

## Focussing Review

# The Use of Fibrous Polymer Materials in Capillary Electrochromatography (Review)

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## Introduction

Miniaturization of analytical systems is the most important issue in recent analytical chemistry, since it realizes high performance and rapid analysis without a large solvent consumption and with low cost operation. These miniaturized high performance/throughput systems are, therefore, environmentally favorable not only economically preferable. There are two major processes in a typical analytical system, a sample preparation procedure and that for the subsequent analysis. In the former stage, the extraction and/or preconcentration of the analyte(s) of interest have been carried out, typically with liquid-liquid extraction (LLE) or solid phase extraction (SPE). In the latter part, the separation of components in the analytical sample, and identification and quantification of each component isolated should be performed by using chromatography and spectroscopy as the hyphenated system. For the miniaturization of the whole analytical systems, micro-scale sample preparation methods and the hyphenated system coupled to micro-scale separation techniques should be developed.

Solid phase microextraction (SPME) is a solventless sample preparation method developed by Pawliszyn *et al.*, and originally employed as a miniaturized sample preparation technique for the analysis of volatile analytes in gas chromatography (GC) [1, 2]. The applications of SPME for the analysis of non-volatile or thermally liable compounds have been reported using a specially designed desorption device [3-13]. The extracted analytes are desorbed from the polymer coatings on the fused-silica SPME rod into a small amount of solvent filled in the desorption device, and then transferred to the injection valve for the subsequent analysis by liquid chromatography (LC) or capillary electrophoresis (CE). More effective coupling of the extraction method to LC was also made by the development of so-called "in-tube" SPME [14-17] and "wire-in-tube" SPME [18]. In these studies, a fused-silica capillary of coated inside was employed as the extraction medium. Therefore, the effective on-line coupling to LC system was accomplished

and also the extraction efficiency was significantly improved by the multiple draw/eject procedure [14-16] or inserting a stainless wire inside of the capillary [18]. On the basis of the extensive investigations as described above, fiber-in-tube SPME [19] was developed recently. Fibrous synthetic polymer was packed into a capillary of fused-silica or polymeric tubing, such as polyetheretherketone (PEEK) or polytetrafluoroethylene (PTFE), and used as the extraction medium. In terms of the miniaturization of the sample preparation process, the fiber-in-tube SPME has a great advantage, because of the several hundreds of fine fibrous materials were packed inside and could have a function as the extraction medium. Consequently, the use of the fibrous polymer materials as the extraction medium enables itself to have the extended applications to the sample preparation for microcolumn separation methods, such as microcolumn LC (micro-LC) and capillary electrochromatography (CEC).

Since mobile phase is driven by electroosmotic flow (EOF) in CEC, peak broadening by the effect of parabolic flow is quite smaller than that in LC. Therefore high separation performance beyond LC can be accomplished in CEC [20]. Although a large number of LC packing materials can be utilized for the CEC stationary phases, column preparation requires very skillful work to achieve a good reproducible column, especially frit preparation that retains the particles in the column is quite difficult. Because the frits are main origin of bubble formation that prevents accurate analysis in CEC [21, 22], pressurizing the buffer vials by using LC pump together with high voltage power supply has been attempted [23, 24]. However, the required instrument is more complicated than simple CE instruments. As an alternative approach to improve the column reproducibility and to avoid bubble formation, various column preparation methods without the requirement of any frit preparation have been reported [25-31]. Fiber-packed column is also one of the promising format.

Jinno and Kiso *et al.* have studied the novel usage of fibrous

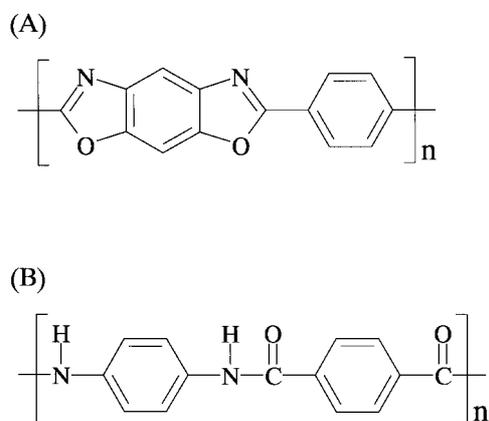
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material as the stationary phase in microcolumn separations [32-33]. The results demonstrated the effectiveness of fibrous stationary phases for the separation of alcohols [32], polycyclic aromatic hydrocarbons (PAHs) [33] in micro-LC. The applications for CEC were also reported with different type of synthetic fibers [34-36]. In these studies, no bubble formation was observed without the pressurization to the column. Taking advantage of the flow profile of CEC, it has been also realized that an improvement over the fiber-packed LC columns, for example, a very fast separation by a short fiber-packed capillary, could be obtained for the CEC separation [35, 36].

