Polyethylene Terephthalate Nanofiber Sheet as the Novel Extraction Medium for the Determination of Phthalates in Water Samples

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A novel extraction medium was developed by packing polyethylene terephthalate (PET) nanofiber sheet having a diameter of 500 nm into a stainless-steel capillary of 0.8 mm inner diameter. The nanofiber was prepared by a carbon dioxide (CO2) laser supersonic multi-drawing method, which has a significantly higher surface area than the original PET fiber. A nanofiber sheet was prepared by winding the nanofibers. Extraction of phthalates in water samples by a PET nanofiber-packed extraction capillary was investigated using a conventional high-performance liquid chromatography (HPLC). Water samples were introduced into the extraction capillary with a low pressure. After extracting the water sample, the extraction capillary was directly connected to a six-port valve of HPLC with a PEEK nut, and the extracted analytes were desorbed, followed by injection to an HPLC system using a small amount of organic solvent. In this manuscript, the fundamental performance of the nanofiber sheet-packed extraction capillary for the extraction of organic compounds in water samples is quantitatively evaluated using a conventional HPLC system.

Keywords PET nanofiber, nanofiber sheet, extraction, phthalates, HPLC

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Introduction

Nanomaterials, such as nanoparticles and nanofibers, have been focused on due to these unique properties and a high surface area.1 Nanofiber is a fiber with a diameter in the nanometer range. The most commonly used process for preparing nanofiber is an electrospinning method because of its versatility.2 Electrospinning is a nanofiber production method based on an electrical force to a polymer solution. However, this method requires the use of an organic solvent, and therefore it has been reported that the existence of a residual solvent in the nanofiber might cause a problem.3 Carbon dioxide (CO2) laser supersonic drawing (CLSD) is an alternative technique to prepare nanofibers.4 In this method, a nanofiber is produced by the irradiation of a CO2 laser on an as-spun fiber in a supersonic jet. The CO2 laser supersonic drawing has been applied to the preparation of various nanofibers, such as poly(l-lactic acid) (PLA),5 polyethylene terephthalate (PET),6 and polypropylene.7 Furthermore, a nanofiber sheet was fabricated by the winding of nanofiber.3

Sample preparation is the most important process for the pretreatment of complex samples prior to analytical processes, such as gas chromatography (GC) or high-performance liquid chromatography (HPLC). Especially, miniaturized extraction devices are one of the most important approaches because they enable reducing the amount of sample and solvent, as well as the analytical time cost.9–11 Our research group have developed a miniaturized extraction capillary for the HPLC analysis of organic compounds. The extraction capillary was prepared by packing a particulate extraction medium into a stainless-steel capillary of 0.8 mm i.d. and 1.6 mm o.d. The extraction capillary can be directly connected to a conventional six-port valve with a poly(ether ether ketone) (PEEK) nut, and extracted analytes were desorbed by a small amount of desorption solvent. Therefore, a sensitive determination of the extracted analytes could be easily accomplished without any contamination from the atmosphere. We had reported on applications of the extraction capillary for the analysis of gaseous formaldehyde12 and aqueous formaldehyde13 in HPLC, and also aqueous formic acid in ion-chromatography.14 A miniaturized extraction device packed with synthetic polymer fiber had been developed.15,16 They introduced a Zylon filament as the extraction medium. The diameter of the Zylon filament was about 11.5 μm, and typically about 310 filaments were packed into PEEK tubing of 0.25 mm i.d.17 Further miniaturized extraction devices were reported.18,19 The extraction medium using nanofibers prepared by the electrospinning method have been also reported by many researchers.20,21 A nanofiber-composite membrane filter prepared by a melt spinning method was also reported for the enrichment of nickel in water samples.22

Phthalates are widely used as plasticizers for increasing the flexibility of polyvinyl chloride (PVC). However, some phthalates are regarded as being endocrine-disrupting compounds. Therefore, many countries have restricted the use of phthalates in children’s toys and child-care products.23–25 In addition, phthalates have been detected from various air26 and

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water samples, such as river water and tap water. The Ministry Health, Labour and Welfare Japan has established reference values for dibutyl phthalate (DBP) of 10 μg L⁻¹ and di(2-ethylhexyl)phthalate (DEHP) of 80 μg L⁻¹, respectively, in tap water (drinking water), and the World Health Organization has established a drinking water guideline for DEHP to be 8 μg L⁻¹.

