Molecular Shape Selectivity for Polycyclic Aromatic Compounds on a Poly(benzoguanamine-co-melamine-co-formaldehyde) Stationary Phase in Reversed-Phase Liquid Chromatography

Ohjiro SUMIYA¹, Koki NAKAGAMI¹, Ikuo UETA², Yoshihiro SAITO*¹

¹Department of Applied Chemistry and Life Science, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi 441-8580, Japan
²Department of Applied Chemistry, University of Yamanashi, 4-3-11 Takeda, Kofu 400-8511, Japan

Abstract
A novel spherical polymeric material has been introduced as a stationary phase in liquid chromatography (LC). Poly(benzoguanamine-co-melamine-co-formaldehyde) (BMF) was packed into a stainless-steel tube having 4.6 mm i.d., 150 mm length, as a particulate separation medium in LC. The retention behavior for polycyclic aromatic compounds (PACs) on the BMF phase was evaluated in reversed-phase LC and the trend was compared with that on a commercially-available octadecylsilica (ODS) phase. The BMF phase showed a unique molecular shape selectivity for PACs that was clearly different from that on typical ODS phases. The BMF stationary phase more strongly retained "rod-like" molecules such as naphthacene than "square-like" molecules with a relatively large molecular weight. From the results, it was considered that the phenyl groups as stationary phase ligands were exposed on the surface of BMF particles, and these phenyl functionalities were apart from each other. Therefore, the BMF phase strongly retained "rod-like" analyte molecules that can interact with more than one phenyl groups simultaneously of the stationary phase.

Keywords: Spherical polymer; Poly(benzoguanamine-co-melamine-co-formaldehyde); Stationary phase; Polycyclic aromatic compounds; Retention behavior; Liquid chromatography

1. Introduction
Liquid chromatography (LC) is one of the most powerful and convenient separation methods, especially for the separation of non-volatile sample matrix in separation science [1-4]. In order to obtain an effective separation of the complex sample mixtures commonly encountered in actual separation problems, the stationary phase plays an essential role [5-8]. Development of novel stationary phases has been regarded as a key process to create more efficient and cost-effective separations. In fact, various types of new stationary phases have been developed in LC [9-14]. 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 [15]. However, typical ODS phases still have some problems to be solved, such as insufficient heat resistance and chemical resistance, along with a limitation on the mobile phase composition. Polymer-based stationary phases having a good heat resistance and also a good chemical resistance have been applied as a stationary phase in LC [16-18]. Polymer-based materials is already employed in some chromatographic methods, especially for the stationary phase in LC and also in size-exclusion chromatography (SEC) as one of the variations in LC separation methods [19-21], as well as in gas chromatography (GC) [22,23]. For example, various types of fibrous polymeric materials were successfully introduced as a stationary phase in GC [23-33] and LC [32-40].

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

Received: 7 January 2022
Accepted: 27 January 2022
J-STAGE Advance Published: 3 February 2022
DOI: 10.15583/jpchrom.2022.001
Recently, our research group introduced novel polymer-based stationary phases, such as a poly(benzoguanamine-co-formaldehyde) (BF) as separation media in LC [17,18]. The BF stationary phase showed a unique molecular shape selectivity for the separation of aromatic compounds. As an extension of these previous investigations, in this work, a poly(benzoguanamine-co-melamine-co-formaldehyde) (BMF) was introduced as an LC stationary phase, where the effect of the chemical structure of the stationary phase on the molecular shape selectivity for polycyclic aromatic compounds (PACs) was systematically studied by comparing the selectivity with that obtained on the BF phase. The retention behavior of the BMF phase for aromatic compounds was also compared with that on typical ODS stationary phases.

2. Experimental

2.1. Reagents and solvents

All of the reagents and solvents, including PACs, 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 BMF column

Spherical particles of the BMF were obtained from Nippon Shokubai, Osaka, Japan. Typical chemical structure of the BMF phase was shown in Fig. 1. The average particle size is about 9.5 μm after removing relatively fine particles using a decantation process with the suspension of the particles in a solvent, as similar to the method described previously [18]. The BMF phase was packed into a blank stainless-steel tube of 4.6 mm i.d., 150 mm length with a slurry packing method. The particle size distribution was somewhat larger than a commercially-available silica-based stationary phase. However, the typical column head pressure was about 2.6 MPa with methanol (1.0 mL/min) as the mobile phase, suggesting a practical use of the BMF column in typical LC conditions.

![Chemical structure of the BMF stationary phase.](image)

2.3. LC measurements

For all the measurements, an LC system consisted of a PU1585 and a UV2075 (Jasco, Tokyo, Japan), a Model 7120 injector (Rheodyne, Cotati, CA, USA) with a 20-μL sample loop volume was employed. Chromatogram was recorded with ChromNAV Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer. As the mobile phase, methanol was used, and the flow-rate was set at 1.0 mL/min unless otherwise specified. UV detection wavelength was individually determined in the preliminary experiments for all the analytes. For the analysis of the retention behavior, a group of PACs were employed along with polyphenyls compounds including o-terphenyl, m-terphenyl, p-terphenyl and triphenylmethane. The chemical structures of these sample analytes are illustrated in Fig. 2.