In this article, the recent use of fibrous polymer materials in microcolumn separation science, especially CEC, and the related techniques has been reviewed. Novel "fiber-in-tube" configuration will be described as a specially designed sample preparation method for the miniaturized separation systems. As the fibrous polymeric material in this article, Zylon<sup>®</sup> and Kevlar<sup>®</sup> were mainly introduced along with their variations: HM-type with heat treatment after the spinning process; and AS-type with out the heat treatment. The applications of these fibrous materials as a stationary phase in microcolumn separations are also shown. The future possibilities for the development of novel fibrous polymers for separation science will be briefly described.

#### Miniaturized sample preparation process using fibrous materials

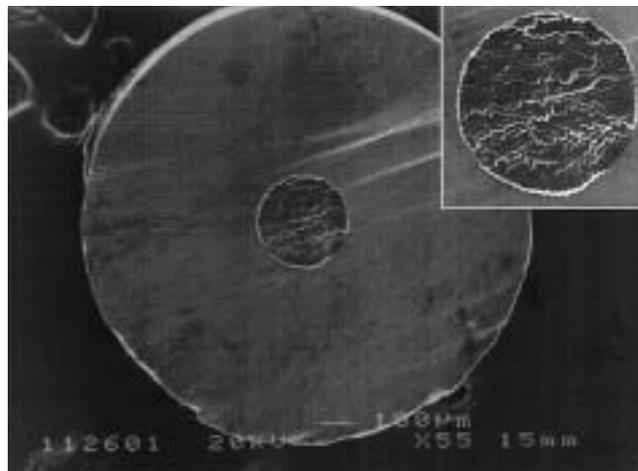
Fiber-in-tube SPME [19] is one of the miniaturized sample preparation method, in which several hundreds of fine polymer filaments play the role as an extraction medium. Figure 1 shows the typical examples of the polymer structures: Zylon<sup>®</sup>, poly(p-phenylene-2,6-benzobisoxazole) (PBO) obtained from Toyobo Co., Ltd., Ohtsu, Japan and Kevlar<sup>®</sup>, which is a typical aromatic amide polymer, obtained from Du Pont-Toray Co., Ltd., Tokyo, Japan. Taking into account the chemical structures, solvent resistance and



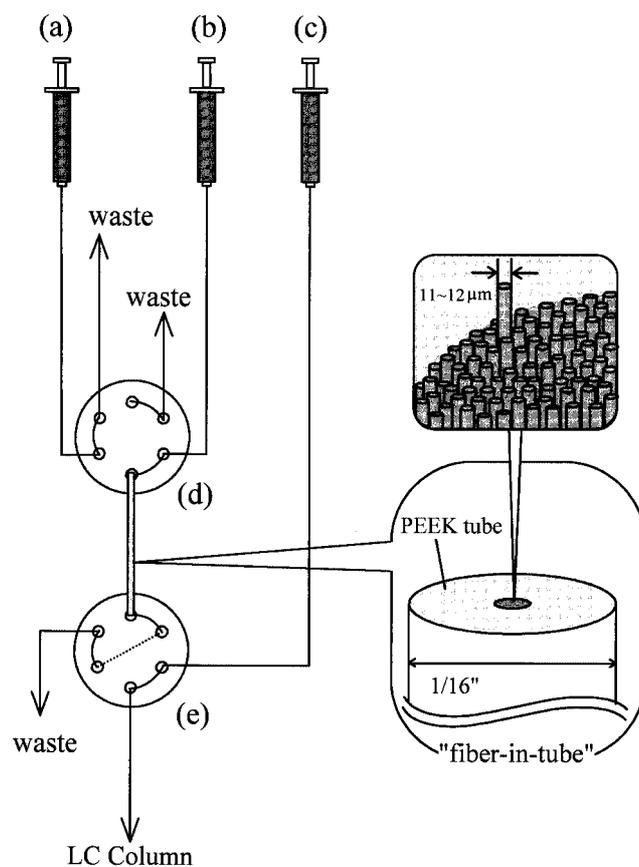
**Figure 1.** Chemical structures of Zylon<sup>®</sup> (A) and Kevlar<sup>®</sup> (B).

