Original Paper

Retention Behavior of Polycyclic Aromatic Compounds in a Novel Polymer-Based Stationary Phase Liquid Chromatography

Ohjiro SUMIYA¹, Koki NAKAGAMI¹, Ryota KOIKE¹, Ikuo UETA², Yoshihiro SAITO^{*1}

¹*Department of Environmental and Life Sciences, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi 441-8580, Japan*

²*Department of Applied Chemistry, University of Yamanashi, 4-3-11 Takeda, Kofu 400-8511, Japan*

Abstract

A novel polymer-based particles was introduced as a stationary phase in liquid chromatography (LC). Spherical particles of a copolymer, poly(benzoguanamine-co-formaldehyde) (BF), was packed into a conventional blank LC column and the retention behavior of polycyclic aromatic compounds (PACs) was evaluated in reversed-phase LC. The trend for the retention was compared with that obtained on commercially-available octadecylsilica (ODS) and phenylbutylsilica (PBS) phases. In terms of the trend on the retentivity for PACs, the BF phase showed a similar behavior as that on the ODS phase, although the selectivity to some critical pairs was somewhat different from that on the ODS phase. The results suggest that the BF phase has a retentivity basically on the basis of hydrophobic interaction between the stationary phase ligands and the solute molecule, however, at the same time, the BF phase has a different selectivity from the PBS phase for the recognition of PACs molecules. On the basis of the comparison with the PBS phase, the unique selectivity of the BF phase can be attributed to the restricted moving of the phenyl ligands.

Keywords: Novel stationary phase; Polymer particle; Polycyclic aromatic compounds; Retention behavior; Liquid chromatography

1. Introduction

Development of novel stationary phases has been regarded as a key approach to create more efficient and cost-effective separations in chromatography. In fact, various types of new stationary phases have been developed in liquid chromatography (LC) [1-10], however there is a still long way to get the final systematic interpretation of the whole LC retention mechanism. In order to understand the retention mechanism in LC, a variety of specially-designed stationary phases were synthesized and evaluated along with the comprehensive analysis of the retention data obtained for various types of sample analytes [11-14].

Polymer-based stationary phases can be classified as one of the cost-effective material if the synthesis is simply done without complicated multi-step reactions. Another advantageous feature of the polymer-based stationary phase

is an easy synthesis of the phase structure on the basis of the design. Compared with a conventional octadecylsilica (ODS) phase, the ligands on the stationary phase surface structure can be easily expected for a polymer-based stationary phase synthesized from a simple polymerization reaction of a well-designed monomer.

As an extension of our previous studies, including the employment of natural and synthetic polymers as the stationary phase in chromatographic techniques [15-29] or the extraction medium in miniaturized sample preparation methods [30-39], spherical particles of a copolymer material, poly(benzoguanamine-co-formaldehyde) (BF), was introduced as a novel LC stationary phase in this work. The retention behavior for polycyclic aromatic compounds (PACs) on the BF stationary phase was studied on the basis of a systematic comparison with that obtained on a conventional ODS phase.

*Corresponding author: Yoshihiro SAITO
Tel: +81-532-44-6803; Fax: +81-532-48-5833
E-mail: saito@ens.tut.ac.jp

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2. Experimental

2.1. Reagents and solvents

All of the reagents and solvents, including polycyclic aromatic hydrocarbons (PAHs), and all polyphenyls used as the sample probes were of analytical reagent grade, and obtained from either Kishida Chemical (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan). Water was purified using a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany).

2.2. Preparation of the BF column

Spherical particles of the BF were obtained from Nippon Shokubai, Osaka, Japan. Typical synthetic scheme of the BF phase is shown in Fig. 1. The average particle size is about 4.7 μm (the standard deviation of about 2.7 μm) after removing relatively fine particles using a decantation process, where the suspension of the particles in a solvent having a similar density as the BF particles was used. The specific surface area of the BF material was 12 m^2/g , suggesting that it was classified as a non-porous material.

Fig. 1. Reaction scheme of benzoguanamine and formaldehyde to form the BF phase.

Fig. 2. Typical SEM image of particulate the BF stationary phase.

