same molecular size (i.e. F number) and similar two-dimensional molecular shape. The selectivity for various planar/non-planar solute pairs and isomers of terphenyl were summarized in Table 1 along with the selectivity for cis-stilbene and trans-stilbene. The results suggest that the P4VP stationary phase has a good selectivity for recognizing planar and non-planar PACs with a similar molecular size. In the P4VP phase, the retention of non-planar analytes was quite smaller than that observed on other three phases, while planar analytes were retained longer as similar to the retention trend on typical polymeric ODS phases. When compared with the PBS stationary phase having aromatic rings on the silica support, the P4VP phase demonstrated a good molecular planarity recognition capability. This result could be attributed as a larger density of the aromatic functionality on the silica support of the P4VP phase.
Table 1. Selectivity for planar/non-planar solute pairs on the P4VP and other three stationary phases.

| Analyte pair | P4VP | Monomeric ODS | Polymeric ODS | PBS |
|--------------|------|---------------|---------------|-----|
|              | 3.58 | 1.01          | 1.21          | 1.07|
|              | 3.63 | 1.13          | 1.87          | 0.991|
|              | 10.4 | 1.57          | 3.04          | 1.20|
|              | 1.01 | 1.12          | 1.54          | 0.929|
|              | 2.90 | 1.56          | 2.51          | 1.12|
|              | 2.86 | 1.39          | 1.63          | 1.21|
|              | 9.84 | 1.35          | 3.22          | 1.05|
|              | 1.05 | 1.17          | 0.994         | 1.14|
|              | 2.03 | 1.00          | 1.37          | 1.02|

Mobile phase: methanol/water = 90/10.

3.2. Retention behavior of alkylbenzenes on the P4VP stationary phase

Introducing a group of alkylbenzenes, with a linear alkyl chain having up to seven (i.e., heptylbenzene) carbon atoms as the sample probes, the retention behavior on the P4VP phase were additionally studied. In Fig. 4, the logarithmic retention factors of alkylbenzenes on each stationary phase were plotted against the number of carbon atoms in the alkyl chain. On the ODS and PBS phases, a linear correlation between \( \ln k \) and the number of carbon atoms in the alkyl functionality was observed. As expected, the results suggest that these phases have a retentivity on the basis of the hydrophobic interaction between the ligands of the stationary phase and the solute molecules. On the P4VP phase, a similar trend was also obtained, however, the slope of the correlation line was somewhat smaller than other phases, suggesting a relatively small contribution of the hydrophobicity to the retention on the P4VP phase. It is probably due to the polarization in the pyridine ring moiety of the P4VP phase.

Fig. 4. Relationship between the number of carbon atoms in alkyl chain and \( \ln k \) of alkylbenzenes on three stationary phases. (A) P4VP, (B) PBS and (C) monomeric ODS. Mobile phase: (A) methanol/water = 70/30, (B) and (C) methanol/water = 90/10.

On the other hand, as mentioned above, the P4VP phase showed a large retentivity for the planar PACs as similar to typical polymeric ODS phase. The trend can be explained by the \( \pi-\pi \) interaction between the pyridine ring in the stationary phase ligand and the PACs. The retention on the P4VP phase could be mainly attributed to the \( \pi-\pi \) interaction, while the contribution of the hydrophobicity of the analytes to the retention is somewhat limited because of the nitrogen atom in the P4VP ligand.

3.3. Selectivity for structural isomers of disubstituted benzenes

Table 2 summarizes the retention factor of the structural isomers of disubstituted benzenes on four stationary phases. For dichlorobenzene and dibromobenzene, the elution order on the typical monomeric ODS, polymeric ODS and PBS phases were ortho, para and then meta. However, on the P4VP phase, the corresponding elution order was para, meta and ortho. The results clearly demonstrated a unique selectivity to the position of these substituents of the analytes when compared with the ODS and PBS phases. The unique selectivity may be due to the dipole-dipole interaction between pyridyl groups in the P4VP ligand and the solute molecules having electron withdrawing substituents, such as dichlorobenzene. Therefore, it is assumed that the molecules having a large dipole moment such as \( o \)-dichlorobenzene, could be interacted more with the P4VP ligands than para-isomers.
Table 2. Retention data for halogenated benzene isomers on the P4VP and other three phases.

