Extraction and Separation Media with Synthetic Polymers for High Performance Chromatographic Analysis

（高性能クロマトグラフィー分析のための抽出・分離用合成高分子媒体）

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Abstract

Recently, the requirements for analytical techniques in separation science have been significantly increased on the basis of the enhanced demands for these methods. Due to the enhanced necessity for developing more powerful and efficient separation technique, a systematic study to create appropriate separation and extraction media designed for the target analytes has been significantly desired in chromatographic analysis. On the basis of the technical and scientific background, in this thesis, several synthetic polymers have been introduced as extraction media in sample preparation and as separation media in chromatographic separation.

In **Chapter 1**, general introduction of this thesis including the aims and scope of the study is described along with the background of this work.

Chapter 2 deals with molecular shape selectivity for polycyclic aromatic compounds (PACs) on poly(4-vinylpyridine) (P4VP) stationary phase in liquid chromatography (LC), and the results was compared with that obtained on conventional chemically-bonded stationary phases. In contrast to a conventional octadecylsilica (ODS) phase, the chemical structure of the P4VP phase is consisted of linear poly(4-vinylpyridine) on a silicagel support. On the P4VP phase, a high linear correlation between the logarithmic retention factor and *F* number was observed for planar PACs, and smaller retention for non-planar PACs was also observed than planar PACs. The selectivity for planar/non-planer analytes on the P4VP phase was significantly better than that of conventional a polymeric ODS.

In **Chapter 3**, another stationary phase on the basis of a different type of synthetic polymer was introduced, where poly(butylene terephthalate) (PBT)-coated silica was applied to the stationary phase in LC. The retention trends for various

aromatic compounds on the PBT phase were studied along with the effect of column temperature on the retention behavior. In the case of the PBT stationary phase, a good selectivity for planar/non-planar analytes was observed for PACs. The trend was quite similar to that of the P4VP stationary phase described above. However, retention trend on the PBT phase showed that "rod-like" molecules were retained longer than "square-like" molecules, suggesting a different separation mechanism between P4VP and PBT phases. In addition, the selectivity for quinoline/isoquinoline on the PBT stationary phase was better than that on the conventional stationary phases. The results suggest that the PBT recognizes the position of the nitrogen atom in the analyte molecule.

The application of fibrous polyimide (PI) as a separation medium in gas chromatography (GC) is described in **Chapter 4**. Fiber-packed capillary column was prepared by packing the PI filaments into a fused-silica capillary and the retention performance as a GC stationary phase was studied. In the PI phase, a good linear relationship between the carbon number and the logarithmic retention factors of alkane analytes was observed along with linear van't Hoff plots where the logarithmic retention factors was plotted against the reciprocal absolute column temperature. Taking advantage of the synthetic PI filaments with a good heat-resistance, temperature-programmed GC separations with the fibrous PI stationary phase was successfully demonstrated.

In **Chapter 5**, the extraction and subsequent chromatographic analysis of monoethanolamine (MEA), one of the toxic volatile organic compounds, with Zylon filaments as the extraction medium is described. Simultaneous extraction and derivatization process was studied with the specially-designed needle-type device

packed with fibrous Zylon as the extraction medium. The needle-type device was further applied to the sample preparation of other volatile amines. Introducing derivatization reaction with cyclohexanone, the sensitivity of MEA was significantly improved, and the method was possible to be applied to the sample preparation of other volatile amines. Taking advantage of the specially-designed needle, the needle was able to be stored for several days at room temperature after the sampling, where the derivatives of the volatile amines were stably trapped on the surface of the Zylon filaments in the needle.

As another application of fine synthetic filaments in separation science, a braided arrangement of Zylon filaments was introduced as a novel extraction medium, in **Chapter 6**. Preconcentration of aromatic compounds in water samples with the braided extraction medium was established. By connecting metal wire inside of the braid to a power supply and applying voltage to the wire, heat-assisted desorption with resistive heating was demonstrated. On the basis of a low voltage application to the metal wire in the center of braided fiber as a extraction medium, the temperature of the extraction capillary was elevated and the desorption efficiency was clearly improved by applying a voltage during the desorption process.

Finally, the over-all conclusion of this thesis is summarized in **Chapter 7**.

Contents

List of Abbreviations

ACN	acetonitrile
EDA	ethylenediamine
FID	frame ionization detector
GC	gas chromatography
L/B	length-to-breadth ratio
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantitation
MEA	monoethanolamine
NBA	*n*-butylamine
ODS	octadecylsilica
P4VP	poly(4-vinylpyridine)
PACs	polycyclic aromatic compounds
PBT	poly(butylene terephthalate)
PDMS	polydimethylsiloxane
PEG	polyethylene glycol
PI	polyimide
PTFE	polytetrafluoroethylene
PVF	poly(vinylidene fluoride)
RSDs	relative standard deviations
SPE	solid-phase extraction
SPME	solid-phase microextraction

Chapter 1

General Introduction

1-1. General Introduction

Chromatography is a high-performance separation technique that is commonly employed in a variety of fields, including the medical, environmental, and food industries [1-3]. Liquid chromatography (LC) is a representative technique of chromatographic methods that uses a liquid, such as organic solvents, as the mobile phase for the separation. In gas chromatography (GC), which is carried out with a gaseous the mobile phase, an efficient separation performance can be achieved especially for the separation of complex sample mixtures consisted of closely-related volatile organic compounds.

The choice of the stationary phase is quite important for efficient separation and accurate quantification of the target analyte in chromatography [4-6]. Silicagel-based stationary phases such as octadecylsilica (ODS) have been mainly employed in LC [7-8]. ODS stationary phase is often the first choice in real sample analysis because of its high separation performance along with reasonable cost. For typical GC separation, fused-silica open-tubular capillary column are widely used for the separation of general samples, where the inner wall of the capillary is coated with a thin film of polydimethylsiloxane (PDMS) as the stationary phase. Although the PDMS stationary phase could be normally regarded as the first choice for the GC separation on the basis of a good universal selectivity to a wide variety of sample matrices, the development of new stationary phase is one of the most important research objectives to make sure the solution of upcoming difficult separation problems [9-15].

On the other hand, sample preparation before the chromatographic separation is regarded as a key step in the analytical process [16-18], because the sample preparation includes pretreatments to improve detection sensitivity by concentrating the target

components and removing interfering substances. In contrast to conventional classic sample preparation techniques such as liquid-liquid extraction, solid-phase extraction (SPE) enables less amount of organic solvent usage in the sample preparation process. However, the SPE method is basically based on the partition theory in LC column, and silicagel-based particulate extraction media are widely employed [19-21].

In order to further improve the extraction performance and also realize the more miniaturized sample preparation method, fine fibrous polymeric materials have been introduced as the extraction media in miniaturized sample preparation. Downsizing the sample preparation, the amount of solvent in the sample preparation can be significantly reduced, while the coupling of the sample preparation to subsequent chromatographic system can be easily made [22-24].

In this thesis, several types synthetic polymers have been introduced as extraction and separation media for high performance chromatographic analysis. As the reference silicagel particles with chemically-bonded polymer ligands and polymer-coated stationary phases were also introduced for LC measurements, and the retention behavior for aromatic compounds were compared with those of conventional stationary phases. In addition, fibrous polymers were introduced as extraction and separation media for GC, and their performance was evaluated. As a further application of fibrous polymers, braid was introduced as a novel extraction medium for preconcentration of aromatic compounds in water samples. Furthermore, heat-assisted desorption by applied voltage to a metal wire inside the braid was also investigated.

1-2. References

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Chapter 2

Retention Behavior for Polycyclic Aromatic Compounds on a Poly(4-vinylpyridine) Stationary Phase in Reversed-Phase Liquid Chromatography

2-1. Introduction

LC is one of the most powerful techniques for the separation of various types of complex mixtures and has been employed in a wide range of scientific fields such as pharmaceutical, analytical and environmental chemistry [1-5]. For the effective LC separation of actual samples, the proper setting of separation conditions, especially the selection of an appropriate stationary phase, is a quite important [6-10]. ODS has been widely employed as a stationary phase for LC because of its advantages such as versatility, high separation performance, widely commercial availability and low cost [11-13]. However, there are still many complex mixtures consisting of many closely-related components that are difficult to be completely separated on the conventional ODS phases [14-21].

Recently, several synthetic polymers with excellent heat and chemical resistance have been applied as separation media for LC and GC [22-28]. In contrast to the conventional silicagel-based stationary phases, polymer-based stationary phases allow the use of harsh separation conditions that cannot be employed in typical ODS phases by introducing the stationary phase ligands into the polymer backbone, such as styrene/divinylbenzene copolymers. Furthermore, fibrous polymer materials has also introduced as extraction media for miniaturized sample preparation, including fiber-packed capillary and needle [29-41].

In this chapter, as an extension of the previous investigations, a poly(4-vinylpyridine) (P4VP) stationary phase [42-44] was introduced as a separation media for LC. The retention behavior of various polycyclic aromatic compounds (PACs) was systematically investigated, and the selectivity of the molecular shape for these analytes was evaluated in comparison with that observed with typical ODS and

phenylbutylsilica (PBS) stationary phases. Alkylbenzenes and structural isomers of disubstituted benzene were also employed for additional investigations to interpret the retention mechanism on the P4VP stationary phase.