In this study, a novel extraction capillary that is packed with a CO₂ laser supersonic drawing PET nanofiber sheet was developed. With the extraction capillary, the extraction of organic compounds in water samples was studied. As the target analytes, two typical phthalates, DBP and DEHP, were investigated.

**Experimental**

**Chemicals**

DBP and DEHP were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Methanol and acetonitrile (ACN) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

**Collection devices**

The nanofiber was prepared by the irradiation of a CO₂ laser (4 W) on as-spun original PET fiber (100 μm diameter) in a supersonic jet. The details for preparing the nanofiber are described elsewhere. The diameter of the nanofiber was approximately 500 nm, and the smooth surface of the nanofiber was observed by a scanning electron microscope image. Seven nanofibers were captured on a winding spool rotated at a constant speed, and a nanofiber sheet was prepared (12 cm wide) (Fig. 1).

An extraction capillary packed with a nanofiber sheet was prepared by a similar procedure as in previous studies (Fig. 2): (1) an appropriate length of poly(vinylidene fluoride) (PVF) fishing line was inserted into a stainless-steel capillary (0.8 mm i.d., 1.6 mm o.d., 15 cm length) as a guide fiber; (2) the nanofiber sheet (1.5 cm length and 12 cm wide, 3.8 mg) was rolled, and the rolled nanofiber sheet was inserted into the loop of the guide fiber; (3) the guide fiber was carefully pulled to introduce the nanofiber sheet into the capillary, and then the guide fiber was removed; (4) the nanofiber sheet was pushed by a stainless-steel rod; (5) finally, the capillary was cut to a 5.0-cm length by removing any unnecessary part. The packing length of the nanofiber sheet was approximately 2.5 – 2.6 cm. The void volume of the extraction capillary was approximately 25 μL.

An original fiber-packed capillary device was also prepared to compare both the extraction and desorption performances. Instead of a nanofiber sheet, 10 original PET fibers of 7.5 cm were inserted into the loop of the guide fiber, and the original fiber-packed capillary was prepared (20 filaments, packing length of 3.7 cm). The weight of the original packed fibers was 7.2 mg and the packing ratio, calculated by the cross section of fibers and the capillary, was 31.2%. The surface area of the nanofiber is theoretically 200 times greater than original fiber per unit weight.

**Collection and elution of the analytes**

For extraction of phthalates in water samples, a water sample of 100 - 1000 μL was collected in a glass syringe. The extraction capillary was connected to a Rheodyne Model 7725 injection valve (Cotati, CA) using a PEEK nut, as illustrated in Fig. 3, and the sample solution was introduced to the extraction capillary at a flowrate of approximately 200 μL min⁻¹. A higher sample loading speed was difficult due to the resistance of the nanofiber sheet. The extraction capillary can also be connected to the syringe using PTFE tubes. After sample loading and extraction, the extraction capillary was connected to a six-port valve (Rheodyne 7010) having 20 μL of the sample loop using another PEEK nut. A syringe containing the desorption solvent was connected to another side of the extraction capillary via the Rheodyne injection valve, and the desorption solvent was introduced into the extraction capillary from the opposite side of
the water sample introduction. Desorption of the extracted analytes as accomplished in two steps: first, 25 μL of the desorption solvent was introduced into the extraction capillary, and held for 5 min. Then, 25 μL of the desorption solvent was additionally loaded, and injection was made by switching a six-port valve. After desorption, the extraction capillary was washed with 100 μL of the desorption solvent, and dried by passing air. The extraction capillary was then reused for the next measurement. By employing this cleaning process, all of the analytes were completely eluted from the extraction capillary, and any analytes were detected in the next measurement. The nanofiber sheet-packed extraction capillary can be reused more than 50 times without any significant decrease of the extraction and desorption performances.

