Original

Retention Behavior of Fluorobenzenes on Fluoro-Derivatized Poly(*p*-phenylene terephthalamide) Fibrous Stationary Phases in Microcolumn Liquid Chromatography

Shingoro Shirai[1], Kenichi Nakane[1], Ikuo Ueta[2] and Yoshihiro Saito[1]*

[1]*Department of Environmental and Life Sciences, Toyohashi University of Technology, Toyohashi 441‑8580, Japan*

[2]*Department of Applied Chemistry, University of Yamanashi, Kofu 400‑8511, Japan*

Abstract

Surface derivatization of poly(*p*-phenylene terephthalamide) (PPTA) fibers was studied to prepare novel stationary phases in liquid chromatography (LC). As one type of surface derivatization of synthetic fibers, alkyl or perfluoroalkyl chains as functional groups were introduced to the surfaces of PPTA fibers. When each functionalized fiber-packed column was used for LC, increases in retention power with respect to a variety of compounds were ascertained in comparison with that prepared by an untreated-fiber as the stationary phase. Furthermore, it was indicated that the perfluoroalkyl group introduced fibrous stationary phase showed a specific retention behavior with respect to halogenated benzenes. These results demonstrated that the derivatization of the surfaces of PPTA fibers was successfully carried out. Selectivity differences between the modified-fiber packed columns were also confirmed through the use of some sample probes.

Keywords : Surface derivatization, Fibrous stationary phase, Fluorobenzenes, Poly(*p*-phenylene terephthalamide), Retention behavior, liquid chromatography

Introduction

High performance liquid chromatography (HPLC) is one of the most important technologies in separation science in fields including agricultural, pharmaceutical, and medical science [1]. In particular, a significant degree of the performance of LC systems is due to columns which can separate complex mixtures, and columns are constantly being improved to obtain optimal separations of an enormous variety of samples. The most popular columns for HPLC are packed columns containing silica packings with octadecyl groups, called octadecylsilica (ODS) columns. The ODS column is appropriate for separations of a wide range of samples, and can be used for the rapid separations under high pressure conditions due to the good toughness of silica packings. Silica-gel particles with smaller sizes have also been developed to obtain the higher column efficiencies, high-sensitivity, and high-throughput

analyses [2-4]. In order to use smaller particles, however, LC pumps need to have high levels of performance to flow mobile phases at higher pressures, due to increased column back pressures. Furthermore, it may somewhat difficult for silica-gel particles to separate samples under excessive basic conditions, due to decreases in theoretical plate numbers and tailing of sample peaks possibly induced by the hydrolysis of silica-surfaces in columns. Hence, silica-based columns might not be appropriate for separations of basic compounds in some cases. For a similar reason, structures composed of silica are not appropriate when using mobile phases consisting of only water alone.

In contrast to these columns, packed columns with fibrous stationary phases have been developed. These fibers are aligned parallel in the column. This arrangement of fiber bundles makes it possible to flow mobile phases at low back pressures. Jinno and co

-workers have described the use of capillaries in which bundles of cellulose acetate fibers running the entire length of the column are inserted in fused-silica capillaries [5-9]. In this approach, the fiber surfaces served as stationary phases for reversed-phase microcolumn LC and capillary electrochromatography. These separations demonstrated the feasibility of using fibers as chromatographic stationary phase in LC separations of such as alcohols and polycyclic aromatic hydrocarbons (PAHs). Marcus and co-workers recently described the use of capillary-channeled polymer (C-CP) fibers for use as stationary phases for reversed-phase liquid chromatographic separations [10-14]. These fibers possess approximately three times the surface area of conventional fibers with circular cross-sections, because the C-CP fibers are manufactured with eight parallel channels on the periphery of each fiber. As a part of these investigations, the C-CP fiber stationary phase for LC was described for separations of a variety of organic compounds including PAHs, amino acids, and proteins using both isocratic and gradient elution methods.

On the other hand, the use of synthetic fine fibrous materials as a sample preparation device for solid phase extraction (SPE) has been demonstrated in our laboratory. Heat-resistant fibers used as an extraction medium possess good chemical resistance and rigidity, hence, the effective hyphenation of miniaturized sample preparation to microscale separation methods such as LC and capillary electrophoresis (CE) was successfully achieved [15-19]. Also, the successful application of an in-needle type sample preparation device using synthetic fibers as an extraction medium for gas chromatography (GC) has been presented due to the good heat resistance of the fibrous materials [20-23]. Owing to the high surface area of these fibers, other applications for heat-resistant fibrous materials were demonstrated through the development of a polymer-coated fiber-packed capillary column for GC [24-26].

