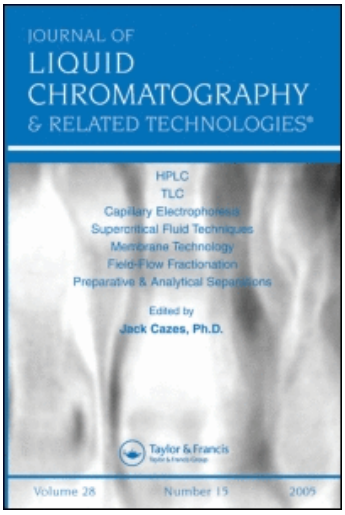

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LIQUID CHROMATOGRAPHIC RETENTION BEHAVIOR OF POLYCYCLIC AROMATIC HYDROCARBONS ON NEWLY-SYNTHESIZED CHITOSAN STATIONARY PHASES CROSS-LINKED WITH LONG ALIPHATIC CHAINS

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ABSTRACT

Novel cross-linked chitosan materials have been introduced as the stationary phase in microcolumn liquid chromatography (micro-LC). Three types of cross-linked chitosan phases were synthesized with changing the degree of cross-linking, and the retention behavior for polycyclic aromatic hydrocarbons (PAHs) was compared with that obtained by various commercially available stationary phases, including monomeric and polymeric octadecylsilicas (ODSs) and chemically modified chitosan phases.

The results clearly showed the applicability of the newly-synthesized cross-linked chitosan phases as the stationary phase

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in LC. From the considerations of the shape selectivity, it was also demonstrated that the cross-linked chitosan phase, synthesized with a higher degree of cross-linking reaction, possessed a strong molecular planarity recognition power over typical polymeric ODS phase.

Key Words: Chitosan; Cross-linking; Stationary; Microcolumn; Liquid chromatography; Polycyclic aromatic hydrocarbons

INTRODUCTION

Chitosan is synthesized by the deacetylation of the acetylamino groups in chitin, which is a natural polymer being contained in the cell walls of crustaceans, such as crabs and shrimps. Not only because of the chemical structure, but also its unique characteristics, for example, biocompatibility and biodegradability, a number of investigations for the chemical modification of chitosan molecules and the applications have been reported. Various types of modified chitosan have been employed as a good adsorbent material for metal ions,[1–3] organic acids,[4,5] and dyes,[6–10] and as a stationary phase in liquid chromatography (LC).[11–17] Most of the chemical modifications, however, have been made by the chemical derivatization of the amino groups in the chitosan molecule. Although, the increased selectivities, such as chiral selectivities, could be obtained by the derivatization of the amino functionalities with various ligands, the stability as a stationary phase is still limited. As the same as other polymer-based stationary phases, for example, polystylene-divinylbenzene-based materials, cross-linking is one of the solutions for the stabilization of chitosan-based adsorbents.

Recently, some cross-liked chitosan-based materials were introduced as an adsorbent for metal ions.[18,19] The results showed that the stability of these cross-linked chitosan materials could be dramatically improved without losing the adsorption power for metal ions. Therefore, with cross-linking by an appropriate reaction, a new stationary phase, having both a unique selectivity and sufficient stability for the operation under the normal LC conditions, could be synthesized.

As an extension of our previous studies,[7–10] three types of novel cross-linked chitosan phases, having different degrees of cross-linking, were synthesized as the stationary phase for LC separation in this work. For the cross-linking, bifunctional reagent, 7-ethyloctadecanedioic acid diglycidyl ester (EOAD, Fig. 1) was used. The cross-linker was reacted with amino functional groups in the chitosan backbone to make cross-linked chitosan stationary phases. As the sample probes, various types of polycyclic aromatic