2-2. Experimental

Reagents and solvents

All solvents were obtained from Kishida Chemical (Osaka, Japan), and the sample analytes including PACs were purchased either from Tokyo Chemical Industry (Tokyo, Japan) or Sigma-Aldrich (St. Louis, MO, USA). The chemical structure of PACs was shown in **Figure 2.1**. These chemicals were of analytical grade, and used without further purification. Water was purified by Milli-Q Water purification system (Merck Millipore, Darmstadt, Germany). Column temperature was controlled at 25ºC.

P4VP stationary phase

The P4VP column was supplied from DAICEL (Osaka, Japan), and the internal diameter of the packed column was 4.6 mm and the length was 150 mm. The average particle size of this phase is 3 μm. Details of the P4VP phase can be found elsewhere [42]. As shown in **Figure 2.2**, the structure of the P4VP phase is consisted of polymeric pyridyl groups on a silicagel.

LC measurements

LC system was consisted of a PU-1585 pump (Jasco, Tokyo, Japan) and a model MD-910 photodiode array detector (Jasco), and a model 7725 injector (Rheodyne, Cotati, CA, USA). Data analysis software used was Borwin PDA (Jasco) running on a

personal computer. As the mobile phase, mixture of methanol/water, acetonitrile (ACN)/water and ACN/20 mM phosphate buffer (pH 7.5) were employed unless otherwise specified, and the flow-rate was set at either 1.0 or 0.50 mL/min. For all the experiments, an injection volume of 20 μL was used. UV detection wavelength was determined in the preliminary experiments for all the analytes in order to make sure the effective detection of each analyte. The relative standard deviations (RSDs) were less than 3% for all the retention time measurements in this work.

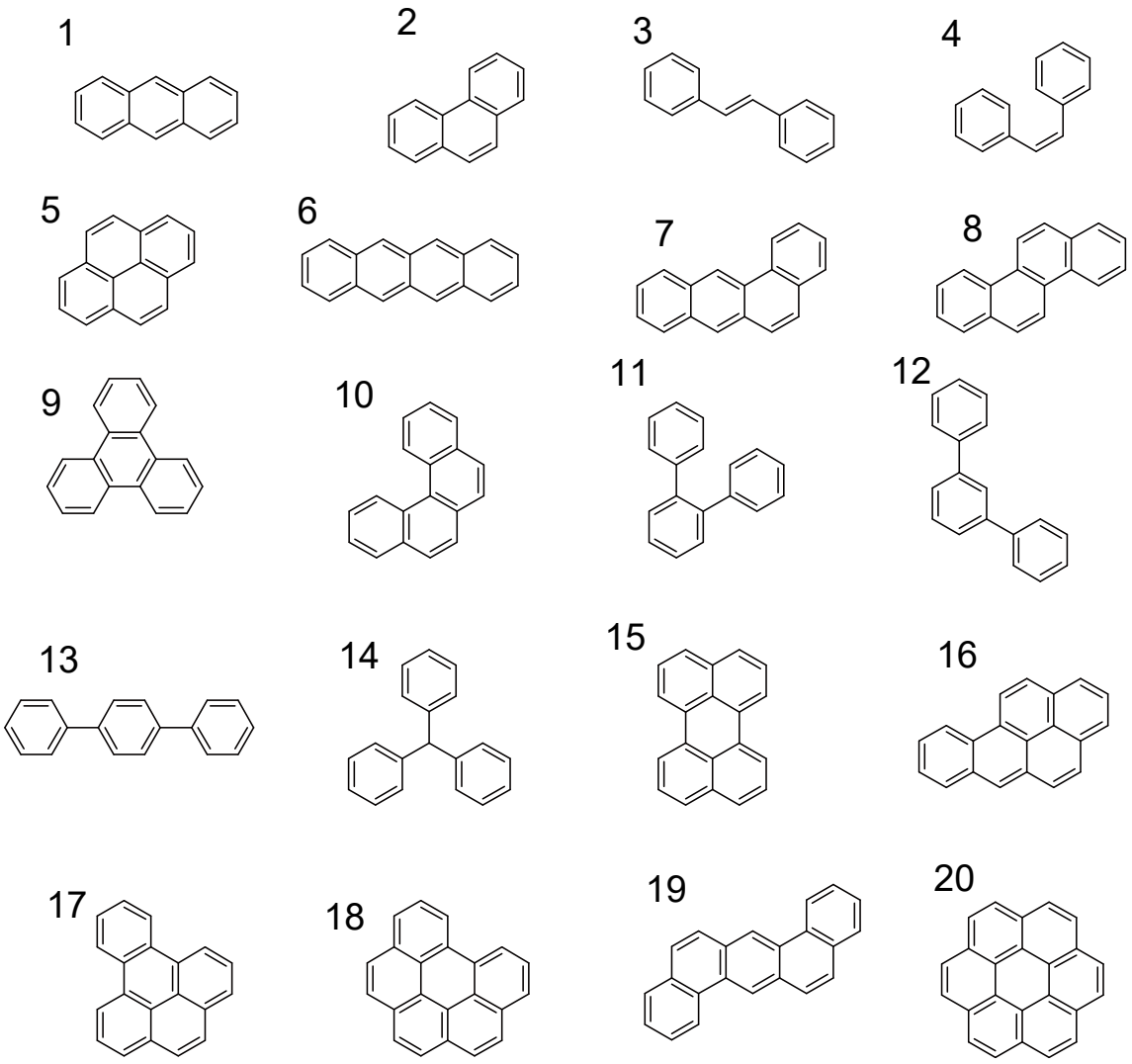


Figure 2.1 Chemical structure of PACs employed as sample probe. 1, anthracene; 2, phenanthrene; 3, *trans*-stilbene; 4, *cis*-stilbene; 5, pyrene; 6, naphthacene; 7, benz[*a*]anthracene; 8, chrysene; 9, triphenylene; 10, benzo[*c*]phenanthrene; 11, *o*-terphenyl; 12, *m*-terphenyl; 13, *p*-terphenyl; 14, triphenylmethane; 15, perylene; 16, benzo[*a*]pyrene; 17, benzo[*e*]pyrene; 18, benzo[*ghi*]perylene; 19, dibenz[*a,h*]anthracene; and 20, coronene.

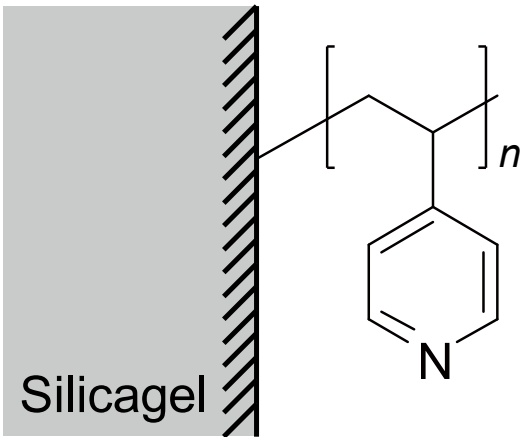


Figure 2.2 Chemical structure of the P4VP stationary phase.

2-3. Results and Discussion

Molecular shape recognition capability for PACs on the P4VP stationary phase

Retention behavior for PACs on the P4VP stationary phase was evaluated in LC, and compared with commercially-available stationary phases. For comparison, following three stationary phases were employed: a Develosil ODS-UG-5 (monomeric ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical, Seto, Japan), a Develosil ODS-A-5 (polymeric ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical) and a YMC-Triart Phenyl (PBS phase; 4.6 mm i.d., 150 mm length; YMC, Kyoto, Japan).

For evaluating retention behavior of PACs, *F* number, which represents a molecular size, was introduced in this work. The *F* number was proposed by Hurtubise *et al.* [45,46] and defined as follows: *F* = (number of double bonds) + (number of primary and secondary carbons) – 0.5 × (number of non-aromatic rings). As shown in **Figure 2.3**, a high linear correlation between the logarithmic retention factor (*ln k*) and *F* number for planar PACs was obtained on the P4VP phase, and similar retention trend was observed on other three phases, monomeric ODS, polymeric ODS and PBS phases [47,48]. For non-planer PACs, however, the retention on the P4VP phase was a significantly small, the trend was similar to on the polymeric ODS phase.

Another parameter for evaluating the retention trend, length-to-breadth (*L/B*) ratio, which proposed by Wise *et al.* and Kaliszan *et al.* [49-51] was also introduced. *L/B* ratio was defined as the maximized length to breadth ratio of the two-dimensional molecules. For example, a molecule with a small *L/B*, such as triphenylene (*L/B* = 1.12), has a "square-like" shape, while the one with a large *L/B*, such as naphthacene (*L/B* = 1.89), has a "rod-like" shape. In other words, *L/B* ratio can be used to classify

molecular shapes for compounds with the same *F* number. Typical chromatograms of planar four-rings PACs with the same *F* number (*F* = 9) were shown in **Figure 2.4**. On the conventional polymeric ODS, a PACs molecule with a large *L/B* ratio has a relatively large retention. The above trend has been thought to be due to the "slot-like" structure of the polymeric ODS stationary phase on the silicagel support, which allows for effective interaction with analyte molecules with large *L/B* ratio [47,52,53]. In the monomeric ODS, the elution order of these PACs was the same as the polymeric ODS, although the molecular shape selectivity of monomeric ODS was smaller than polymeric ODS due to the relatively low ligand density of octadecyl functional groups on the silica support [47].

On the other hand, retention trend on the P4VP phase showed that "square-like" molecules were retained longer than "rod-like" molecules, and this tendency was clearly different from that observed on typical ODS phases. These results can be suggested that a relatively shallow "slot-like" structure is formed on the surface of the P4VP phase. Since there are many pyridyl groups in the ligand of P4VP, these pyridyl groups can easily interact with each other, and the interaction between pyridyl groups in adjacent ligands is also possible. Therefore, a shallow "slot-like" structure was formed, which might be contribute to the retention of the molecule.