mechanical strength, these synthetic fibers were selected. The typical outer diameter of each filament is about 11-12  $\mu\text{m}$  and these filaments were packed as shown in Figure 2.

The overview of the fiber-in-tube SPME/micro-LC system is



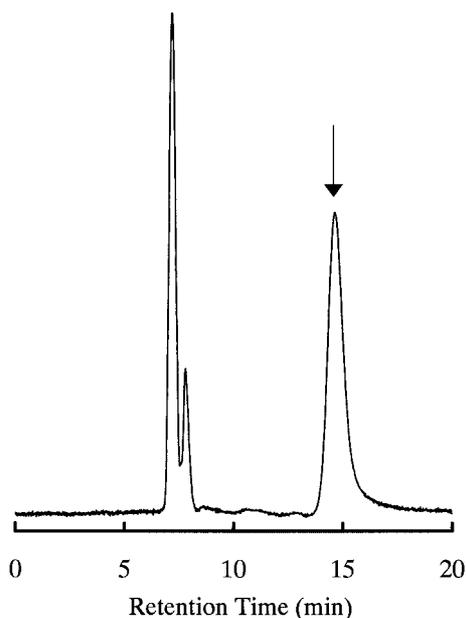
**Figure 2.** SEM images of typical "fiber-in-tube".



**Figure 3.** Schematic diagram of fiber-in-tube SPME/micro-LC. Syringe pumps for sample solution (a), desorption solvent (b) and mobile phase (c), and switching (d) and injection (e) valves.

depicted in Figure 3 and the resulting chromatogram for the analysis of *n*-butyl phthalate in water sample is shown in Figure 4. The extraction capillary was placed between the switching valve and the injection valve. During the extraction process, an aqueous sample solution was delivered to the extraction tube by a syringe pump, and the analyte was adsorbed onto the fibers. Next a certain amount of the desorption solvent was also pumped after changing the switching valve. At the same time, the injector was switched to fill the sample loop by the effluent from the extraction tube. The injection was made after the sample loading. The preconcentration factor of this method was calculated by injecting the standard solution in a conventional way, and was estimated about 100 for the chromatogram in Figure 4.

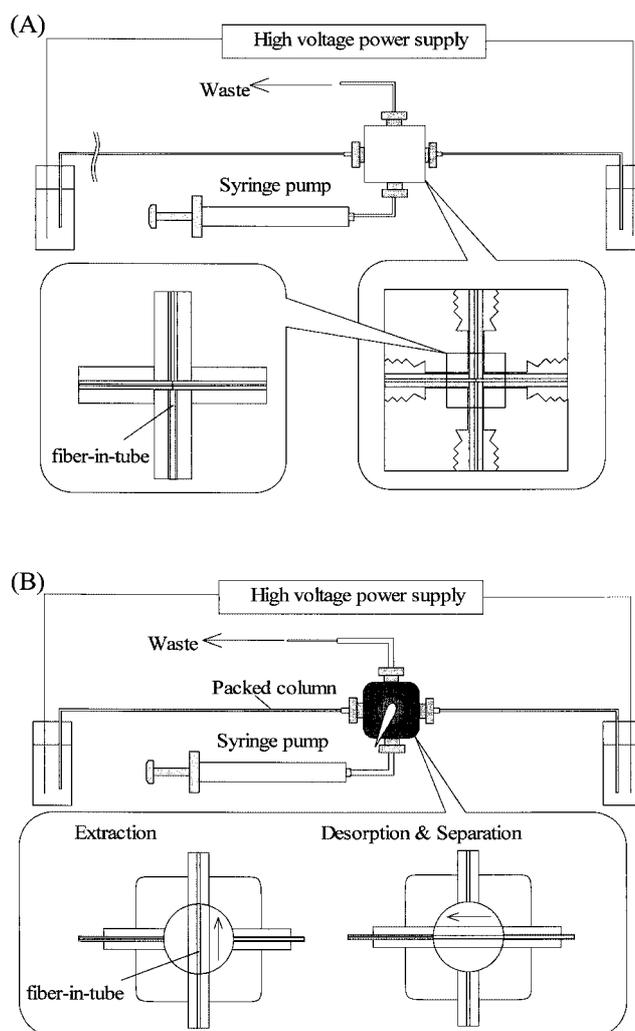
For the on-line coupling of the fiber-in-tube SPME to electrokinetic separation methods, two types of laboratory-made interfaces, shown in Figure 5, have been developed. In Figure 5 A, a short extraction capillary was incorporated in a modified cross connector. The extraction capillary was prepared with a DB-5 inner-



