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Polymer-coated synthetic fibers designed for miniaturized sample preparation process

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Abstract

Miniaturized sample preparation technique for complex sample matrices has been developed with a polymer-coated fibrous extraction medium. Several hundreds of fine fibrous materials were packed longitudinally into a fused-silica capillary followed by a polymeric coating on it to prepare the extraction capillary. The extraction capillary was installed in a liquid chromatograph as a sample loop of the injection valve. The on-line coupled sample preparation/separation system demonstrated a good validity for the analysis of phthalates in real river and wastewater samples. The lowest limits of quantification for several phthalates were less than 1 ng/ml. The effect of polymeric coating to the filaments on the extraction power was also investigated.

Keywords: Polymer-coated synthetic fibres; Miniaturization; Water analysis; Extraction capillary

1. Introduction

Because of the increasing requirements for recent analytical systems, such as selective/specific detection with high sensitivities, high throughput processing, as well as an environmentally friendly feature of the systems, the miniaturization and automation of the whole analytical instruments have been an important project in analytical chemistry. Sample handling process prior to the analysis and the subsequent chromatographic separation are the typical examples. For the development of the microscale separations, many attempts have been made to downsize the components in the separation systems, such as for injectors, columns and detectors [1,2].

In contrast to the intensive studies on the miniaturization of these instruments, the investigation for the downsizing of the sample preparation process has been somewhat limited. In the typical sample preparation process, traditional liquid–liquid extraction (LLE) method, which needs a large volume of organic solvents, has been employed for the preconcentration of the analytes and the elimination of the interferences from the sample matrix. To reduce the solvent consumption during the sample preparation process, solid-phase extraction (SPE) has been developed using a cartridge packed typically with a stationary phase for liquid chromatography (LC), such as octadecylsilica (ODS) phases. Comparing with the conventional LLE method, a significant

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reduction of the solvent usage can be accomplished with the SPE techniques; however, it still requires organic solvents of ml order. Furthermore, an effective on-line coupling of the SPE method with chromatographic separation seems to be quite difficult because of the limitation of the sample loading volume to the separation columns [3].

Solid-phase microextraction (SPME) was developed by Pawliszyn and co-workers [4–6] as a solventless sample preparation method for the GC analysis of volatile compounds, and the concept of the extraction process was then applied to in-tube SPME, in which an open-tubular GC column was employed as the extraction tube [7–11]. Recently, further miniaturization of the extraction tube has been demonstrated by Saito et al. with fibrous extraction media longitudinally packed in the tube, so-called ''fiber-in-tube'' SPE [12–16]. With a fine fibrous polymeric material as the extraction medium, an improved extraction power was obtained. Moreover, the preliminary results for the use of the fibrous stationary phase in GC also showed that various kinds of coating onto the surface of the packed filaments in the extraction tube could be accomplished for a selective extraction with an increased extraction power [17].

In this work, a polymeric coating onto the fibers, which were longitudinally packed in the extraction capillary, was investigated with a polymeric material normally used as the liquid-phase of GC capillary columns, and the extraction power of the polymer-coated fiber-packed capillary was studied as a sample preparation technique for the analysis of phthalates in wastewater samples by microcolumn LC (micro-LC). As the fibrous material, Zylon® fiber was employed taking into account the heat resistance, solvent resistance and the physical strength for the convenience in the column packing and polymer-coating processes.

2. Experimental

2.1. Materials and reagents

Di-*n*-hexyl phthalate (DHP), di-2-ethyl-*n*-hexyl phthalate (DEHP), and di-*n*-octyl phthalate (DOP) were purchased from Tokyo Kasei (Tokyo, Japan) and all other reagents and solvents were obtained from Kishida Chemical (Osaka, Japan). These reagents and solvents were of analytical reagent grade and used without any purification process. For the preparation of mobile phase and standard sample solutions, water was purified with a Milli-Q water purification system (Millipore, Tokyo, Japan).