|                  | o-  | m-  | p-  | mobile phase |
|------------------|-----|-----|-----|--------------|
| dichloro-        |     |     |     |              |
| benzene         |     |     |     |              |
| P4VP             | 4.79| 3.81| 3.37| (A)          |
| monomeric       | 4.19| 5.59| 4.72| (B)          |
| polymeric       | 4.27| 5.29| 4.36| (B)          |
| PBS             | 2.99| 3.35| 3.15| (B)          |
| dibromo-        |     |     |     |              |
| benzene         |     |     |     |              |
| P4VP             | 7.75| 6.46| 5.51| (A)          |
| monomeric       | 5.15| 7.32| 6.40| (B)          |
| polymeric       | 5.20| 6.86| 5.75| (B)          |
| PBS             | 3.73| 4.40| 4.19| (B)          |

Mobile phase: (A) methanol/water = 40/60, (B) methanol/water = 70/30.

Table 3. Retention data for disubstituted benzene isomers on the P4VP and other three phases.

|                  | o-  | m-  | p-  | mobile phase |
|------------------|-----|-----|-----|--------------|
| dimethoxy-       |     |     |     |              |
| benzene         |     |     |     |              |
| P4VP             | 0.888| 1.59| 1.32| (A)          |
| monomeric       | 1.41| 2.71| 2.32| (B)          |
| polymeric       | 1.51| 2.27| 2.26| (B)          |
| PBS             | 1.62| 2.52| 2.47| (B)          |
| phenylene-      |     |     |     |              |
| diamine         |     |     |     |              |
| P4VP             | 0.622| 0.484| 0.131| (D)         |
| monomeric       | 1.80| 0.627| 0.043| (D)         |
| polymeric       | 3.66| 1.65| 0.892| (D)         |
| PBS             | 2.30| 1.46| 0.504| (D)         |

Mobile phase: (A) ACN/water = 30/70, (B) ACN/water = 50/50, (C) methanol/water = 60/40, (D) ACN/20 mM phosphate buffer (pH 7.5) = 10/90.

As shown in Table 3, the above interpretation is additionally confirmed with the results for dimethoxybenzene and phenylenediamine, where the elution order on the P4VP phase was the same as observed on the ODS and PBS phases. The results suggested that the structural isomers having electron donating substituents, such as methoxy group and amino group, may not have any significant effect to the specific retention behavior on the P4VP phase. On the basis of the above results, it can be considered that the P4VP phase has a unique molecular shape recognition capability for structural isomers having electron withdrawing substituents probably on the basis of the dipole-dipole interaction, although a further systematic investigation is necessary to derive the final conclusion for the retention mechanism from these retention data.

4. Conclusions
In this work, a poly(4-vinylpyridine) stationary phase was introduced as a novel stationary phase in LC. The P4VP phase demonstrated a specific molecular shape recognition for planar PACs, where "square-like" analyte molecules such as triphenylene were strongly retained than the analytes having "rod-like" shape. Compared to the typical ODS and PBS phases, the P4VP phase showed a good selectivity for planar/non-planar PACs with a similar molecular size. On the P4VP phase, the retention of non-planar PACs was quite smaller than other stationary phases. From the results, it might be concluded that the P4VP phase have a strong molecular shape recognition capability for the PACs having different molecular planarity, although further investigation should be carried out in terms of selectivity.

In contrast to typical ODS and PBS phases, only a small retention of alkylbenzenes was found on the P4VP phase. The results suggested that the P4VP phase has a small contribution of hydrophobicity due to the polarization in pyridine ring. In addition, unique molecular shape selectivity for the structural isomers of dichlorobenzene and dibromobenzene was confirmed. The results can be interpreted as the dipole-dipole interaction between the solute molecules having electron withdrawing substituents and the nitrogen atoms in the P4VP ligands.

Above results suggested a possibility of the P4VP phase as a novel stationary phase having a unique selectivity for various aromatic compounds in LC. Further systematic investigation should be made for the interpretation of the retention mechanism on the P4VP stationary phase. These studies are currently underway in our laboratory including the interpretation of the effect on the nitrogen atoms in the P4VP ligands to the selectivity using a set of heterocyclic compounds such as pyridines and the derivatives.

Acknowledgements
Technical Support from Mr. Atsushi Ohnishi of Daicel Corporation is acknowledged. A part of this study was financially supported by JSPS KAKENHI (Grant Number 18K05169). OS acknowledges the support from Toyohashi University of Technology.

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