Focusing Review

Miniaturization of Separation Systems and Its Applications

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Abstract

Development of miniaturized separation system consisted of microscale extraction and liquid phase separation processes has been reviewed. Various types of novel bonded stationary phases have been developed on the basis of the systematic analysis for the retention behavior of polycyclic aromatic hydrocarbons on experimentally synthesized phases. In this review, the miniaturization of microscale sample preparation technique and the effective on−line coupling to microcolumn liquid phase separations are also described especially focusing on the approach by the author's group. The novel use of synthetic polymer filaments as the stationary phase and extraction media in those microscale separation systems will be introduced along with the applications in gas chromatographic separation.

Keywords: miniaturization; sample preparation; microcolumn; on−line coupling; fibrous materials; liquid chromatography; gas chromatography

1. Introduction

Miniaturization of separation systems has been increasingly focused because it must provide a promising solution to the recent requirements such as high performance, rapid analysis with a reduced running cost and without environmental pollution [1−4]. Down−sizing of the separation columns is also one of the most effective approach to develop novel stationary phases in liquid chromatography (LC) because the evaluation of the limited amount of experimentally synthesized stationary phases could be accomplished [5,6]. With microscale LC the characteristics of commercially available stationary phases such as octadecylsilicas (ODSs) have been investigated, and various novel bonded stationary phases have been synthesized and evaluated based on the systematic analysis of the retention behaviors of probe solutes on those conventional stationary phases [7−29]. To realize the miniaturized separation system, the development of microscale sample preparation process can be regarded as a key innovation in separation science. However, the reports for the miniaturized sample preparation technique, which was specially designed for microscale separation systems, have been still limited mainly because of the complexity and difficulties for the on−line coupling and the operation [1,2].

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In this article, several recent results, especially on the miniaturization of separation columns in liquid chromatography and extraction cartridges in sample preparation process will be briefly reviewed. Novel use of fibrous materials in microscale separation systems, as a separation medium and an extraction medium, will be also described along with some applications for the analysis of real complex sample mixtures.

2. Miniaturization of Columns in Liquid Chromatography

On the successful applications in wide scientific fields, LC method has been recognized as one of the most powerful and versatile separation techniques. In contrast to the wide applicability, however, there is still a long way to explain the whole separation mechanism in LC, because many parameters are controlling the molecular recognition in the chromatographic process. A large portion of LC separations in reversed−phase (RP) conditions have been carried out with commercially available ODS phases, and those phases could be basically divided into two types depending on the bonding chemistry: one is polymeric, which is typically synthesized with trifunctional silanes as the starting material in aqueous synthetic conditions, and another is monomeric synthesized

from monofunctional silanes in non−aqueous conditions. Although the correlation between the synthetic methods and the selectivities has been reported [30−35], the theoretical interpretation for the molecular−molecular interaction between solute molecule and bonded phase ligand(s) has not been well established, mainly due to the difficulties in the surface characterization of these ODS stationary phases in real chromatographic conditions. However, various novel bonded phases have been synthesized and introduced as the stationary phases by the recent innovations in synthetic and bonding chemistries [7−23]. Since the design of these novel phases, having a desirable separation characteristics and performance, could be accomplished by the systematic analysis of retention behaviors of experimentally synthesized phases of a limited amount, it can be said that microcolumn separation is a most powerful and inevitable technique during the bonded phase developments.

Figure 1 shows typical microcolumns prepared by fused−silica capillaries of 0.32 and 0.53 mm i.d. These columns can be packed with a preliminary stationary phase of less than 50 mg by a slurry method and operated with a typical flow−rate of less than 10 to 20 μL/min, indicating the advantageous features of the microscale separation [5,6]. The introduction of microcolumn LC system also enables the use of valuable solutes as the sample probes [36].

Jinno and Saito *et al*. designed several groups of novel bonded stationary phases based on the molecular shape recognition concept in chromatographic process [36,37]. Taking advantage of the miniaturized system, these experimentally synthesized phases, as illustrated in Figure 2, have been chromatographically characterized by the systematic investigation using polycyclic aromatic hydrocarbons (PAHs) and fullerenes as the solute molecules. Although the PAHs have many structural isomers, these relatively simple molecular structures than real samples to be separated in LC applications are quite suitable for the systematic analysis of the separation mechanism based on the molecular shape recognition concept. In addition the combination of the results obtained from the retention behavior studies for PAHs with that from fullerenes could offer more systematic and comprehensive interpretations about molecular shape recognition process on the stationary phase, because the fullerene molecules, such as C_{60} and C_{70}, can be regarded as very large PAHs with specific shape and size [9,37]. Introducing these spherical model solutes, it has been confirmed that the interval of the bonded ligands on the silica gel support and the ordering of these bonded ligands play a role to generate an effective interaction with the solute molecules [25−27]. From the retention behaviors of these sample probes on various multi−legged and C_{60} bonded phases, it has been also demonstrated that an interactive structure of the bonded ligand on the support surface can be well−designed by the introduction of novel synthetic reactions to the

