Retention Behavior of Various Aromatic Compounds on Poly(butylene terephthalate) Stationary Phase in Liquid Chromatography

Koki NAKAGAMI¹, Misato AMIYA¹, Keiichi SHIMIZU¹, Ohjiro SUMIYA¹, Ryota KOIKE¹, Ikuo UETA², Yoshihiro SAITO*¹

¹Department of Applied Chemistry and Life Science, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi 44-1-8580, Japan
²Department of Applied Chemistry, University of Yamanashi, 4-3-11 Takeda, Kofu 400-8511, Japan

Abstract
Poly(butylene terephthalate)-coated silica (PBT) have been introduced as a stationary phase in liquid chromatography (LC) 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 two types of commercially-available octadecylsilica (ODS) phases and phenylbutylsilica (PBS) phase. A good linear relationship between molecular size of planar PACs and the corresponding logarithmic retention factor was confirmed on the PBT stationary phase, and the trend is quite similar to that obtained on a conventional polymeric ODS stationary phase. In addition, a good molecular shape recognition capability of the PBT stationary phase was confirmed for several solute pairs consisted of planar and non-planar PACs with a similar two-dimensional molecular size. The selectivities to some planar/non-planar solute pairs on the PBT stationary phase were significantly better than conventional ODS phases, even when compared with that of typical polymeric ODS stationary phases operated in a similar experimental condition. In the case of structural isomers of dichlorobenzene and dibromobenzene, the elution order on the PBT stationary phase was o-, m- and p-, however the corresponding elution order in typical ODS phases, and PBS phase was different, o-, p- and m-. The results can be explained on the basis of the molecular-molecular interaction between the stationary phase ligand and the analyte molecule, because the PBT stationary phase has a similar partial chemical structure to these p-isomers on the silica support.

Keywords: Stationary phase; Poly(butylene terephthalate); Polycyclic aromatic compounds; Retention behavior; Liquid chromatography

1. Introduction
Liquid chromatography (LC) is one of the most widely used methods for the effective separation and isolation of various complex mixtures, because of the powerful separation capability and the applicability to wide variety of sample matrices [1-4]. Therefore, LC has been employed in various scientific fields, such as pharmaceutical, environmental and biomedical analyses as well as routine analytical situations [4-8]. As majority of the LC separations are carried out using a reversed-phase condition [9], development of chemically modified silica stationary phase is regarded as a key step for improving the separation of complex sample matrices consisted of many components [9-14].

In order to improve the separation process, various types of chemically-bonded stationary phases have been developed during the past several decades [15-20], and octadecylsilica (ODS) phases are the most dominant stationary phase in LC [21-24] due to the good separation performance and the wide availability. The ODS stationary phases can be divided into two types, depending on the bonding chemistry during the phase synthesis [4,9].
One is “polymeric,” which is typically synthesized with trifunctional (or difunctional) silanes as the starting material in an aqueous condition and another is “monomeric” synthesized from monofunctional silanes in a non-aqueous synthetic condition [25-27]. In contrast to the numerous number of reports for the selectivity and the phase ordering differences between polymeric and monomeric ODS phases [28-32], however, the systematic understanding and the resulting fundamental theory for the intermolecular interaction between solutes and bonded phase ligands in LC has not been established well. In the LC separation for a particular separation problem, a wide variety of stationary phases has been synthesized and commercialized [9,33], however, there is still a long way to get a universal chemically-bonded stationary phase that could offer a complete separation for real sample matrices within a reasonable analysis time [33-38], as typically found in the separation of structural isomers [39]. This is because a complexity in the actual retention process on the bonded phase, where many parameters should be taken into account to systematically understand the retention of particular analyte on the stationary phase.

Development of novel stationary phases, especially polymer-based stationary phase, has also been desired to create more efficient and cost-effective separations in chromatography [10,40]. Taking advantage of a good stability to a mobile phase of highly-acidic and highly-basic conditions, various applications have been reported [40]. Another advantageous feature of the polymer-based phase in separation science is a simple phase structure design and the relatively easy synthesis of the phase, allowing a cost-effective development of novel stationary phases [41]. Recently, various types of polymeric fibers have been reported as a novel stationary phase. Fibrous polymers having a good chemical resistance, heat resistance and mechanical strength have been applied as stationary phase in LC [42,43] and gas chromatography (GC) [44-50]. These fibers showed not only a good performance as a stationary phase, but it also showed a good concentration performance as an extraction medium in sample preparation process [51-56].