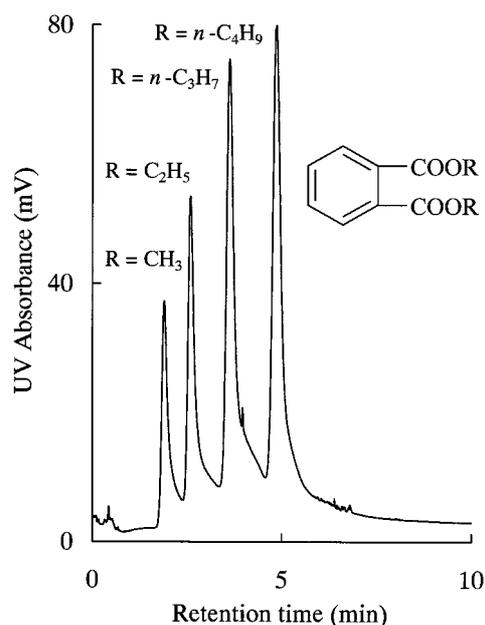
**Figure 4.** Typical chromatogram for the analysis of di-*n*-butyl phthalate in water samples with fiber-in-tube SPME/micro-LC system. SPME conditions: SPME tube, PEEK tube (0.25 mm i.d.  $\times$  24 mm) packed with Zylon<sup>®</sup> (HM) fibers; extraction flow-rate and time, 16  $\mu$ L/min  $\times$  30 min; desorption flow-rate and time, 2  $\mu$ L/min  $\times$  4 min; desorption solvent, methanol. Micro-LC conditions: column, fused-silica capillary (0.53 mm i.d.  $\times$  200 mm) packed with Develosil ODS-5 (Nomura Chemical, Seto, Japan); mobile phase, methanol/water = 90/10 (4  $\mu$ L/min); injection volume, 1  $\mu$ L; detection, UV at 254 nm.

wall-coated capillary (J & W Scientific, Folsom, CA, USA) and fibrous packing materials. For the sample preparation, the sample solution was pumped by a syringe and then the desorption solvent was supplied to transfer the concentrated sample zone into the cross-section of the separation capillaries. Next, the voltage was applied between the vials for the separation of preconcentrated analytes. Combining the inner-coated capillary and fibrous packings, an improved extraction power was obtained for the mixture of antidepressant drugs having different polarity. The volume of required solvent was less than about 2  $\mu$ L for each run and, more than 200 times preconcentration was demonstrated with the total sample volume of 1.0 mL and the extraction time of about 10 min.