As proposed by Tanaka *et al.* and Jinno *et al.*, triphenylene with a planar shape and *o*-terphenyl with a non-planar shape were introduced as the sample probes for the investigation of the molecular planarity recognition capability [54,55] of the P4VP phase. The pair of solute molecules is usually employed for evaluation the molecular planarity recognition capability because they have the same *F* number and similar two-dimensional molecular shape. The selectivity for planar/non-planar solute pairs

was summarized in **Table 2.1**, and the results suggest that the P4VP stationary phase has a good selectivity for recognizing planar and non-planar PACs of similar molecular size. The retention of the P4VP stationary phase for non-planar analytes was observed quite smaller than that of the typical ODS stationary phase even compared to the PBS stationary phase having aromatic rings. The result shows excellent molecular planarity recognition capability for the P4VP stationary phase and it could be considered as the large density of aromatic functional groups on the silica support of the P4VP phase.

Retention tendency of alkylbenzenes on the P4VP phase

The retention behavior for a group of alkylbenzenes as the sample probes on the P4VP stationary phase was also studied. As shown in **Figure 2.5**, the logarithmic retention factors of alkylbenzenes on each stationary phase were plotted against the number of carbon atoms in the alkyl chain. A linear correlation was found between *ln k* and the number of carbon atoms of the alkyl functional group on the ODS and PBS phases, and the result suggests that these phases have retentivity based on hydrophobic interactions between the ligands in the stationary phase and solute molecules.

A similar retention trend was observed on the P4VP phase, although the slope of the correlation line was smaller than other phases, suggesting that the contribution of hydrophobicity to retention was relatively small on the P4VP phase. This is probably due to the polarization of the pyridine ring in the P4VP phase. However, as mentioned above, the P4VP phase showed a large retention to planar PACs as similar to the typical polymeric ODS phase. The trend can be interpreted by the π-π interaction between the pyridine ring in the P4VP ligand and the PACs. Therefore, the retention on the P4VP phase could be mainly attributed to the π-π interaction, but its contribution of the

hydrophobicity of the analyte to the retention was limited because the ligand of P4VP contains nitrogen atoms.

Selectivity for structural isomers of disubstituted benzenes on the P4VP phase

Table 2.2 summarizes the retention factor of the structural isomers of disubstituted benzene on the four stationary phases. For the elution order of dichlorobenzene and dibromobenzene, the order on P4VP phase was different from other phases. The results indicate that there is a unique selectivity in the position of these substituents in the analyte compared to the ODS and PBS phases. This selectivity might be due to the dipole-dipole interaction between the pyridyl group of the P4VP ligands and solute molecules with electron withdrawing substituents such as dichlorobenzene. Therefore, a molecule with a large dipole moment, such as *o*-dichlorobenzene, is expected to interact more with the P4VP ligands than the *p*-isomer.

For dimethoxybenzene and phenylenediamine, the elution order on the P4VP phase was the same as that on the ODS and PBS phases, as shown in **Table 2.3**. From the results, it was indicated that the structural isomers with electron-donating substituents such as methoxy and amino groups might not significantly affect the unique retention behavior on the P4VP phase. Therefore, these results suggest that the P4VP phase has a unique molecular shape recognition capability for structural isomers with electron withdrawing substituents, probably based on dipole-dipole interactions, although further systematic investigation is necessary to derive a final conclusion for the retention mechanism of the P4VP stationary phase.

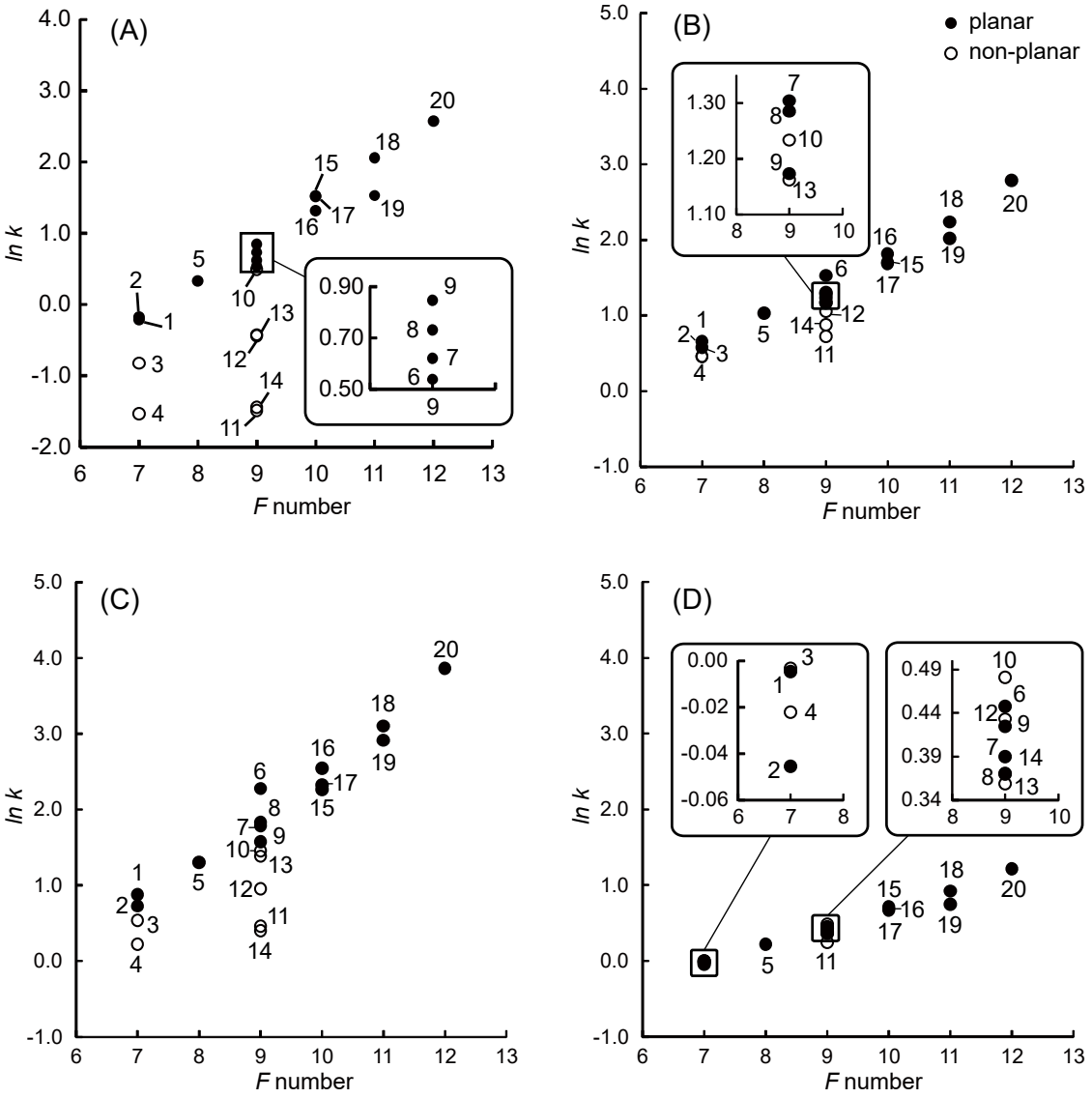


Figure 2.3 Relationship between *F* number and *ln k* for PACs on (A) P4VP, (B) monomeric ODS, (C) polymeric ODS and (D) PBS phase. Analyte: 1, anthracene; 2, phenanthrene; 3, *trans*-stilbene; 4, *cis*-stilbene; 5, pyrene; 6, naphthacene; 7, benz[*a*]anthracene; 8, chrysene; 9, triphenylene; 10, benzo[*c*]phenanthrene; 11, *o*-terphenyl; 12, *m*-terphenyl; 13, *p*-terphenyl; 14, triphenylmethane; 15, perylene; 16, benzo[*a*]pyrene; 17, benzo[*e*]pyrene; 18, benzo[*ghi*]perylene; 19, dibenz[*a,h*]anthracene; and 20, coronene. Mobile phase: methanol/water = 90/10.