As an extension of these previous investigations, in this work, poly(butylene terephthalate)-coated silica (PBT) were introduced as the stationary phase in LC, where the retention behavior of the PBT for a group of polycyclic aromatic compounds (PACs) was evaluated along with a comparison with the observed trends on typical ODS and phenylbutylic silica (PBS) phases.

2. Experimental

2.1. Reagents and solvents

All of the reagents and solvents including PACs were obtained from either Kishida Chemical (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan). These chemicals were of analytical grade, and used without further purification. Water was purified by Milli-Q Water purification system (Merck Millipore, Darmstadt, Germany).

2.2. PBT stationary phase

The PBT-packed column was obtained from DAICEL, Osaka, Japan. Fig. 1 shows the chemical structure of the PBT stationary phase, and the structure of the PBT stationary phase is consisted of a polymer chain having a planar phenyl group and two ester groups in the monomer units [57,58]. In this phase, fine silica particles of 3 µm o.d. were used as the support.

2.3. LC measurements

For all the measurements, an LC system consisted of a PU-1585 pump and a model MD-910 Photodiode Array Detector (Jasco, Tokyo, Japan), and a Model 7725 injector (Rheodyne, Cotati, CA, USA) was used. Column temperatures between 15 and 40ºC were controlled by Low Temperature Thermostatic Water Bath T-10L (THOMAS Kagaku, Tokyo, Japan). Data analysis software used was Borwin PDA (Jasco) running on a personal computer. As the mobile phase, a mixture of methanol/water was used, and the flow-rate was set at either 1.0 or 0.50 mL/min. For all 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.

3. Results and discussion

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

The retention behavior for various PACs on the PBT stationary phase was investigated, and compared with commercially available stationary phases. For comparison, three commercially available phase, 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 stationary phase (PBS phase; 4.6 mm i.d., 150 mm length; YMC, Kyoto, Japan) were also introduced.
In this work, $F$ number, which represents the two-dimensional molecular size of PACs, was introduced for the analysis of the retention behavior of the PACs [59,60]. The molecular size descriptor $F$ 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 as shown in Fig. 2, a high linear correlation was obtained between the logarithmic retention factor ($ln\ k$) of PACs and the $F$ number on typical ODS stationary phases [61-63]. The trend on the PBS phase can be regarded as basically similar to that of the monomeric and polymeric ODS phases with some exceptions. From the plots in Fig. 2, it can be observed a good correlation for planar PACs on the PBT stationary phase, as similarly found on the monomeric and the polymeric ODS stationary phases.
|        | \(k\) | \(a\) |
|--------|-------|-------|
| PBT    | 9.80  | 4.19  |
|        | 2.34  | 2.26  |
|        | 4.33  | 1.89  |
|        | 5.17  | 4.07  |
|        | 2.41  | 1.57  |
|        | 4.19  | 0.991 |
|        | 2.26  | 1.07  |
|        | 1.89  | 1.05  |

Mobile phase: methanol/water = 90/10.

Compared to the PBS phase and the ODS stationary phases, a large retention factor for planar molecules on the PBT stationary phase was observed. In contrast, the PBS phase exhibited relatively smaller retention factors for PACs. Previous studies demonstrated that the molecular shape selectivity of the ODS phases can be attributed to the actual surface structure consisted of a dense brush-type phase structure formed by the bonded alkyl functionalities on the silica support. Especially a “slot-like” structure could be expected on a polymeric ODS phase, allowing an effective interaction with planar PACs. However, the estimated surface structure of the PBT phase is different from the ODS phase, because the silica support is surrounded by long polymeric chains of the PBT as a thin layer. Therefore, the observed good planarity recognition power of the PBT phase for planar analytes can be interpreted as an effective interaction between the planar analytes and the stationary phase ligand, i.e. the polymeric chain, surrounding the silica support.

3.2. Molecular shape recognition capability of the PBT stationary phase

Table 1 summarizes the selectivity data for triphenylene, terphenyls and triphenylmethane on four stationary phases, where the selectivity (\(a\)) for planar/non-planar PACs was compared. The results demonstrated that the PBT stationary phase, as well as polymeric ODS stationary phase, was able to recognize the molecular planarity of those compounds, while the retentivity for non-planar analytes were relatively smaller on the PBT phase. The limited retentivity toward the non-planar analytes can be interpreted on the basis of the limited interaction of the non-planar aromatic molecule with the surface structure of the stationary phase.

In the ODS phases, non-planar analytes could be retained to some extent on the basis of the interaction between the alkyl ligands on the surface of the silica support, although the retentivity is relatively smaller than that for planar analytes. The interaction of the PBT stationary phase with non-planar analytes is assumed to be quite limited due to the surface structure that is suitable for the interaction with planar PACs as described above. Table 1 demonstrated that some of non-planar PACs were retained longer than planar PACs on the PBS phase. The results indicated a characteristic molecular shape selectivity of the PBS phase, which is different from other stationary phases compared in this work.