The BF phase was packed into a blank stainless-steel column of 4.6 mm i.d., 150 mm length with a slurry packing method. As can be seen from the photograph, the particle size distribution was larger than a commercially available silica-based stationary phase. However, the typical column pressure was about 8.0 MPa with methanol (1.0 mL/min) as the mobile phase, suggesting a practical use of the BF column in typical LC conditions.

Fig. 3. Chemical structures of PACs used as the sample analytes. (1) *o*-terphenyl; (2) *m*-terphenyl; (3) *p*-terphenyl; (4) triphenylmethane; (5) phenanthrene; (6) anthracene; (7) pyrene; (8) triphenylene; (9) benzo[*c*]phenanthrene; (10) benz[*a*]anthracene; (11) chrysene; (12) naphthacene; (13) benzo[*e*]pyrene; (14) perylene; (15) benzo[*a*]pyrene; (16) benzo[*ghi*]perylene; (17) dibenz[*a,h*]anthracene and (18) coronene.

2.3. LC measurements

For all the measurements, a LC system consisted of a PU1585 and a UV2075 (Jasco, Tokyo, Japan), a Model 7120 injector (20- μL sample loop volume) (Rheodyne, Cotati, CA, USA) was used. Chromatogram was recorded with ChromNAV Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer. As the mobile phase, methanol or mixtures of methanol/water were used, and the flow-rate was set at 1.0 mL/min unless otherwise specified. UV detection wavelength was determined in the preliminary experiments for all the analytes.

For the analysis of the retention behavior, a group of PAHs were employed along with polyphenyl compounds including *o*-terphenyl, *m*-terphenyl, *p*-terphenyl and triphenylmethane. The chemical structures of these sample analytes are illustrated in Fig. 3.

3. Results and discussion

3.1. Evaluation of retention behavior of the BF stationary phase for various PACs

The trend in the retention of PACs on the BF stationary phase was evaluated. For comparison, two commercially available phase, a Develosil ODS UG-5 phase (monomeric-type ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical, Seto, Japan) and a YMC-Triart Phenyl phase (phenylbutylsilica (PBS) phase; 4.6 mm i.d., 150 mm length; YMC, Kyoto, Japan) were also studied. In order to consider the size of the PAC molecule, one of the structural parameter, *F* number, was introduced. The *F* number is a molecular size descriptor proposed by Hurtubise *et al.* [40] which is defined as follows: $F = (\text{number of double bonds}) + (\text{number of primary and secondary carbons}) - 0.5 \times (\text{number of non-aromatic rings})$, and a high linear correlation between the logarithmic retention factor and *F* number has been found for a typical monomeric ODS phase in aqueous LC [12]. Typical plots for these three phases

are shown in Fig. 4. As expected for a monomeric ODS phase, a good linear correlation between logarithmic retention factor and the *F* number for the planar PACs on the ODS phase was observed in Fig. 4a, although a negative deviation for non-planar analytes and a positive deviation for the analytes having "rod-like" shapes were also observed. The trend on the PBS phase can be regarded as basically similar to that of the ODS phase with some exceptions.

The selectivity for the two-dimensional shape of PACs has been further considered with introducing another molecular descriptor, length-to-breadth (*L/B*) ratio. This parameter was proposed by Wise *et al.* [41] and Kaliszan *et al.* [42], and defined as the length-to-breadth ratio of the two-dimensional molecular shape projected on the flat surface. That means, the *L/B* ratio indicates a kind of two-dimensional shape of the molecule, and it can quantitatively classify "rod-like" molecules and "square-like" molecules. This results have a good agreement with previous results [12], suggesting that the retention is basically increasing with increasing the molecular size of the analytes, and also that the analytes having a similar molecular size (*i.e.* the same *F* number) but having a larger *L/B* ratio (*i.e.* a "rod-like" shape) retained longer than the analytes with a smaller *L/B* ratio.