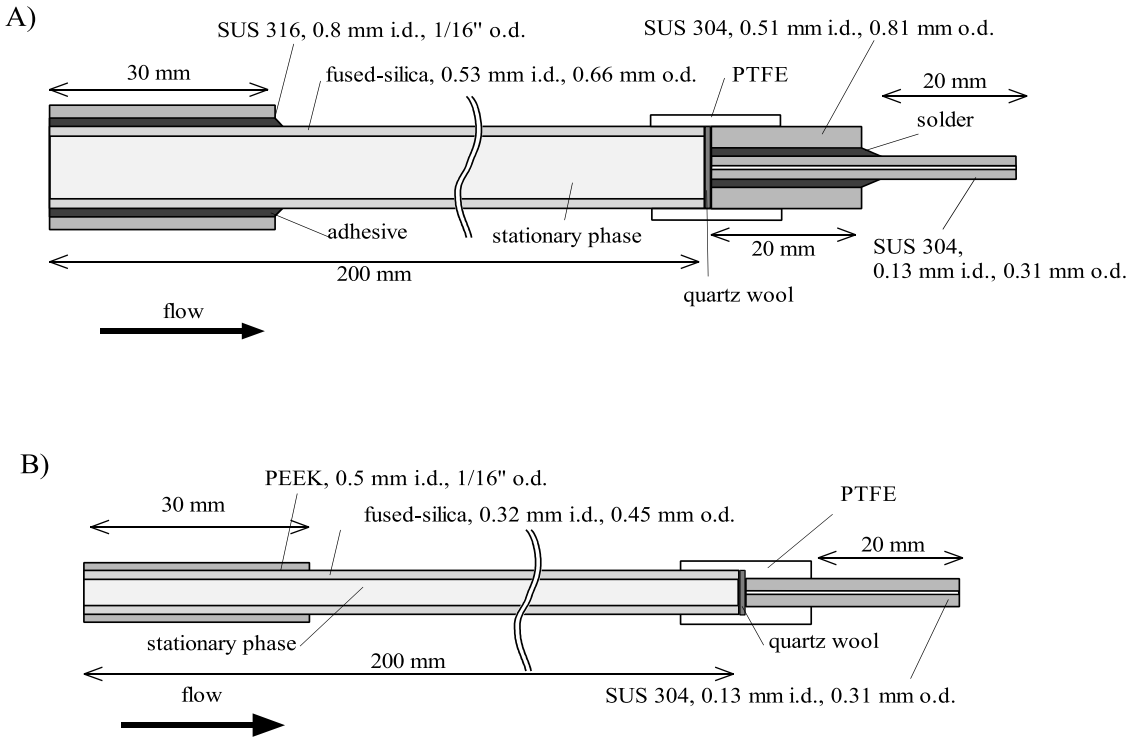

Figure 1. Illustrations of typical laboratory made microcolumns for bonded phase evaluation. A) 0.53 mm i.d. and B) 0.32 mm i.d.