There is potential value in the through study of fibrous stationary phases not only for GC, but also for LC. This is because the numbers of samples that can be separated using LC are large in comparison with GC. Microcolumn LC enables the evaluation of small amounts of experimentally synthesized bonded phases. Furthermore, the trend towards miniaturization of separation systems has been boosted by the promise of high performance, rapid analyses with low running costs, and minimum environmental pollution [27,28]. The poly(*p*-phenylene terephthalamide) (PPTA) fibers used in this study consisted of aromatic amide fibers containing amide groups. PPTA fibers show promise as a material for stationary phases for LC due to good chemical resistance, heat resistance, and durability. However, sufficient retention small molecules cannot be obtained on columns packed with parent PPTA fibers. In this article, we attempted to derivatize the surfaces of PPTA fibers in order to develop a fiber packed column. It has already been re-

ported that the addition of other functional groups onto the nitrogen atoms contained in the amide groups can be carried out by chemical derivatization [29,30]. Additionally, it has been confirmed that the separation characteristics of the fibrous stationary phase can be tuned by changing the functional group on the surface of the PPTA fiber [30].

The development of chromatographic packing materials modified by fluorine-containing functional groups has been investigated earlier [31]. It was reported that these packings possess the ability to separate fluorine-containing compounds. Recently, fluorine-containing compounds have been gathering attention as medicines possessing good bioactivity, and the synthesis of drug intermediates for these medicines is being studied in the field of organic chemistry [32]. These facts indicate that it is important to develop stationary phases with fluorine functional groups. Based on these previous reports, the introduction of alkyl or perfluoroalkyl functional groups onto the fibrous material, and retention properties with respect to alkylbenzenes and halogenated benzenes as probes were tested.

Experimental

Materials and methods

The fibrous material, Kevlar 29 (filament diameter: ca. 12.5 μm), was provided by Du Pont-Toray (Tokyo, Japan). The fibers were washed with acetone and water repeatedly, and dried at 120 °C before use. Dimethylsulfoxide (DMSO) was purchased from Kishida Chemical (Osaka, Japan), and was distilled over calcium hydride in a vacuum prior to use. Sodium hydride (in oil, 60%), bromobutane, nonafluorobutyl iodide, and all other chemicals were obtained from either Tokyo Chemical Industries (Tokyo, Japan) or Kanto Chemicals (Tokyo, Japan). For the preparation of mobile phase and standard sample solutions, water was purified with a Milli-Q water purification system (Millipore, Tokyo, Japan).

The chemically modified filaments were packed into stainless steel tubes and employed as a stationary phase in LC. Each column was prepared by longitudinally packing approximately 1,800 filaments of reacted or untreated fibers into a stainless steel tube (0.8 mm i.d., 150 mm length). The packing procedure was the same as that reported previously [33,34]. The packing densities of the prepared fiber-packed columns were calculated, and the typical value was approximately 43.9% in this work. The LC system consisted of a micro 21PU-01 pump, a DG-2080-45 degasser, an MX-2080-32 mixer, a UV-2075 UV/Vis detector (Jasco, Tokyo, Japan), an Agilent 6890 gas chromatograph column oven (Yokogawa Analytical Systems, Tokyo, Japan), and a Model 7520 injector (Rheodyne, Cotati, CA, USA) with a 0.2 μL injection loop.

LC measurements were performed using an acetonitrile (ACN)/water mobile phase at a flow-rate of 100 μL min⁻¹ at 35°C.

Mobile phase was flowed through the column for at least 20 min prior to the measurements to ensure stable baselines. The detection of samples was carried out at a wavelength of 210 nm except for specific conditions. Uracil was used for estimation of the column void time. All LC measurements were performed at least three times, and the relative standard deviations (RSDs) for the retention times were less than 3.0%. Data collection was performed using Borwin Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer.