3.3. Selectivity for two-dimensional shape of PACs on the PBT phase

The selectivity for the two-dimensional shape of PACs has also been considered using another molecular descriptor, length-to-breadth ratio (\(L/B\)) [66,67]. This parameter is defined as the maximized length-to-breadth ratio of the two-dimensional molecule projected on the flat surface. That means, a PAC molecule with a large \(L/B\) value has a “rod-like” shape, as similar to naphthacene, and the one with a small \(L/B\) value has a “square-like” shape, as similar to triphenylene. In Fig. 3, logarithmic retention factors for planar four-ring PACs on these four stationary phases were plotted against the corresponding \(L/B\) values. From the plots in Fig. 3, one can conclude that the PBT stationary phase has a good shape selectivity to these isomeric PACs, i.e. “rod-like” molecules retained longer than “square-like” molecules.
Table 2. Selectivity for halogenated benzene isomers.

|                  | k   |       |       |       | α     |       |       |       |
|------------------|-----|-------|-------|-------|-------|-------|-------|-------|
|                  | o-  | m-    | p-    | m-/o- | p-/m- | p-/o- |
| dichlorobenzene  | PBT 5.04 | 5.78  | 6.13  | 1.15  | 1.06  | 1.22  |
|                  | monomeric 4.19 | 5.59  | 4.72  | 1.33  | 0.844 | 1.13  |
|                  | polymeric 4.27 | 5.29  | 4.36  | 1.24  | 0.824 | 1.02  |
|                  | PBS 2.99 | 3.35  | 3.15  | 1.12  | 0.940 | 1.05  |
| dibromobenzene   | PBT 6.94 | 8.43  | 9.26  | 1.21  | 1.10  | 1.33  |
|                  | monomeric 5.15 | 7.32  | 6.41  | 1.42  | 0.876 | 1.24  |
|                  | polymeric 5.20 | 6.86  | 5.75  | 1.32  | 0.838 | 1.11  |
|                  | PBS 3.73 | 4.40  | 4.19  | 1.18  | 0.952 | 1.12  |

Mobile phase: methanol/water = 75/25.

Table 3. Selectivity for diacetylbenzenes and dimethyl phthalates.

|                  | k   |       |       |       | α     |       |       |       |
|------------------|-----|-------|-------|-------|-------|-------|-------|-------|
|                  | o-  | m-    | p-    | m-/o- | p-/m- | p-/o- |
| dimethyl phthalate* | PBT 1.02 | 2.70  | 5.45  | 2.66  | 2.02  | 5.37  |
|                  | monomeric 0.407 | 0.915 | 1.06  | 2.25  | 1.16  | 2.60  |
|                  | polymeric 0.447 | 1.07  | 1.35  | 2.39  | 1.26  | 3.02  |
|                  | PBS 0.720 | 1.23  | 1.26  | 1.71  | 1.02  | 1.75  |
| diacetylbenzene** | PBT 2.45 | 5.59  | 6.98  | 2.28  | 1.25  | 2.85  |
|                  | monomeric 0.923 | 1.60  | 1.70  | 1.73  | 1.06  | 1.84  |
|                  | polymeric 1.15 | 2.28  | 2.49  | 1.98  | 1.10  | 2.17  |
|                  | PBS 1.62 | 2.52  | 2.47  | 1.56  | 0.980 | 1.53  |

*Mobile phase: methanol/water = 70/30. ** Mobile phase: methanol/water = 50/50.

The trend is quite similar to the shape selectivity trend on a polymeric ODS stationary phase, although the shape selectivity on the PBT phase is slightly less than that of the polymeric ODS stationary phase. The results are quite consistent with the molecular shape recognition capability of the PBT stationary phase on the basis of the surface structure of the stationary phase.

3.4. Selectivity for disubstituted benzene isomers on the PBT phase

Table 2 summarizes retention factor (k) and selectivity (α) values for dichlorobenzenes and dibromobenzenes on four stationary phases, and Fig. 4 shows typical chromatograms of dibromobenzenes on these stationary phases. For dichlorobenzenes and dibromobenzenes, the elution in order on the PBT stationary phase was o-, m- and p-; however, the elution order was o-, p- and m- on ODS stationary phases and the PBS phase. This trend clearly showed a unique selectivity for these structural isomers on the PBT stationary phase. The results can be explained on the basis of the molecular-molecular interaction between the analyte molecule and the PBT stationary phase ligand having a similar partial chemical structure to these para-isomers, such as p-dichlorobenzene.