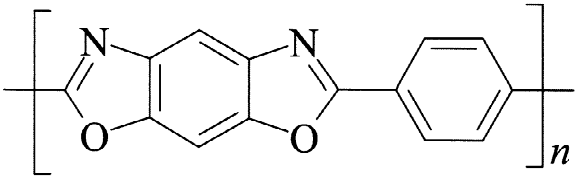

Fig. 1. Chemical structure of Zylon fiber.

Zylon®, poly(*p*-phenylene-2,6-benzobisoxazole), fiber (AS-type) was obtained from Toyobo (Ohtsu, Japan). The chemical structure of Zylon® fiber is shown in Fig. 1.

2.2. Preparation of extraction tubes

In order to prepare the extraction capillaries, about 330 filaments (11.5 μm O.D.) of the Zylon® fiber were packed longitudinally into fused-silica capillaries of 0.32 mm I.D.×300 mm length (GL Sciences, Tokyo, Japan). After the packing process, a commercially available polymeric material for capillary GC columns, HR-52 (Fig. 2; 5%-phenyl-95%-methyl-polysiloxane; Shinwa Chemical Industries, Kyoto, Japan) was used as the coating reagent.

The coating procedure was similar to the preliminary experiments [17] as described below. First, a fiber-packed capillary described above was con-

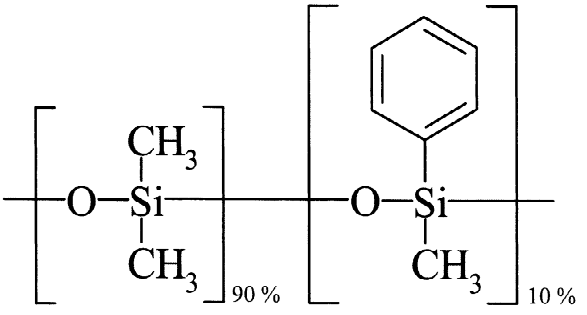

Fig. 2. Chemical structure of HR-52 polymeric coating material.

nected to the pressure-proof vessel containing 10 ml of acetone and washed with the solvent pumped by N_2 gas at the pressure of 500 kPa. The suitable number of filaments packed for the preparation of coated-fiber packed capillary was determined by the preliminary experiments. After the same volume of the following solvents, water, acetone and chloroform were pumped in the similar manner, the capillary was let it dry at room temperature for about 2 h using N_2 gas flow. Second, the capillary was subject to the heating in GC oven (HP 5890-II GC, Yokogawa Analytical Systems, Musashino, Japan) with the flow of N_2 gas. The temperature was programmed from room temperature to 300 °C at 2 deg/min and then held for about 10 h. Next, 6.5% solution of the polymeric coating material (HR-52) in *n*-hexane–acetone (90:10) containing a cross-linking reagent was pumped through the packed-capillary. After the total volume of the polymer solution (0.5 ml) was pumped, the N_2 flow was maintained for more than 5 h. For the cross-linking and chemical bonding reaction, the column was installed in the GC oven again and the programmed heating was carried out as follows: from 40 to 280 °C at 0.5 deg/min and then held more than 48 h to make sure the complete reaction.

The extraction capillary was directly connected to the injection valve with two modified zero dead-volume unions (Valco Instruments, Houston, TX, USA), as shown in Fig. 3.

2.3. LC measurements

All LC measurements were carried out with LC-10A HPLC System (Shimadzu, Kyoto, Japan) consisted of two pumps, a diode array detector, two motor-drive six-port valves and data analysis system. For the pumping of water sample, a syringe pump (Microfeeder MF-2, Azuma Denki Kogyo, Tokyo, Japan) was employed. Another syringe pump was also used for the re-conditioning of the extraction tube, if needed. These microflow pumps were connected to the switching valve. The chromatographic separations were carried out with a Speriorex ODS