No desorption solvent is needed for the "in-valve" configuration depicted in Figure 5 B. A small commercially available 2-way-4-ports valve (Model HV 4-1, Hamilton, Reno, NV, USA) was employed for this system. Fiber-packed PTFE capillary of 0.25 mm i.d.  $\times$  5.0 mm was inserted into the rotor. The number of packed fila-



**Figure 5.** Schematic diagram of fiber-in-tube SPME/CE (A) and fiber-in-tube SPME/CEC (B) system.

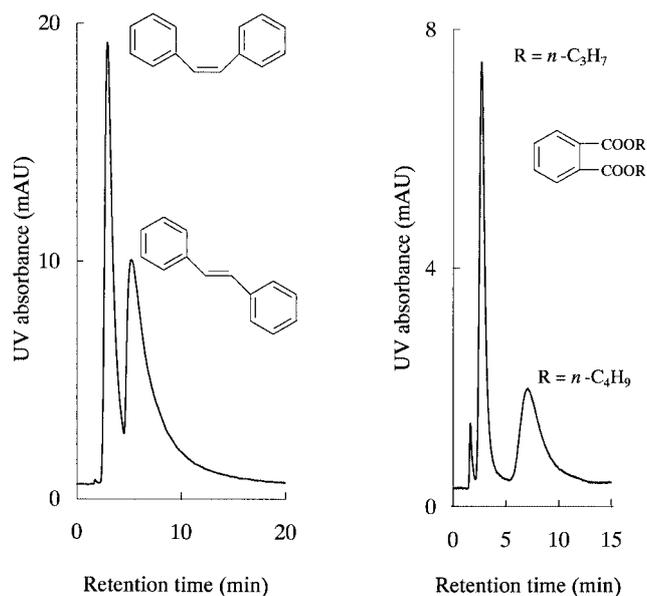


**Figure 6.** Typical CEC separation of di-*n*-alkyl phthalates in water samples with fiber-in-tube SPME preconcentration. SPME conditions : SPME tube, PTFE tube (0.25 mm i.d.  $\times$  5 mm) packed with Zylon<sup>®</sup> (HM) fibers ; extraction flow-rate and time, 4  $\mu$ L/min  $\times$  5 min. CEC conditions : column, fused-silica packed with Superiorex ODS (Shiseido, Yokohama, Japan, 0.15 mm i.d.  $\times$  50 mm ; total length 350 mm) ; mobile phase, methanol/water = 95/5 (Tris 0.5 mM) ; applied voltage, 30 kV ; detection, UV at 230 nm.

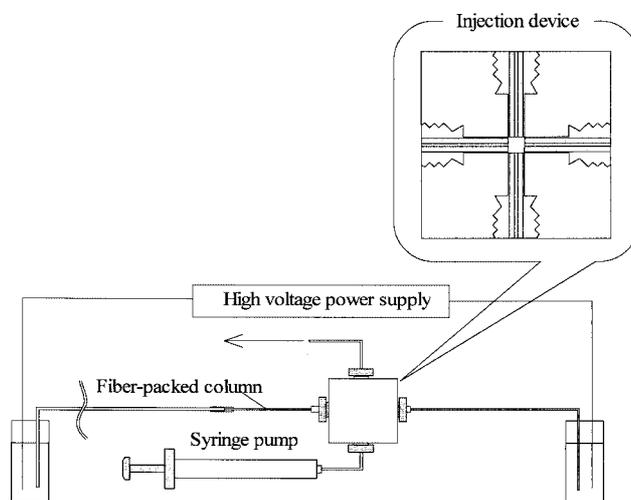
ments was about 380, and the packing density was about 80%. Figure 6 shows the typical chromatogram of a phthalates mixture. The calculated preconcentration factors for these analytes were more than 60 for the extraction of 20- $\mu$ L sample volume (4  $\mu$ L/min for 5 min).

#### The use of fiber-packed capillaries in liquid-phase separations

For the use of fibrous cellulose acetate (CA) in chromatographic process, Jinno and Kiso *et al.* reported the possibility of CA as a stationary phase for LC [37, 38], and demonstrated that the application for the separation of alcohols [32] and polycyclic aromatic hydrocarbons [33]. Other synthetic fibers, such as Zylon<sup>®</sup> and Kevlar<sup>®</sup>, were also introduced as the stationary phase in liquid-phase separation techniques. Figure 7 illustrates the typical chromatograms for the separation of aromatic compounds in micro-LC, in which about 1,300 filaments of Zylon<sup>®</sup> (HM) are packed into a PEEK capillary of 0.50 mm i.d.  $\times$  600 mm length. Although the efficiency was not sufficient as a column for LC separation, it was shown that the fiber materials might have a possibility to be a novel stationary phase in liquid-phase separations.