In order to study the correlation between modified-fiber packed columns, five alkylbenzenes: toluene, ethylbenzene, propylbenzene, butylbenzene and pentylbenzene; and five fluorinated benzenes : fluorobenzene , 1,2-difluorobenzene , 1,2,3-trifluorobenzene, 1,2,3,4-tetrafluorobenzene and pentafluorobenzene were used as sample probes.

Surface derivatization of PPTA fibers

Surface derivatization was conducted using a similar scheme as reported previously [29]. The reaction scheme is illustrated in Figure 1. Under a nitrogen atmosphere, sodium hydride was reacted with an excess amount of DMSO and stirred at 30℃ for 20 min. Then, the solution was heated to 70℃ and the reaction between sodium hydride and DMSO was allowed to proceed for 1 h. Upon immersing the bundle of PPTA filaments (approximately 150 filaments, 2.0 m length) into the solution, the metalation reaction was initiated at 30℃ and allowed to continue for 10 min. The metalation reaction was confirmed by the change in the color of the fibers from red to gold [29,30,35]. Next, the fiber bundle was reacted with a halogenated compound, R-X, as can be seen in Figure 1, at the same temperature for 4 h. During this step, it was confirmed that the color of the fiber surface turned back to the original golden yellow, indicating both deactivation of the amide anion and the end of the substitution reaction. Finally, the filaments were removed from the reaction bath, were cleaned with acetone and water repeatedly, and then dried at 120℃ for 2 h. With the above derivatization process, two types of surface-derivatized fibrous materials, C4 and C4-F in Figure 1, were synthesized, and employed as the stationary phase in microcolumn LC. Successful chemical modification of the filaments was confirmed by increases in the retention factors of several standard solutes.

Results and Discussion

Retention behavior of alkyl-derivatized fibrous materials as LC stationary phases

To confirm differences between stationary phases, it is common practice to investigate the retention factors or selectivities for various compounds. Also, the values of retention factors are dependent on the densities of functional groups on the support surfaces of stationary phases. Hence, before evaluations of retention properties, the densities of functional groups per unit area of packing material are generally evaluated. Amounts of functional groups on the surfaces of chromatography packing materials can be easily calculated from amounts of carbon dioxide generated by pyrolysis in an elemental analyzer if the packings are silica-based materials. However, the fibers used in this study consist mainly of carbon. Trace amounts of functional groups on the surfaces of fibers make measurements of the densities of functional groups difficult. On the other hand, inverse chromatography can be used as a method to measure the surface properties of stationary phases and other materials [36]. This technique can be used to evaluate the surface characteristics of a sample from the intensity of the interaction between a probe and a sample in a column. On the basis of this principle, the chromatographic properties of the modified fibrous stationary phase were first assessed.

In order to evaluate the performance of the alkyl-functionalized fibers as an LC stationary phase, the retention behavior was determined using a variety of compounds as sample probes. The effects on the retention behaviors of probes according to mobile phase composition were also tested (Figure 2). This figure illustrates that the retention factors for alkylbenzenes as probes
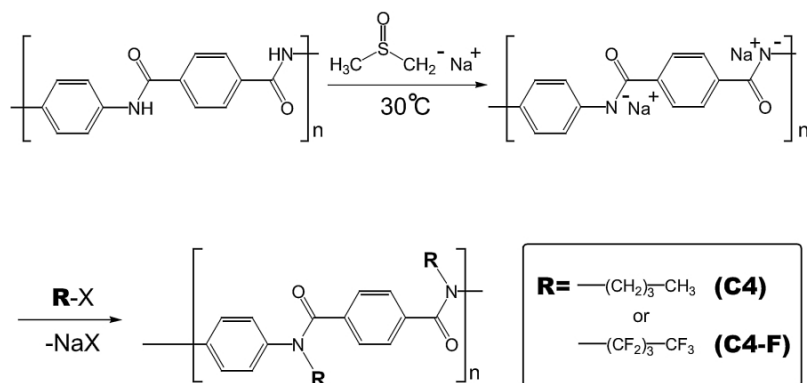

