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Review

Miniaturized sample preparation combined with liquid phase separations

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Abstract

Miniaturized sample preparation methods designed as the sample pretreatment for liquid phase separations, such as liquid chromatography, capillary electrophoresis and capillary electrochromatography, have been reviewed especially for the on-line coupling of the sample preparation process and the separation process. The development of the desorption interfaces for the effective combining of the sample preparation and subsequent liquid phase separations is briefly described along with the applications of the combined analytical systems to the analysis of complex sample mixtures such as biological and environmental matrices. Novel use of fine polymeric filaments as the extraction medium for microscale liquid phase separation methods are investigated and a comparison is made with other sample preparation techniques. Polymer coating onto the fibrous material is also introduced to further develop microscale sample preparation methods with improved extraction performance. Several other microscale sample preparation methods having a potential compatibility to the liquid phase separations are also described for future applications of these techniques.

Keywords: Reviews; Sample preparation; Solid-phase microextraction; Microcolumns; Miniaturization

Contents

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1. Introduction

Miniaturized sample preparation methods have been regarded as the most attractive techniques for the pretreatment of complex sample mixtures prior to the chromatographic process, especially in microscale separation systems. An effective on-line coupling of the miniaturized sample preparation and the microcolumn separation enables to take various advantageous features of the combined system, such as:

(1) high speed analysis with high efficiency;

(2) low cost operation due to extremely low or no solvent consumption;

(3) development of environmentally friendly analytical procedure; and

(4) highly selective analysis by developing tailored systems designed for particular applications.

Considering the above advantages and also the recent requirements for the analytical process, the miniaturization of the analytical methods and instrumentation should be studied for a wide range of applications such as environmental analysis, toxicological and forensic drug analysis, and other analytical situations.

As a microscale sample preparation method for gas chromatographic (GC) analysis of volatile compounds, one of the typical examples is solid-phase microextraction (SPME) developed by Pawliszyn et al. [1–5]. In the technique, a fused-silica rod with a polymeric coating on the surface is employed as the extraction medium for the extraction of volatile analytes from aqueous sample solution or the headspace of the solution. Desorption of extracted analytes can be carried out by the heating of the SPME fiber in a conventional GC injector, allowing the SPME device to have a good compatibility with GC system. For the analysis of nonvolatile and/or thermally liable compounds, however, a specially designed desorption device is needed to accomplish the coupling of the SPME to liquid-phase separation systems, such as liquid chromatography (LC) [6–14], supercritical fluid chromatography (SFC) [15,16], capillary electrophoresis (CE) [17] and micellar electrokinetic chromatography (MEKC) [18,19]. Introducing a section of open-tubular capillary GC column as the extraction medium, in-tube SPME has been developed for an effective on-line coupling of sample preparation and LC separation and the automation of the hyphenated system has been also reported for the analysis of various classes of compounds [31–47], although the employment of packed [20–23] and open-tubular capillaries [24–30] in the extraction process was reported earlier. In addition to several conventional coatings used widely as GC stationary phases, novel polymeric coating materials have been developed for a selective extraction [48–55].

For the on-line coupling of sample preparation and microcolumn separations, a novel miniaturized sample preparation method has been developed recently by Jinno and co-workers [56–64]. In the newly developed method, fiber-in-tube solid-phase extraction (FIT-SPE), the extraction was accomplished in a short capillary, which is packed longitudinally with several hundred filaments of synthetic polymer as the extraction medium. Because of the parallel arrangement of the filaments to the outer tubing, a number of coaxial narrow channels are formed in the capillary. Therefore, the FIT-SPE device shows a reduced pressure drop during the extraction and desorption comparing with conventional particle-packed SPE cartridge, and also the undesirable plugging from insoluble and/or particulate materials in real sample matrices can be significantly reduced in the FIT method. It has been also demonstrated that an effective interaction of the sample solution with a number of the fine fibrous extraction media in the extraction capillary could enable further miniaturized as a microscale sample preconcentration device. Further downsizing of the extraction device will also allow the direct coupling of the extraction process

with microcolumn separation methods, but without any disadvantages such as overloaded sample injection and poor resolution during the chromatographic separations.

In this review, the on-line coupling of miniaturized sample preparation techniques and microcolumn liquid phase separation methods, such as micro-LC, CE and CEC has been overviewed along with the typical applications of these combined systems for the analysis of complex sample mixtures, such as biological and environmental matrices.

2. SPME preconcentration for liquid chromatographic and electrodriven separation methods

2.1. Desorption interface

Immersing the SPME fiber in the sample solution, the extraction analytes of interest from the matrix is typically carried out as shown in Fig. 1. The SPME rod with a polymeric coating on the surface can be stored in the needle of the device to protect the coating and the rod from damage during the insertion to a septum of GC injection port and the extraction vial. Although the solution should be thermostated along with the continuous stirring for the reproducible extraction, the optimization of the extraction condition including the selection of the fiber coating can be made systematically as the preliminary experiments [5]. For the extraction of volatile analytes, headspace sampling is also possible, where the SPME fiber is inserted into the headspace of the sample vial during the extraction. Because the desorption of extracted analytes is accomplished easily by the heating of the SPME fiber in the GC injector, the SPME device have a good compatibility with GC separation system without an additional desorption device [1–4]. In contrast to the above successful application for volatile compounds analysis by GC, however, a specially designed desorption interface should be introduced to the LC analysis of nonvolatile and thermally liable compounds with SPME preconcentration.