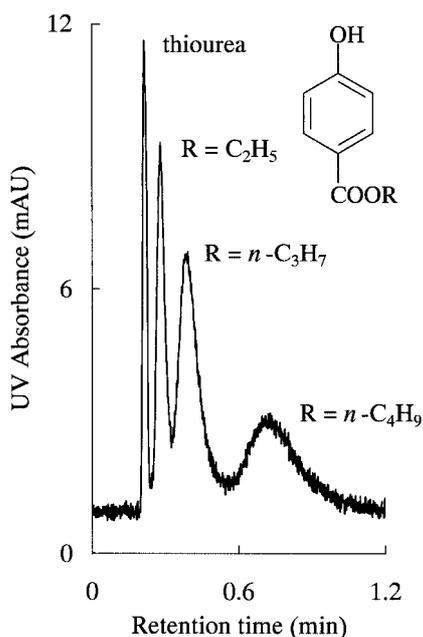


**Figure 7.** Typical chromatograms for the separation of cis-/trans-stilbene mixture (A) and di-*n*-alkyl phthalates (B) with fiber-packed column in LC. Conditions : mobile phase, methanol/water = 50/50 (A), 30/70 (B) at the flow-rate of 20  $\mu$ L/min ; injection volume 1  $\mu$ L ; detection, UV at 254 nm.



**Figure 8.** Overview of the fiber-packed CEC system.

Recently the applications of the fiber-packed capillary column in CEC have been reported [35, 36]. With a specially designed sample injection device, shown in Figure 8, a short fiber-packed capillary was connected to a blank capillary for mobile phase delivery during the separation [36]. A reproducible sample injection to fiber-packed column could be made with the combination of electrokinetic injection and micro-flow pumping. Taking an advantage of the flat-flow profile of CEC, better separation performance over



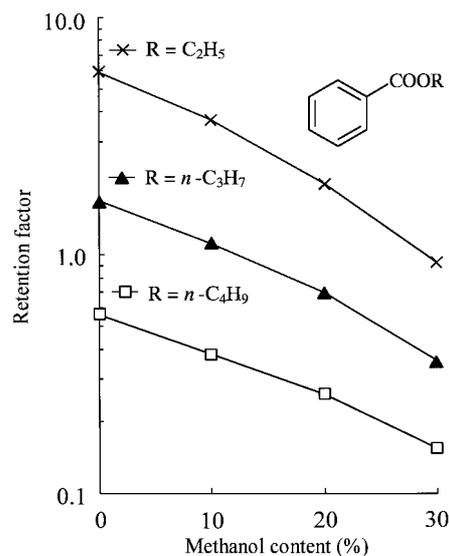
**Figure 9.** Chromatogram for the separation of *p*-hydroxy-*n*-alkyl benzoates with fiber-packed column. CEC conditions : column, fused-silica (0.20 mm i.d. × 50 mm ; total length 700 mm) packed with Kevlar<sup>®</sup> (AS) fibers ; mobile phase, 2.5 mM sodium phosphate buffer (pH = 7.0) ; injection 5 kV (5 sec) ; applied voltage, 20 kV ; detection, UV at 254 nm.

LC was obtained with the column effective length of only 50 mm. Another advantage of the fiber-packed CEC is its short analysis time. As shown in Figure 9, the time required for this particular separation is just 1 min. Even though more resolution should be needed, the total analysis time will be less than a few minutes with a decreased applied voltage.

Furthermore, no frit will be required for the fibrous packings. As described above, the frits are main origin of bubble formation that prevents accurate analysis in CEC [21, 22], and it has been attempted to pressurize the buffer vials by using LC pump [23, 24]. Therefore, it can be considered that the fiber-packed capillary should have a wide application as a separation media in CEC without the requirements for bubble elimination techniques.