Figure 1   Reaction scheme for poly(*p*-phenylene terephthalamide) (PPTA) fibers.
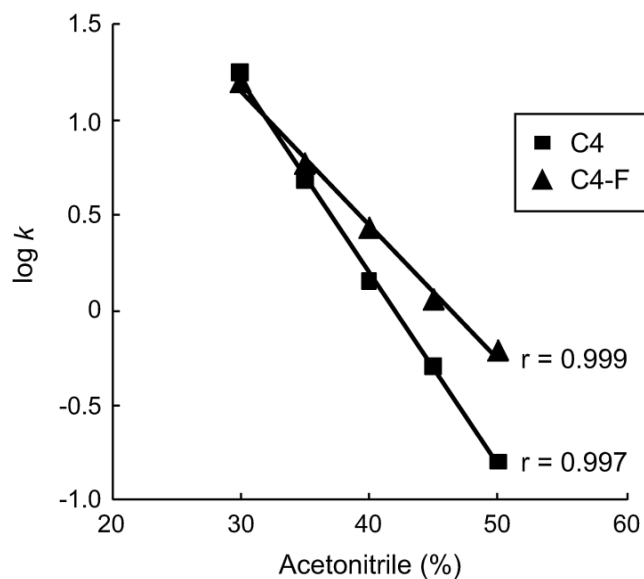
Figure 2 Retention data for modified fiber-packed columns in various mobile phase compositions. Chromatographic conditions: analyte, hexylbenzene; mobile phase, ACN/ water; column temperature, 35°C; detection, UV at 210 nm.

increased together with decreases in the ratio of ACN in the mobile phase. It was also confirmed that hexylbenzene as a solute possessed good linear behavior at mobile phase compositions on each stationary phase. From the retention behavior with respect to mobile phase composition, it was shown that the surface-derivatized fiber packed column works as a reversed-phase-type stationary phase. However, the results showed that the linear correlation had a different slope for the equation for retention behavior on each fiber packed column. This fact is consistent with the theoretical relationship between the retention factor and the mobile phase composition described by Snyder and coworkers [37]. Also, the slope for a butyl -modified fiber packed column (C4) is higher than that for a perfluorobutyl-modified fiber packed column (C4-F). In their article, Snyder and coworkers argue that the value of the slope is determined by factors such as the type of solute and stationary phase, etc. If the ratio of ACN in the mobile phase decreases, the greater the degree of hydrophobicity of the stationary phase becomes, and the more strongly hydrophobic probes are adsorbed on the stationary phase. Taking into account the slopes in this figure, it should be noted that the interaction between the C4-F column and hexylbenzene is weaker in comparison with the interaction between the C4 column and hexylbenzene. It is generally known that fluorine containing materials possess low surface energies [38]. In other words, this fact suggests that perfluoroalkyl chains possessing low surface energies were introduced onto the surfaces of fibers. However, more quantitatively detailed analyses of the amounts of derivatized functional groups on the surfaces of the stationary phase are re-

quired.

*Back pressure of the fiber packed column*

It was previously been reported that one of the merits of fiber packed columns is low back pressures on LC systems [14]. However, this characteristic may be lost as a result of surface derivatization, because of the relatively rough surface of the derivatized fibers as found previously [29,30]. Therefore, back pressure measurements were performed on conventional particle packed and fiber packed columns. Back pressure values were investigated over the flow-rate range 10 to 200 μL/min. The results show that the back pressures for fiber packed columns are significantly lower than those for a commercially available ODS column of 1.0 mm i.d., 150 mm length, packed with typical 5 μm particle. With a methanol/water=(50/50) mobile phase at a flow-rate of 200 μL/min, the back pressure for typical ODS column was about 14.8 MPa, whereas that for the fiber packed column was 0.5 MPa, which was a very low value. Also, similar back pressures were measured on the modified fiber packed columns. Low back pressures on the fiber packed columns make it possible to achieve high flow-rates if the column diameter is similar. This phenomenon was also confirmed on the derivatized-fiber packed columns. From these results it can be seen that the pressure characteristics of fiber packed columns are not lost as a result of the surface derivatization of PPTA fibers. The good permeability of these columns suggests that chemically modified fibers can be used as a sample preparation device designed to extract target compounds.