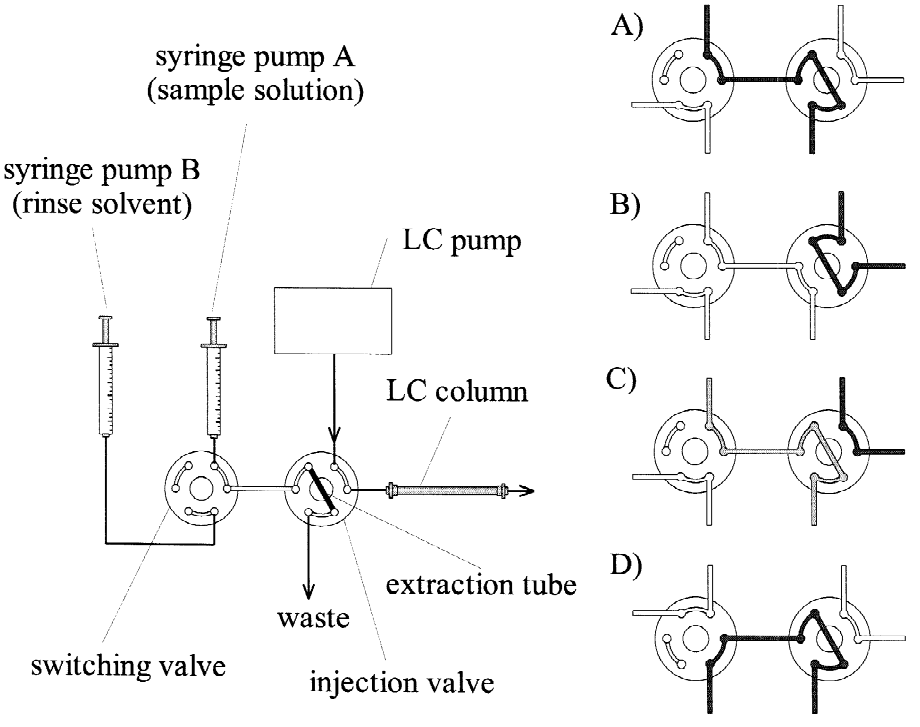

Fig. 3. On-line coupling system of the fiber-packed extraction capillary and micro-LC. (A) Extraction; (B) desorption and injection; (C) separation and the next extraction; and (D) re-conditioning processes.

(1.0 mm I.D.×150 mm, 5-μm particle size; Shiseido, Yokohama, Japan). As the mobile phase, a mixture of methanol–water (90:10) was used, and the typical flow-rate of the mobile phase was set at 50 μl/min. During the chromatographic run, the UV–Vis spectra were collected for the wavelength between 210 and 800 nm, while the real-time monitoring was made at 254 nm. All measurements were carried out at the room temperature (22.0±0.5 °C) for at least three times and the relative standard deviations (RSDs) for the retention times were less than 1.0%.

2.4. Extraction process

For the extraction process (Fig. 3A), a sample solution was pumped to through the extraction tube by one of the syringe pumps at a typical flow-rate between 20 and 40 μl/min. As shown in Fig. 3B, the desorption of the extracted analytes was carried out with a flow of mobile phase solvent. The separation and the next extraction can be processed simultaneously by changing the injection valve after a certain period of time for injection (Fig. 3C), which is an advantageous feature for the analysis of multiple samples with an automated system.

The reproducibility for the preparation of the extraction tubing was studied in the preliminary experiments and the results demonstrated that the RSDs for these capillaries, as determined by the extraction power, were less than 3% for five extraction capillaries. These extraction capillaries also showed a good stability for repeatable use, typically more than 30 runs without any significant problems, such as a decrease in the extraction power and an increase in the pressure drop through the capillaries. If, in case the performance was slightly decreased after the consecutive extraction of more than 10 times, a simple washing process with a typical organic solvent (Fig. 3D), such as methanol or acetonitrile, could be employed to make sure the reproducible results in the next 10 extractions. Furthermore, no statistical variation was observed between the replicates of the same extraction tube within the same day and between days.

