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Development of miniaturized sample preparation with fibrous extraction media

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Abstract

Introducing fine polymeric filaments as the extraction medium, a miniaturized sample preparation technique for micro-column liquid chromatography (micro-LC) has been developed along with the investigation of a reproducible preparation scheme of the extraction capillary. The polymeric filaments were packed longitudinally into either a fused-silica capillary or a polyether ether ketone (PEEK) capillary of appropriate dimensions, and the extraction capillary was installed to the injection valve in micro-LC system. The number of packed filaments should be precisely counted before the packing process to make sure the reproducible preparation of the extraction capillary. With conventional stationary phase materials for open-tubular gas chromatography, polymeric coating to the surface of the filaments was also studied in order to further enhance the extraction performance and selectivity. Coated with the polymeric material suitable for the extraction of particular analyte, a dramatic improvement on the extraction power was obtained. The results suggest that the future possibility of novel tailored fibrous extraction medium with an appropriate coating on it, especially for the analysis of complex sample matrices.

Keywords: Instrumentation; Sample preparation; Water analysis; Extraction methods; Phthalates

1. Introduction

As to the increasing importance of the development of environmentally favorable analytical systems, miniaturization of the sample preparation process and the subsequent chromatographic separation process has been regarded as one of the key technologies including an effective on-line coupling of these two major analytical processes [1,2]. Compared with traditional solvent-solvent extraction, miniaturized sample preparation techniques could significantly reduce the usage of organic solvents as well as other chemicals. Downsizing of the chromatographic separation should be accomplished at the same time to maximize the advantageous feature of these miniaturized sample preparation methods.

As a miniaturized sample preparation technique for liquid phase separation methods, in-tube solid-phase micro-extraction (SPME) has been developed [3,4], where a section of open-tubular capillary column for gas chromatography (GC) was introduced as the extraction tube. The typical size of the extraction capillary is 0.25 mm, i.d. and 400 mm length. While the sample solution is pumped through the capillary, the analytes are extracted onto the coating inside of the capillary. Then, a small amount of the desorption solvent or mobile phase will be pumped, and the desorbed analytes are introduced to the separation column. A variety of open-tubular capillary columns have been studied as the extraction capillary [5–7] and the combination of the extraction method with liquid chromatography (LC) has been also reported for the reproducible analysis [8–12]. Due to the relatively large phase-ratio of in-tube capillary as the extraction medium, however, the extraction efficiency should be improved for the quantitative extraction [11,12].

In order to increase the extraction efficiency and reduce the solvent volume needed for each analysis, a modified extraction capillary by inserting a stainless steel wire inside has been reported [13]. With the modification, the extraction efficiency was dramatically improved, and also the on-line coupling of the extraction method and micro-column separation was accomplished. On the basis of successful introduction of the modified extraction capillary, the authors have further developed a new type of extraction method, so-called

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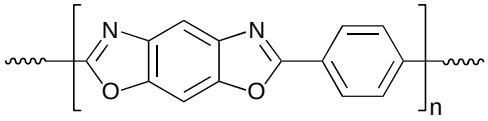

Fig. 1. Chemical structure of Zylon, poly(*p*-phenylene-2,6-benzobisoxazole), fiber.

"fiber-in-tube" solid-phase extraction (FIT-SPE) [14–20], where several hundreds of fine filaments of synthetic fiber were packed longitudinally into a capillary of polyether ether ketone (PEEK) or polytetrafluoroethylene (PTFE).

In this article, the development of the fiber-packed capillary and the applications as the miniaturized sample preparation device are described especially focusing on the reproducible preparation of the extraction capillary. Not only the on-line coupling of the FIT-SPE with micro-column separation methods, but also the polymeric coating onto the packed-filaments are studied for the effective and selective extraction of particular class of compounds from complex sample matrices.

2. Experimental

2.1. Reagents and materials

Dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-propyl phthalate (DPP), di-*n*-butyl phthalate (DBP), di-*n*-hexyl phthalate (DHP), di-2-ethyl-*n*-hexyl phthalate (DEHP), and di-*n*-octyl phthalate (DOP) were purchased from Tokyo Chemical Industries (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).