Derivatization of perfluoroalkyl groups on the surfaces of PPTA fibers

In past references, it is reported that halogenated benzenes as samples for obtaining retention data were used in order to confirm the surface properties of fluorinated particles used as packing materials [31,39]. Therefore, the fibrous stationary phase was evaluated using the same method. Table 1 illustrates the selectivity of halogenated benzenes with respect to benzene on each modified-fiber packed column. In Table 1, it is shown that the selectivities for halogenated benzenes on the C4-F column are higher than those on the C4 column. These results indicate that the retention behavior based on the type of halogen or the number of halogen atoms in the sample compound was induced by the perfluorobutyl-modified fiber packed column. This fact is attributed to the difference in the functional groups on the surface of the fibrous stationary phase.

Additionally, the retention behavior of fluorinated benzene regioisomers was tested using the same method (Table 2). Di-, tri- and tetrafluorobenzenes were used as sample probes. On the butyl-modified fiber packed column, the retention times for each pair of regioisomers were the same. However, selectivity with respect to

Table 1 Selectivity between benzene and halogenated benzenes on butyl- (C4) and perfluorobutyl-modified (C4-F) fiber-packed columns.

Solute pair	Selectivity	
	C4	C4-F
fluorobenzene / benzene	1.11	1.13
chlorobenzene / benzene	1.34	1.92
bromobenzene / benzene	1.73	2.28
iodobenzene / benzene	2.15	3.01
difluorobenzene / benzene	1.11	1.19
trifluorobenzene / benzene	1.11	1.28
tetrafluorobenzene / benzene	1.23	1.45
pentafluorobenzene / benzene	1.23	1.59
dichlorobenzene / benzene	1.49	2.22
trichlorobenzene / benzene	2.29	3.86
tetrachlorobenzene / benzene	4.82	8.15
pentachlorobenzene / benzene	10.06	16.96

Conditions: column temperature, 35°C; detection, UV at 210 nm; mobile phase, ACN/water=(40/60).

Table 2 Selectivity for several regioisomers of fluorinated benzenes on surface-modified fiber-packed columns.

Solute pair	Selectivity	
	C4	C4-F
1,2-difluorobenzene / 1,4-difluorobenzene	1.00	1.07
1,2,3-trifluorobenzene / 1,2,4-trifluorobenzene	1.00	1.06
1,2,3,4-tetrafluorobenzene / 1,2,4,5-tetrafluorobenzene	1.00	1.12

Conditions: column temperature, 35°C; detection, UV at 210 nm; mobile phase, ACN/water=(40/60). Other conditions are the same as in Table 1.

Table 3 Correlation coefficient between retention data obtained with C4 and C4-F fibrous stationary phases.

Analyte	Correlation coefficient
Alkylbenzenes	0.999
Fluorinated benzenes	0.779

Column temperature: 35°C; mobile phase: ACN/water=(30/70). Other conditions are in the text.

these compounds was confirmed on the perfluorobutyl-modified column. Interactions between fluorinated stationary phases and fluorine-containing compounds occur as a result of the general principle "*like dissolves like*" as reported earlier for the retention behavior of fluorinated analytes on fluorinated stationary phases [31]. With regard to difluorobenzenes, the fluorine atoms on 1,2-difluorobenzene are more closely arranged in comparison with 1,4-difluorobenzene. Based on the positions of the fluorine atoms, it is thought that the retention of 1,2-difluorobenzene is stronger than that of 1,4-difluorobenzene due to the ability of the fluorine atoms of the former to more easily approach the fluorine atoms on the alkyl chain. This tendency in selectivity is similar to fluorinated packing materials which have been reported previously. Therefore, this fact suggests that perfluoroalkyl functional groups were successfully introduced onto the surfaces of the PPTA fibers [31].

Correlation between alkyl - and perfluoroalkyl -modified fibers

Differences in the surface properties of stationary phases result in changes in retention behavior with respect to compounds [40]. Therefore, calculations of the correlation coefficients between columns are carried out in order to evaluate differences in the surface properties of two surface-derivatized fibrous packing materials, as shown in Table 3. The sample probes were classified into two groups containing five alkylbenzenes: toluene, ethylbenzene, propylbenzene, butylbenzene and pentylbenzene; and five fluorinated benzenes: fluorobenzene, 1,2-difluorobenzene, 1,2,3-trifluorobenzene, 1,2,3,4-tetrafluorobenzene and pentafluorobenzene. The results indicated a high correlation factor between the retention data for alkylbenzenes on these two modified fibrous phases was observed. However, the values for fluorobenzenes' retention data were relatively low in comparison with that for the data for alkylbenzenes. In other words, it was confirmed that the specific characteristics with respect to halogenated compounds on the C4-F column were different to those on the C4 column, whereas the hydrophobic properties on the C4-F column are similar to those on the C4 column. These results demonstrated that the surface properties of the fibrous stationary phase can be tuned by surface derivatization on the parent PPTA fibers. However, a detailed investigation of the modified fiber packed column is needed in order to improve the column efficiency of the fibrous stationary

phase.