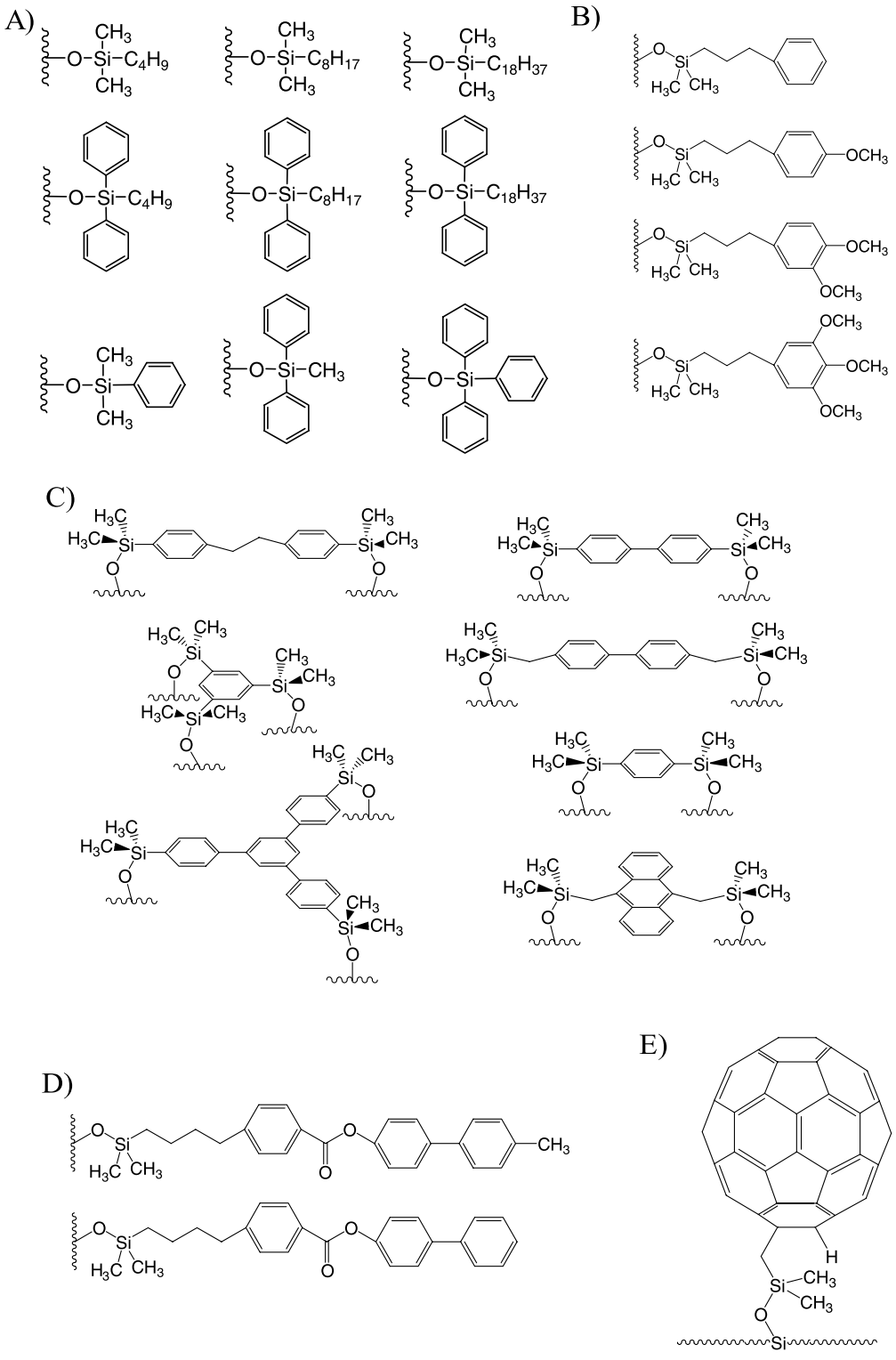

Figure 2. Novel stationary phases synthesized by the molecular shape recognition concept. A) alkyl, alkyldiphenyl and triphenyl bonded phases; B) phenylpropyl and methoxylated phenylpropyl bonded phases; C) multi−legged phenyl bonded phases; D) liquid−crystal bonded phases and E) C_{60} bonded phase.

bonded phase preparation.

3. Miniaturization of Sample Preparation Device for Liquid Phase Separations

3.1 Coupling of SPME and micro−LC

As a microscale sample preparation method for the analysis of volatile and semi−volatile compounds in gas chromatography (GC), Pawliszyn *et al.* developed solid−phase microextraction (SPME) [38−40]. In the SPME method, a fused−silica rod with a polymeric coating on the surface is employed as the medium for the extraction of volatile analytes from aqueous sample solution or the headspace of the sample solution. Desorption of extracted analytes can be made with the heating of the SPME fiber in a conventional GC injection port, allowing the SPME device to have a good compatibility with GC system. In order to apply the SPME method for the analysis of non−volatile and/or thermally labile compounds, however, a specially designed desorption device is needed to accomplish the coupling of the SPME to liquid−phase separation systems, such as LC [41−47], supercritical fluid chromatography (SFC) [48,49], capillary electrophoresis (CE) [50] and micellar electrokinetic chromatography (MEKC) [51,52]. As the desorption interface between SPME and micro−LC, Jinno and Saito *et al*. [44−47] developed a desorption device (Figure 3), where 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 void volume in the device. The SPME fiber is inserted from the top of the desorption interface and a small amount of the desorption solvent is supplied from the port positioned just above the inserted SPME fiber using a micro−flow pump. The desorbed analytes are transferred to the loop of the LC injector. 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 Salleh *et al*. and Pawliszyn *et al*. to accomplish the desorption under the pressurized conditions [48,49].

With the desorption interface shown in Figure 3, the determination of common pesticides in environmental water samples has been reported [44]. The effect of the extraction conditions, such as the type of the fiber coating, the extraction time and temperature, the effect of the agitation have been investigated along with the effect of additives into the sample matrix to improve the extraction efficiency of the analytes. The desorption conditions, such as the type of desorption solvent, the flow−rate for dynamic desorption and the desorption time have been also systematically studied taking into account an effective on−line coupling with subsequent LC separation process. After the preliminary optimization process, 10 pesticides in surface water samples obtained near a local golf course were determined successfully, indicating the powerful preconcentration ability [44].