As the desorption interface between SPME and micro-LC, Jinno and co-workers [11–16] developed a specially designed desorption device (Fig. 2), in which a modified T-shape connector was combined with a union and several tubes having an appropriate size in order to maximize the desorption performance and the compatibility to microscale separation systems, but to minimize undesirable extra volume

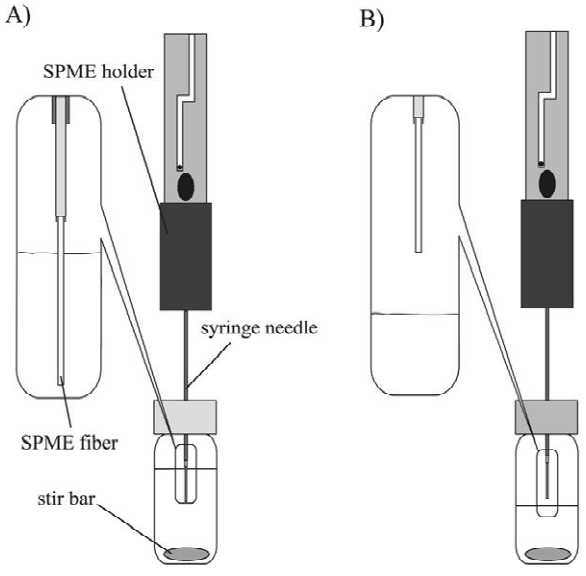


Fig. 1. Extraction from aqueous sample solution by conventional SPME device. (A) Liquid phase sampling and (B) headspace sampling.

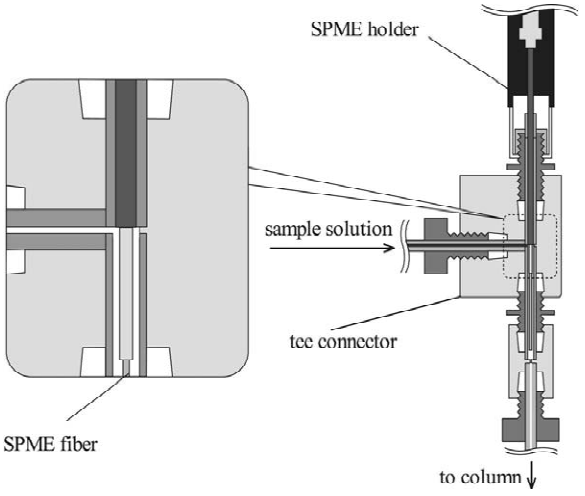


Fig. 2. Specially-designed desorption interface for the coupling of SPME and micro-LC [11–14].

during the desorption process. The SPME fiber is inserted from the top of the interface and a small amount of the desorption solvent is supplied from the port just above the SPME fiber using either a microsyringe or a micro-flow pump. The desorbed analytes are transferred to the loop of the LC injector. The desorption conditions, such as the volume of extraction solvent and the flow-rate, can be optimized as well as the selection of the desorption solvent. For the desorption by the flow of the LC mobile phase or a highly pressurized fluid, such as supercritical fluid (SF), other types of desorption interface have been developed by Pawliszyn and co-workers to accomplish the desorption under the pressurized conditions [6–10].

2.2. Applications of SPME to various complex sample matrices

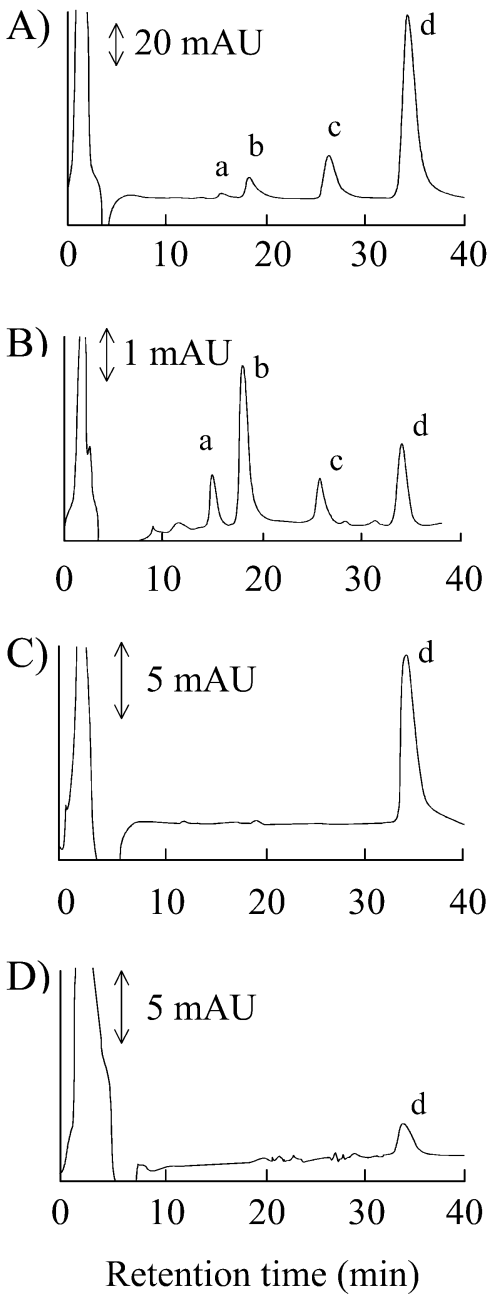
Typical examples of the SPME as the sample preparation method for liquid phase separations are summarized in Table 1, although numerous applications have been reported in several review articles and books mainly published by Pawliszyn and co-workers [5,32,74–80]. These extensive applications range over various scientific fields, such as pharmaceutical, environmental, agricultural and medical sciences as well as separation science. One of the main applications is the determination of trace amount of environmental pollutants such as polycyclic aromatic compounds (PAHs), pesticides and other chemical compounds. Alpendurada et al. [65] reported the determination of several PAHs spiked into a waste water sample by SPME–LC with photodiode-array detection. The extraction of aromatic amines and hydroxyaromatic compounds from lake water samples has been demonstrated by Huang et al. [66,67]. In order to obtain more sensitive detection and rapid identification, Pawliszyn et al. [9] introduced SPME–LC system with mass spectrometric (MS) detection for the analysis of water-soluble components in sludge and sediments. The determination of common pesticides in river water has been reported by Jinno et al. [11] using the SPME–LC

Table 1
Typical applications of SPME pretreatment for the analysis of complex mixtures by liquid phase separation methods[a]