Fig. 4. Retention of various PACs on three types of stationary phases. (a) BF, (b) ODS and (c) PBS phases. Mobile phase: methanol. (To be continued to the next page)

Fig. 4. (Continued)

In contrast to the ODS phase, the BF phase showed a somewhat different trend in the retentivity for PACs. Compared to the ODS phase, the selectivity to the molecular size, F number, is not clearly observed for these PACs. In the BF phase, a large analyte with "square-like" shape, that is the molecule having both a large F number and a small L/B , such as coronene and benzo[ghi]perylene, showed a smaller retention when compared to the ODS phase.

3.2. Molecular shape recognition capability of the BF stationary phase

Planarity of the analytes should also be considered for the interpretation of the molecular shape recognition process. Table 1 summarizes the selectivities to planar/non-planar solute pairs including a set of triphenylene and *o*-terphenyl proposed by Tanaka *et al.* [43] and Jinno *et al.* [44]. The results clearly indicate a unique selectivity of the BF phase for planar/non-planar solutes. Taking into account the retention trend observed in Fig. 4, the excellent selectivity to triphenylene and *o*-terphenyl on the BF stationary phase can be regarded mainly as a significantly smaller retention to non-planar analyte, *o*-terphenyl.

Table 1. Selectivity for triphenylene and terphenyls.

Solute Pair [L/B]	Selectivity		
	BF	ODS	PBS
[1.12] / [1.11]	24.6	1.66	1.26
[1.43] / [1.11]	25.8	1.26	1.07
[2.34] / [1.11]	39.7	1.42	1.02

Mobile phase: methanol.

In Table 2, the selectivities to some *ortho*-fused four-ring PAHs are summarized, where the F number of all the solutes are the same. The L/B of all compounds are also shown in the table along with these chemical structures. From the results, it can be confirmed a larger retention for a "rod-like" molecule on the BF phase, because a larger retention was observed for the analytes having a larger L/B ratio. This trend is also found on the ODS phase as reported before, however, the selectivity on the BF phase is significantly larger than that on the ODS phase. On the basis of the comparison with the PBS phase, it can be assumed a different retention mechanism of the BF phase. Since the

selectivities for these isomeric PAHs on the PBS phase have a good agreement with other stationary phases with phenyl functional group in the chemical structure [8,45], the reason in the difference in the selectivity can be interpreted as a different mobility of the phenyl functional groups. In the PBS phase, the phenyl groups on the silica support is able to move on the basis of a flexibility of the alkyl spacer between the support and phenyl group, while the BF phase can have rigid phenyl ligands on the surface.

Table 2. Selectivity for various four-ring PAHs.

Solute Pair [L/B]	Selectivity		
	BF	ODS	PBS
[1.89] / [1.72]	1.21	1.20	1.03
[1.89] / [1.58]	1.60	1.25	1.06
[1.89] / [1.22]	2.69	1.27	0.95
[1.89] / [1.12]	3.29	1.31	0.72
[1.72] / [1.58]	1.32	1.04	1.03
[1.72] / [1.22]	2.21	1.06	0.93
[1.72] / [1.12]	2.70	1.09	0.70
[1.58] / [1.22]	1.68	1.06	0.90
[1.58] / [1.12]	2.05	1.04	0.68
[1.22] / [1.12]	1.22	1.03	0.75

Mobile phase: methanol.

4. Conclusions

In this work, a new polymer-based stationary phase, a copolymer of benzoguanamine and formaldehyde, was introduced in LC. The retention behavior of PACs on the phase was evaluated along with a comparison of selectivities with that obtained on conventional monomeric ODS and phenylbutylsilica phases.

The results suggest a possibility to the BF material as the stationary phase having a unique selectivity on the basis of the rigid phenyl ligands on the stationary phase surface, although a more systematic investigation should be carried out to derive the final conclusion for the interpretation of the retention behavior. The effect of the nitrogen atoms at the root of the phenyl ligands on the retention behavior of the BF stationary phase should be further estimated using a set of polar analytes. These studies are currently underway in our laboratory including the interpretation of the retention mechanism on the basis of a molecular simulation [46-48]. Taking into account a good heat resistance of the BF phase up to 300°C and a good solvent resistance, the possibilities of the BF phase to the materials as the stationary phase in gas chromatography [49,50] and the extraction medium in sample preparation [51,52] are also investigated.

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