Zylon, poly(*p*-phenylene-2,6-benzobisoxazole), fiber (Toyobo, Ohtsu, Japan) [21] was selected based on the previous results [15,17], where practical suitability of the Zylon fiber for the extraction of phthalates was suggested mainly due to a better polarity matching to the target analytes than other solvent-resistant fibers such as aromatic amides. The chemical structure of Zylon fiber is illustrated in Fig. 1. As the guide fiber for packing Zylon filaments, poly(vinylidene fluoride) (PVF) fishing line (52 μm o.d.; Kureha Chemical Industry, Tokyo, Japan) was used taking into account the flexibility and strength needed for the packing process. All PEEK and PTFE tubing was purchased from GL Sciences (Tokyo, Japan).

2.2. LC measurements

The micro-LC system was consisted of a Micro-Tech Scientific Ultra-Plus II Capillary LC Pump (Yamato Scientific, Tokyo, Japan), a UV 2075 Plus UV-Vis detector (JASCO, Tokyo Japan), and two Model 7000 six-port valves (Rheodyne, Cotati, CA, USA). One of these valves was used as the switching valve and the other was employed as the injection valve. For the pumping of water sample, a syringe pump (Microfeeder MF-2, Azuma Denki Kogyo, Tokyo, Japan) was connected to the switching valve as reported earlier [14–16]. Another syringe pump was also used for the re-conditioning of the extraction tube, if needed. The chromatographic separation was performed either with a commercially available octadecylsilica (ODS) phase, Capcell Pak C_{18} MG (150 mm × 1.0 mm, i.d., 5 μm particle size, Shiseido, Yokohama, Japan) or with a laboratory-made packed-capillary column [20,22] packed with an commercially available ODS phase, Develosil ODS-UG-5 (150 mm × 0.53 mm, i.d., 5 μm particle size, Nomura Chemical, Seto, Japan) As the mobile phase, a mixture of methanol and water was used, and the typical flowrate of the mobile phase was set at 50 and 10 μl/min for 1.0 and 0.53 mm, i.d. columns, respectively.

2.3. Data processing

For data acquisition and processing, Borwin chromatography data handling software (JASCO, Tokyo, Japan) running on the computer was employed. All measurements were performed at the room temperature (22.0 ± 0.5 °C) for at least three times and the relative standard deviation (R.S.D.) for the retention times were <2.0%.

2.4. Water samples

After sampling, water samples were filtrated immediately through a glass fiber filter (GA100, pore size: 1.0 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 sample [19]. Prior to the filtration these filters were washed with methanol and pure water for complete clean up. These water samples were analyzed immediately, while a portion of each water sample was stored in a refrigerator at 4 °C for 48 h to monitor the variation during the storage of the samples. The stock samples were re-analyzed at every 24 h, and no statistical difference was observed between the fresh and stored samples for the determination of phthalates.

3. Results and discussion

3.1. Reproducible preparation of the extraction capillary

In order to establish the reproducible preparation procedure of fiber-packed extraction capillary, several preliminary experiments have been carried out especially taking into account the precise control of (a) number of packed fila-

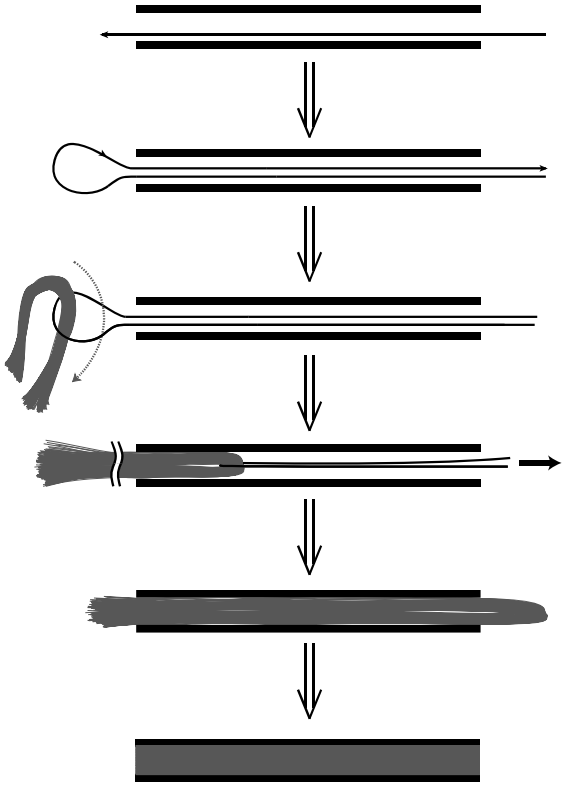

Fig. 2. Preparation scheme for fiber-packed capillary.