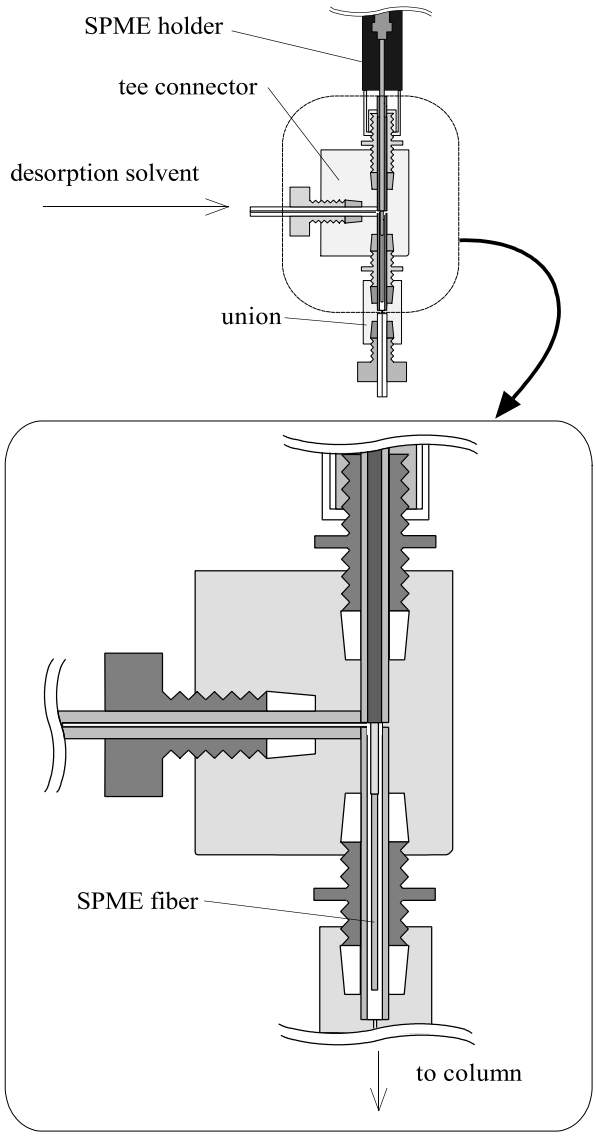

Figure 3. Specially designed desorption interface for the coupling of SPME and micro−LC.

The determination of pharmaceutical compounds in biological fluids has also demonstrated with the desorption interface, where the extraction of benzodiazepines and tricyclic antidepressants (TCAs) from human urine samples by SPME and the separation in micro−LC have been reported [47]. Although the extraction power for each TCAs with a SPME fiber is different mainly due to the polarity, a good extraction performance was demonstrated for the analysis of human urine samples, and the detection limits for common TCA drugs such as amitriptyline, nortriptyline and imipramine, were determined to be at ng/mL level, showing a quite acceptable detection performance in clinical and forensic situations. 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.

3.2 In−tube SPME and wire−in−tube SPME as the microscale sample preparation method

Introducing a short section of open−tubular GC column as the extraction device, in−tube SPME method has been developed by Pawliszyn *et al*. for an effective on−line coupling of sample preparation and LC separation [53,54]. The automation of the hyphenated analytical system has been also reported for the analysis of various classes of compounds in complex matrices [55−58]. In addition to several commercially available coatings used widely as GC stationary phases, novel polymeric coating materials have been developed for a selective extraction [59−61], however, the extraction efficiency of the in−tube method is generally lower than that can be obtained with conventional solid phase extraction (SPE) method. This is mainly due to the large phase ratio between the "solid phase" (viscous polymeric material on the inner capillary surface) and the "liquid phase" (aqueous sample solution passed through the capillary), and therefore, the extraction should be repeatedly carried out with pumping the sample solution by so−called "draw/eject" procedures [53−58] to get an improved extraction efficiency.

Based on the above results with in−tube SPME, Saito *et al*. have developed wire−in−tube SPME [62] by inserting a stainless steel wire into the extraction capillary in in−tube SPME. As shown in Figure 4, the internal volume of the extraction capillary could be significantly reduced while the surface area of the polymeric coating material contacting the sample solution is maintained as same as the vacant capillary having the same dimensions. After the insertion of the stainless 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 the capillary of 0.25 mm i.d. x 40 cm, producing the phase ratio changed from about 500 to 180 with a polymeric coating of 0.25 μm thickness [63,64]. In the wire−in−tube configuration, more effective extraction could be obtained and more efficient on−line coupling of the extraction process to subsequent microcolumn separation process has been established. With this modification it was reported that the successful applications for environmental analysis such as determination of phthalates in surface and waste water samples [63,64], and for biological analysis such as determination of TCAs in biological fluids [62]. The results also demonstrated that more effective preconcentration than the conventional in−tube SPME method could be accomplished with this modification, and that the hyphenated system consisted of wire−in−tube SPME and micro−LC should present a great potential for the fast analysis of various organic compounds in other complex sample matrices.

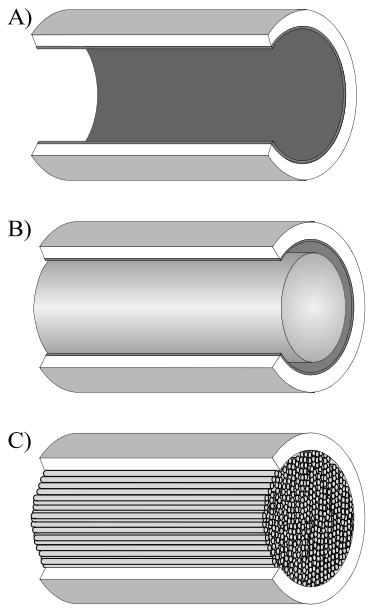

Figure 4. Schematic representation of three types of extraction capillary [63]. A) In−tube; B) wire−in−tube; C) fiber−in−tube.