Analyte	Sample matrix	Type of SPME fiber[b]	Separation–detection	Ref.
Polycyclic aromatic hydrocarbons	Water	PDMS	LC–UV	[6]
Polycyclic aromatic hydrocarbons	Waste water	PDMS	LC–UV	[65]
Aromatic amines	Surface water	PDMS–DVB, PA, CW–TPR, CW–DVB	LC–UV	[66]
Hydroxyaromatic compounds	Surface water	PDMS–DVB, CW–TPR	LC–UV	[67]
Alkylphenol ethoxylate surfactants	Water	PDMS, PA and several experimentally prepared	LC–UV	[7]
Nonionic surfactant	Water	PDMS–DVB	LC–UV	[68]
Diethylphthalates	Water	PDMS–DVB, CW–TPR	LC–UV	[69]
Phenols	Water	PA	CE–UV, MEKC–UV	[17]
Pesticides	Surface water	PA	LC–UV	[11]
Pesticides	Water	PA	SFE–LC–UV	[16]
Water soluble organic compounds	Sludge and sediments	CW	LC–MS	[9]
Hg(II) ion	Water	Polypropyrene microporous hollow fiber	LC–UV	[8]
Explosives	Water	CW–TPR	LC–UV	[70]
Benzodiazepines	Water	PA	MEKC–UV	[18]
Benzodiazepines	Human urine	PA, PDMS	LC–UV, LC–MS	[12]
Benzodiazepines	Human urine	PA, CW–TPR and sol–gel C_{11}PDMS	Micro-LC–UV	[13]
Tricyclic antidepressants	Human urine	PDMS	Micro-LC–UV	[14]
Lidocaine	Human urine	PDMS, PA	LC–UV	[71]
Corticosteroids	Human urine	CW–DVB	LC–MS	[72]
Erythromycin A	Water	PDMS–DVB	LC–MS	[73]

[a] A number of other of applications can be found in several reviews and books [5,32,74–79].
[b] Abbreviations: PDMS, polydimethylsiloxane; PA, polyacrylate; DVB, divinylbenzene; CW, carbowax; TPR, templated resin.

system coupled by the specially designed desorption interface as described above. In these studies, the effect of the extraction conditions, such as the type of the (extraction) fiber coating, the extraction time and temperature, the effect of the agitation have been systematically investigated along with the effect of additives into the sample matrix to improve the extraction efficiency. The desorption conditions, such as the type of desorption solvent, the flow-rate for dynamic desorption and the desorption time have been also studied taking into account an effective on-line coupling with subsequent chromatographic separation process.



Another major field of application could be found in the extraction of pharmaceutical compounds from biological fluids. Koster et al. [71] reported the analysis of lidocaine in urine by SPME–LC, and Volmer et al. [72] published the determination of eleven corticosteroids and two steroid conjugates in urine samples in SPME–LC–MS system. Jinno et al. have demonstrated the extraction of benzodiazepines and tricyclic antidepressants (TCAs) from human urine samples by SPME and the separation in microscale liquid phase separation methods such as micro-LC and MEKC [12,13,18]. Fig. 3 shows typical chromatograms for the separation of TCA drugs with the sample preconcentration by SPME prior to the separation. For the separation, a micro-column packed with polymer-coated octadecylsilica (Capcell Pak C_{18} UG, 5-μm particle size, 1.0 mm I.D.×150 mm length; Shiseido, Yokohama, Japan) was employed. The patient's urine sample was diluted 15-fold with a sodium borate buffer solution (5 m*M*, pH 9.0) in a 20-ml vial. Then, the solution was extracted by a SPME fiber coated with polydimethylsiloxane (PDMS) of 100-μm film thickness. The extraction and desorption conditions, such as extraction time and temperature, pH value of sample

Fig. 3. Typical chromatograms for the analysis of TCAs [14]. Separation of a standard sample containing 500 ng/ml each of four TCAs with (A) and without (B) SPME preconcentration. Chromatogram for a standard sample of amitriptyline (100 ng/ml) (C) and for a human urine sample containing amitriptyline (D) with SPME preconcentration. SPME conditions: fiber coating, PDMS; extraction time and temperature 180 min at 40 °C; pH of matrix, 9.0; desorption solvent and time, acetonitrile for 30 min. Micro-LC conditions: column Capcell Pak C_{18} UG80 (1.0 mm I.D.×150 mm; Shiseido, Yokohama, Japan); mobile phase composition and flow-rate, acetonitrile–water (50:50) containing 0.18% triethylamine at 50 μl/min; column temperature, 40 °C. Detection was made by a UV–Vis absorption detector typically monitored at 240 nm. Other conditions are in the text. Peaks: a=desipramine; b=nortriptyline; c=imipramine and d=amitriptyline.

matrix, salt concentration, volume of desorption solvent and desorption time, were systematically determined by the preliminary experiments [14]. Comparing the chromatograms obtained with SPME (Fig. 3A) and without SPME (Fig. 3B), one can observe a good preconcentration power of this method. Although the extraction power for each TCAs with PDMS is different mainly due to the polarity, a good extraction performance was demonstrated for the analysis of human urine samples as shown in Fig. 3D, where the concentration of amitriptyline in the original urine sample was determined as 0.49 μg/ml taking into account the dilution prior to the extraction process. The detection limits for some other common TCA drugs such as nortriptyline and imipramine were determined to be at the ng/ml level, which is quite acceptable detection performance in clinical and/or forensic situations. Because of the advantageous feature of the microscale system, the total solvent consumption in this particular example was less than 1.5 ml even including the mobile phase required for the micro-LC separation.

An extensive review of SPME pretreatment for drug analysis was published by Lord et al. [76], in which various parameters of the extraction and desorption conditions, type of the SPME fibers and their extraction characteristics, and calibration methods required for reproducible quantification were described as well as the applications for drug analysis. Kataoka et al. [79] also reviewed the applications of the SPME techniques for food analysis by GC and LC. Other applications can be found elsewhere [77,78].

3. On-line coupling of in-tube SPME and liquid phase separations

3.1. In-tube SPME

With commercially available open tubular GC columns, an alternative SPME method, in-tube SPME, which is enabled to be directly coupled with LC separation system without any specially-designed interfaces, has been developed by Pawliszyn and co-workers [31–33]. In their approach, a section of GC capillary column is employed as the extraction medium, and the analytes in an aqueous solution are extracted to the polymeric material coated onto the inner wall of the capillary by passing the sample solution through the capillary using a micro-flow pump. The desorption process is carried out by passing a small amount of organic solvent (or mobile phase) into the capillary in a similar manner. The absence of the desorption interface [6–19], which was needed for the transfer of the extracted solutes to the subsequent liquid phase separation system, allowed the elimination of a difficult manual handling process and further reduced the volume of the organic solvent required for the conventional desorption process in SPME–LC.