2.5. Water samples

Domestic wastewater samples were obtained, as raw wastewater, from the primary sedimentation tank at Wastewater Treatment Facility of Toyohashi University of Technology (Toyohashi, Japan). Two river water samples were obtained from Toyogawa River and Umedagawa River in Toyohashi. These water samples were filtered immediately through a glass fiber filter (GA100, pore size: 1 μm; Advantec, Tokyo, Japan), and then the filtrate was further filtrated through a finer glass fiber filter (GA75, pore size, 0.3 μm; Advantec) to prepare the final water sample [12,13]. Prior to the filtration these filters were washed thoroughly with methanol and pure water for clean up. These water samples were analyzed immediately, while a portion of each water samples were stored in a refrigerator at 4 °C for 36 h to monitor the variation during the storage of the samples. The stock samples were re-analyzed at every 12 h, and no statistical difference was observed between the fresh and stored samples for the determination of phthalates.

3. Results and discussion

Fig. 4 shows the effect of the sample volume pumped and the flow-rate on the peak area counts of DEHP (1 ng/ml) observed in the chromatogram. The area counts increased linearly with increasing the total sample volume pumped through the extraction capillary; however, no effect of the pumping flow-rate on the extraction efficiency was found for the flow-rate between 20 and 40 μl/min. Good correlation coefficients were obtained for linear calibration curves between the sample volume (0–800 μl) and the area counts, $r=0.998$ and 0.999 at the extraction flow-rate of 20 and 40 μl/min, respectively. The RSDs for the area counts in these conditions were less than 3.0% ($n=5$). For the sample flow-rate below 20 μl/min, an extended extraction time should be needed to obtain the same quantitation power, and the flow-rate much more than 40 μl/min is also impractical taking into account the pressure drop in the extraction capillary. Then, the extraction flow-rate of 40 μl/min was selected for the following experiments in this work.

A typical chromatogram for the analysis of a standard sample containing 1 ng/ml each of three

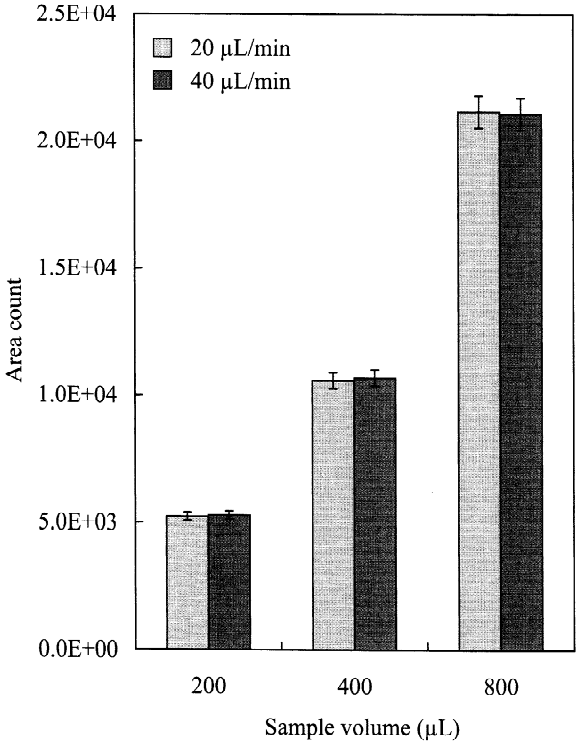

Fig. 4. Effect of extraction flow-rate and time for the extraction of aqueous DEHP solution (1 ng/ml).