Conclusions

In this study, derivatization onto the surfaces of the PPTA fibers designed for LC applications was successfully carried out. From the retention behavior of alkylbenzenes in ACN/water as mobile phase, it was confirmed that the modified fibrous stationary phases worked as reversed-phase types. Furthermore, from the method of evaluation employing various sample probes to determine the surface characteristics of the stationary phases, it was confirmed that chemical modification of the PPTA fibers is available to introduce various functional groups to the surfaces of the parent PPTA fibers, and that the surface properties of the fibrous materials can be tuned through the introduction of functional groups on the surfaces of the PPTA fibers. These results are important for the future development of the fiber packed column. Furthermore, derivatized fibers are expected to play roles as extraction media for specific compounds. However, in order to obtain sufficient column efficiencies, further research is required with respect to appropriate conditions for separations of compounds, as well as the types and lengths of functional groups introduced on the PPTA fibers. Additionally, to obtain sufficient theoretical plate numbers, it is necessary to conduct research into packing methods that can achieve high fiber densities. All of these investigations, including more selective separations for specific samples, are currently underway in our laboratory.

Acknowledgments

References

[1]   Miller, J. M. *Chromatography: Concepts and Contrasts, 2nd Edition*, Wiley: Hoboken, NJ, USA, **2004**.

[2]   Badoud, F.; Grata, E.; Perrenoud, L.; Saugy, M.; Rudaz, S.; Veuthey, J. -L. *J. Chromatogr. A* **2010**, *1217*, 4109-4119.

[3]   Cai, S. -S.; Syage, J. A.; Hanold, K. A.; Balogh, M. P. *Anal. Chem.* **2009**, *81*, 2123-2128.

[4]   Ai, F.; Li, L.; Ng, S. -C.; Tan, T. T. Y. *J. Chromatogr. A* **2010**, *1217*, 7502-7506.

[5]   Kiso, Y.; Takayama, K.; Jinno, K. *J. High Resolut. Chromatogr.* **1989**, *12*, 169-173.

[6]   Kiso, Y.; Jinno, K.; Nagoshi, T. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1986**, *9*, 763-764.

[7]   Jinno, K.; Watanabe, H.; Saito, Y.; Takeichi, T. *Electrophoresis* **2001**, *22*, 3371-3376.

[8]   Jinno, K.; Wu, J.; Sawada, H. *J. High Resolut. Chromatogr.* **1998**, *21*, 617-619.

[9]   Jinno, K.; Watanabe, H.; Kiso, Y. *J. Biochem. Biophys. Methods* **2000**, *48*, 209-218.

[10] Marcus, R. K.; Davis, W. C.; Knippel, B. C.; LaMotte, L.; Hill, T. A.; Perahia, D.; Jenkins, J. D. *J. Chromatogr. A* **2003**, *986*, 17-31.

[11] Stanelle, R. D.; Sander, L. C.; Marcus, R. K. *J. Chromatogr. A* **2005**, *1100*, 68-75.

[12] Nelson, D. K.; Marcus, R. K. *J. Chromatogr. Sci.* **2003**, *41*, 475-479.

[13] Stanelle, R. D.; Mignanelli, M.; Brown, P.; Marcus, R. K. *Anal. Bioanal. Chem.* **2006**, *384*, 250-258.

[14] Nelson, D. M.; Marcus, R. K. *Anal. Chem.* **2006**, *78*, 8462-8471.

[15] Saito, Y.; Imaizumi, M.; Ban, K.; Tahara, A.; Wada, H.; Jinno, K. *J. Chromatogr. A* **2004**, *1025*, 27-32.

[16] Imaizumi, M.; Saito, Y.; Ban, K.; Wada, H.; Hayashida, M.; Jinno, K. *Chromatographia* **2004**, *60*, 619-623.