3.3 Fiber−in−tube SPE: a novel use of polymer filaments as the extraction medium

More recently, the use of polymer filaments as the extraction medium has been also reported. The novel technique, fiber−in−tube SPE (FIT−SPE) [65], where several hundreds of fine filaments of polymeric material are packed longitudinally into a short capillary of polyetheretherketone (PEEK) or polytetrafluoroethylene (PTFE), has been developed on the basis of the successful applications of wire−in−tube extraction. Not only significantly reduce the internal void volume of the extraction capillary (Figure 4), those fine polymer filaments can also be employed as the extraction medium. A rigid−rod heterocyclic polymer, Zylon® (Toyobo, Ohtsu, Japan) was firstly chosen [66] taking into account its chemical structure, the solvent resistance for the mobile phase and mechanical strength during the packing process of the fiber, although a certain effectiveness of other several solvent− and heat−resistant fibers was confirmed for the extraction of other class of compounds [67]. For the preparation of the extraction tube, the fiber was typically cut to 100−mm length and packed longitudinally into the same length of PEEK tube (0.25 mm i.d., 1/16" 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. Because of the parallel arrangement of the filaments to the outer tubing, a number of coaxial narrow channels have been formed parallel to the axis of the capillary therein. Therefore, the FIT−SPE device shows a reduced pressure drop during the extraction and desorption comparing with conventional SPE cartridge,

and also the undesirable plugging from insoluble and/or particulate materials in real sample matrices can be significantly reduced in the FIT format.

In the developed FIT−SPE/LC method [65], the extraction capillary 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 10 to 50 μL/min. After changing the position of the switching valve, a certain volume of the desorption solvent was also pumped by another syringe pump to desorb the extracted analytes and simultaneously transfer them into the sample loop of the injection valve. The successful quantification of di−2−ethylhexyl phthalate (DEHP) in waste water samples has been reported with the FIT−SPE tubing of 0.25 mm i.d. x 10−cm length [65], showing the powerful sample preparation performance for complex sample matrices.

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 itself to be further miniaturized as a microscale sample preconcentration device. Down−sizing of the extraction device also allows the direct coupling of the extraction process with microcolumn separation methods, but without any disadvantages such as over−loading during sample injection and poor resolution in the chromatographic separations.

4. Miniaturized Separation System Using Novel Fibrous Materials

4.1 Miniaturized FIT−SPE for microcolumn separations

Further miniaturization of the extraction tube has been demonstrated by Saito and Jinno *et al*. [67,68] for the direct coupling of FIT−SPE to packed capillary LC. Figure 5 shows the miniaturized FIT−SPE cartridge installed in the rotor of the Rheodyne Model 7520 micro−injector (Rheodyne, Cotati, CA, USA) [67]. The flow channel for the sample loading (the center hole in the rotor) was enlarged and the FIT cartridge was inserted therein. The extraction cartridge was prepared with a PEEK tube of 0.50 mm i.d. x 5.0 mm packed with Zylon® of about 1500 filaments in a similar manner as described above. The miniaturized extraction cartridge was then sandwiched by two pieces of short blank PEEK capillaries, and installed into the hole in the modified rotor. Inserting appropriate size of several PEEK capillaries to other flow−channels in the valve, the injector was further modified to reduce any undesirable extra void volume.

In the extraction process, the sample solution from a syringe pump is passed through the extraction cartridge, while the analytes in the sample solution are extracted onto the packed filaments. Next, the position of the injector is changed to “desorption/injection” to make desorption and injection simultaneously by the mobile phase flow for micro−LC separation. Therefore it can be said that no desorption solvent is needed for the sample preparation process. As the same as longer FIT capillary, the mini−cartridge can be prepared with a good reproducibility, and these cartridges also have a good stability for repeatable use, normally more than