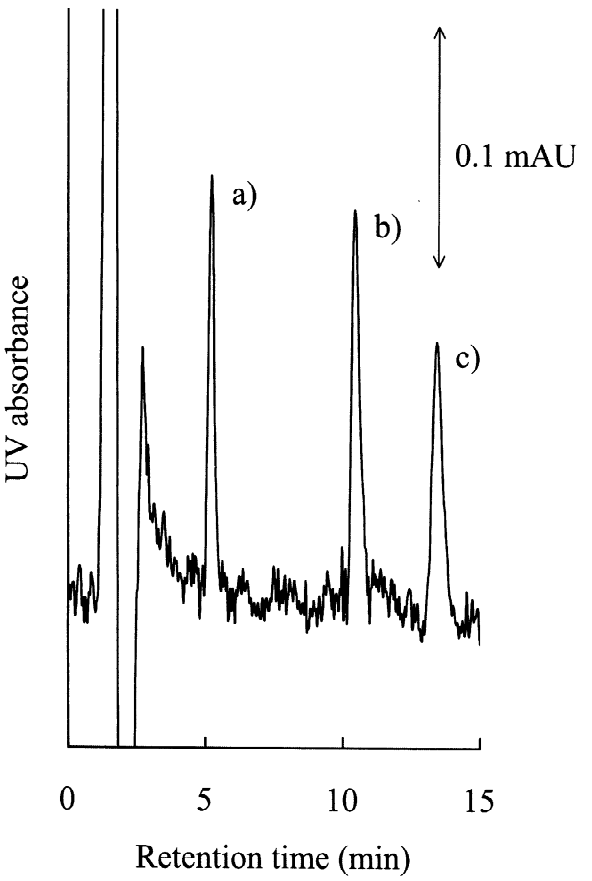

Fig. 5. Typical chromatogram for the analysis of a standard sample. Sample: an aqueous solution of phthalate mixture containing 1 ng/ml each of (a) DHP, (b) DEHP, and (c) DOP.

phthalates is shown in Fig. 5. The extraction was carried out for 12.5 min at the flow-rate of 40 μl/min, therefore, the total volume of pumped water sample was 500 μl. The extraction efficiency for these phthalates is summarized in Table 1 along with the results obtained using bare fiber-packed extraction capillary (non-polymer-coated). The extraction efficiency was calculated by comparison with direct LC analysis of using no pre-concentration process. Comparing with non-coated fiber-packed capillary, the extraction efficiency obtained with coated one was dramatically improved, especially for DEHP. The results clearly demonstrate the effect of polymer-coating to the packed-fibers on the extraction power. With polymer-coated capillary, however, the extraction efficiency for DHP and DOP was somewhat smaller than DEHP, which was quantitatively extracted in the same conditions. This trend has a good agreement with the previous results, where a correlation between the hydrophobicity of the analyte and the extraction efficiency was found

Table 1
Extraction efficiencies for phthalates using fiber-packed extraction media with and without polymer coating

	Extraction efficiency (%)[a,b]	
	With coating[c]	Without coating
DHP	63.1	19.8
DEHP	101	21.2
DOP	66.4	21.1

[a] Calculated for the extraction of a phthalate mixture (1 ng/ml each in water) at the flow-rate of 40 μl/min for 12.5 min.

[b] All RSDs ($n=5$) for the extraction efficiency were less than 3.0%.

[c] Coated with HR-52.

using fiber-packed extraction media [14,15]. Similar results were also observed for the extraction of di-*n*-butyl phthalate (DBP) with different kinds of polymer-coated fused-silica capillaries [13]. Although the effect of the chemical structure and the polarity of the polymeric coating should be further studied, the results demonstrate the suitability of the HR-52 coating for the quantitative extraction of DEHP from aqueous sample matrix.

In Fig. 6, the chromatograms for two river water samples are shown. After the simple filtration process as described above, these water samples were analyzed with the preconcentration process by HR-52-coated fibrous extraction medium. These data clearly show the effectiveness of this on-line sample preparation technique for the analysis of real sample matrix. As one of more complex sample matrix, wastewater samples were also analyzed with this system. Fig. 7 shows a typical chromatogram for the wastewater analysis. The wastewater samples were analyzed after the simple filtration process as described in Section 2. The concentration of DEHP in the wastewater was determined as 1.38 ng/ml from the peak area of the chromatogram.

