Retention Behavior of Aromatic Hydrocarbons on a Novel Chitosan-Based Stationary Phase Synthesized with a Bifunctional Crosslinking Reagent Having Aliphatic and Aromatic Functionalities

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Abstract
A novel cross-linked chitosan phase was synthesized with a novel cross linking reagent having both aliphatic and aromatic functionalities. Retention behavior for polycyclic aromatic hydrocarbons (PAHs) were compared with that obtained on several commercially available stationary phases including monomeric and polymeric octadecylsilicas (ODSs) in liquid chromatography (LC). The results clearly demonstrated the applicability of the newly-synthesized chitosan phase as the stationary phase in LC, especially for the separation of PAHs having different planarities, and as novel wall-paint or wall-paper materials for reducing volatile organic compounds in indoor air environment.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); Chitosan; Crosslinking; Stationary phase; Liquid chromatography; Retention behavior

1. Introduction
Polycyclic aromatic hydrocarbons (PAHs) have been widely employed as the sample probes to systematically analyze the retention behavior of various stationary phases in liquid chromatography (LC), especially focused on the molecular shape recognition mechanism [1-7]. Because of the unique molecular shape and structure, and the availability of many critical pairs, which are quite valuable for analyzing the interaction mechanism between the solutes and the ligands of stationary phases, a group of PAHs can be considered to be one of the essential probes to evaluate a novel stationary phase [7-9].

On the other hand, chitosan is synthesized by the deacetylation reaction of the acetylamino functional groups in chitin, which is a natural polymer in the cell walls of crustaceans, such as crabs and shrimps. Based on the characteristic chemical structure and the unique characteristics such as the biocompatibility and biodegradability, a number of reports have been published for the chemical modification of chitosan molecules and the applications for example, an adsorbent material for metal ions [10-14] and dyes [15], and as a stationary phase in LC [16-18]. In contrast to the wide range of applications, however, most of the reported chemical modifications were carried out by the chemical derivatization of the amino groups in the chitosan molecule, therefore, the resulting chemically-modified chitosan materials only show a limited stability as the stationary phase, although the increased selectivities, such as chiral selectivities, could be obtained by the derivatization of the amino functionalities with various ligands.

Introducing a crosslinking reaction, it has been shown
that the stability of the resulting chitosan materials could be dramatically improved without losing the adsorption power for metal ions [19-22] and dyes [23-27]. The results also suggest the possible applications of the crosslinked chitosan as a novel stationary phase in LC. Crosslinked with an appropriate reaction, a new stationary phase having both a unique selectivity and sufficient stability for the operation under a conventional LC conditions could be synthesized.

As an extension of our previous publications [26-29], novel crosslinked chitosan material was synthesized with a crosslinking reagent having aromatic and aliphatic functionality in the structure, and the resulting cross-linked chitosan materials was evaluated as a stationary phase in micro-LC. With two phenyl groups in the crosslinker, an enhanced retentivity to typical aromatic compound. Taking advantage of the wide availability of the sample probes with the closely-related molecular structure, several pair of the PAHs have been employed as the solutes for the study on the systematic retention behavior. Commercially available monomeric and polymeric octadecylsilica (ODS) stationary phases were also used for comparison.

2. Experimental
2.1. Materials and reagents
Chitosan was obtained from Koyo Chemical Co., Osaka, Japan and the reagent for crosslinking reaction, 8,9-diphenylhexadecanoic diacid diglycidyl (DPHG) was supplied from Okamura Oil Mill Ltd., Kashiwara, Japan. The chemical structure of the DPHG is illustrated in Fig. 1. All solvents were of analytical grade and obtained from Kishida Chemical, Osaka, Japan, and all PAHs and aromatic sample probes (Fig. 2) were purchased from Tokyo Chemical Industries, Tokyo, Japan. Fused-silica capillaries for microcolumns were obtained from Shinwa Chemical Industries Ltd., Kyoto, Japan. Water was purified by a Milli-Q Water Purification System (Millipore, Tokyo, Japan). For comparison, two types of ODS phase, Develosil ODS-UG-5 (monomeric-type; Nomura Chemical, Seto, Japan) and Vydac 201 TPB-5 (polymeric-type; Separations Group, Hesperia, CA, USA) were also used.

2.2. Micro-LC system
Micro-LC was consisted of an Ultra-Plus II Capillary LC pumping system (Micro-Tech Scientific Inc., Vista, CA, USA), a UV/Vis absorption detector (Model 875-UV, Jasco, Tokyo, Japan) with a home-made flow-cell of about 0.3-µL volume. The detection wavelength was typically set at 254 nm unless otherwise specified. As the injector, Model 7520 micro-injector (Rheodyne, Cotati, CA, USA) with a sample loop volume of 0.2 µL was employed. A laboratory-made packed capillary column (fused-silica of 150 mm x 0.32 mm I.D.) was prepared with a slurry packing method.