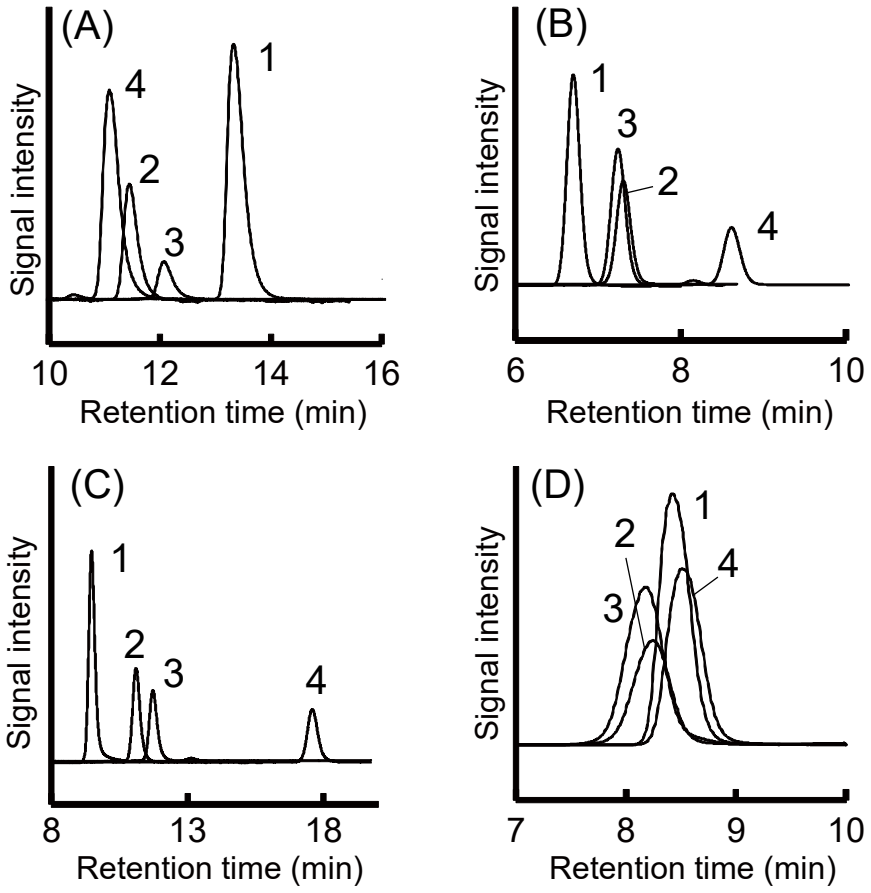
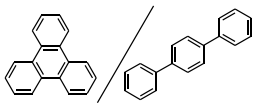
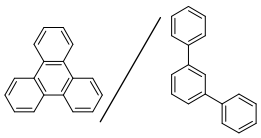
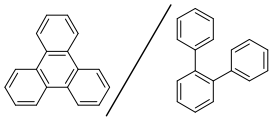
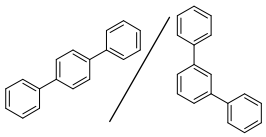
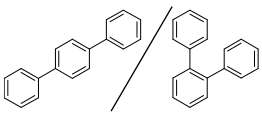
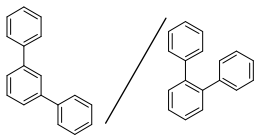
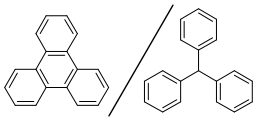
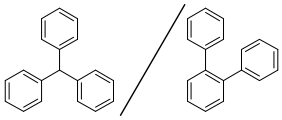
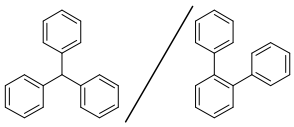


Figure 2.4 Typical chromatograms of planar four-rings PACs on (A) P4VP, (B) monomeric ODS, (C) polymeric ODS and (D) PBS phases. Analyte: 1, triphenylene (*L/B* = 1.12); 2, benz[*a*]anthracene (*L/B* = 1.58); 3, chrysene (*L/B* = 1.72); and 4, naphthacene (*L/B* = 1.89). Mobile phase: methanol/water = 90/10.

Table 2.1 Selectivity for planar/non-planar solute pairs on the P4VP and other three stationary phases.

analyte pair	selectivity (α)			
	P4VP	monomeric ODS	polymeric ODS	PBS
	3.58	1.01	1.21	1.07
	3.63	1.13	1.87	0.991
	10.4	1.57	3.04	1.20
	1.01	1.12	1.54	0.929
	2.90	1.56	2.51	1.12
	2.86	1.39	1.63	1.21
	9.84	1.35	3.22	1.05
	1.05	1.17	0.994	1.14
	2.03	1.00	1.37	1.02

Mobile phase: methanol/water = 90/10.

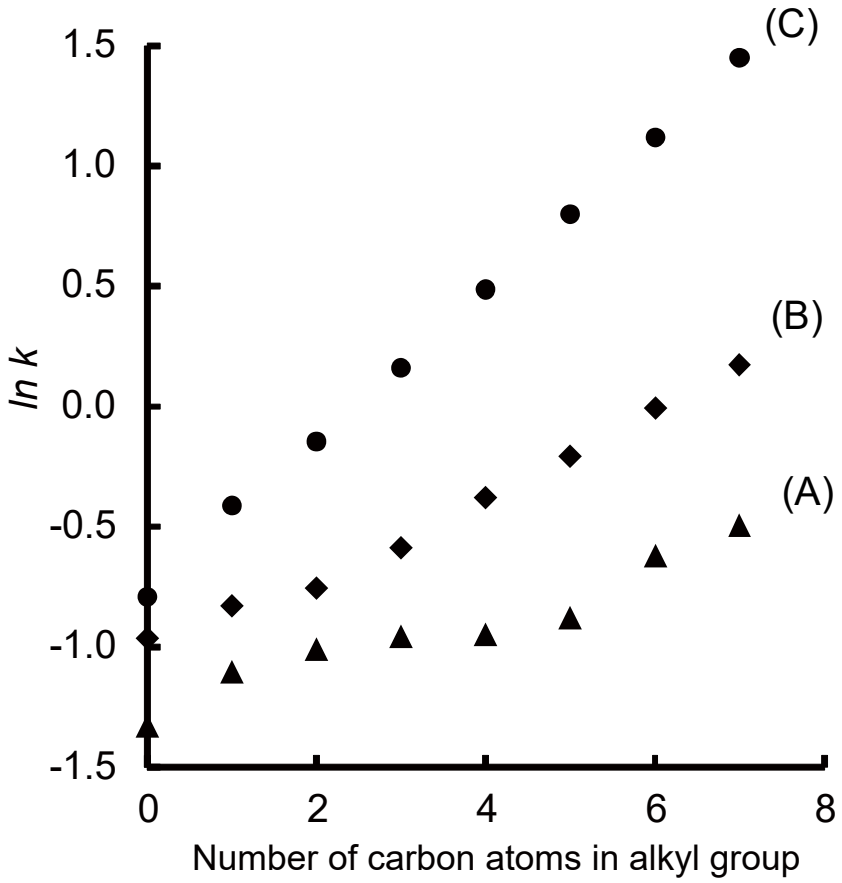


Figure 2.5 Relationship between the number of carbon atoms in alkyl chain and *ln k* of alkylbenzenes on three stationary phases. (A) P4VP, (B) PBS and (C) monomeric ODS. Mobile phase: (A) methanol/water = 70/30, (B) and (C) methanol/water = 90/10.

Table 2.2 Retention data for halogenated benzene isomers on the P4VP and other three phases.

		retention factor (*k*)			mobile phase
		o-	*m*-	*p*-	
dichlorobenzene	P4VP	4.79	3.81	3.37	(A)
	monomeric	4.19	5.59	4.72	(B)
	polymeric	4.27	5.29	4.36	(B)
	PBS	2.99	3.35	3.15	(B)
dibromobenzene	P4VP	7.75	6.46	5.51	(A)
	monomeric	5.15	7.32	6.40	(B)
	polymeric	5.20	6.86	5.75	(B)
	PBS	3.73	4.40	4.19	(B)

Mobile phase: (A) methanol/water = 40/60, (B) methanol/water = 70/30.

Table 2.3 Retention data for disubstituted benzene isomers on the P4VP and other three phases.

		retention factor (*k*)			mobile phase
		o-	*m*-	*p*-	
dimethoxybenzene	P4VP	0.888	1.59	1.32	(A)
	monomeric	1.41	2.71	2.32	(B)
	polymeric	1.51	2.27	2.26	(B)
	PBS	1.62	2.52	2.47	(C)
phenylenediamine	P4VP	0.622	0.484	0.131	(D)
	monomeric	1.80	0.627	0.043	(D)
	polymeric	3.66	1.65	0.892	(D)
	PBS	2.30	1.46	0.504	(D)

Mobile phase: (A) ACN/water = 30/70, (B) ACN/water = 50/50, (C) methanol/water = 60/40, (D) ACN/20 mM phosphate buffer (pH 7.5) = 10/90.

2-4. Conclusions

In this chapter, P4VP stationary phase was introduced as a separation medium in LC. The P4VP phase showed specific molecular shape selectivity for planar PACs, where "square-like" analyte molecules were retained more strongly than "rod-like" analyte molecules. Compared to the typical ODS and PBS phases, the P4VP phase showed a good selectivity for planar/non-planar PACs of similar molecular sizes. In contrast to the typical ODS and PBS phases, only a small amount of alkylbenzene was retained on the P4VP phase. This result suggested that the hydrophobic contribution from the polarization of the pyridine ring is small in the P4VP phase. Furthermore, a unique molecular shape selectivity for the structural isomers of dichlorobenzene and dibromobenzene was confirmed. The result could be interpreted as a dipole-dipole interaction between the solute molecule with an electron withdrawing substituent and the nitrogen atoms of the P4VP ligands.

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Chapter 3

Molecular Shape Recognition of Various Aromatic Compounds on a Poly(butylene terephthalate) Stationary Phase in Liquid Chromatography

3-1. Introduction

In LC, ODS stationary phase has been the most dominant stationary phase as described in the previous chapter. This is because its good separation performance and wide availability [1-4], although various types of chemically bonded stationary phases have been developed in the past decades to improve the separation process [5-10]. ODS stationary phases can be classified into two types from the bonding chemistry during phase synthesis [11,12]. One is the "polymeric-type", which is typically synthesized from trifunctional (or bifunctional) silanes as starting materials under aqueous conditions, and another is the "monomeric-type", which is synthesized from monofunctional silanes under non-aqueous synthetic conditions [13-15]. The differences in the selectivity and phase order of the polymeric and monomeric ODS phases was investigated [16-20], but the fundamental theory of retention mechanism has not been well established.