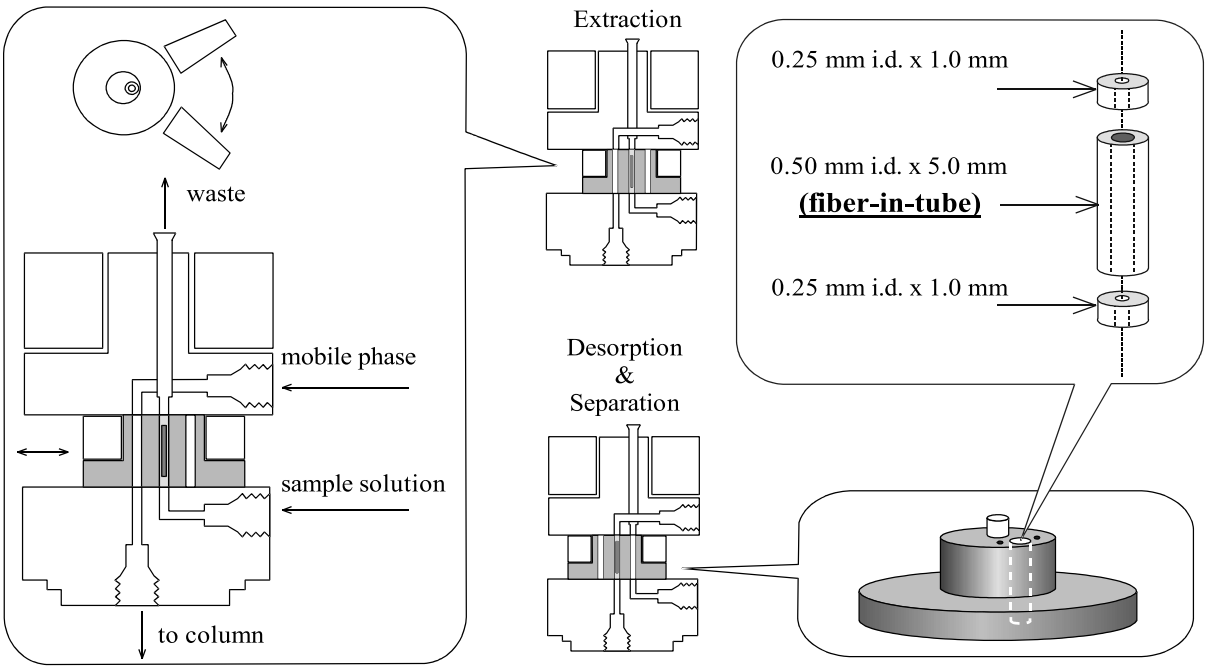

Figure 5. Miniaturized FIT−SPE cartridge installed in modified Rheodyne 7520 microinjector.

50 runs without any significant problems, such as an increase in the pressure drop over the cartridge 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 simple washing and/or re−conditioning processes using an organic solvent could be employed to make sure the reproducible results during the next 50 consecutive runs. With a LC separation using a packed capillary column of 0.53 mm i.d x 20 cm, the total solvent volume required for the typical analysis of phthalates in a waste water sample is less than 40 μL even including the solvent as the mobile phase component [67].

For the on−line coupling of the fiber−in−tube SPME to electrokinetic separation methods, two types of laboratory−made interfaces, shown in Figure 6, have been developed [69]. Figure 6 A shows the overview of the FIT−SPE/CE system and the close−up of the specially designed extraction capillary installed in a modified cross−connector [69,70]. The extraction capillary was prepared with a short section of a GC column (DB−5, 5%−phenyl−95%−methyl−polysiloxane; J & W Scientific, Folsom, CA, USA) and fibrous (250 filaments of Zylon®) packing materials. 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 (*i.e.* the total sample volume pumped was 1.0 mL) during the extraction process. Next, the syringe was replaced by another syringe containing acetonitrile as the desorption solvent. By pumping an appropriate volume of the solvent, the desorbed analytes were transferred to the space in the modified cross connector. When the zone containing the desorbed analytes was reached to the cross section of the separation capillary, the separation was started by applying the voltage. The volume of the desorption solvent and the flow−rate could be optimized easily in simple preliminary experiments. Combining the inner−coated capillary and fibrous packings, an improved extraction power was obtained for the mixture of TCAs having different polarity. The volume of required solvent was less than about 2.0 μL for each run and, typical preconcentration factor of more than 200 times was demonstrated with the total sample volume of 1.0 mL and the extraction time of about 10 min [70].