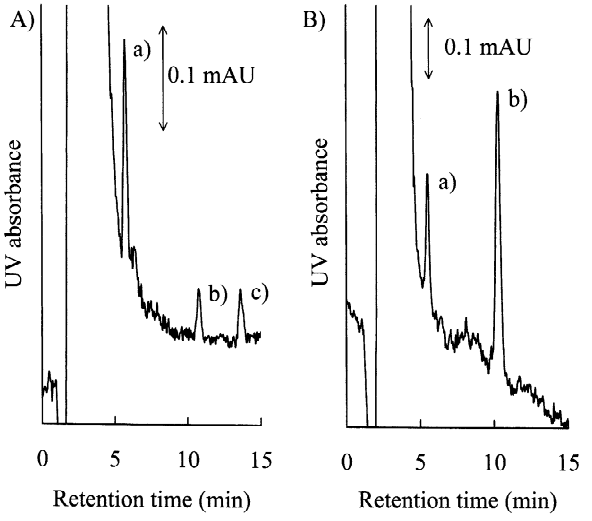

Fig. 6. Chromatogram for the analysis of phthalates in river water samples. (A) Toyogawa River: (a) DHP (0.73 ng/ml), (b) DEHP (0.17 ng/ml), and (c) DOP (0.24 ng/ml). (B) Umedagawa River: (a) DHP (0.69 ng/ml); and (b) DEHP (1.63 ng/ml). Extraction conditions are the same as in Table 1 except for the extraction time of 20 min (total sample volume extracted was 800 μl). Other conditions are in the text.

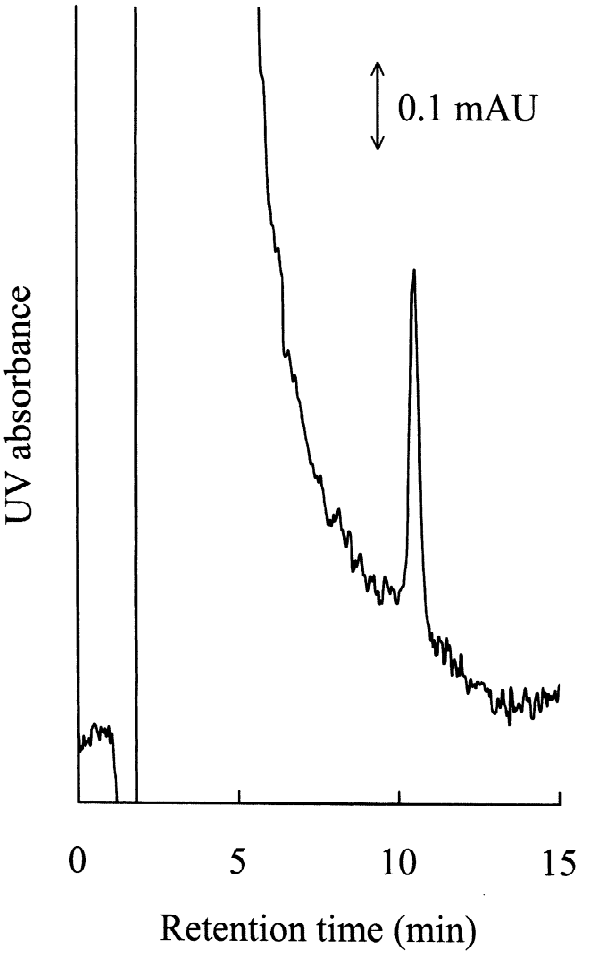

Fig. 7. Determination of DEHP in a wastewater sample. The original concentration of DEHP (peak at 10.7 min) in the wastewater sample was determined as 1.38 ng/ml. Conditions are the same as in Fig. 6.

The lowest limits of quantification for the determination of phthalates in wastewater are summarized in Table 2, where the data obtained by the fiber-packed extraction capillary (without polymer-coating) are also tabulated for comparison. These results demonstrate the validity of the on-line sample preparation method as the sample pretreatment technique for wastewater analysis. In contrast to the river water analysis, however, a periodical re-conditioning process seems to be needed for wastewater samples, typically after more than 10 consecutive extractions, in order to maintain the extraction power and to avoid undesirable pressure drop of the extraction

Table 2
Comparison of the limits of quantification for three phthalates in wastewater sample using fiber-packed extraction capillaries with and without polymeric coating

	Lowest limit of quantification[a]	
	With coating[b]	Without coating
DHP	0.15	0.5
DEHP	0.10	0.5
DOP	0.20	0.7

[a] Calculated for 20-min extraction of typical wastewater sample at the extraction flow-rate of 40 μl/min.
[b] Coated with HR-52.

tube. With the washing process shown in Fig. 3D, the re-conditioning of the extraction capillary can be made without any time-consuming procedure, i.e., a simple rinse by an organic solvent. To make sure the reproducible extraction for wastewater analysis, the re-conditioning process was incorporated in the operation program of the system. The RSD for the determination of DEHP (as shown in Fig. 7) was less than 3.0% for consecutive 15 runs with the re-conditioning process every 5 runs using 200 μl of methanol (50 μl/min for 4 min).