The development of novel stationary phases, especially polymer-based stationary phases, has been also desired to develop more efficient and cost-effective separations in chromatography [21,22]. Another advantageous feature of the polymer-based phase in separation science is a simple phase structure design and the relatively easy synthesis of the phase, allowing a cost-effective development of novel stationary phases [23].

As an extension of previous studies, a poly(butylene terephthalate)-coated silica (PBT) was introduced as a stationary phase for LC in this chapter. Retention behavior for a group of PACs on the PBT stationary phase was evaluated in comparison with the trends observed on typical ODS and phenylbutylsilica (PBS) phases. Furthermore, the retention trend for quinoline and disubstituted benzene isomers was

investigated on the PBT phase, and the effect of column temperature for the retention tendency was also evaluated.

3-2. Experimental

Reagents and solvents

All solvents were obtained from Kishida Chemical (Osaka, Japan), and the sample analytes including PACs were purchased either from Tokyo Chemical Industry (Tokyo, Japan). These chemicals were of analytical grade, and used without further purification. Water was purified by Milli-Q Water purification system (Merck Millipore, Darmstadt, Germany).

PBT stationary phase

The PBT-packed column was obtained from DAICEL (Osaka, Japan) and the internal diameter of the packed column was 4.6 mm and the length was 150 mm. In this phase, fine silica particles of 3 μm o.d. were used as the support. **Figure 3.1** shows the chemical structure of the PBT stationary phase, and the structure of the PBT stationary phase is consisted of a polymer chain having a planar phenyl group and two ester groups in the monomer units [24,25].

LC measurements

LC system consisted of a PU-1585 pump and a model MD-910 Photodiode Array Detector (Jasco, Tokyo, Japan), and a Model 7725 injector (Rheodyne, Cotati, CA, USA) was employed. Data analysis software used was Borwin PDA (Jasco) running on a personal computer. As the mobile phase, a mixture of methanol/water was used,

and the flow-rate was set at either 1.0 or 0.50 mL/min. For all experiments, an injection volume of 20 μL was used. UV detection wavelength was determined in the preliminary experiments for all the analytes in order to make sure the effective detection. Column temperatures between 15 and 40ºC were controlled by Low Temperature Thermostatic Water Bath T-10L (THOMAS Kagaku, Tokyo, Japan).

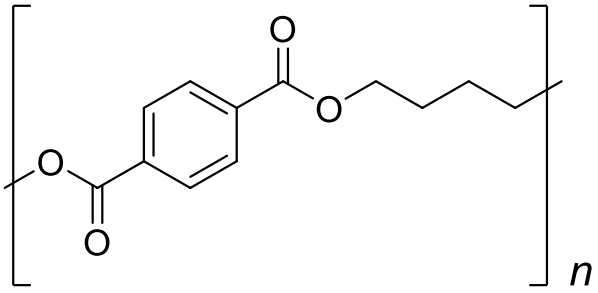


Figure 3.1 Chemical structure of PBT stationary phase.

3-3. Results and Discussion

Retention behavior of the PBT stationary phase for PACs

The retention behavior for PACs on the PBT stationary phase was investigated in LC, and compared with commercially available stationary phases. For comparison, following three stationary phases were employed: a Develosil ODS-UG-5 (monomeric ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical, Seto, Japan), a Develosil ODS-A-5 (polymeric ODS phase; 4.6 mm i.d., 150 mm length; Nomura Chemical), and a YMC-Triart Phenyl stationary phase (PBS phase; 4.6 mm i.d., 150 mm length; YMC, Kyoto, Japan).

F number, which represents the molecular size of PACs, was introduced for the analysis of the retention behavior of the PACs [26,27]. The molecular size descriptor *F* is defined as follows: *F* = (number of double bonds) + (number of primary and secondary carbons) - 0.5 × (number of non-aromatic rings). As shown in **Figure 3.2**, a high linear correlations was obtained between the logarithmic retention factor (*ln k*) and *F* numbers of PACs on the ODS and PBS stationary phases [28-30]. In the case of PBT phase, a linear correlation was also observed between *ln k* and *F* numbers of planer PACs. Previous studies have shown that the molecular shape selectivity of the ODS stationary phase could be attributed to the actual surface structure, which consists of a dense brush-type phase structure formed by octadecyl groups bonded on the silica support. Especially on the polymeric ODS phase, which allows effective interaction with planar PACs, "slot-like" structure is predicted [31,32]. On the other hand, the assumed surface structure of the PBT phase is considered to be a thin layer surrounded by long polymer chains of PBT. Therefore, the good planarity recognition capability of the PBT phase for planar analytes can be interpreted as an effective interaction

between the planar analytes and the polymer chains on the silica support.

Molecular shape recognition capability of the PBT phase

Table 3.1. summarizes the selectivity (α) for a solute pair of planar/non-planer PACs on four stationary phases. The PBT stationary phase was able to recognize the molecular planarity of these compounds as well as the polymeric ODS stationary phase, although the retention for non-planar analytes was relatively small. The limited retention for non-planar compounds on the PBT stationary phase could be construed as due to the limited interaction between the non-planar aromatic molecules and the surface structure of the stationary phase. In the ODS phase, the interaction with the alkyl ligands on the surface of the silica support allows for some retention of non-planar analytes, but the retention is relatively smaller than that for planar analytes. The interaction of the PBT stationary phase with non-planar analytes is considered to be quite limited due to the surface structure suitable for interaction with planar PACs.

Selectivity for two-dimensional shape of PACs on the PBT phase

The selectivity for the two-dimensional shape of PACs on the PBT stationary phase has also been investigated with another molecular descriptor, length-to-breadth ratio (*L/B*) [33,34]. The parameter is defined as the maximized length-to-breadth ratio of the two-dimensional molecule projected on the flat surface. The logarithmic retention factor of planar four-ring PACs with the same *F* number on these four stationary phases were plotted against the corresponding *L/B* values as shown in **Figure 3.3**. The PBT stationary phase seems to have a good molecular shape selectivity for these isomeric PACs, with the "rod-like" molecules retained longer than the

"square-like" molecules. The trend was quite similar to that of the molecular shape selectivity on the polymeric ODS stationary phase, but the shape selectivity on the PBT stationary phase was slightly lower than that on the polymeric ODS phase.

Selectivity for disubstituted benzene isomers on the PBT phase

Table 3.2 summarizes the retention factor (k) and selectivity (α) values of dichlorobenzenes and dibromobenzenes on the four stationary phases, and **Figure 3.4** shows typical chromatograms of dibromobenzenes on these phases. The elution order of the isomers of dichlorobenzenes and dibromobenzenes on the PBT stationary phase was different from that on the other phases, and the trend clearly shows the unique selectivity for these structural isomers on the PBT stationary phase. The result could be explained on the basis of intermolecular interactions between the analyte molecules and PBT stationary phase ligands with similar partial chemical structures to these *para*-isomers, such as *p*-dichlorobenzene.

In the case of structural isomers of dimethyl phthalate and diacetylbenzene, the retention data on the four stationary phases were summarized in **Table 3.3**, and typical chromatograms of these compounds were shown in **Figure 3.5**. The elution order of dimethyl phthalate on the PBT stationary phase was the same as that on the ODS stationary phase, but the selectivity for these isomers was greatly improved, as confirmed in **Table 3.3**. The PBT stationary phase showed a good selectivity for dimethyl phthalate.

Furthermore, the selectivity of structural isomers of heterocyclic compounds on the PBT stationary phase was also investigated. Typical chromatograms of quinolines on four stationary phases were shown in **Figure 3.6**. For the PBT phase, a large α

values were observed compared with on the ODS stationary phase, and above results might be attributed to the higher recognition capability of these analytes for the position of the nitrogen atom. The elution order of quinoline and isoquinoline on the PBT stationary phase was the same as that on the ODS stationary phase, but greater selectivity for these isomers was obtained on the PBT stationary phase. The result could be interpreted that the slight difference in these *log P* values on the PBT stationary phase resulted in better selectivity.

Effect of column temperature on retention behaver

In the PBT stationary phase, the analyte retention improved with decreasing the column temperature. The trend was the same as for the typical ODS stationary phase. The effect of column temperature was further analyzed using van't Hoff plots [35,36]. For the PBT stationary phase as well as the ODS stationary phase, a good linear relationship of the logarithmic retention factor (*ln k*) against the reciprocal absolute column temperature (1/T) was clearly shown in the temperature range considered. The linear correlation coefficient of these plots was more than 0.99. The good linear relationship between 1/T and *ln k* indicated that the retention mechanism of PBT phase was kept constant in the range of column temperature from 20 to 40°C.

The ΔH calculated from the slope on the van't Hoff plots for phthalates on these three stationary phases was summarized in **Table 3.4**, showing the excellent retention based on the large enthalpy value during solute transfer from the mobile phase to the PBT stationary phase. On the PBT phase, the enthalpy difference between *p*-isomer and *m*-isomer was significantly larger than that on the ODS phase.