The variety of commercially available polymer coatings, i.e. liquid phases of the GC capillary is another advantageous feature of in-tube SPME as well as the compatibility to an automated separation system which makes the system useful for the extraction of particular classes of compounds [35–38].

3.2. Applications of in-tube SPME

Upon successful development of the automated in-tube SPME–LC system by Pawliszyn and co-workers [31–33], various applications have been published. The preconcentration of several pharmaceutical compounds from biological fluids and the determination with the hyphenated system was reported by Kataoka and co-workers along with the on-line coupled MS detection [34–37]. Applications for food analysis have also been reported [38,39,79,80]. Gou and co-workers [40–42] described the reproducible extraction of carbamate pesticides from water with an automated in-tube SPME–LC system, and Takino et al. [81] studied the determination of several common herbicides in environmental water samples by in-tube SPME–LC–MS. With mass-selective detection, Mester and co-workers [43–45] investigated the preconcentration, separation and determination of organolead and organotin compounds in environmental water samples.

The development of novel polymer coatings for the extraction capillary has been also reported. Wu and co-workers [48–54] introduced a polypyrrole (PPY)-coated capillary as the extraction capillary for

the determination of β-blockers in human urine and serum samples, and aromatic compounds in drinking and lake water samples. They also reported the speciation of organoarsentic compounds by in-tube SPME–LC–MS system with PPY-coated extraction capillary [51], as well reviewing the preparation and application of PPY-coated capillaries for in-tube SPME [53]. Further selectivity enhancement was demonstrated by Mullet and co-workers [54,55] by using a molecularly imprinted polymer (MIP) as the extraction phase material. A comprehensive review of the in-tube SPME applications for various sample matrices was also published by Kataoka [82].

4. Miniaturization of extraction device and the effective on-line coupling to microscale liquid phase separation methods

4.1. Wire-in-tube

By inserting a stainless steel wire into the extraction capillary of in-tube SPME, the internal volume of the capillary could be significantly reduced while the surface area of the polymeric coating material contacting the sample solution was maintained as the same as shown in Fig. 4. After the insertion of the wire of 0.20 mm O.D., the internal volume of the extraction capillary was reduced from 19.6 μl to 7.1 μl for a capillary of 40 cm×0.25 mm I.D., leading the change in the phase ratio from about 500 to 180 with a polymeric coating of 0.25 μm thickness. With this wire-in-tube configuration (Fig. 4B), more effective extraction could be obtained. More efficient on-line coupling of the extraction process to microcolumn separation process has been established by Saito et al. The authors reported its successful application to environmental analysis [60], such as the determination of phthalates in river water and waste water, and to biological analysis [47] such as the identification and quantification of TCAs in human fluids. The results have also indicated that further preconcentration compared to the conventional in-tube SPME method could be obtained with this modification and that the on-line wire-in-tube SPME–micro-LC system has a great potential for the fast analysis of various organic compounds in other complex sample matrices.

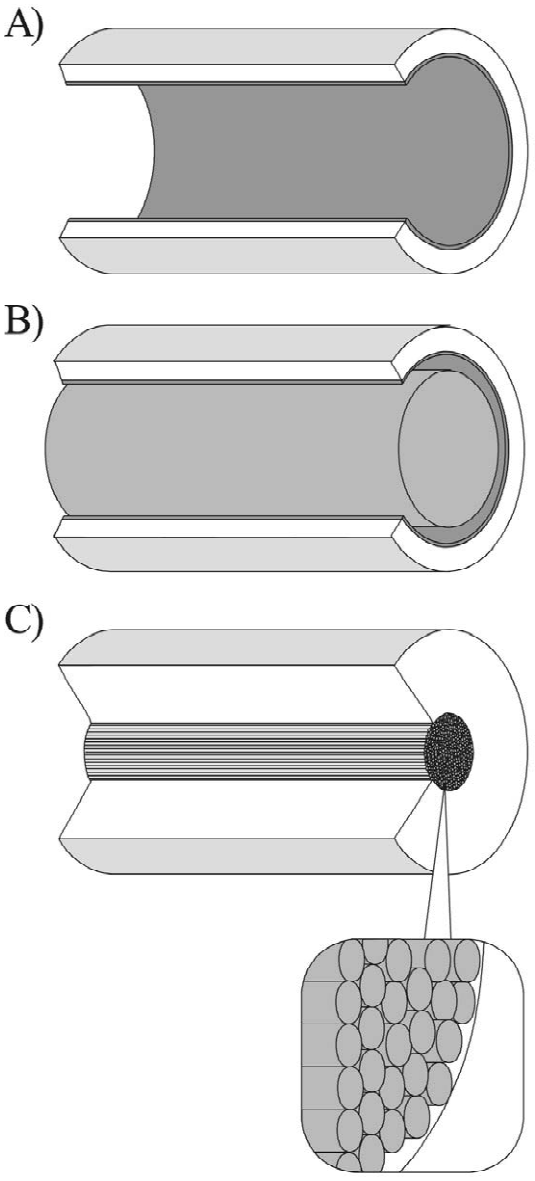


Fig. 4. Illustrations of three types of extraction capillaries. (A) In-tube [31], (B) wire-in-tube [47] and (C) fiber-in-tube [56].

4.2. Fiber-in-tube

Recently, the use of polymer filaments as the extraction medium has been reported. The technique, FIT-SPE, in which several hundreds of fine filaments of polymeric material are packed longitudinally into a short capillary of polyetheretherketone (PEEK) or

polytetrafluoroethylene (PTFE), is developed on the basis of the successful applications of wire-in-tube extraction tube [47,60]. Not only to reduce the internal void volume of the extraction tube, fine polymer filaments can also be employed as the extraction media as shown in Fig. 4C. Taking into account the chemical structure, the solvent resistance for the mobile phase and mechanical strength during the packing process of the fiber, a rigid-rod heterocyclic polymer, Zylon® (Toyobo, Ohtsu, Japan) was firstly selected as the fibrous extraction medium [56], although the effectiveness of other several solvent-resistant fibers was confirmed for other classes of compounds [64]. To prepare the typical extraction tube, the fiber was cut to 100-mm lengths and packed longitudinally into the same length of PEEK tube (0.25 mm I.D., 1/16 inch O.D.). The diameter of each filament of the fiber is about 11.5 μm and the typical number of the filaments packed in the PEEK tubing of 0.25 mm I.D. is about 310.