**HPLC measurements**

Chromatographic separation was performed using two JASCO PU-980 pumps (JASCO, Tokyo, Japan), a DG-980-50 degasser, a CO-265 Plus column oven, and a PU-2080 Plus UV-Vis detector (JASCO). The detection wave length was set at 230 nm. A Chromato-PRO data integrator (Run Time Instruments, Tokyo, Japan) was used for data acquisition. For separation, an octadecylsilica analytical column (5 μm particle size, 4.6 mm diameter, 150 mm long; Shinwa Chemical Industries, Kyoto, Japan) was employed. A six-port valve with 20 μL of the sample loop was used for sample injection. As the mobile phase, 100% acetonitrile was used at a flow rate of 1 mL min⁻¹. The column temperature was kept at 35°C. A low-volume precolumn filter (0.5 μm frit, GL Sciences, Tokyo, Japan) was placed in front of the separation column.

**Results and Discussion**

**Evaluation of extraction and desorption performances**

The extraction ratio of the two extraction capillaries (nanofiber sheet-packed and original fiber-packed capillaries) for the target analytes was investigated. First the extraction capillary was connected to the six-port valves, as illustrated in Fig. 3. Then, 100 μL of the standard aqueous sample solution, including 0.5 mg L⁻¹ of DBP and 1.0 mg L⁻¹ of DEHP, was introduced into the extraction capillary. The sample solution that passed through the extraction capillary was then analyzed by HPLC. The extraction ratio was calculated on the basis of a comparison of the peak areas obtained by the extraction capillary passed solution and that by direct injection of the same sample solution (without sample loading to the extraction capillary). If analytes were not detected from the extraction capillary passed solution, the extraction ratio of the capillary was calculated to be 100%. The results of the extraction ratios of DBP and DEHP using two extraction capillaries are given in Table 1. Although the original fiber-packed extraction capillary showed an insufficient extraction ratio for the analyte phthalates, these compounds were completely extracted by the nanofiber sheet-packed extraction capillary. This result could be interpreted as an advantageous feature of the nanofiber having an extremely high surface area. In addition, a mesh-patterned sheet could contribute to increasing the extraction ratios due to increasing the contact opportunities of the analytes to the nanofiber.

The desorption recovery of the extracted analytes from the nanofiber sheet-packed extraction capillary was calculated by the peak area obtained in the first desorption to the total peak areas obtained in the first, second and third desorptions from the extraction capillary. The volume of the desorption solvent and the desorption time were optimized in a preliminary experiment.

| Extraction recoveries of extraction capillaries for analytes | Extraction recovery, % |
|-------------------------------------------------------------|------------------------|
| Original fiber packed                                      | Nanofiber sheet packed |
| DBP                                                         | 74.2                   | 100                    |
| DEHP                                                        | 36.9                   | 100                    |

| Desorption recoveries of extraction capillaries for analytes | Desorption recovery, % |
|-------------------------------------------------------------|------------------------|
| Methanol                                                   | ACN                    |
| DBP                                                        | 88.8                   | 100                    |
| DEHP                                                       | 50.0                   | 63.4                   |

Sample: 100 μL of 1 mg L⁻¹ of DBP and 0.5 mg L⁻¹ of DEHP solution.

The volume of the desorption solvent of more than 50 μL led to decreasing the peak area of the analytes. The second desorption was made after the 10 min (analytical time of the analytes in the first desorption) from the first desorption. Table 2 gives desorption recoveries of the analytes using methanol and ACN as the desorption solvent. Because ACN showed a better desorption recovery than methanol, ACN was chosen as the desorption solvent. Before sample loading, no compounds were eluted from the nanofiber sheet-packed extraction capillary by loading ACN; however, DEHP was continuously eluted from original fiber-packed extraction capillary by loading ACN. This DEHP elution means that DEHP was originally contained in the original fiber, and it was not adsorbed from the surface of the original fiber. On the other hand, DEHP was not eluted from the nanofiber sheet-packed extraction capillary. This could be due to the evaporation of DEHP by upon CO₂ laser irradiation. The results also suggest that other volatile compounds included in the original PET fiber might be evaporated during CO₂ laser irradiation and therefore, they could not be included in nanofibers after irradiation.