[17] Imaizumi, M.; Saito, Y.; Hayashida, M.; Takeichi, T.; Wada, H.; Jinno, K. *J. Pharm. Biomed. Anal.* **2003**, *30*, 1801-1808.

[18] Saito, Y.; Nakao, Y.; Imaizumi, M.; Morishima, Y.; Kiso, Y.; Jinno, K. *Anal. Bioanal. Chem.* **2002**, *373*, 81-86.

[19] Saito, Y.; Imaizumi, M.; Takeichi, T.; Jinno, K. *Anal. Bioanal. Chem.* **2002**, *372*, 164-168.

[20] Saito, Y.; Ueta, I.; Ogawa, M.; Jinno, K. *Anal. Bioanal. Chem.* **2006**, *386*, 725-732.

[21] Ogawa, M.; Saito, Y.; Ueta, I.; Jinno, K. *Anal. Bioanal. Chem.* **2007**, *388*, 619-625.

[22] Saito, Y.; Ueta, I.; Ogawa, M.; Hayashida, M.; Jinno, K. *J. Pharm. Biomed. Anal.* **2007**, *44*, 1-7.

[23] Ogawa, M.; Saito, Y.; Shirai, S.; Kiso, Y.; Jinno, K. *Chromatographia* **2009**, *69*, 685-690.

[24] Ogawa, M.; Saito, Y.; Imaizumi, M.; Wada, H.; Jinno, K. *Chromatographia* **2006**, *63*, 459-463.

[25] Saito, Y.; Ogawa, M.; Imaizumi, M.; Ban, K.; Abe, A.; Takeichi, T.; Wada, H.; Jinno, K. *Anal. Bioanal. Chem.* **2005**, *382*, 825-829.

[26] Saito, Y.; Tahara, A.; Ogawa, M.; Imaizumi, M.; Ban, K.; Wada, H.; Jinno, K. *Anal. Sci.* **2004**, *20*, 335-339.

[27] Kato, M.; Inaba, M.; Tsukahara, T.; Mawatari, K.; Hibara, A.; Kitamori, T. *Anal. Chem.* **2010**, *82*, 543-547.

[28] Kiefer, P.; Delmotte, N.; Vorholt, A. *Anal. Chem.* **2011**, *83*, 850-855.

[29] Abe, A.; Saito, Y.; Imaizumi, M.; Ogawa, M.; Takeichi, T.; Jinno, K. *J. Sep. Sci.* **2005**, *28*, 2413-2418.

[30] Shirai, S.; Saito, Y.; Sakurai, Y.; Ueta, I.; Jinno, K. *Anal.*

Sci. **2010**, *26*, 1011-1014.

[31]   Kamiusuki, T.; Monde, T.; Yano, K. *J. Chromatogr. Sci.* **1999**, *37*, 388-394.

[32]   Shibatomi, K. *Synthesis* **2010**, *16*, 2679-2702.

[33]   Saito, Y.; Tahara, A.; Imaizumi, M.; Takeichi, T.; Wada, H.; Jinno, K. *Anal. Chem.* **2003**, *75*, 5525-5531.

[34]   Saito, Y.; Ogawa, M.; Imaizumi, M.; Ban, K.; Abe, A.; Takeichi, T.; Wada, H.; Jinno, K. *J. Chromatogr. Sci.* **2005**, *43*, 536-541.

[35]   Takayanagi, M.; Katayose, T. *J. Polym. Sci. Polym. Chem. Ed.* **1981**, *19*, 1133-1145.

[36]   Perruchot, C., Chehimi, M. M., Delamar, M., Dardoize, F. *J. Chromatogr. A* **2002**, *969*, 167-180.

[37]   Snyder, L. R.; Dolan, J. W.; Gant, J. R. *J. Chromatogr.* **1979**, *165*, 3-30.

[38]   Kamiusuki, T.; Monde, T.; Nakayama, N.; Yano, K.; Yoko, T.; Konakahara, T. *J. Colloid Interf. Sci.* **1999**, *220*, 123-127.

[39]   Jinno, K.; Nakamura, H. *Chromatographia* **1994**, *39*, 285-293.

[40]   Sun, Y.; Cabovska, B.; Evans, C. E.; Ridgway, T. H. *Anal. Bioanal. Chem.* **2005**, *382*, 728-734.