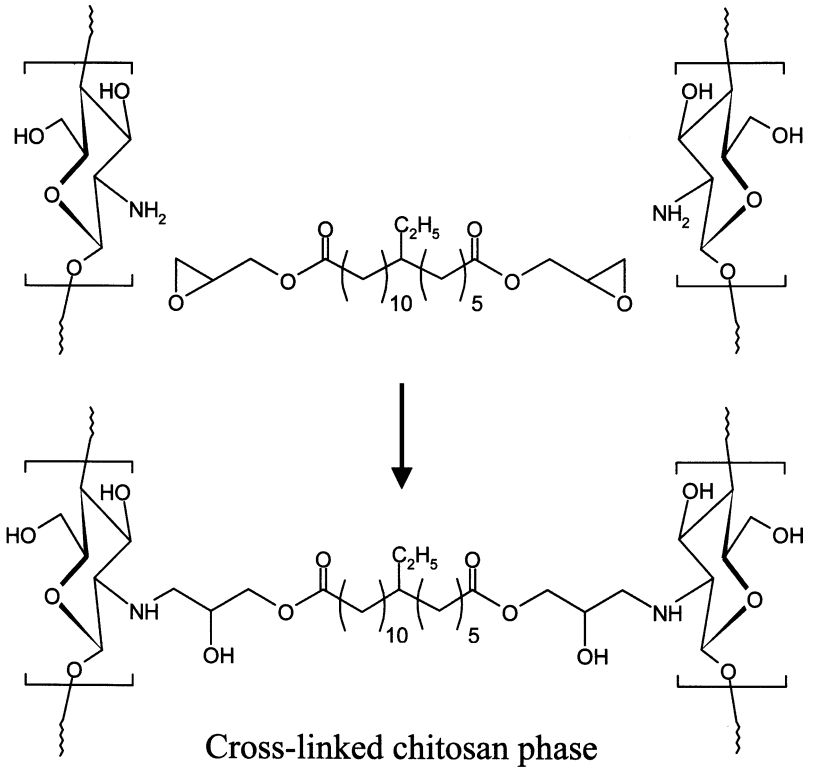


Figure 1. Synthetic scheme for cross-linked chitosan stationary phases.

hydrocarbons (PAHs, Fig. 2) were employed and the retention behaviors on these chitosan phases in microcolumn LC (micro-LC) were studied in this investigation.

EXPERIMENTAL

Reagents and Materials

Chitosan was obtained from Koyo Chemical, Tokyo, Japan. The cross-linking reagent, EOAD (Fig. 1), was donated from Okamura Oil Mill Ltd., Osaka, Japan. All solvents were of analytical grade and obtained from Kishida Chemical, Osaka, Japan and all PAHs 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

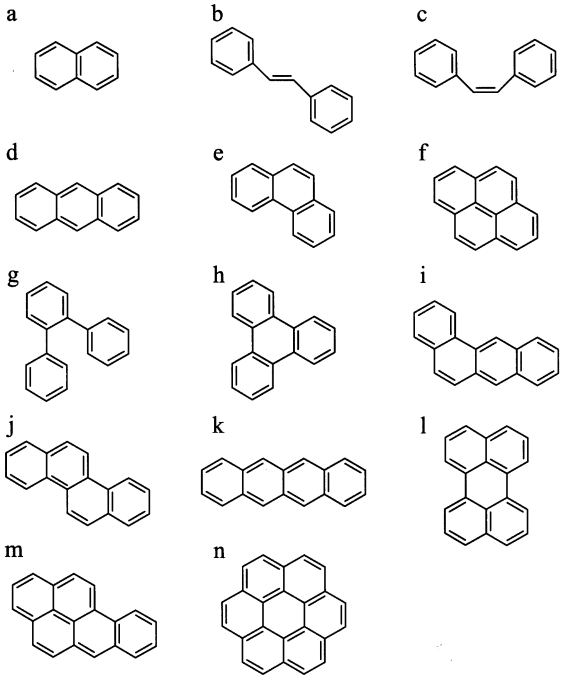


Figure 2. Chemical structures of various PAHs used as the sample probes. (a) naphthalene; (b) *trans*-stilbene; (c) *cis*-stilbene; (d) anthracene; (e) phenanthrene; (f) pyrene; (g) *o*-terphenyl; (h) triphenylene; (i) benz[*a*]anthracene; (j) chrysene; (k) naphthacene; (l) perylene; (m) benzo[*a*]pyrene; and (n) coronene. The UV detection was made at 273 and 300 nm for naphthacene and coronene, respectively.

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), and two commercially available chitosan-based (synthesized by the derivatization of amino groups) phases, Chitopearl BT-01 and Chitopearl PH-01 phases (Fig. 3; Fuji Spinning Co., Tokyo, Japan) were also used.