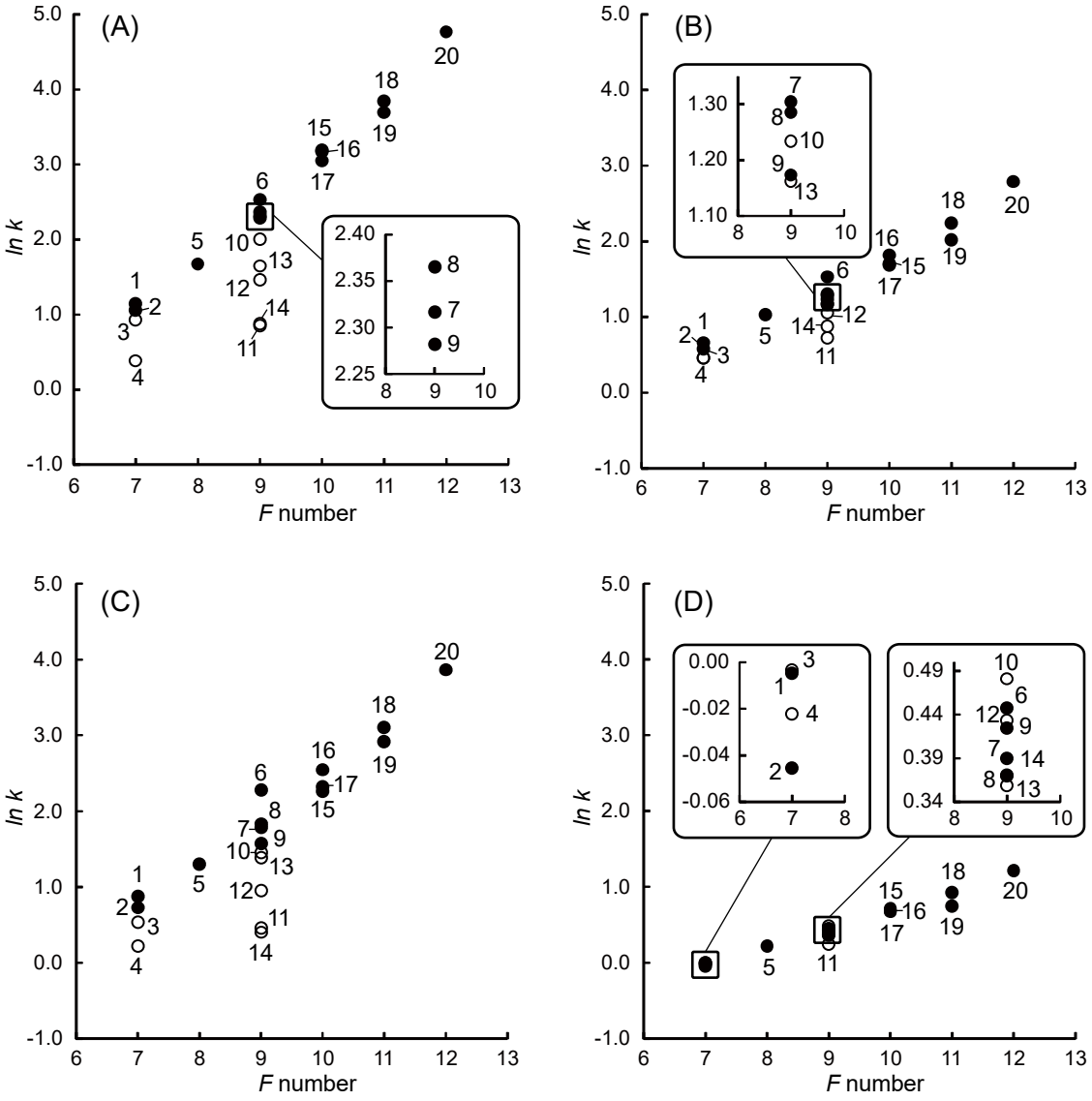
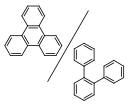
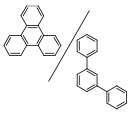
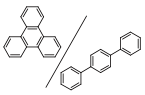
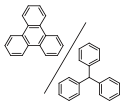


Figure 3.2 Relationship between *F* number and *ln k* for PACs on (A) PBT, (B) monomeric, (C) polymeric and (D) PBS phase. Analyte: 1, anthracene; 2, phenanthrene; 3, *trans*-stilbene; 4, *cis*-stilbene; 5, pyrene; 6, naphthacene; 7, benz[*a*]anthracene; 8, chrysene; 9, triphenylene; 10, benzo[*c*]phenanthrene; 11, *o*-terphenyl; 12, *m*-terphenyl; 13, *p*-terphenyl; 14, triphenylmethane; 15, perylene; 16, benzo[*a*]pyrene; 17, benzo[*e*]pyrene; 18, benzo[*ghi*]perylene; 19, dibenz[*a,h*]anthracene; and 20, coronene. Mobile phase: methanol/water = 90/10.

Table 3.1 Retention data for triphenylene, terphenyls and triphenylmethane.

	selectivity (α)			
				
PBT	4.19	2.26	1.89	4.07
monomeric	1.57	1.13	1.01	1.35
polymeric	3.04	1.87	1.21	3.22
PBS	1.20	0.991	1.07	1.05

Mobile phase: methanol/water = 90/10.

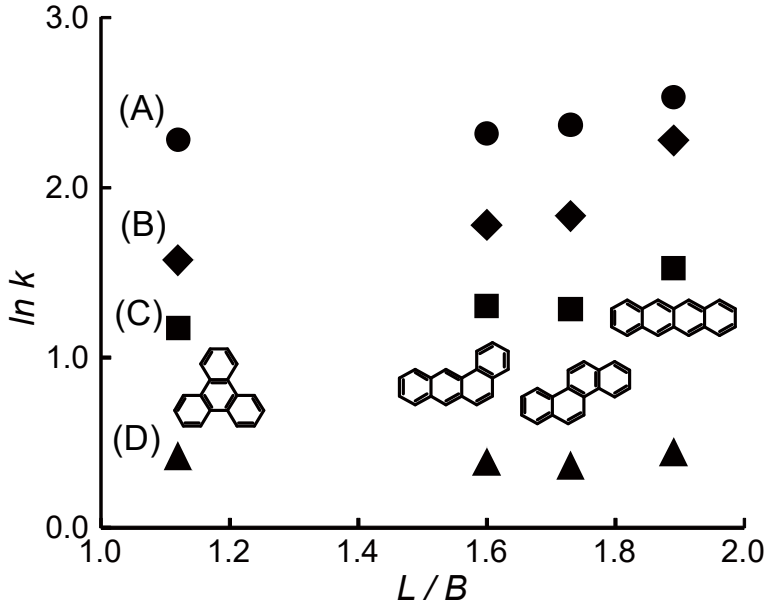


Figure 3.3 Correlation between *L/B* and *ln k* for planar four-ring PACs on four stationary phase. (A) PBT, (B) polymeric, (C) monomeric and (D) PBS. Mobile phase: methanol/water = 90/10.

Table 3.2 Selectivity for halogenated benzene isomers.

		retention factor (*k*)			selectivity (α)		
		o-	*m*-	*p*-	*m*- / *o*-	*p*- / *m*-	*p*- / *o*-
dichlorobenzene	PBT	5.04	5.78	6.13	1.15	1.06	1.22
	monomeric	4.19	5.59	4.72	1.33	0.844	1.13
	polymeric	4.27	5.29	4.36	1.24	0.824	1.02
	PBS	2.99	3.35	3.15	1.12	0.940	1.05
dibromobenzene	PBT	6.94	8.43	9.26	1.21	1.10	1.33
	monomeric	5.15	7.32	6.41	1.42	0.876	1.24
	polymeric	5.20	6.86	5.75	1.32	0.838	1.11
	PBS	3.73	4.40	4.19	1.18	0.952	1.12

Mobile phase: methanol/water = 75/25.

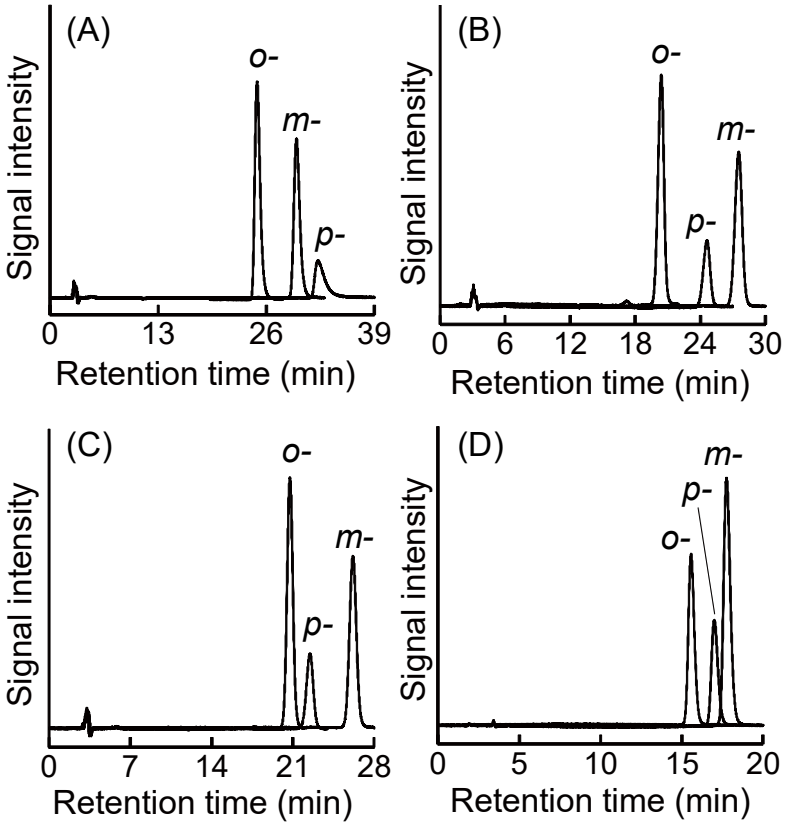


Figure 3.4 Chromatograms for the separation of dibromobenzenes. (A) PBT, (B) monomeric, (C) polymeric and (D) PBS. Mobile phase: methanol/water = 70/30.