ments in the capillary and (b) uniform packing over the total length of the capillary. Based on the results, the packing procedure of Zylon filaments (Fig. 2) has been developed as follows:

1. Insert the PVF guide fiber into an appropriate length of a PEEK capillary.
2. The end of the guide fiber through the PEEK capillary is inserted into the capillary again, while the guide fiber should have an extra length to form a loop at the outside of the capillary.
3. The bundle of the filaments to be packed is inserted into the loop of the guide fiber as described above, where the front-end of the bundle should be appropriately bended to make sure the smooth introduction.
4. Pull the guide fiber from another side of the capillary with careful attention to the uniform introduction of the bundle.
5. Cut the extra length of the packed filaments just at the both ends of the capillary with a sharp razor blade to adjust to the same length of the capillary.

Prior to the packing process, the total number of the filaments in a bundle should be manually counted with a help of conventional magnifier, and it has been confirmed that the R.S.D. ($n = 10$) of the total number should be less than about 3% to ensure the reproducible extraction performance. The suitable number of packed filaments for different size of capillaries has been calculated to maintain a similar

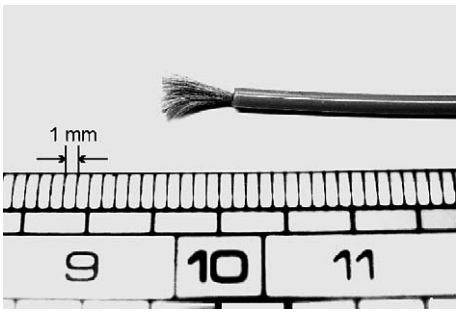

Fig. 3. Typical photograph of fiber-packed PEEK capillary of 0.25 mm i.d. × 1.6 mm o.d.

packing density, where the packing density is calculated based on the percentage of the cross-section occupied by the packed filaments and the opening cross-section of the capillary [15]. For a PEEK capillary of 10 cm × 0.25 mm, i.d., (Fig. 3), the total number of packed Zylon filaments (11.5 μm, o.d.) should be about 300–350, corresponding the packing density of 63–74%, to maximize the extraction performance without any undesirable pressure drop over the extraction tube during the pumping of the sample solution.

Fig. 4 shows the overview of FIT-SPE–LC system. The extraction capillary, fiber-in-tube, was directly connected to the injection valve with two modified zero dead-volume unions (Valco, Houston, TX, USA) as like a sample loop. For the extraction process, the water sample was pumped to through the extraction tube by the syringe pump at a typical flow-rate between 10 and 50 μl/min. Changing the position of injection valve, the desorption of the extracted analytes was carried out with the flow of mobile phase solvent. The separation and the next extraction can be processed simultaneously by returning the position of the injection valve after a certain period of time for sample injection, which is an

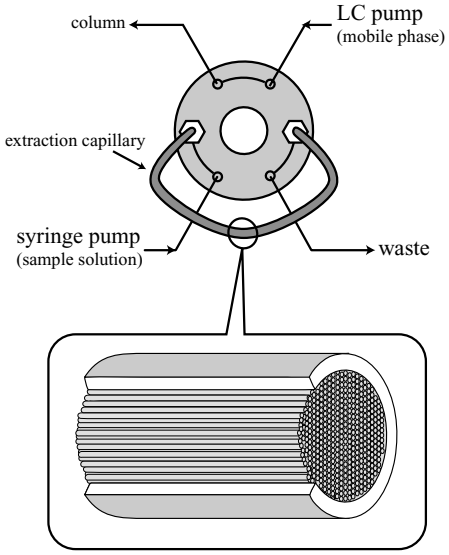

Fig. 4. Schematic representation of the connection around the injection valve of on-line coupled FIT-SPE–LC system.