In order to further investigate the molecular shape selectivity toward structural isomers, two other analytes, dimethyl phthalates and diacetylbenzenes, were introduced. Table 3 summarized retention data for dimethyl phthalates and diacetylbenzenes on four stationary phases, where the typical chromatograms for these compounds were depicted.
on Fig. 5. The elution order to dimethyl phthalates on the PBT stationary phase was the same as that of ODS phases, however, significantly improved selectivities for these isomers were confirmed in Table 3. The PBT stationary phase showed a good selectivity for dimethyl phthalates.

Additional consideration was carried out about the selectivity for structural isomers of heterocyclic compounds on the PBT stationary phase. Fig. 6 shows typical chromatograms of quinolines on these stationary phases. Compared with ODS stationary phases, a large α value on the PBT stationary phase can be observed, and this is probably due to a good recognition capability to the position of nitrogen atom of these analytes. The elution order for quinoline \((\log P: 2.03)\) and isoquinoline \((\log P: 2.08)\) [68] on the PBT stationary phase was the same as that of ODS stationary phases, however a larger selectivity for these isomers was obtained on the PBT stationary phase. The data could be interpreted as a better selectivity for the slight difference of these \(\log P\) values on the PBT stationary phase, although a further investigation should be scheduled to clearly conclude the effect of the position of nitrogen atom in these compounds.

3.5. Effect of column temperature on retention

With the PBT stationary phase, the effect of the column temperature on the retention for dimethyl phthalates was studied. As expected, the retention of the analytes was increased with decreasing the column temperature on the PBT stationary phase. The trend was the same as that of typical ODS stationary phases. The effect of the column temperature was further analyzed with the van’t Hoff plots [69,70]. The PBT stationary phase as well as ODS stationary phases clearly showed a good linear relationship for logarithmic retention factor \((\ln k)\) against the reciprocal absolute column temperature \((1/T)\) within the studied temperature range. The linear correlation factors for these plots were higher than 0.99. A good linear relationship between 1/T and \(\ln k\) showed that the retention mechanism in the PBT phases was kept constant in the column temperature range between 20 and 40°C.

**Table 4.** Enthalpy for the phase transfer for dimethyl phthalates.

|          | \(\Delta H\) (kJ/mol) |
|----------|---------------------|
|          | o-                  | m-                  | p-                  |
| PBT      | -12.4               | -21.4               | -32.4               |
| monomeric| -5.56               | -9.38               | -10.7               |
| polymeric| -7.38               | -11.8               | -14.4               |

Mobile phase: methanol/water = 75/25.

From the slope on the van’t Hoff plots, \(\Delta H\) could be calculated. Table 4 summarizes the \(\Delta H\) values for phthalates on these three stationary phases, where an excellent retentivity on the basis of large enthalpy value during the solute transfer from the mobile phase to the PBT stationary phase can be confirmed. In the case of the PBT phase, the difference between the enthalpies for dimethyl terephthalate (\(p\)-isomer) and dimethyl isophthalate (\(m\)-isomer), and also for dimethyl terephthalate and
dimethyl phthalate, were significantly larger than that obtained on typical monomeric and polymeric ODS phases. Especially, the enthalpy difference between the p- and m-isomers on the PBT phase was remarkably larger than that on the ODS phases.

4. Conclusions

In this work, the retention behavior of PACs on PBT-coated silica phase was studied. The results have a good agreement with the estimated retention mechanism for the PBT phase consisted of: 1) hydrophobic interaction between the analyte and stationary phase ligand, 2) π-π interaction between the phenyl ring of the analyte and that in the stationary ligand, and also 3) polar interaction between the ester functionalities and that in the bonded phase ligand. A more polar interaction could be expected when the position of the polar functional groups in the analyte is similar to that in the bonded phase structure, that means, para-isomer of the analyte could preferentially interact with the bonded phase ligand in addition to the hydrophobic interaction and the π-π interaction. Although further investigations on the retention mechanism of the PBT phase is still needed to fully understand the retention behavior, the above results suggest the future possibility of the PBT phase as a stationary phase in LC for the separation of other class of structural isomers having slightly different polarities each other [71-73].

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.