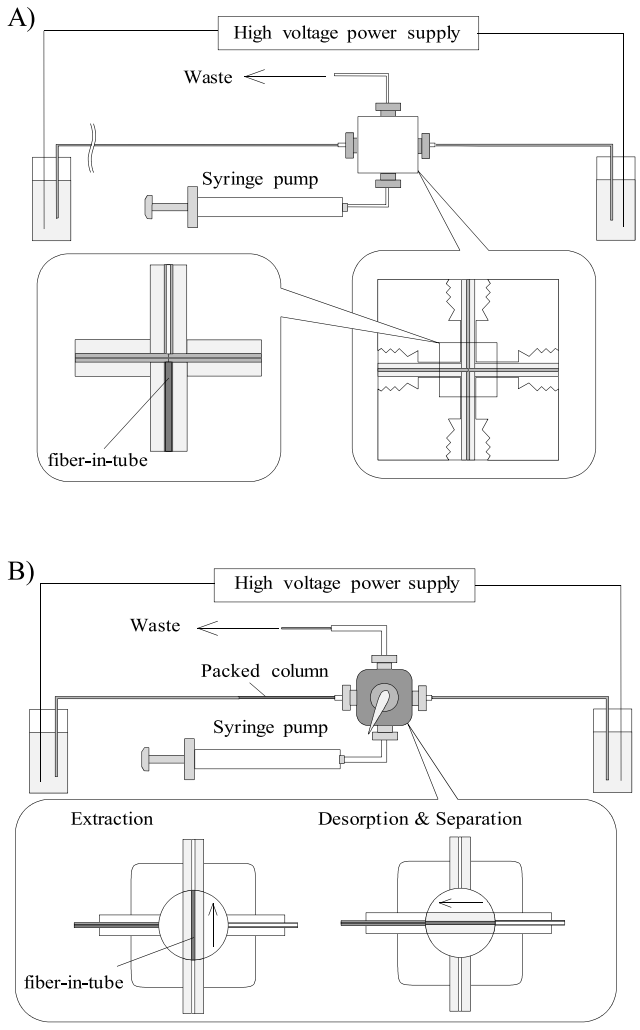

Figure 6. System overview of FIT−SPE/CE (A) and FIT−SPE/CEC (B). (Reproduced from reference [69])

On−line coupled FIT−SPE/CEC system was also developed with the "in−valve" configuration depicted in Figure 6 B, where a commercially available 4−port 2−way valve (Model HV 4−1, Hamilton, Reno, NV, USA) was employed as an interface housing [68,69]. About 380 pieces of Zylon® filaments were packed (packing density was about 80%) longitudinally into a PTFE tubing of 0.25 mm i.d. x 5.0 mm, and then the tube was installed into the rotor of the valve as the miniaturized extraction cartridge. As can be seen from the structure, no desorption solvent is needed for the sample preparation process, and the calculated preconcentration factors for the typical phthalates in aqueous samples were more than 60 for the extraction of 20−μL sample volume (at the flow−rate of 4 μL/min for 5 min). The total volume of the organic 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 [68].

4.2 Fibrous stationary phases for microcolumn separations

The possibility of the fibrous stationary phase in LC was reported more than a decade ago [71−74]. In these studies, it was shown that the fibrous materials might have a possibility to be a novel stationary phase in liquid−phase separations, although the efficiency was not sufficient as a column for LC separation. Taking an advantage of the flat−flow profile of CEC, recently, a better separation performance over LC was obtained with the fiber−packed column having the effective length of only 50 mm. As the fibrous materials, synthetic fibers such as Zylon® and Kevlar® (Du

Pont−Toray Co., Ltd., Tokyo, Japan) were also introduced to prepare the columns [75], and rapid separation of aromatic compounds has been demonstrated without frits that might be an origin of bubble formation. Therefore, it can be considered that the fiber−packed capillary may have a wide application as a separation media in CEC without the requirements for bubble elimination techniques.

Successful introduction of the fibrous stationary phases was demonstrated not only in liquid−phase separations but also in gas−phase separations [76]. About 330 and 600 filaments of heat resistant polymers were packed longitudinally into fused−silica capillaries of 0.32 and 0.53 mm i.d., respectively, and the separation of several test mixtures, such as *n*−alkanes, was carried out with these fiber−packed capillary columns in GC. Figure 7 shows typical cross−section photograph of a fiber−packed GC capillary column. The separation of alkylbenzenes, alkanes, alcohols and PAHs has been carried out and the data demonstrated that the fibrous stationary phase has a function as the stationary phase for the GC analysis of volatile compounds, although the difference in retention power between different fibrous stationary phases should be further investigated. The linear relationship between the carbon number of these homologous mixtures and the corresponding logarithmic retention factors was observed, indicating that temperature−programmed separation with these fiber−packed columns could be a powerful tool even for the low−volatile compounds having a higher boiling point [76]. The results also demonstrated that the fiber−packed columns possessed the practical sample loading capacity over a conventional open−tubular capillary column. Actually, an increased sensitivity was obtained especially for low−volatile compounds because no splitter should be needed for most of the separations on fiber−packed columns.

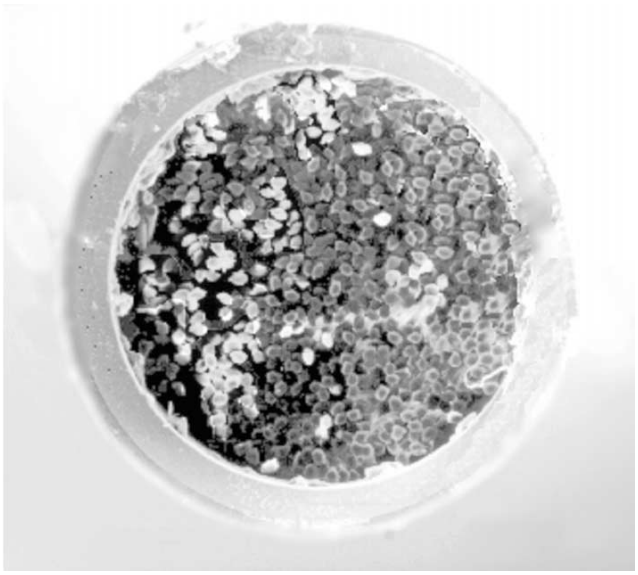

Figure 7. SEM image of the cross−section of fiber−packed capillary GC column (0.53 mm i.d.).