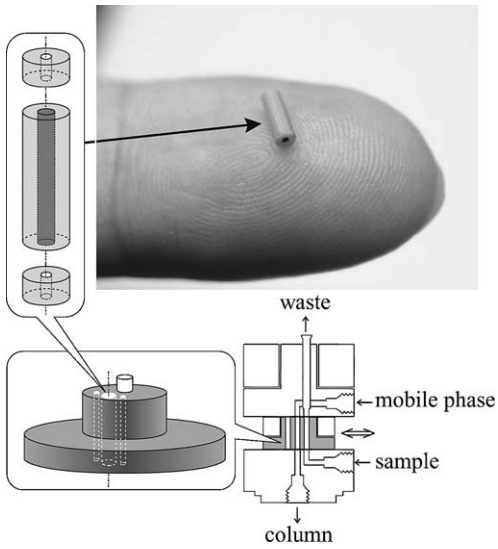

Fig. 5. Miniaturized fiber-packed extraction cartridge installed into a Rheodyne 7520 micro-injector.

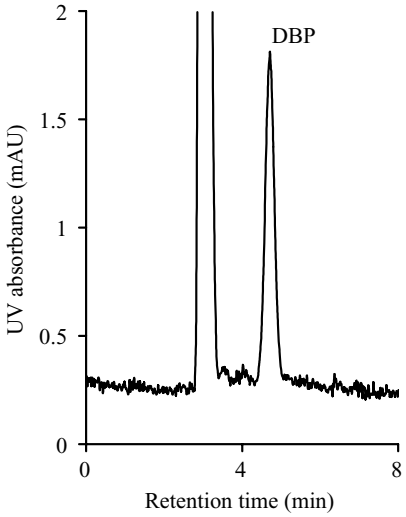

Fig. 6. Typical chromatogram of DBP obtained with the preconcentration process by the mini-extraction cartridge packed with Zylon filaments. Extraction conditions: extraction flowrate and time, 32 μl/min for 7.5 min. Chromatographic conditions: column, fused-silica capillary (150 mm × 0.53 mm, i.d.) packed with Develosil ODS-UG-5; mobile phase composition and flowrate, methanol–water (95:5) at 10 μl/min; detection wavelength, 254 nm. Sample: DBP (1 μg/ml) spiked in water. Other conditions are in the text.

advantageous feature for the analysis of multiple samples with an automated system. With the hyphenated system, the obtained preconcentration factor [14,23], defined as the ratio of the peak areas obtained with the extraction process and that with direct analysis, was more than 100 for the typical 10 min extraction of DBP from waste water samples at the extraction flow-rate of 50 μl/min. The lowest limit of quantification (LLQ) for DBP in waste water was about 0.1 ng/ml, indicating the sufficient quantification performance for routine checking in waste water treatment facility. These extraction capillaries also exhibited a good stability for repeatable use, typically more than 20–30 runs without any significant problems, such as a decrease in the extraction power and an increase in the pressure drop through the capillaries. Furthermore, even in case slight decrease of the extraction performance was observed after the consecutive extraction of more than 10 times, especially for the extraction of complex samples, a simple washing process with a typical organic solvent (such as methanol or acetonitrile) could be employed to ensure the reproducible results in the next 10 extractions.

Miniaturized FIT extraction cartridge [15,19] has been also developed for further down-sizing of the extraction process. As can be seen in Fig. 5, the size of the cartridge is even smaller than “a piece of rice” and it can be installed into a modified micro-injector. The number of the packed filaments were about 1500 for a PEEK capillary of 5.0 mm × 0.50 mm, i.d., 1.6 mm o.d. On-line coupling of the preconcentration device and packed-capillary LC has been demonstrated for the analysis of phthalates in water samples, and it has been also confirmed that the mini-extraction cartridge showed quite comparable preconcentration power to the longer FIT extraction capillary mentioned earlier, except for the total extraction capacity. However, the total extraction capacity of mini-cartridge for a typical phthalate, DBP, was still more than 700 ng, clearly showing a practical acceptance of the device as a method for typical river water analysis [19]. A typical chromatogram of DBP in a water sample is shown in Fig. 6, where a preconcentration of about 70 times is obtained using the miniaturized extraction cartridge.