The sample loading capacity of the nanofiber sheet-packed extraction capillary was investigated by introducing a standard aqueous solution (0.5 mg L⁻¹ of DBP and 1.0 mg L⁻¹ of DEHP) into the extraction capillary. The peak areas for both DBP and DEHP linearly increased with increasing sample loading volume from 100 to 1000 μL with a correlation coefficient (r) of more than 0.99, and a satisfactory sample loading capacity of the nanofiber sheet-packed extraction capillary was confirmed. The sample loading time needed for loading 1000 μL of an aqueous solution was approximately 5 min.

**Evaluation of the method**

The limit of detection (LOD) and the limit of quantification (LOQ) of the method were determined as to be a signal-to-noise ratio of 3 and 10, respectively. The LODs and LOQs for a sample loading volume of 1000 μL are given in Table 3. For a comparison of the obtained LODs, the LODs obtained by direct injection into HPLC are also listed in the table. Obviously, greater sensitivities were obtained by the simply extraction process using the nanofiber sheet-packed extraction capillary, and the proposed method could have acceptable sensitivity for monitoring of pollution by DBP and DEHP in real samples.
Linear calibration curves ($r > 0.99$) in the range from LOQs to 1000 $\mu$g L$^{-1}$ were obtained for both DBP and DEHP. The relative standard deviations of the peak area of DBP and DEHP on five consecutive injections were 5.3 and 11%, respectively for extraction of 1000 $\mu$L of the standard solution (10 $\mu$g L$^{-1}$ of DBP and 50 $\mu$g L$^{-1}$ of DEHP). Since the proposed method employing the on-line desorption and injection of the extracted analytes, risk of the pollution of the analytes can be minimized, and a sensitive analysis be achieved.

The additional recoveries of spiked DBP and DEHP from tap-water and river-water samples were investigated. Both analytes were not detected from the two tap-water samples (Kofu, Japan). In addition, these compounds were not detected in two river-water samples (Kofu, Japan) either. One of the reasons for this could be due to avoiding the use of phthalates in greenhouse covers. Then, the recovery was calculated by comparing the peak area of the analytes obtained by the standard solution and spiked samples (DBP: 10 and 2 $\mu$g L$^{-1}$; DEHP 50 and 10 $\mu$g L$^{-1}$). The additional recoveries of DBP and DEHP from tap-water samples were from 97.2 to 102%, and river-water samples were from 94.6 to 102%, and quantitative recoveries were obtained. Figure 4 indicates the typical chromatogram for the determination of spiked DBP and DEHP in a river sample (A). As shown in the figure, no interference peak was observed, and spiked analytes were quantitatively determined from the river-water sample.

### Conclusions

A novel extraction capillary packed with a nanofiber sheet prepared by CLSD was developed, and its fundamental extraction and desorption performances for DBP and DEHP in water samples were quantitatively evaluated. Because of an extremely high surface area of nanofiber, the successful extraction of analytes and a high sample loading capacity were confirmed. Furthermore, good sensitivity and repeatability were obtained owing to reducing contamination risk by on-line extraction/desorption. The elution of contaminants from the nanofiber sheet was not observed due to the evacuation of contaminants by CO$_2$ laser irradiation. Since preparing nanofibers by CLSD does not use any organic solvents, the method is beneficial to the environment and health risks. Because of an easy and inexpensive preparation, the device is regarded as being a disposable device. Further application of the nanofiber sheet-packed extraction capillary could be expected for the extraction of various organic compounds from water samples, such as polycyclic aromatic hydrocarbons and pesticides, including the use of other types of nanofiber sheet, like PLA.