![Chemical structures of PACs used as the sample analytes.](image)

3. Results and discussion

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

Fundamental trend in the retention of PACs on the BMF stationary phase was evaluated. For comparison, a commercially-available stationary phase, a Develosil ODS UG-5 (monomeric-type ODS phase; 4.6 mm i.d., 150 mm length, 5 μm particle size; Nomura Chemical, Seto, Japan) was also employed.
In order to consider the size of the PAC molecules, one of the structural parameters, $F$ number, was introduced. The $F$ number is a molecular size descriptor proposed by Hurtubise et al. [41] 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 the $F$ number has been found for a typical monomeric ODS phase in aqueous LC conditions [42].

Typical plots for the relationship between the $F$ number and the logarithmic retention factor on the BMF phase were shown in Fig. 3. In contrast to the retention tendency reported on the ODS stationary phase, no clear correlation could be confirmed for the BMF stationary phase as found on the BF phase previously [18]. Especially, the retention for PACs with a large molecular weight such as coronene was relatively small, suggesting a significantly unique retention trend for PACs on the BMF stationary phase. In the retention mechanism of the BMF phase, one can assume that the phenyl groups on the surface of the BMF particles have a remarkable contribution, where the solute molecules interacted with these phenyl groups in the BMF phase were effectively retained on the BMF stationary phase. Since these phenyl groups could be located around the surface of the stationary phase, it is considered that most of the PACs with large molecular weight could not be effectively interacted with the stationary phase ligands since majority of the analytes molecule could not be interacted during the interaction of a part of the analyte molecule with the stationary phase ligand.

3.2. Molecular shape recognition capability of the BMF stationary phase for PACs

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. [43] and Kaliszhan et al. [44] and defined as the maximized length-to-breadth ratio of the two-dimensional molecular shape projected on a flat surface. That means, the $L/B$ ratio indicates a kind of two-dimensional shape of the PACs molecule, and it can quantitatively classify "rod-like" molecules and "square-like" molecules. On the ODS and the BF phases, the data suggested that the retention was 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 [18].

In Fig. 4, logarithmic retention factors for planar four-ring PACs with the same $F$ number on three stationary phases were plotted against the corresponding $L/B$ values. From the plot, it can be observed a relatively large retention for "rod-like" analytes such as naphthacene on the BMF phase. However, the BMF phase showed quite smaller retention for "square-like" PACs, such as triphenylene, when compared to the corresponding retention on the BF and the ODS phases.

The chemical structure of the BMF phase was similar to that of the BF phase, while the density of phenyl groups on the surface of the synthesized BMF particle was smaller than that of the BF particle due to the copolymerization of the melamine moiety in the polymer chain, suggesting that there was a certain distance between these phenyl groups each other on the BMF phase. It is considered that the "rod-like" PACs were retained longer than the "square-like" PACs, because the PACs having "rod-like" molecular shape could simultaneously interact with multiple phenyl groups on the surface of the BMF phase. This interpretation was additionally supported by the results for triphenylene having a smaller retention on the BMF phase. Because the molecular length of triphenylene is somewhat shorter than other four-ring PACs with the same $F$ number.

Fig. 3. Relationship between $F$ number and ln $k$ for PACs on the BMF stationary phase. Assignments are the same as in Fig. 2. Mobile phase: methanol.

Fig. 4. Relationship between the $L/B$ and ln $k$ of four-rings PACs on three stationary phases. Mobile phase: methanol. Retention data on the BF phase was adopted from previous study [18].
4. Conclusion

In this work, a poly(benzoguanamine-co-melamine-co-formaldehyde) stationary phase was introduced as a novel polymer-based stationary phase in LC. The retention behavior of various PACs on the BMF phase was evaluated along with a comparison of the selectivity with that obtained on conventional monomeric ODS phases. The results suggest a possibility to the BMF material as the stationary phase having a unique selectivity. The effect of the nitrogen atoms at the root of the phenyl ligands on the retention behavior of the BMF stationary phase should be further estimated using a set of polar analytes. However, the BMF stationary phase shows significantly smaller retention than the BF stationary phase [18] for planer “square-type” analytes, probably due to the phase structure of the BMF having a relatively lower phenyl ligand density than the BF phase due to the copolymerization of non-aromatic component in the synthesis.

Taking into account a good heat resistance of the BMF phase up to 300°C and also a good solvent resistance, the possibilities of the BMF phase to the materials as the stationary phase in GC and the extraction medium in sample preparation are also promising [45-53].

Acknowledgements

The authors thank Mr. K. Matsukawa of Nippon Shokubai for supplying the BMF particles. A part of this study was financially supported by JSPS KAKENHI (18K05169 and 21K05110). One of the authors, OS acknowledges the financial support from Toyohashi University of Technology.

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