Fig. 5 illustrates the on-line coupling of FIT-SPE and micro-LC, where the extraction tube (FIT) is installed between the switching valve and the injection valve, and two syringe pumps (one for sample solution and another for desorption solvent) are connected to the switching valve. For the extraction, the sample solution was pumped through the extraction tube by one of the syringe pumps, typically at the flow-rate of 16 μl/min. After changing the position of the switching valve, the desorption solvent was also pumped by another syringe pump. Then, the injection was made. In Fig. 6, typical chromatograms for the analysis of di-2-ethylhexyl phthalate (DEHP) in waste water samples are shown. With FIT-SPE preconcentration, the peak

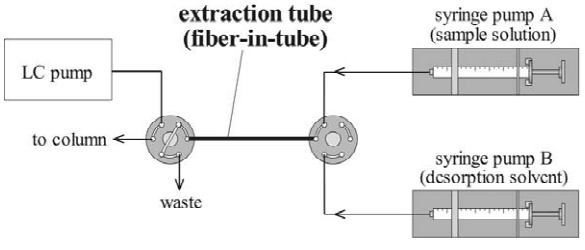


Fig. 5. On-line coupled analytical system of fiber-in-tube SPE and micro-LC [56,59].

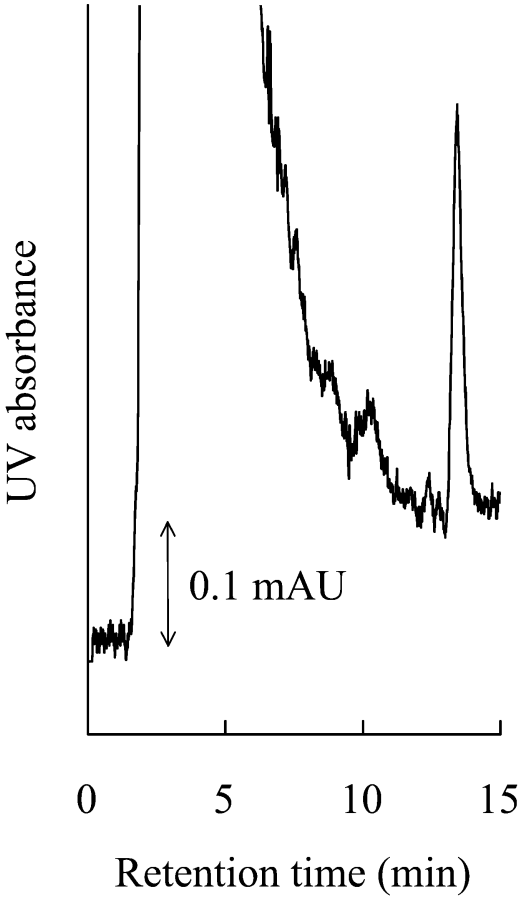


Fig. 6. Chromatogram for the determination of DEHP in a waste water sample [59,60]. Extraction conditions: extraction capillary, PEEK tubing of 0.25 mm I.D.×100 mm packed with Zylon® filaments of about 330 (packing density: 70%); extraction flow-rate and time, 8 μl/min×40 min; desorption flow-rate and time, 4 μl/min×1.5 min; desorption solvent, methanol. Micro-LC conditions: column, Superiorex ODS (1.0 mm I.D.×150 mm; Shiseido); mobile phase composition and flow-rate, methanol–water (90:10) at 50 μl/min; detection, UV at 254 nm. Based on the analysis of standard samples, the concentration of DEHP (peak at 13.4 min) was determined as 1.40 ng/ml.

of DEHP was clearly observed and the peak was identified by the UV–Vis spectrum measurements [58,59]. The original concentration in the waste water was determined as 6.8 ng/ml by comparing the peak area of the standard sample. In this particular case, the direct injection was carried out with the standard sample of 1.0 μg/ml because no measurable peak was obtained without preconcen-

tration. The results clearly indicate that the fibrous extraction medium has a strong preconcentration power for the phthalates.

4.3. Miniaturization of FIT cartridge

Further miniaturization of the extraction medium has been investigated by Saito and Jinno and coworkers [57–61]. Fig. 7 shows the miniaturized FIT-SPE cartridge installed in the rotor of the Rheodyne Model 7520 injector (Rheodyne, Cotati, CA, USA) [53]. The microflow channel for the sample loading (the center hole in the rotor) was enlarged and the FIT cartridge was inserted. The extraction cartridge was prepared with a PEEK tube of 0.50 mm I.D.× 5.0-mm length packed by Zylon® of about 1500 filaments. The miniextraction cartridge was then sandwiched by two pieces of short blank PEEK capillaries, and installed into the hole in the modified rotor. By inserting appropriate size of PEEK capillaries to other flow-passes of the valve, the microinjector was further modified to reduce any undesirable extra volume. In the extraction process (Fig. 7A), the sample solution from a syringe pump is passed through the extraction cartridge, while the analytes in the sample solution are extracted onto the filaments packed therein. Next, the position of the injector is changed to ''desorption–injection'' as shown in Fig. 7B. The desorption and injection are made simultaneously by the flow of the mobile phase for micro-LC separation. Therefore, technically, no desorption solvent is needed for the sample preparation process.