3.2. Polymer coating onto the packed filaments

Polymer-coating to the packed filaments can be regarded as an effective post-treatment to enhance the extraction performance of the fiber-packed capillary [13,24]. Coated with a conventional polysiloxane-based liquid-phase for GC open-tubular capillary onto the packed filaments, the retentivity as a GC column was significantly improved than that obtained with fibrous packing material only. Furthermore the selectivity of the polymer-coated fiber-packed capillary column could be tuned with different type of polymeric coatings [13,19]. In order to apply the polymeric coating onto the packed filaments, a bundle of Zylon is packed into a fused-silica capillary followed by the polymer-coating process that is quite similar to the preparation of conventional GC open-tubular capillary columns as reported previously [24].

First, a fiber-packed fused-silica capillary of 1.0 m length (either 0.32 or 0.53 mm, i.d.) was connected 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 to be about 170 and 330 for 0.32 and 0.53 mm, i.d. columns. 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 3 h using N_2 flow. Second, the capillary was subject to the heating in GC oven with the flow of N_2 gas. The temperature was programmed from room temperature to 300 °C at 2°/min and then held for about 10 h. Next, a solution of the polymeric coating material in *n*-hexane–acetone (90:10) containing a cross-linking reagent was pumped through the packed-capillary. As the coating reagent, four types of polymeric materials have been employed: HR-1, 100% methylpolysiloxane; HR-52, 5% phenyl 95% methylpolysiloxane; HR-1701, 7% phenyl–7% cyanopropyl–86% methylpolysiloxane; and HR-17, 50% phenyl–50% methylpolysiloxane (Shinwa Chemical Industries). The concentration of polymer in the coating solution was set at 5.0% based on the preliminary results. 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 made as follows: from 40 to 280 °C at 0.5°/min and then held about 48 h to make sure the complete reaction.

The good reproducibility of the coating (<2.0% R.S.D.; $n = 5$) has been confirmed by using the resulting fiber-packed capillaries as a short column in GC, where the separation of several standard mixtures have been carried out as well as the comparison of the polymer-coated fiber-packed capillary with conventional open-tubular capillary columns of the same polymeric coating. Compare with a commercially available open-tubular capillary column of the same dimensions (1.0 mm × 0.32 mm, i.d., d_f: 0.25 μm, Shinwa Chemical), the retention factors of three alkanes (undecane, dodecane and tridecane) on the polymer-coated fiber-packed capillary were about 100 times larger. The results also shown that the retention factors for these solutes in GC have been enhanced up to about 10–20 times with the polymeric coating onto the packed filaments as reported earlier [24]. The film thickness estimated by the comparison with the conventional capillary GC column was about 1.7 μm, assuming the uniform coating onto the all the filaments packed and coating onto the internal surface of fused-silica capillary. The resulting polymer-coated fiber-packed capillaries were cut into several pieces and the central parts of the capillary were employed as the extraction capillary. Fig. 7 shows the typical cross-section photograph of the fiber-packed capillary taken by scanning electron microscope (JSM-5900LV, JEOL, Tokyo, Japan). The capillary was installed to the injection valve of the micro-LC system, as shown in Fig. 4, where an appropriate size of PEEK tube and the modified zero dead-volume union were employed.

As expected from the positive results in GC studies [24,25], the extraction efficiency for phthalates was significantly improved with the polymer coating onto the packed-filaments. For example, quantitative extraction of DEHP with HR-52 coating has been accomplished (as shown in Table 1), while the extraction efficiency with non-coated fiber-packed capillary (bare fiber-in-tube) of the same dimensions and fibrous material was about 20% in the same extraction conditions [2]. The results demonstrate not only a well-suitable polarity of HR-52 polymer for DEHP molecule as suggested in our previous investigations [19], but also that the uniform coating onto the surface of the filaments could significantly enhance the extraction power. The chromatogram for a typical waste water analysis is shown in Fig. 8. For the extraction the polymer-coated (HR-52) fiber-packed capillary (150 mm × 0.32 mm, i.d.) was used at the extraction of 0.5 ml sample volume. The LLQ for DEHP in the waste water was calculated (with S/N = 10) as <0.1 ng/ml (Table 1).