Synthesis of Cross-Linked Chitosan Stationary Phases (Figure 1)

First, chitosan (2.50 g) was dissolved into 100 mL of aqueous acetic acid solution (5%), and then methanol (100 mL) was added. To the resulting solution, 100 mL of EOAD solution (in methanol) was gradually added with stirring over the period of 30 min at the temperature of 65°C. To obtain chitosan phases having

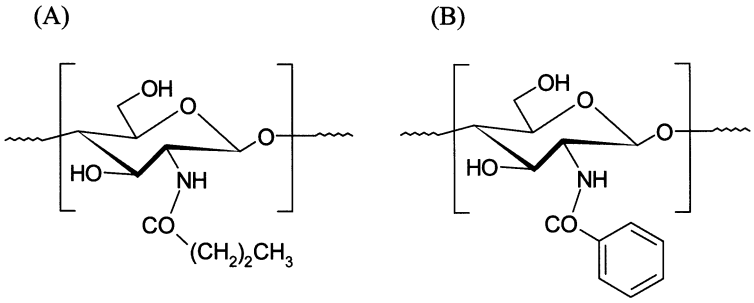


Figure 3. Chemical structures of Chitopearl BT-01 (A) and Chitopearl PH-01 (B).

different degree of cross-linking, as shown in Table 1, the amount of EOAD was adjusted based on the ratio of epoxy-/amino-functionalities. The solution was then stirred for 20 h at the same temperature. The time for complete reaction was determined by the preliminary experiments. After the cross-linking, the reaction mixture was neutralized with an aqueous solution of KOH (5%), and then mixed with 500 mL of acetone. With a qualitative filter (Advantec, Tokyo, Japan), the product was filtrated, and sequentially washed with acetone (200 mL) and diethyl ether (200 mL). After the drying *in vacuo*, the resulting solid was ground to a powder of a typical diameter of about 20–30 μm.

Microcolumn Liquid Chromatography System

Microcolumn liquid chromatography consisted of a Micro-Tech Scientific Ultra-Plus II Capillary LC pumping system (Yamato Scientific Co., Ltd., Tokyo, Japan), a UV/Vis absorption detector (Model 875-UV, Jasco, Tokyo, Japan) with

Table 1. Three Types of Cross Linked Chitosan Stationary Phases Synthesized

Cross-Linked Chitosan Phase	Amount Used for the Reaction (g)		Reacted Amino Group (%)
	Chitosan	EOAD	
Chitosan-I	2.50	1.86	25
Chitosan-II	2.50	3.73	50
Chitosan-III	2.50	7.08	95

Other synthetic conditions are in the experimental section.

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 microinjector (Rheodyne, Cotati, CA, USA) with a sample loop volume of 0.2 μL was used. A laboratory-made, packed capillary column (fused-silica of 150 mm × 0.53 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 flowrate was set at 2 μL/min. For the column dead volume measurements, the peak of dichloromethane, which was added into the sample solvent, was used. The column temperature ($22.0 \pm 0.5^{\circ}C$) was controlled by the air circulation of a thermostated laboratory.

Data Processing

As the data acquisition and processing, Borwin Chromatography Data Handling Software (Jasco) running on a personal computer was used. All chromatographic measurements were carried out at least three times and the relative standard deviations (RSDs) were less than 3% for all runs.

RESULTS AND DISCUSSION

For the evaluation of the basic separation performance of the chitosan phases as a stationary phase in LC, the retentivities for four typical PAHs, naphthalene, phenanthrene, anthracene, and pyrene were evaluated in the same chromatographic conditions. Table 2 shows the retention data obtained with three

Table 2. Retention Factors for PAHs with Various Stationary Phases

	Retention Factor[a] (k)			
	Napthalene	Phenanthrene	Anthracene	Pyrene
Chitosan-I	0.09	0.27	0.27	0.45
Chitosan-II	0.59	1.64	1.65	3.01
Chitosan-III	1.05	2.78	2.89	5.12
Chitopearl BT-01	—[b]	0.11	0.09	0.10
Chitopearl PH-01	—[b]	0.06	0.07	0.08
Develosil ODS-UG-5 (monomeric ODS)	1.53	3.56	4.08	6.49
Vydac 201 TPB-5 (polymeric ODS)	0.53	1.48	1.75	2.78

[a]Mobile phase, methanol/water = 80/20.
[b]Not retained.

cross-linked chitosan phases. For comparison, the data with two commercially available chitosan phases, and with typical ODS phases, are also tabulated. The results indicate that the retentivity is dramatically improved with increasing the degree of cross-linking. Compared with commercially available chitosan phases (Fig. 3), which were synthesized by the chemical modification of amino functional groups, it is clearly demonstrated that the dominant contribution is by the cross-linking to the retention power for these PAHs. Therefore, one can assume the major hydrophobic interaction between the long aliphatic chains in the cross-linked chitosan stationary phase and analytes, and in fact, the retention power of Chitosan-III phase is almost comparable with that of a monomeric ODS phase, which normally possesses a higher retentivitiy than a polymeric one.[20–25] In addition, the retention factors for all PAHs used in this work were increased with increasing the water content in the mobile phase as shown in Fig. 4, where good linear relationships were obtained between the logarithmic retention factor and the mobile phase composition. The trend is quite similar to the reversed-phase (RP) behavior obtained with a hydrophobic stationary phase, such as ODS, and a hydrophilic mobile phase, typically a mixture of methanol and water. Then, it can be said that the hydrophobic interaction between the long aliphatic cross-linker and the solute is the main driving force for the retention of PAHs. Typical chromatograms for the separation of PAHs are shown in Fig. 5. Although,