### References

1. Kenry and C. T. Lim, *Prog. Polym. Sci.*, 2017, 70, 1.
2. N. Bhardwaj and S. C. Kundu, *Biotechnol. Adv.*, 2010, 28, 325.
3. K. Morikawa, M. Green, and M. Naraghi, *Procedia Manuf.*, 2018, 26, 205.
4. A. Suzuki and T. Okano, *J. Appl. Polym. Sci.*, 2004, 92, 2989.
5. A. Suzuki and K. Aoki, *Eur. Polym. J.*, 2008, 44, 2499.
6. A. Suzuki and K. Tanizawa, *Polymer*, 2009, 50, 913.
7. A. Suzuki and K. Arino, *Eur. Polym. J.*, 2012, 48, 1169.
8. A. Suzuki, K. Hosoi, and K. Miyagi, *Polymer*, 2015, 60, 252.
9. Y. Saito and I. Ueta, *Chromatography*, 2017, 38, 85.
10. I. Ueta, Y. Nakamura, S. Kawakubo, and Y. Saito, *Anal. Sci.*, 2018, 34, 201.
11. I. Ueta, R. Takenaka, K. Fujimura, S. Narukami, T. Sasaki, and T. Maeda, *Anal. Sci.*, 2019, 35, 855.
12. I. Ueta, S. Mochizuki, S. Kawakubo, T. Kuwabara, K. Jinno, and Y. Saito, *Anal. Bioanal. Chem.*, 2015, 407, 899.
13. I. Ueta, S. Mochizuki, S. Kawakubo, T. Kuwabara, and Y. Saito, *Anal. Sci.*, 2015, 31, 99.
14. I. Ueta, M. Kajimoto, and Y. Saito, *Chromatography*, 2019, 40, 33.
15. K. Jinno, M. Ogawa, I. Ueta, and Y. Saito, *TrAC, Trends Anal. Chem.*, 2007, 26, 27.
16. Y. Saito, M. Kawazoe, M. Imaizumi, Y. Morishima, Y. Nakao, K. Hatano, M. Hayashida, and K. Jinno, *Anal. Sci.*, 2002, 18, 7.
17. Y. Saito, Y. Nakao, M. Imaizumi, Y. Morishima, Y. Kiso, and K. Jinno, *Anal. Bioanal. Chem.*, 2002, 373, 81.
18. Y. Saito, M. Imaizumi, T. Takeichi, and K. Jinno, Anal. Bioanal. Chem., 2002, 372, 164.
19. Y. Saito, M. Nojiri, M. Imaizumi, Y. Nakao, Y. Morishima, H. Kanehara, H. Matsuura, K. Kotera, H. Wada, and K. Jinno, J. Chromatogr. A., 2002, 975, 105.
20. S. Chigome and N. Torto, TrAC, Trends Anal. Chem., 2012, 38, 21.
21. M. Háková, L. C. Havlíková, J. Chvojka, F. Švec, P. Solich, and D. Šatínský, Anal. Chim. Acta, 2018, 1018, 26.
22. H. Mizuguchi, R. Ishida, Y. Kouno, T. Tachibana, T. Honda, T. Kujima, Y. Yamamoto, and T. Takayanagi, Anal. Sci., 2018, 34, 907.
23. “Commission Conclusions on the Review Clause of REACH Annex XVII, Entry 51 (DEHP, DBP, BBP)”, European Commission, 2014, Brussels.
24. “Prohibition of Children’s Toys and Child Care Articles Containing Specified Phthalates”, Consumer Product Safety Commission, 2008, Washington, D.C.
25. “Specifications and Standards for Foods, Food Additives, etc. (MHLW Notification No. 336)”, Ministry of Health, Labour and Welfare (MHLW), 2010, Tokyo.
26. I. Ueta, R. Takenaka, K. Fujimura, T. Yoshimura, S. Narukami, S. Mochizuki, T. Sasaki, and T. Maeda, Anal. Sci., 2018, 34, 1149.
27. K. Hashizume, J. Nanya, C. Toda, T. Yasui, H. Nagano, and N. Kojima, Biol. Pharm. Bull., 2002, 25, 209.
28. J. Santana, C. Giraudi, E. Marengo, E. Robotti, S. Pires, I. Nunes, and E. M. Gaspar, Environ. Sci. Pollut. Res., 2014, 21, 1380.
29. “Drinking Water Guideline”, Ministry of Health, Labour and Welfare (MHLW), Tokyo.
30. “Guidelines for Drinking-water Quality, Fourth Edition Incorporating the First Addendum”, World Health Organization, 2017.
31. Y. Saito, I. Ueta, M. Ogawa, A. Abe, K. Yogo, S. Shirai, and K. Jinno, Anal. Bioanal. Chem., 2009, 393, 861.
32. L. He, G. Gielen, N. S. Bolan, X. Zhang, H. Qin, H. Huang, and H. Wang, Agron. Sustain. Dev., 2015, 35, 519.