The mobile phases were pure methanol and mixtures of methanol and water, and the typical flow-rate was set at 5.0 µL/min. For the column dead volume measurements, the peak of dichloromethane, which was added into the sample solvent, was used. The column temperature (24.0 ± 1.0°C) was controlled by the air circulation of a thermostatted laboratory.

![Fig. 1. Synthetic scheme of crosslinked chitosan stationary phase.](image-url)

3. Results and discussion
3.1. Synthesis of crosslinked chitosan stationary phase
The crosslinking reaction (Fig. 1) was carried out by a similar procedure as described previously [25,26]. First, chitosan (2.50 g) was dissolved in 100 mL of 5% acetic acid and diluted with methanol (100 mL). To the solution, 100 mL of methanol solution containing DPHG was added dropwise under stirring for 30 minutes at 65°C, and then, the temperature was maintained for 48 hours. Changing the amount of DPHG for the reaction, three types of DPHG-crosslinked chitosan phases (DCCs), having different molar ratios for the epoxy- and amino-functionalities, were prepared as shown in Table 1.

After the reaction, the solution was neutralized by an aqueous solution of 5% potassium hydroxide. Then the contents were poured into 500 mL of acetone. The precipitate was filtrated, and sequentially washed with
acetone and ether, and finally dried in vacuo. Other synthetic conditions were determined by the preliminary experiments to ensure the successful reaction between the chitosan and the crosslinking reagent.

Table 1. Three types of crosslinked chitosan phases synthesized in this work.

<table>
<thead>
<tr>
<th>Crosslinked chitosan phase</th>
<th>Amount used for crosslinking epoxy/amino ratio</th>
<th>Reactivity (%)</th>
</tr>
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<tbody>
<tr>
<td>DCC-1</td>
<td>2.5, 8.23, 2.00</td>
<td>31.3</td>
</tr>
<tr>
<td>DCC-2</td>
<td>2.5, 4.13, 1.00</td>
<td>13.5</td>
</tr>
<tr>
<td>DCC-3</td>
<td>2.5, 2.07, 0.50</td>
<td>10.6</td>
</tr>
</tbody>
</table>

*a* Calculated from the amount of chitosan and DPHG for the crosslinking reaction.

*b* Determined by the elemental analysis after the crosslinking reaction.

With the infrared spectroscopic analysis and the solubility measurements in aqueous acetic acid solution (10%), the formation of the crosslinked structure was confirmed as a similar way as described previously [24,25]. By the elemental analysis of the resulting DCCs, it was also confirmed that about 30% of amino groups in chitosan were reacted with DPHG when the reaction was carried out using the epoxy/amino-ratio of 2.00 (DCC-1). However, at the same time, the other two crosslinked chitosan materials (DCC-2 and DCC-3) showed quite low reactivities, suggesting a limited retentivity, as similarly found in our previous work, when employed as a stationary phase in LC [24,28,29]. Therefore, DCC-1 was used in the following experiments to ensure that the evaluation of the effect of DPHG-crosslinking on the retention behavior. Prior to the LC column preparation, the solid of DCC-1 was ground to powder with a mean diameter of about 10-20 µm and packed into a laboratory-made fused-silica capillary column as described elsewhere [30].

3.2. Retention behavior of PAHs on the crosslinked chitosan phase

To evaluate basic retentivity of DCC-1 as the stationary phase in LC, three typical PAHs, naphthalene, anthracene and pyrene, were separated. Figure 3 shows the representative chromatogram obtained with a packed-capillary column of 150-mm length. Although the efficiency is not comparable to that of commercially available ODS phases, a good separation was obtained, suggesting the practical retentivity as a novel stationary phase in LC.
Comparing the retentivity for these analytes on DCC-1 to that observed on other crosslinked chitosan phases reported earlier [28], a contribution of aromatic functional groups in the crosslinker was clearly found. The retentivity on DCC-1 was quite comparable to that on a crosslinked chitosan phase previously developed [28]. The selectivity for planar/non-planar solute pairs are summarized in Table 2, where the data on commercially available monomeric and polymeric ODSs are also tabulated for comparison. Among these selectivities, especially the selectivity (α) for triphenylene/o-terphenyl has been well confirmed by Tanaka et al. [32] and Jinno et al. [1] as a good indicator of the planarity recognition power of the stationary phases in RPLC.

Figure 5 shows typical chromatograms for the separation of o-terphenyl and triphenylene on DCC-1 phase. In general, typical polymeric ODS phases give a value of about 2.0-3.0, where typical monomeric phases show the value about 1.0-2.0 in RPLC conditions. Taking into account these typical value for the selectivity and also the data for ODS phases, it can be said that DCC-1 phase has an excellent molecular planarity recognition ability over typical polymeric ODS phases. The selectivity for triphenylene/o-terphenyl on DCC-1 phase was even higher than a crosslinked chitosan phase previously developed [28]. Because DCC-1 phase is assumed to be three-dimensionally bridged by DPHG-crosslinker, the intervals between chitosan backbones linked together should be similar, resulting a uniform ordered phase having a certain size of space for interaction with aromatic functionalities therein.