#### Characteristics of fiber-packed column in CEC

Figure 10 shows the logarithmic plots of retention factor to the organic solvent content in the mobile phase on Zylon<sup>®</sup> (AS) column. As the sample probes, *p*-hydroxy-*n*-alkyl benzoate homologues having different alkyl-chain length were employed. The retention factors for these solutes decrease with increasing the methanol content, and the results are quite similar to that obtained with CA-packed columns [35]. The results also have a good agreement



**Figure 10.** Effect of methanol content in the mobile phase on the retention on alkyl benzoates. Conditions : column, fused-silica (0.20 mm i.d. × 50 mm ; total length 500 mm) packed with Zylon<sup>®</sup> (HM) fibers, mobile phase, methanol/Tris-HCl buffer (2.5 mM, pH = 8.6). Other conditions are the same as in Figure 9.

**Table 1.** Retention factor of *n*-alkyl benzoates with different fibers.

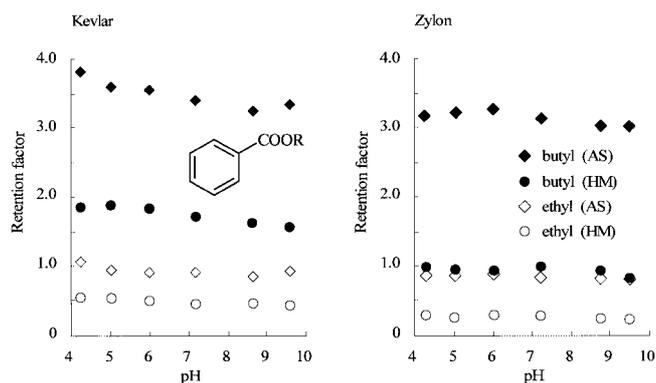
Fiber		Retention factor	
		Ethyl	Butyl
Kevlar	HM	0.91	3.41
	AS	0.49	1.73
Zylon	HM	0.86	3.16
	AS	0.29	0.97

Mobile phase, 2.5 mM Tris-HCl buffer (pH=8.6). Other conditions are the same as in Figure 11.

with the retention factors for benzoates with different types of fiber-packed columns, as summarized in Table 1.

As to the retentivity, HM-type always offers larger values than that of AS-type, and Kevlar<sup>®</sup> fibers always mark longer retentions than Zylon<sup>®</sup>. Although the more extensive investigations should be needed for the differences among these fibers including the contribution of the heat treatment after the spinning process, it is clearly shown that the fiber-packed column has a function similar to conventional reversed-phase packing materials.

Figure 11 shows the pH dependence of the retention factor for

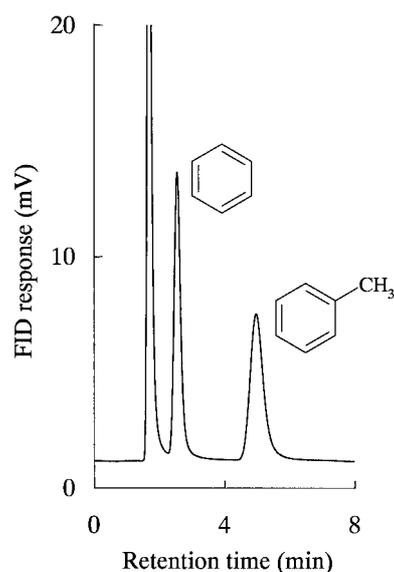


**Figure 11.** Effect of pH on the retention of *n*-alkyl benzoates. Conditions : column, fused-silica (0.20 mm i.d.  $\times$  50 mm ; total length 700 mm) packed with different fibers. Mobile phase was prepared with various buffer solutions having different pH values.

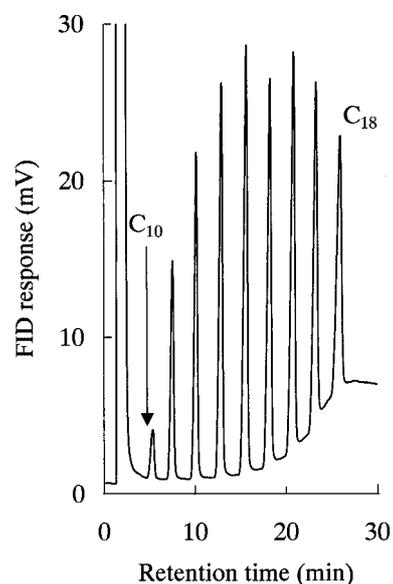
benzoates. Although a slight decrease of the retention factors was observed for all fibers with increasing the pH value of the mobile phase, the retention factors were almost the same over the pH range studied. The results indicate that the surface of each filament is not affected by the pH value of the surrounding liquid. These plots also demonstrate the excellent stability of these fibrous polymers from acidic to basic environments.