Table 3.3 Selectivity for diacetylbenzenes and dimethyl phthalates.

		retention factor (*k*)			selectivity (α)		
		o-	*m-*	*p-*	*m-* / *o-*	*p-* / *m-*	*p-* / *o-*
dimethyl phthalate*	PBT	1.02	2.70	5.45	2.66	2.02	5.37
	monomeric	0.407	0.915	1.06	2.25	1.16	2.60
	polymeric	0.447	1.07	1.35	2.39	1.26	3.02
	PBS	0.720	1.23	1.26	1.71	1.02	1.75
diacetylbenzene**	PBT	2.45	5.59	6.98	2.28	1.25	2.85
	monomeric	0.923	1.60	1.70	1.73	1.06	1.84
	polymeric	1.15	2.28	2.49	1.98	1.10	2.17
	PBS	1.62	2.52	2.47	1.56	0.980	1.53

*Mobile phase: methanol/water = 70/30. ** Mobile phase: methanol/water = 50/50.

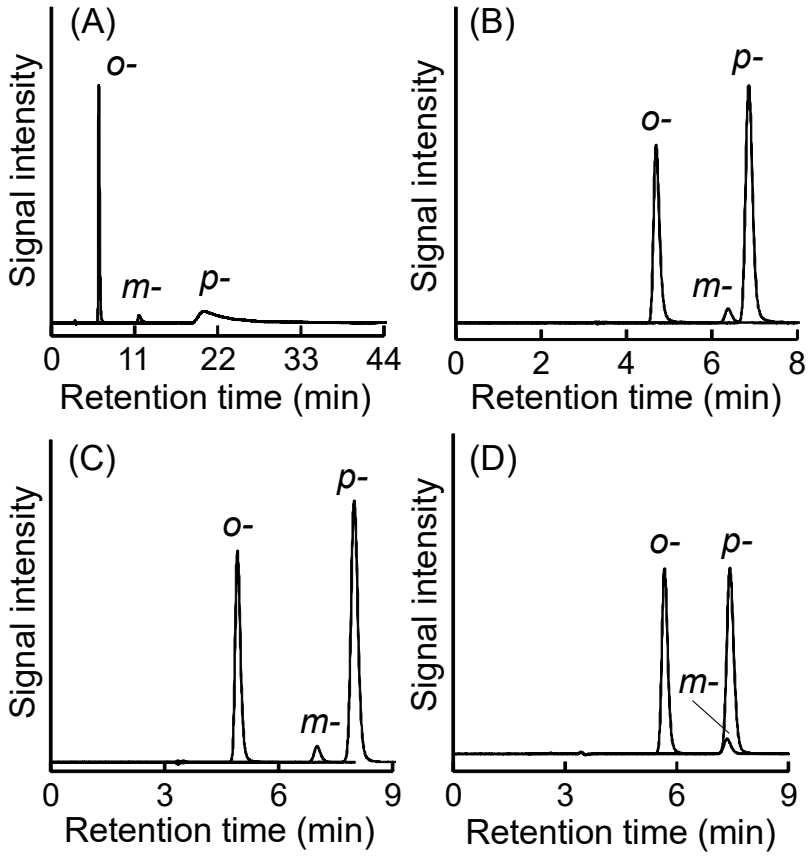


Figure 3.5 Chromatograms for the separation of dimethyl phthalates. (A) PBT, (B) monomeric, (C) polymeric and (D) PBS. Mobile phase: methanol/water = 75/25.

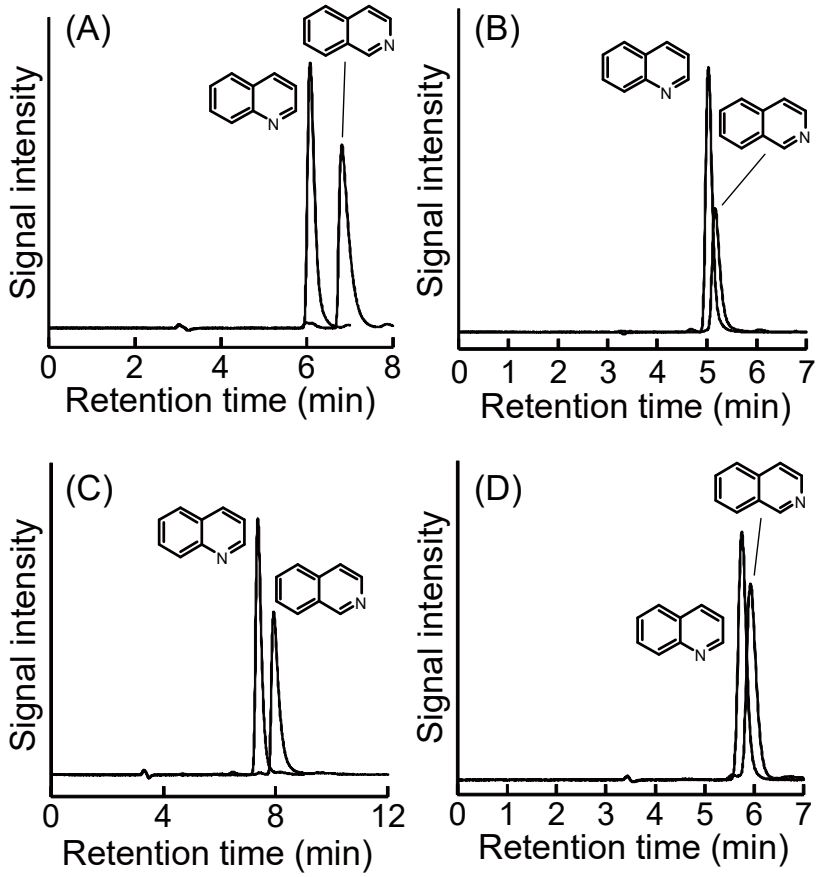


Figure 3.6 Typical chromatograms of quinoline and isoquinoline on four stationary phases. (A) PBT, (B) monomeric, (C) polymeric and (D) PBS. Mobile phase: methanol/water = 75/25.

Table 3.4 Enthalpy for the phase transfer for dimethyl phthalates.

	ΔH (kJ/mol)		
	o-	*m*-	*p*-
PBT	-12.4	-21.4	-32.4
monomeric	-5.56	-9.38	-10.7
polymeric	-7.38	-11.8	-14.4

Mobile phase: methanol/water = 75/25.

3-4. Conclusions

The retention behavior of PACs on PBT-coated silica stationary phase was studied. The results have a good agreement with the estimated retention mechanism for the PBT phase consisted of: 1) hydrophobic interaction between the analyte and stationary phase ligand, 2) π-π interaction between the phenyl ring of the analyte and that in the stationary ligand, and also 3) polar interaction between the ester functionalities and that in the bonded phase ligand. Although further investigation is needed to fully understand the retention mechanism of the PBT phase, the above results suggest the future potential of the PBT phase as a stationary phase in LC for the separation of other class of structural isomers with slightly different polarities from each other.

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Chapter 4

Fibrous Polyimide Material as a Novel Stationary Phase in Packed-Capillary Gas Chromatography

4-1. Introduction

GC is one of the most powerful and popular techniques for the separation of volatile organic compounds. Various types of stationary phases have been developed and commercialized for effective separation of complex mixture in GC along with specially-designed detectors and injectors for GC instruments [1-3]. Another approach for more effective separations was miniaturization of chromatographic system, and down-sizing of separation systems has been studied along with appropriate combinations with modern sample preparation techniques [4-12].

Recently, various types fibrous polymeric materials have been introduced for novel separation methods and corresponding appropriate sample preparation techniques [13-19]. Bundles of fibrous synthetic polymers were packed into short extraction cartridges for sample preparation of gaseous [20-25] and liquid [26-35] sample matrices. The application of fibrous materials as stationary phase was investigated in chromatographic techniques such as GC [36-42], capillary electrochromatography [43-45], and LC [46-52]. Furthermore, fiber-packed capillaries have also been studied as a miniaturized interface for two-dimensional separations [53,54]. Taking advantage of their good heat resistance, several synthetic polymer filaments were employed as stationary phases for high temperature separations in GC, on the other hands, since the filaments also have a good solvent resistance, liquid sample preparation was carried out in mini-cartridges packed with bundles of these filaments [32-37].

In a previous study [55], polyimide (PI) particles has been successfully introduced as a sample preparation medium for gaseous sample, suggesting the PI materials could be applied as a new stationary phase in chromatographic separations. As an extension of the previous investigation, in the present study, fine fibrous PI

materials were introduced as a novel stationary phase for packed capillary GC, in this chapter. The retention behavior on the fibrous PI stationary phase was evaluated with test analytes including alkanes and corresponding alcohols. Taking advantage of the excellent heat resistance of fibrous PI material, a temperature-programmed separation was also investigated.

4-2. Experimental

Reagents

All the alkanes and the corresponding alcohols were obtained from Tokyo Chemical Industry (Tokyo, Japan), and hexane was obtained from Kishida Chemical (Osaka, Japan). For the evaluation of the retentivity, a set of analytes consisted of benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene (i.e. BTEX) was obtained from Wako Pure Chemical (Osaka, Japan). All of the reagents and solvents were of analytical reagent grade.