The minicartridge can be prepared with a good reproducibility (normally the RSDs for the extraction power were less than 3.0%), and these cartridges also showed a good stability for repeatable use, typically more than 50 runs without any significant problems, such as an increase in the pressure drop and a decrease in the extraction power. In case, the extraction performance was slightly decreased after the sequential sample extraction of more than 50 times, a brief washing–reconditioning processes using an organic solvent could be carried out to ensure the reproducible results during the next 50 analyses. Taking the advantage of the microscale system with a packed capillary column of 0.53 mm I.D., the total solvent volume required for the typical analysis of phthalates in waste water is less than

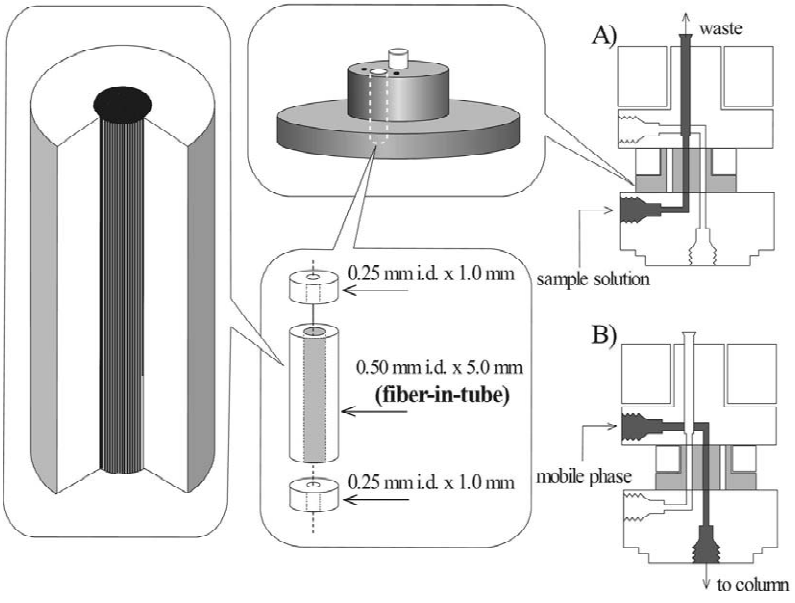


Fig. 7. Miniaturized fiber-in-tube extraction cartridge installed in a conventional microinjector (Model 7520, Rheodyne, Cotati, CA, USA) [59,60]. (A) Extraction and (B) desorption–injection processes.

40 μl including the solvent as the mobile phase component [59].

Similar miniaturization for the extraction tube has been studied in the sample preparation process in CE [57,60] and CEC [59,60]. Fig. 8A shows the overview of the FIT-SPE–CE system and the close-up of the specially designed extraction capillary installed in a cross-connector. In order to prepare the extraction capillary, about 250 filaments of Zylon® (10 mm length) were packed longitudinally into a DB-5 (5%-phenyl-polydimethylsiloxane) coated capillary (0.25 mm I.D.) of the same length, and the extraction capillary was then put into the end of the PTFE tubing which is connected to the fused-silica capillary from the syringe pump for sample pumping. For the analysis of TCAs in human urine, the sample solution was continuously supplied from the syringe, typically at the flow-rate of 80 μl/min for 12.5 min (therefore, the total sample volume pumped was 1.0 ml), during the extraction process. Next, the syringe was replaced by another syringe containing the desorption solvent, acetonitrile. By pumping an appropriate volume of acetonitrile, the desorbed analytes were transferred to the space in the modified cross connector as illustrated in Fig. 8A. When the zone containing the desorbed analytes was reached to the cross section of the separation capillary, the separation voltage was applied. The volume of the desorption solvent and the flow-rate could be optimized easily by several preliminary experiments. The calculated preconcentration factors for four TCAs, desipramine, nortriptyline, imipramine and amitriptyline, were more than 200 (Fig. 9). The

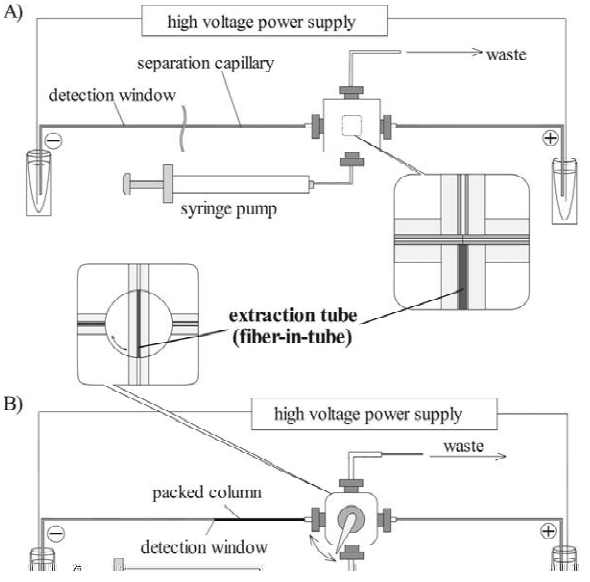


Fig. 8. On-line coupling of miniaturized fiber-in-tube SPE with electrodriven separation methods. The coupling with (A) CE [57] and (B) CEC [59].