4. Conclusions

With polymer-coated fiber-packed capillary, a miniaturized sample preparation technique has been developed for the analysis of several phthalates in environmental complex mixtures, such as wastewater. The on-line coupling of the microscale sample preparation step with a micro-LC made it possible not only to reduce a significant volume of the solvent consumption but also to improve the quantification limits based on its higher extraction efficiency. Although the preparation conditions of extraction tube, such as the combination of fibers and coatings should be studied more, the polymer-coated fibrous packing material will have some future developments as a novel sample preparation medium along with the applications as a packing material in various chromatographic methods [17–20].

Taking into account the applications of the extraction technique as a high throughput sample handling method, further reduction of the extraction time should be needed. The study for more miniaturized sample preparation cartridge packed with coated-fiber, including as an extraction medium for trace amount of organics in complex sample mixture [13,14], is currently underway in our laboratory along with the investigations for the rapid extraction from various sample matrices [21,22].

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References

[1] D. Ishii (Ed.), Introduction to Microscale High-Performance Liquid Chromatography, VCH, New York, NY, USA, 1988.
[2] Microbore Column Chromatography, in: F.J. Yang (Ed.), Chromatographic Science Series, Vol. 45, Marcel Dekker, New York, 1989.
[3] J.S. Fritz, in: Analytical Solid-Phase Extraction, Wiley–VCH, New York, 1999.
[4] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[5] J. Pawliszyn, in: Solid Phase Microextraction: Theory and Practice, Wiley–VCH, New York, 1997.
[6] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, The Royal Society of Chemistry, Cambridge, 1999.
[7] R. Eisert, J. Pawliszyn, Anal. Chem. 69 (1997) 3140.
[8] H. Kataoka, S. Narimatsu, H.L. Lord, J. Pawliszyn, Anal. Chem. 71 (1999) 4237.
[9] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[10] J. Wu, J. Pawliszyn, J. Chromatogr. A 909 (2001) 37.
[11] Y. Saito, M. Kawazoe, M. Hayashida, K. Jinno, Analyst 125 (2000) 807.

[12] Y. Saito, Y. Nakao, M. Imaizumi, T. Takeichi, Y. Kiso, K. Jinno, Fresenius J. Anal. Chem. 368 (2000) 641.
[13] Y. Saito, Y. Nakao, M. Imaizumi, Y. Morishima, Y. Kiso, K. Jinno, Anal. Bioanal. Chem. 373 (2002) 81.
[14] Y. Saito, M. Imaizumi, T. Takeichi, K. Jinno, Anal. Bioanal. Chem. 372 (2002) 164.
[15] K. Jinno, M. Kawazoe, Y. Saito, T. Takeichi, M. Hayashida, Electrophoresis 22 (2001) 3785.
[16] Y. Saito, M. Kawazoe, M. Imaizumi, Y. Morishima, Y. Nakao, K. Hatano, M. Hayashida, K. Jinno, Anal. Sci. 18 (2002) 7.
[17] Y. Saito, M. Imaizumi, K. Nakata, T. Takeichi, K. Kotera, H. Wada, K. Jinno, J. Microcol. Sep. 13 (2001) 259.
[18] K. Jinno, H. Watanabe, Y. Saito, T. Takeichi, Electrophoresis 22 (2001) 3371.
[19] K. Jinno, H. Watanabe, Y. Kiso, J. Biochem. Biophys. Methods 48 (2001) 209.
[20] A.J.H. Louter, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 842 (1999) 391.
[21] Y. Saito, K. Jinno, Anal. Bioanal. Chem. 373 (2002) 325.
[22] M. Imaizumi, Y. Saito, M. Hayashida, T. Takeichi, H. Wada, K. Jinno, J. Pharm. Biomed. Anal. (in press).