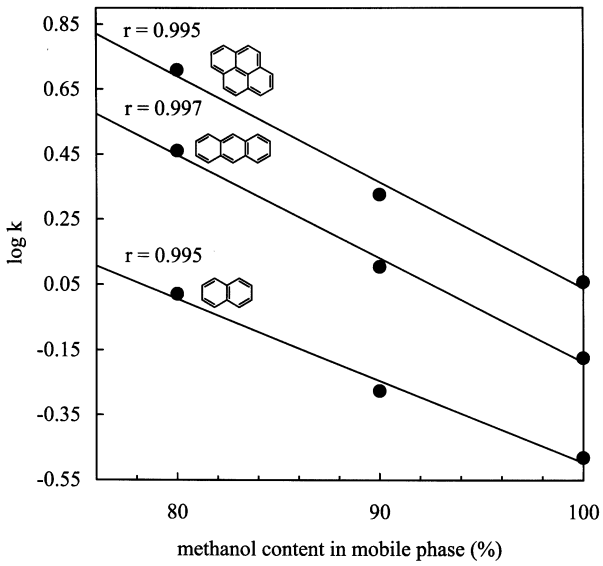


Figure 4. Relationship between logarithmic retention factor (log k) for three PAHs and mobile phase composition with Chitosan-III phase. Mobile phase, methanol/water.

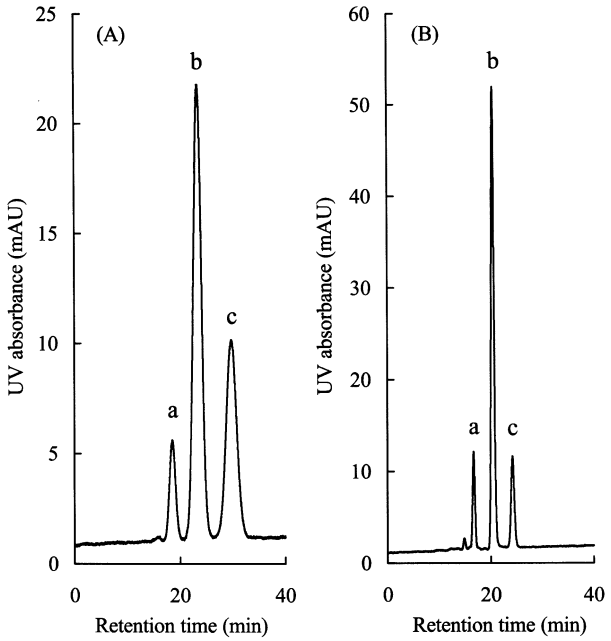


Figure 5. Chromatograms for the separation of three PAHs with Chitosan-III phase (A) and a commercially available monomeric ODS phase (B). Mobile phase, methanol. Peaks: (a) naphthalene; (b) anthracene; and (c) pyrene.

relatively broad peak width was observed with Chitosan-III phase, probably due to the large particle diameter and irregularity of the stationary phase material, a good separation performance was demonstrated. Because of the limited retentivities for PAHs with other cross-linked chitosan phases, especially with Chitosan-I (lowest degree of cross-linking), Chitosan-III was mainly employed in the following experiments.

Further evaluation of the molecular size and shape selectivities for PAHs on the cross-linked chitosan phase was carried out by measuring the retention factors of PAHs having different molecular size and shape. As a molecular size descriptor for PAHs, *F*-number was introduced. The descriptor, *F*, is defined by Hurtbise et al.[26] as follows: $F = (\text{number of double bonds}) + (\text{number of primary and secondary carbons}) - 0.5 \times (\text{number of non-aromatic rings})$, and 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 RPLC.[27,28] In Fig. 6, the logarithmic retention data for various PAHs were plotted against their *F*-number. The plot indicates a linear correlation between