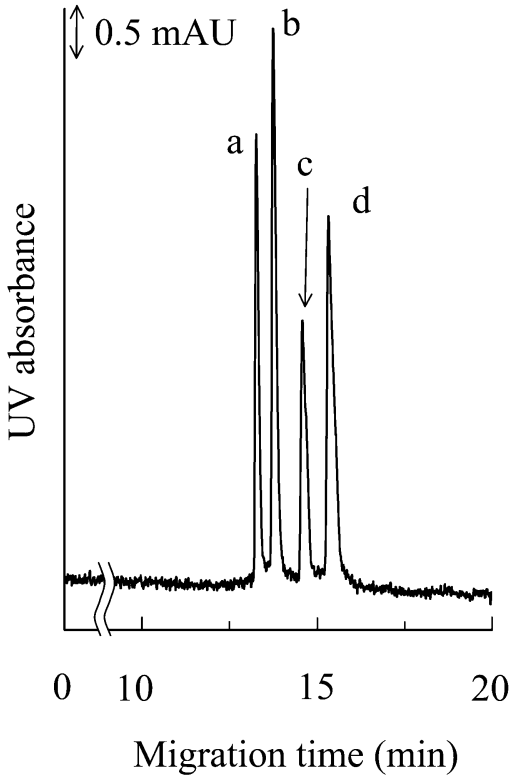


Fig. 9. Electropherogram for typical TCAs analysis with on-line coupled FIT SPE–CE system [57,60]. Extraction conditions: extraction capillary, DB-5 capillary (0.25 mm I.D.×10 mm, d_f: 0.25 μm; J&W Scientific, Folsom, CA, USA) packed with Zylon® filaments of 265 filaments; extraction flow-rate and time 80 μl/min for 12.5 min; desorption flow-rate and time, 4.0 μl/min×0.45 min; desorption solvent, acetonitrile; injection volume (calculated), about 4 nl. CE conditions: separation capillary, 0.075 mm I.D.×1200 mm (with effective length of 800 mm); precapillary, 0.075 mm I.D.×300 mm; applied field, 150 V/cm; buffer solution for the separation, acetonitrile–buffer=(20/80) where the buffer solution contained 20 m*M* Na_2HPO_4 (pH 9.3) and 0.6 m*M* β-cyclodextrin. Detection: UV at 210 nm. Other conditions are in the text. Peaks: a=desipramine; b=nortriptyline; c=imipramine; d=amitriptyline.

increased sensitivity was also demonstrated for the real biological samples [57]. The total organic solvent required for the analysis was less than a few microliters.

As shown in Fig. 8B, another device was designed for the coupling to CEC, in which a commercially available 4-port 2-way valve (HV4-1 Plug Valve, Hamilton, Reno, NV, USA) was employed as an interface housing [59]. About 380 pieces of Zylon® filaments were packed longitudinally into a PTFE tubing of 0.25 mm I.D.×5.0 mm length, and then the tube was installed into the rotor of the valve as the miniextraction cartridge. As the separation column, a fused-silica capillary 0.15 mm I.D. packed with an octadecylsilica (ODS) was connected to the valve. The packed length was 50 mm (total length from the valve to the cathodic vial was 200 mm) and a window for on-column detection was opened at just after the mid-frit. To the valve port on the other side of the separation column, a precolumn capillary of 0.25 mm I.D.×150 mm length was connected to supply the mobile phase from anodic vial during the CEC separation. To the other two ports, a PTFE tube from syringe pump was linked to deliver the sample solution during the extraction process, and a short fused-silica capillary of 0.030 mm I.D.×50 mm length was connected as the waste line. Since the desorption was made with the flow of the mobile phase, no organic solvent was required for the sample preparation step, and therefore, the total usage of the solvent for each analysis was only about 2.5 μl as the mobile phase component for most of the applications such as the analysis of phthalate mixtures in water samples [59].

4.4. Polymer-coated fiber-packed capillary as a powerful extraction medium

Polymeric coating onto the packed filaments was also studied to enhance the selectivity and the extraction efficiency. According to our previous investigations for the applications of fiber-packed capillaries to the separation columns in various chromatographic methods, such as in LC, CEC and GC [83–88], these polymer-coated fibrous materials can be employed as the extraction media [88]. The results are quite consistent with the results obtained with in-tube SPME method, where different extraction characteristics are reported with different types of polymeric coatings to the capillary wall [47,82]. In order to prepare the polymer-coated fiber-packed capillary, some solvent and heat resistant fibers have been selected and packed into the fused-silica capillary as a similar manner to prepare the FIT capillary. Next, the polymeric coating process was made similar to the preparation of conventional open-tubular capillary GC columns [62,63,88]. The system configuration is almost the same as in Fig. 5 except for the position of the extraction capillary, i.e. the extraction capillary is installed as the sample loop of the injection valve, allowing the simultaneous processing of desorption and injection by the flow of the mobile phase. Consequently, no desorption solvent should be prepared for the preconcentration process.

The application of the polymer-coated fibrous material to the analysis of phthalates is shown in Fig. 10. A typical chromatogram for a standard sample of

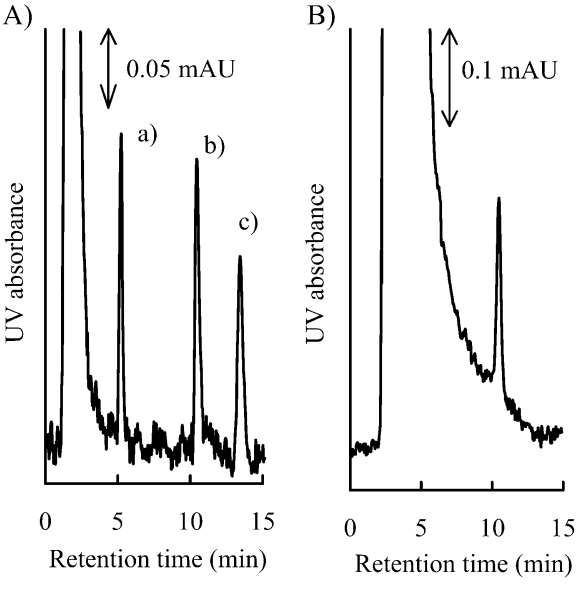


Fig. 10. Application of polymer-coated fiber-packed capillary for the analysis of waste water. (A) Standard water sample [62], and (B) waste water sample. Micro-LC conditions: column, Speriorex ODS (1.0 mm I.D.×150 mm); mobile phase composition and flow-rate, methanol–water (90:10) at 50 μl/min. Detection: UV at 254 nm. Extraction conditions for (A) and (B) are the same except for the extraction time of 20 min to (B). Other conditions are in the text. Peaks: a=DHP; b=DEHP; c=DOP. The concentration of DEHP in the waste water was determined as 1.40 ng/ml from the peak area of the chromatogram.