4.3 Polymer−coated fibrous materials for miniaturized separation systems

Polymer−coating with typical GC stationary phases (liquid phases) onto the packed filaments has been also investigated to develop the stationary phase in short column GC separation and the extraction medium in miniaturized sample preparation process. The study was originally started to confirm the contribution of the chemical structure of different fibrous materials and the coatings toward the separation and extraction performances. As expected, the separation characteristics can be tuned with different types of polymeric coating onto the packed fibers [76], although the compatibility of the polymeric materials to the fibrous materials should be systematically studied more. The results also showed that the retentions for solutes were significantly increased with the polymeric coating onto the packed−fibers. The coating on the fiber surface was very stable for the solvent washing, for example, even after the column washing with 20 mL of acetone, a slight decrease in the retention time (less than 4 % for all solutes) was only observed once, and no further retentivity was decreased with subsequent solvent washing. Therefore, as can be seen from the typical chromatogram illustrated in Figure 8, it is quite clear that the packed−fibers have a function as the support for the coating as well as the stationary phase itself, because a conventional wall−coated capillary with the same size and coating reagent do not show any measurable retention for the same analytes in the same separation conditions.

The extraction power of the fiber−packed capillary was also significantly improved with the polymeric coating [77,78]. The extraction efficiencies for the standard solution containing 1.0 ng/mL each di−*n*−hexyl phthalate (DHP) and DEHP with the polymer−coated fiber−packed capillary (coated with HR−52, 5%−phenyl−95%−methyl−polysiloxane; Shinwa Chemical, Kyoto, Japan) were about 63 and 101 %, respectively; while the extraction efficiencies with non−polymer−coated type were 20 and 21 %, respectively [78]. 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 [64,70]. Comparing with non−coated fiber−packed capillary, the extraction efficiency obtained with coated one was dramatically improved, especially for DEHP, which was quantitatively extracted [77]. 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 [66,67]. Although the contribution of the chemical structure and the polarity of the polymeric coating should be investigated more, the results indicated the excellent suitability of the HR−52 coating for the

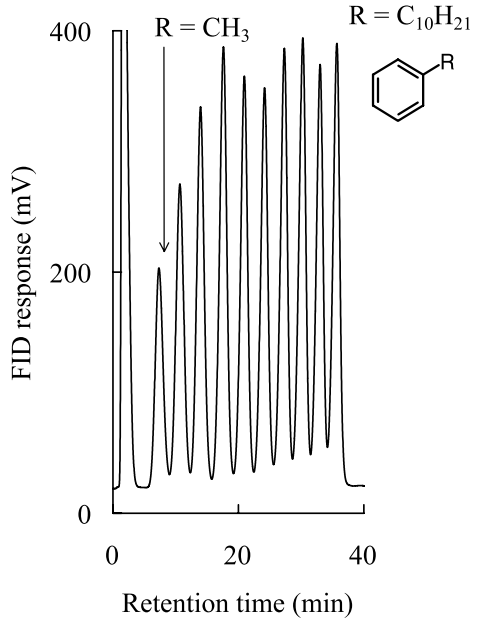

Figure 8. Typical chromatogram for the separation of alkylbenzenes on a polymer−coated fiber−packed capillary column. Conditions: column, fused−silica (0.32 mm i. d. x 2 m) packed with HR−1 (polydimethylsiloxane; Shinwa Chemical) coated Zylon®; packing density, 18 %; carrier gas, N_2; column inlet pressure, 10 kPa; N_2 flow−rate, 1 mL/min; column temperature, 50℃ (1.5 min) to 230℃ at 5 deg/min; injection and detection temperature, 250℃. Injection (1 μL of standard sample containing 0.1 % each of alkylbenzenes in CH_2Cl_2) was made with splitless mode.

quantitative extraction of DEHP from aqueous sample matrix. The lowest limits of quantification for DEHP in waste water was 0.10 ng/mL; while the data obtained by the fiber−packed extraction capillary (without polymer−coating) was 0.50 ng/mL [77].

The polymer−coated fiber−packed capillary was further applied as the sample preparation medium for biological samples. As a typical analysis of biological matrix, a successful sample preparation for the determination of TCA drugs in urine samples has been demonstrated [78], where the lowest limit of quantification was quite acceptable (less than 2.0 ng/mL) for the analysis of TCAs in clinical and forensic situations.

5. Conclusions

Miniaturized separation systems consisted of liquid phase separation methods and sample preparation techniques have been reviewed along with the introduction of typical applications of these hyphenated systems. The author believes that the hyphenation of microscale sample preparation and microscale liquid phase separation will realize the promising future applications in various fields in separation science, especially for the trace amount of the analytes in complex mixtures such as environmental and biological samples.

The applications of polymer−coated and surface−derivatized fibrous materials in miniaturized sample preparation process are currently being studied as well as the employment of similar fibrous separation media as the new format of the stationary phase materials in various chromatographic techniques. Novel fibrous extraction/separation media having different chemical structures and different functionalities on the surface can be specially designed in the near future based on the concept of molecular shape recognition.

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