Table 2. Comparison of molecular shape selectivity of cross-linked chitosan phase (DCC-1) and commercially available monomeric and polymeric ODS phases.

<table>
<thead>
<tr>
<th>stationary phase</th>
<th>mobile phase (methanol/water)</th>
<th>α (k planar / k non-planar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cross-linked chitosan</td>
<td>90/10</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>80/20</td>
<td>2.27</td>
</tr>
<tr>
<td>Develosil</td>
<td>90/10</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>80/20</td>
<td>0.98</td>
</tr>
<tr>
<td>ODS-UG-5 a</td>
<td>90/10</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>80/20</td>
<td>1.46</td>
</tr>
<tr>
<td>Vydar 201 b</td>
<td>90/10</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>80/20</td>
<td>1.46</td>
</tr>
</tbody>
</table>

a Monomeric ODS.
b Polymeric ODS.

With the sample probes shown in Fig. 2, further evaluation of the molecular size and shape selectivities for PAHs, having different molecular size and shape, on the cross-linked chitosan phase was carried out. As a molecular size descriptor for PAHs, F-number was introduced. The descriptor, F, is defined by Hurtubise et al. [31] as follows: \( F = \text{(number of double bonds)} + \text{(number of primary and secondary carbons)} - 0.5 \times \text{(number of non-aromatic rings)} \). A high linear correlation between logarithmic retention factor and F-number was reported for the retention behavior of PAHs with monomeric ODS phases in aqueous reversed-phase (RP) LC [7]. The logarithmic retention data for various PAHs were plotted against their F-number in Fig. 4. The plot clearly indicates a linear correlation between \( \log k \) and F-number for planar PAHs, where the linear correlation coefficient for planar analytes was 0.996. The results demonstrate that DCC-1 stationary phase has a selectivity based on the F-number for planar PAH molecules, that means, the planar solutes were mainly separated according to these molecular size on DCC-1 phase.

In contrast to a high linear correlation for planar solutes, however, a negative deviation from the line was observed for all the non-planar solutes such as diphenylmethane, triphenylmethane and o-terphenyl. Introducing three pairs of PAHs, having a similar molecular size and a different planarity, such as triphenylene and o-terphenyl, further evaluation of the planarity recognition was carried out. The selectivities for planar/non-planar solute pairs are tabulated in Table 2, where the data on commercially available monomeric and polymeric ODSs are also tabulated for comparison. Among these selectivities, especially the selectivity (α) for triphenylene/o-terphenyl has been well confirmed by Tanaka et al. [32] and Jinno et al. [1] as a good indicator of the planarity recognition power of the stationary phases in RPLC.

Fig. 4. Relationship between logarithmic retention factor (log k) and F-number for various PAHs with the crosslinked chitosan phase (DCC-1). Conditions are the same as in Fig. 3, except for the detection wavelength for perylene and coronene at 300 nm. All the solute assignments are the same as in Fig. 2.

\[ F = \text{(number of double bonds)} + \text{(number of primary and secondary carbons)} - 0.5 \times \text{(number of non-aromatic rings)} \]
As reported in our previous studies [28,29], the excellent molecular planarity recognition power of the DCC-1 phase can be explained by the “slot-model” proposed by Wise et al. [1-3]. They proposed a “slot-like” structure to interpret the strong molecular shape recognition capabilities of polymeric ODS phases, in which the bonded ligands were partially cross-linked together on the surface of the silica gel. For the present crosslinked chitosan phase, a similar model can be proposed, because the cross-linked chitosan phase should form a kind of three dimensional network structure having deep “slot-like” space for the interaction with planar PAHs.

4. Conclusion

Introducing a crosslinking compound having aromatic and aliphatic functionality in the structure, novel crosslinked chitosan phase was synthesized, and the resulting cross-linked chitosan materials was evaluated as a stationary phase in micro-LC. The newly synthesized cross-linked chitosan stationary phase possessed a strong molecular planarity recognition power over typical ODS stationary phases. The contribution of aromatic functionality in the cross-linking reagent to the retention has been clearly confirmed.

The results also suggest that the molecular shape selectivity can be tuned by changing the chemical structure of the cross-linking reagent and that the further development of chitosan-based stationary phases can be expected in various separation techniques such as an extraction medium in sample preparation [33-37] and a stationary phase in gas chromatography [38,39]. Taking advantage of the biocompatibility and the characteristic adsorption behavior, a further applications to develop a more "human-friendly" wall-paint or wall-paper materials for reducing volatile organic compounds [36,37] in indoor air could be expected as well.

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