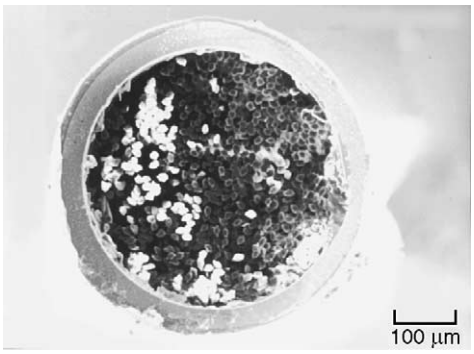

Fig. 7. Scanning electron microscopy image of the cross-section of the fiber-packed fused-silica capillary (0.53 mm, i.d.).

The extraction efficiency for all phthalates studied was improved with polymer coating treatment to the surface of packed-filaments. Since the selective extraction has been also reported for the extraction of other class of compounds, such as tricyclic antidepressants (TCAs) having different polarities [13,18], the selectivity is probably due to a good combination of the polarities of the polymer coating and analyte [15]. These results clearly suggest that a selective extraction can be established with an appropriate type of polymer coating, because a similar trend was already reported during the comparison of various coatings for in-tube SPME [19].

Table 1
Lowest limit of quantification (LLQ) and recovery for phthalates in water sample using HR-52 polymer-coated fiber-packed capillary (150 mm × 0.32 mm, i.d.)

Analyte	LLQ (ng/ml)	Recovery (%) ($n = 5$)
DEP	0.15	28.2 ± 2.0
DPP	0.15	46.4 ± 2.3
DBP	0.10	48.7 ± 1.8
DHP	0.12	61.3 ± 2.5
DEHP	0.09	100.1 ± 2.0
DOP	0.18	65.8 ± 2.8

Conditions: extraction flowrate and time, 40 μl/min for 12.5 min; column, Develosil ODS-UG-5 (150 mm × 0.53 mm, i.d.); detection, UV at 230 nm. Mobile phase (methanol–water) for DEP, DPP and DBP was (90:10), while for DHP, DEHP and DOP it was (95:5). Other conditions are in the text.

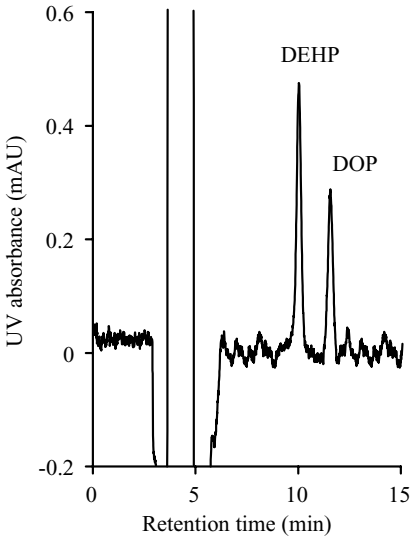

Fig. 8. Chromatogram of a waste water sample. Extraction conditions: extraction flowrate and time, 40 μl/min for 12.5 min. Chromatographic conditions: column, Speriorex ODS (150 mm × 1.0 mm, i.d., Shiseido); mobile phase composition and flowrate, methanol–water (90:10) at 50 μl/min; detection wavelength, 230 nm. Other conditions are in the text. The concentrations of DEHP and DOP were determined as about 1.5 and 2.0 ng/ml, respectively.

4. Conclusion

Miniaturized sample preparation with fiber-packed capillary has been developed for the sample preparation in micro-scale liquid phase separation methods. Because of the well-designed size and configuration, the miniaturized extraction cartridge could be even installed into the rotor of a commercially available micro-injector. The developed FIT-SPE technique demonstrates a practical applicability for the sample preparation of complex sample matrices such as waste water.

The coating by typical open-tubular GC stationary phases to the packed filaments was also studied. With an appropriate type of polymeric coating onto the fibrous material, further enhancement of extraction performance has been demonstrated. Although the preparation conditions of the extraction capillary and the extraction conditions for other class of sample matrices should be further investigated for various coated fiber packed capillaries, the results in the present study clearly showed the future development of novel tailored-made fibrous extraction media along with the another possibility as the separation media. The applications of polymer-coated and/or surface-derivatized fibrous materials in miniaturized sample preparation process are currently being investigated along with the employment of similar fibrous separation media as the new type of the stationary phase materials in various chromatographic techniques such as GC [24,25].

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