References

[1] Saito, T.; Saito, Y.; Kobayashi, A.; Ueta, I. Chromatography 2018, 39, 67-74.
[2] Shirai, S.; Nakane, K.; Ueta, I.; Saito, Y. Chromatography 2011, 32, 127-133.
[3] Motono, T.; Kitagawa, S.; Ohtani, H. J. Chromatogr. A 2017, 1503, 32-37.
[4] Saito, Y.; Ohta, H.; Jinno, K. J. Sep. Sci. 2003, 26, 225-241.
[5] Ban, K.; Saito, Y.; Jinno, K. Anal. Sci. 2005, 21, 397-402.
[6] Jinno, K.; Tanabe, K.; Saito, Y.; Nagashima, H.; Treengove, R. D. Anal. Commun. 1997, 34, 175-177.
[7] Jinno, K.; Quiming, N. S.; Defiola, N. L.; Saito, Y. Anal. Bioanal. Chem. 2009, 393, 137-153.
[8] Lou, D.-W.; Saito, Y.; Zarzycki, P. K.; Ogawa, M.; Jinno, K. Anal. Bioanal. Chem. 2006, 385, 96-104.
[9] Saito, Y.; Nakagami, K.; Sumiya, O.; Ueta, I. Fullerenes and Polycyclic Aromatic Hydrocarbons in Separation Science (Chapter 12) in Zarzycki, P. K. (ed.), Pure and Functionalized Carbon Based Nanomaterials: Analytical, Biomedical, Civil and Environmental Engineering Application, CRC press, Boca Raton, FL, USA, 2020, pp. 272-296.
[10] Sumiya, O.; Nakagami, K.; Koike, R.; Ueta, I.; Saito, Y. Chromatography 2018, 39, 97-103.
[11] Chester, T. L. Anal. Chem. 2013, 85, 579-589.
[12] Jinno, K.; Tanabe, K.; Saito, Y.; Nagashima, H. Analyst 1997, 122, 787-791.
[13] Saito, Y.; Ohta, H.; Nagashima, H.; Itoh, K.; Jinno, K.; Pesek, J. J. J. Microcol. Sep. 1995, 7, 41-49.
[14] Krzemińska, K.; Dembek, M.; Bocian, S. J. Sep. Sci. 2018, 41, 4296-4303.
[15] Saito, Y.; Ueta, I. Chromatography 2017, 38, 85-94.
[16] Ohta, H.; Saito, Y.; Nagae, N.; Pesek, J. J.; Matsyska, M. T.; Jinno, K. J. Chromatogr. A 2000, 883, 55-66.
[17] Sakai-Kato, K.; Nanjo, K.; Goda, Y. Chem. Pharm. Bull. 2018, 66, 805-809.
[18] Saito, Y.; Ohta, H.; Nagashima, H.; Itoh, K.; Jinno, K.; Okamoto, M.; Chen, W.-L.; Luehr, G.; Archer, J. J. Liq. Chromatogr. Relat. Technol. 1995, 18, 1897-1908.
[19] Jinno, K.; Nakagawa, K.; Saito, Y.; Ohta, H.; Nagashima, H.; Itoh, K.; Archer, J.; Chen, Y.-L. J. Chromatogr. A 1995, 691, 91-99.
[20] Saito, Y.; Ohta, H.; Terasaki, H.; Katoh, Y.; Nagashima, H.; Jinno, K.; Itoh, K. J. High. Resolut. Chromatogr. 1995, 18, 569-572.
[21] Kirkland, J. J. J. Chromatogr. A 2004, 1060, 9-21.
[22] Rimmer, C. A.; Sander, L. C.; Wise, S. A.; Dorsey, J. G. J. Chromatogr. A 2003, 1007, 11-20.
[23] Ohta, H.; Jinno, K.; Saito, Y.; Fetzer, J. C.; Biggs, W. R.; Pesek, J. J.; Matsyska, M. T.; Chen, Y.-L. Chromatographia 1996, 42, 56-62.
[24] Ban, K.; Saito, Y.; Jinno, K. Anal. Sci. 2004, 20, 1403-1408.
[25] Sander, L. C.; Wise, S. A. Anal. Chem. 1984, 56, 504-510.
[26] Wise, S. A.; Sander, L. C. J. Sep. Sci. 1985, 8, 248-255.
[27] Sander, L. C.; Wise, S. A. Retention and selectivity for polycyclic aromatic hydrocarbons in reversed-phase liquid chromatography (Chapter 10) in Smith, R. M. (ed.), Retention and Selectivity in Liquid Chromatography (Journal of Chromatography Library, Vol. 57), Elsevier Science B. V., Amsterdam, The Netherlands, 1995, pp. 337-369.
[28] Wilson, M. B.; Wise, S. A.; Sander, L. C. Chromatographia 2019, 82, 499-508.
[29] Wise, S. A.; Sander, L. C.; May, W. E. J. Liq. Chromatogr. 1983, 6, 2709-2721.