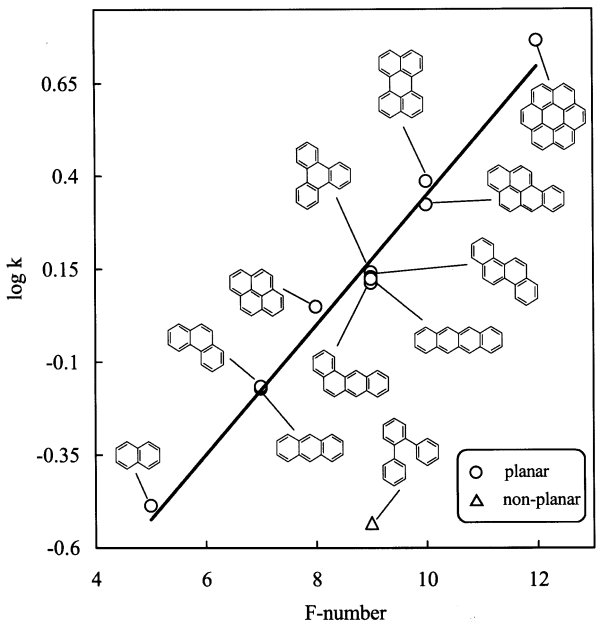
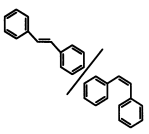
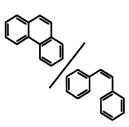


Figure 6. Relationship between logarithmic retention factor (log *k*) and *F*-number for various PAHs with Chitosan-III phase. Mobile phase, methanol.

log *k* and *F*-number for planar PAHs and the linear correlation coefficient for planar analytes was 0.990. Consequently, it can be said from the results, that Chitosan-III phase shows a selectivity based on the *F*-number for planar PAH molecules, i.e., only based on these molecular sizes.

However, as can be seen in Fig. 6, a large negative deviation from the line was observed for *o*-terphenyl (non-planar). In order to investigate the planarity recognition capability of the cross-linked chitosan phase, the selectivity for three pairs of PAHs, having a similar molecular size and a different planarity, such as triphenylene and *o*-terphenyl, were evaluated. The selectivities for planar/non-planar PAH pairs were summarized in Table 3. Similar trend was observed for these three pairs of analytes. Among these selectivities, especially for triphenylene/*o*-terphenyl, it has been confirmed by Tanaka et al.[29–31] and Jinno et al.[32–35] to be a good indicator of the planarity recognition power of the stationary phases in RPLC; generally, polymeric ODS phases give a value of about 2–3, and monomeric phases about 1–2 in RPLC conditions. Therefore, it is quite clear that Chitosan-III has an excellent molecular planarity recognition ability over typical polymeric ODS phases. The value of 4.34 is even comparable to that obtained with a liquid-crystal bonded phase in the same chromatographic conditions, as reported

Table 3. Retention Data for Planar/Non-planar PAHs with Various Stationary Phases

	α ($k_{planar}/k_{non\text{-}planar}$		
			
Chitosan-III	1.52	2.52	4.34
Develosil ODS-UG-5 (monomeric ODS)	1.08	1.30	1.84
Vydac 201 TPB-5 (polymeric ODS)	1.36	1.91	2.80

Mobile phase, methanol.

previously.[33–36] In these studies, the liquid-crystal phase (silica-based chemically bonded stationary phase) demonstrated excellent solute shape and size selectivity over typical polymeric ODS phases, because of the highly ordered phase structure of the bonded phase on the silica surface. Because Chitosan-III phase is assumed to be three-dimensionally bridged by EOAD, the intervals between chitosan backbones linked together should be similar, resulting in a uniform ordered phase with a certain space size for interaction.

Wise et al. proposed "slot-like" structure[20–23] 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 cross-linked chitosan phase with a higher degree of cross-linking, 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.

CONCLUSIONS

Retention behaviors of various PAHs, having different molecular size and shape on novel chitosan phases cross-linked with long aliphatic chains, have been studied in LC. With increasing the degree of cross-linking, the retentivity of the phases was also increased, and the phase having highest degree of cross-linking showed excellent molecular planarity recognition capability over a typical polymeric ODS phase, as well as good mechanical strength as a stationary phase in LC separations.

The results clearly demonstrated the applicability of the newly-synthesized cross-linked chitosan phases, as novel stationary phases for the separation of PAHs with a different molecular planarity, although, further investigation should be needed for the analysis of the retention mechanism on these cross-linked chitosan phases, especially for polar solutes. The study for more extensive applications of the cross-linked chitosan phases, including a stationary phase for capillary electrochromatography (CEC) and an extraction medium for trace amount of organics in complex sample mixtures,[37–42] is currently underway in our laboratory.

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