phthalates mixture (containing 1 ng/ml each) is depicted in Fig. 10A, where total sample volume pumped through the extraction was 500 μl (12.5 min extraction at the flow-rate of 40 μl/min). The extraction efficiencies for di-*n*-hexyl phthalate (DHP), di-2-ethyl-*n*-hexyl phthalate (DEHP), and di-*n*-octyl phthalate (DOP) with the polymer-coated fiber-packed capillary (coated with HR-52; 5%-phenyl–95%-methyl-polysiloxane; Shinwa, Kyoto, Japan) were about 63, 101 and 66%, respectively; while the extraction efficiencies with nonpolymer-coated type (FIT capillary) were 20, 21 and 21%, respectively [62]. The extraction efficiency was calculated by comparison with direct LC analysis of using no preconcentration process. Compared with the noncoated fiber-packed capillary, the extraction efficiency obtained with the coated one was dramatically improved, especially for DEHP. The results clearly demonstrate the contribution of the polymer coating to the packed-filaments on the extraction ability. However, the extraction power of the polymer-coated extraction device for other two phthalates, DHP and DOP, was somewhat smaller than that for DEHP, which was quantitatively extracted under the same experimental conditions. The trend has a good agreement with the results in the previous investigations, where a good correlation between the hydrophobicity of the analyte and the extraction efficiency was found for the extraction by fiber-packed extraction media [58,59]. Similar results were also monitored for the extraction behavior of di-*n*-butyl phthalate (DBP) with fused-silica capillaries having different kinds of polymeric coatings on it [58]. Although the contribution of the chemical structure and the polarity of the polymeric coating should be further studied, the results indicated the excellent suitability of the HR-52 coating for the quantitative extraction of DEHP from aqueous sample matrix, as shown in Fig. 10B [62].

The waste water samples were analyzed after the simple filtration process by glass-fiber filter [56]. In contrast to the river water analysis, a periodical reconditioning process seems to be needed for waste water samples, typically after more than ten consecutive extractions, in order to maintain the extraction power and to avoid any undesirable pressure drop of the extraction tube. The reconditioning of the extraction capillary can be made without any time-consuming procedure, i.e. a simple rinse by a typical organic solvent. To make sure the reproducible extraction for waste water analysis, the reconditioning process could be incorporated in the operational program of the system. The RSD for the determination of DEHP in waste water samples was less than 3.0% for 15 consecutive runs with the reconditioning process every 5 runs using 200 μl of methanol (at a flow-rate of 50 μl/min for 4 min).

Under these conditions, a good linear calibration from 1.0 to 800 ng/ml (for the extraction volume of 1 ml) was observed with a correlation coefficient of more than 0.998. Furthermore, no statistical difference was found between the extraction flow-rate of 20 and 40 μl/min. The lowest limits of quantification for DHP, DEHP and DOP in waste water were 0.15, 0.10 and 0.20 ng/ml, respectively; while the data obtained by the fiber-packed extraction capillary (without polymer-coating) were 0.5, 0.5 and 0.7 ng/ml, respectively [62]. These results clearly demonstrate the validity of the on-line sample preparation method as the sample pretreatment technique for waste water analysis.

The applications of polymer-coated fiber-packed capillary as the sample preparation medium for biological sample have been also reported recently [63]. Imaizumi et al. introduced the extraction capillary for the preconcentration of typical TCA drugs, amitriptyline, imipramine, nortriptyline and desipramine, in urine samples. Taking into account the polarity of these TCAs [47,57], the polymer coating was carried out with HR-52 phase as described earlier [88]. The results demonstrated the good applicability of the method for biological sample matrices. The lowest limits of quantification (LOQs) for these TCAs were less than about 2.0 ng/ml, indicating that the sample preparation method seemed to be quite acceptable for the rapid analysis of these drugs in clinical and forensic situations.

5. Other miniaturized sample preparation techniques designed for liquid phase separations

Tomlinson et al. reported the development of the ''preconcentration-CE'' (PC-CE) method [89–91] as an on-line coupled sample preconcentration process to CE system. The preconcentration was carried out

in a small section of packed tubing (typically a few millimeters length of C_{18} particle-packed section in a PTFE tubing of 400 μm I.D.), which was directly connected to the CE separation capillary. On the basis of the results, Tomlinson et al. have further developed another miniaturized sample preparation technique designed for the on-line coupling to CE separation [92,93]. In the developed method, a piece of a commercially available polymeric membrane (copolymer of styrene–divinyl benzene) was employed as the adsorptive membrane and the successful applications of the method have been published [94–100].

Liquid-phase microextraction (LPME) technique, in which a porous polypropylene hollow fiber was employed as an extraction device, has been developed by Rasmussen et al. [101–106]. LPME was performed in a conventional sample vial (4 ml volume) containing a small piece of polypropylene hollow fiber having a typical size of 600 μm I.D.×8-cm length with the wall thickness of 200 μm and 0.2-μm pore size. The analytes were extracted from the sample into an organic solvent, immobilized in the pores of the hollow fiber, and then into an aqueous acceptor solution filled inside the hollow fiber. The high phase ratio between the sample volume and the acceptor volume (for typical CZE applications, the sample volume and the acceptor volume were 25 μl and 1- to 4-ml, respectively) provides an excellent sample preconcentration factor. Combination with GC and LC separation systems was also reported for the analysis of several classes of drugs in biological matrix as well as the coupling with CE separation system [105].

Several sample preparation techniques developed especially for GC analyses could be applied as the sample preparation for liquid phase separation methods. For example, a miniaturized extraction cartridge developed by Fritz et al. [107,108], where the use of a small resin disks (1.2 mm in height×0.7 mm diameter) has been employed as the extraction medium, and a droplet sampling–extraction method developed by Dasgupta and co-workers [109–112], in which the extraction was made with a single droplet of the collection liquid at the top of the capillary, should have a great possibilities as the sample preparation for liquid phase separations. Baltussen et al. have developed so-called stir bar sorptive extraction (SBSE), in which a small stir bar is coated with polydimethylsiloxane on the surface, and the extraction is carried out by rotating the bar in the sample solution [113,114]. In addition to the combination of the sample preparation with the SBSE method and GC separation [113–118], successful applications have been also reported recently for the coupling to an LC system [119].

6. Conclusions

The recent developments of microscale sample preparation method for the liquid phase separation systems have been briefly reviewed. The combination of microscale sample preparation and microscale liquid phase separation promises good future applications in various fields in separation science, especially for the trace amount of the analytes in complex sample matrices. However, continuous innovations in the extraction materials and the integrated analytical system are also needed to find the complete solution for solving various separation problems.

The authors believe a promising possibility for the new fibrous extraction media having different chemical structures, and different polymeric coatings and different functionalities on the fiber surface, which can be specially designed based on the concept of molecular shape recognition. The applications of polymer-coated and 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 format of the stationary phase materials in various chromatographic techniques.

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