#### Future possibilities of fibrous polymer materials in separation science

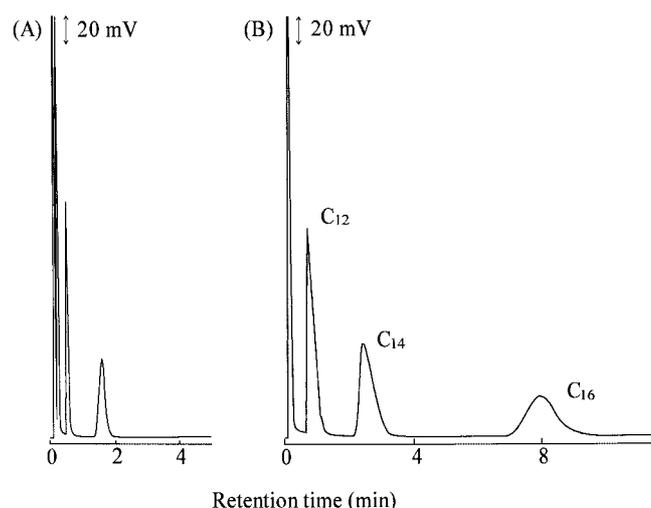
The successful use of the fibrous stationary phases was shown not only in liquid-phase separations but in gas-phase separations [40]. About 330 and 600 filaments of the polymer were packed longitudinally into fused-silica capillaries of 0.32 and 0.53 mm i.d., respectively, and the separation of several test mixtures, such as *n*-alkanes, was carried out with these fiber-packed capillary columns in GC. In Figure 12, typical separation of benzene and toluene with fiber-packed capillary column is depicted. The separation was carried out isothermally at the column temperature of 50 . Temperature-programmed separations of *n*-alkane mixtures, as shown in Figure 13, were also carried out with the fibrous packing materials. The excellent separation performance was demonstrated with only the column length of 2 m, and the retention on these columns are quite reproducible because of the heat resistance of the fiber. Coated-fiber packings, in which a conventional polymer coating process was applied to the packed-filaments, were also evaluated to confirm the contribution of the fibers and the coatings to the separation characteristics. Using the coated- and noncoated-fiber as the stationary phase, isothermal GC separation of *n*-alkans was carried out. With the coating onto the fiber the retentivity was dramatically improved as shown in Figure 14.



**Figure 12.** Typical GC separation of benzene (a) and toluene (b) with Zylon<sup>®</sup> (HM) fiber-packed column. Conditions : column size, 0.32 mm i.d.  $\times$  2 m ; column head pressure, 100 kPa ; column temperature, 50 ; injector and detector temperature, 250 ; split ratio, (50 : 1).



**Figure 13.** Typical chromatograms for the separation of *n*-alkanes (C<sub>10</sub>-C<sub>18</sub>) mixture with Zylon<sup>®</sup> (HM) packed column. Conditions : column size, 0.32 mm i.d.  $\times$  2 m ; column temperature, 80 to 200 at 5 /min ; split ratio, (50 : 1) ; column head pressure, 200 kPa ; injector and detector temperature, 300 .



**Figure 14.** Comparison of fiber-packed column (A) and coated-fiber-packed column (B). For the fiber coating, HR-1701 (Shinwa Chemical, Kyoto, Japan) was used. Other conditions were the same as in Figure 12, except for the column length of 1 m and the temperature at 80 (isothermal).

The results clearly indicated that the coated-fiber-packed capillary columns have a great potential as the separation media not only for volatile compounds but also for a thermally liable or non-volatile compounds. Moreover, as the sample preparation medium, a wide application will be expected for fibrous polymers, especially for polymer-coated fibrous materials. The synthesis of novel tailored polymer fiber to separate particular class of compounds will be possible based on the specific selectivity by the chemical structure of the fibrous polymer materials.

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