Preparation of fiber-packed capillary

A bundle of P84 filaments [56] as the fibrous PI stationary phase was obtained from Toyobo (Otsu, Japan). The chemical structure of P84 is shown in **Figure 4.1**. To prepare of the capillary column, a bundle P84 filaments (consisted of 160 filaments) was packed into a fused-silica capillary with 0.32 mm i.d., 1.0 m length (Shinwa Chemical Industry, Kyoto, Japan). The average cross-section area of the P84 filaments was *ca*. 1.6×10^{-4} mm^2, and diameter of the filament was *ca*. 14.3 μm if a perfectly circular cross-section is assumed. The packing density based on the total cross-section area of the filaments and the cross-sectional area of the capillary opening was calculated

to be about 32%.

In order to ensure parallel alignment of all P84 filaments in the capillary, the packing process was carried out in a similar manner described previously [28]. First, an appropriate length of poly(vinylidene fluoride) (PVF) fishing fiber (64 μm o.d., Kureha, Tokyo, Japan) was inserted as a guide into the fused-silica capillary. The end of the PVF guide fiber has an extra length to form a loop the outside of the capillary. Second PVF fiber was inserted into the loop of the first guide fiber, and then the first guide fiber was pulled from the other end of the capillary. The bundle of precounted P84 filaments (with a pre-cut length of *ca*. 2.4 m for a 1.0-m packed section) to be packed was inserted into the loop of the second guide fiber, where the front-end of the bundle should be appropriately bent to make sure a smooth introduction into the capillary. The second PVF guide fiber is carefully pulled from the other side of the capillary to produce uniform introduction of the bundle into the capillary. Finally, the second guide fiber is pulled out of the capillary and cut off the extra length of the filaments to prepare a fiber-packed capillary column. The typical photograph of PI fiber-packed capillary column was shown in **Figure 4.2**.

GC measurements

A 6890N gas chromatograph (Agilent, Santa Clara, CA, USA) with a split/split-less injection port and a frame ionization detector (FID) was used for all the GC measurements. All the measurements were carried out by a split mode with a typical ratio of 50:1. Injector temperature and detector temperature were typically set at 300ºC. N_2 was used as the carrier gas. An appropriate preconditioning was performed on the PI fiber-packed column before evaluating the retention behavior.

Chromatogram was recorded with ChromNAV Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer.

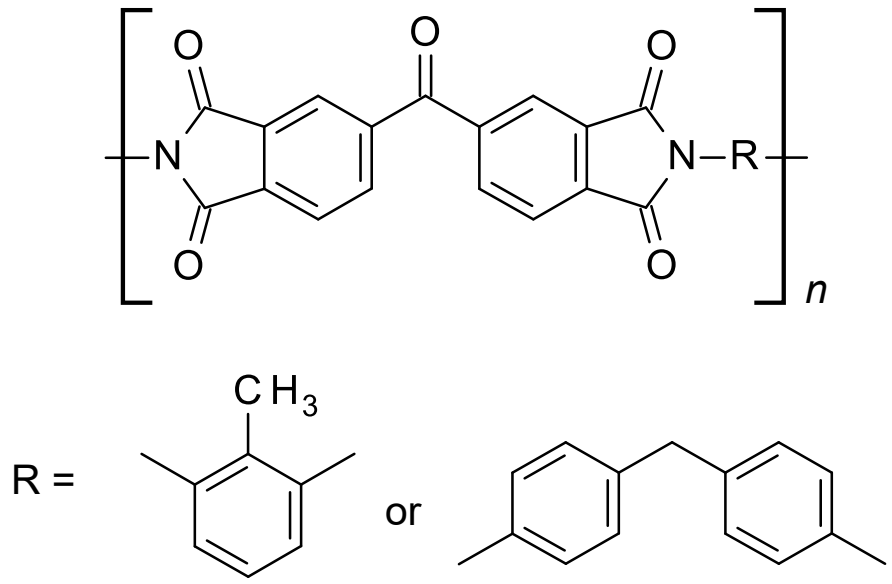

Figure 4.1 Chemical structure of P84 PI [56].

Figure 4.2 Typical photograph of PI fiber-packed capillary column.

4-3. Results and Discussion

Retention behavior of alkanes on the fibrous PI stationary phase

The retention behavior of alkanes in the fibrous PI stationary phase was evaluated. **Figure 4.3** shows the retention trend of alkanes on the fibrous PI stationary phase. A good linear relationship between the logarithmic retention factor and the number of carbon atoms was observed for all alkanes, the linear correlation coefficient of these plots was larger than 0.99. Above results have a good agreement with that obtained for conventional non-polar GC stationary phases such as polydimethylsiloxane (PDMS). For the separation of alkanes on the PI stationary phase, similar separations could be expected to that based on the general selectivity of a typical PDMS stationary phase. On the PI stationary phase, the separation of alkanes was mainly based on the number of carbon atoms in the analyte, but the polar functional groups of the PI structure were expected to contribute to the retention mechanism.

van't Hoff plots of alkanes for the retention on the PI phase

For many GC stationary phases, a linear correlation is obtained when the logarithmic retention factor was plotted against the reciprocal absolute column temperature [57,58]. The van't Hoff plots obtained for the PI stationary phase showed a good linear relationship for all analytes, as shown in **Figure 4.4**, suggesting that the retention mechanism was consistent the temperature range studied. The retention trends for the alkanes were similar to that observed on conventional non-polar stationary phases. The result indicates the possibility of easily optimizing the temperature-programmed separation in the PI stationary phase.

Figure 4.5 shows comparison of the van't Hoff plots of two alkanes and

corresponding alcohol with the same number of carbon atom. In the case of the retention behavior for alcohol on the PI phase, linear van't Hoff plots were obtained for all the alcohols studied. However, their retentions were much larger than that of the corresponding alkanes, which have the same number of carbon atoms. From the result, the enthalpies of solute transfer from the carrier gas to the stationary phase were calculated to be -38.0, -42.0, -60.5, and -61.8 kJ/mol for nonane, decane, 1-nonanol, and 1-decanol, respectively. The contribution of the hydroxyl groups of the alcohols to the retention on the PI stationary phase was significantly larger than that of these alkane skeletons, suggesting the contribution of polar interactions between the alcohols and the fibrous PI stationary phase.

Selectivity for BTEX on the PI phase

As shown in **Figure 4.6**, the retention of BTEX on the fibrous PI stationary phase was compared with that obtained with a commercially available open-tubular DB-WAX capillary column (0.25 mm i.d., 15 m length, 0.25 μm film thickness; J and W Scientific, Folsom, CA, USA). The selectivity of the fibrous PI phase for BTEX was similar to that obtained for the DB-WAX phase, polyethylene glycol (PEG) phase, especially in the elution order of the xylene isomers. This result also suggests that the polar moieties, carbonyl and imide groups, contribute to the chemical structure of the PI stationary phase.

Temperature-programmed separation on the PI column

On the fibrous PI stationary phase, temperature-programmed separation was successfully carried out for a sample of alkane mixture as shown in **Figure 4.7**. As

other fibrous stationary phases reported previously [36-38], the theoretical plate number was not sufficient compared to conventional open capillary columns. This might be mainly due to the partial uniformity of the packed filaments in the capillary. Furthermore, the column was used for temperature-programmed separations up to 300ºC for typically more than 100 times, without any significant loss in retention performance.

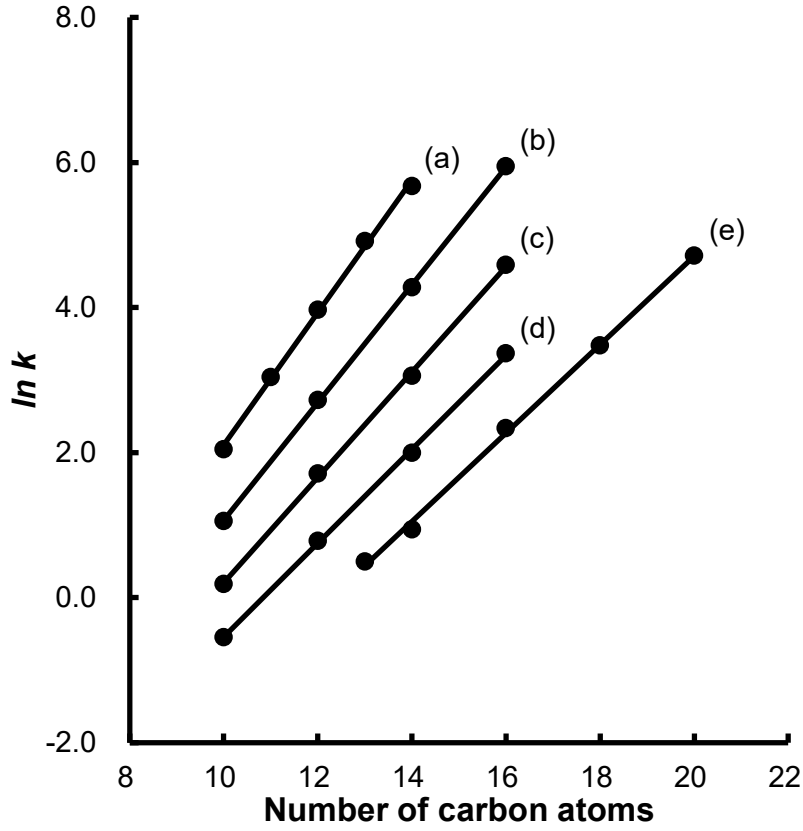

Figure 4.3 Retentivity for normal alkanes having different carbon numbers at various column temperatures on the fibrous PI stationary phase. GC conditions; column head pressure, 100 kPa; injection mode, split; split ratio, 50:1. (a) 40ºC, (b) 60ºC, (c) 80ºC, (d) 100ºC and (e) 120ºC. Other conditions are in the text.

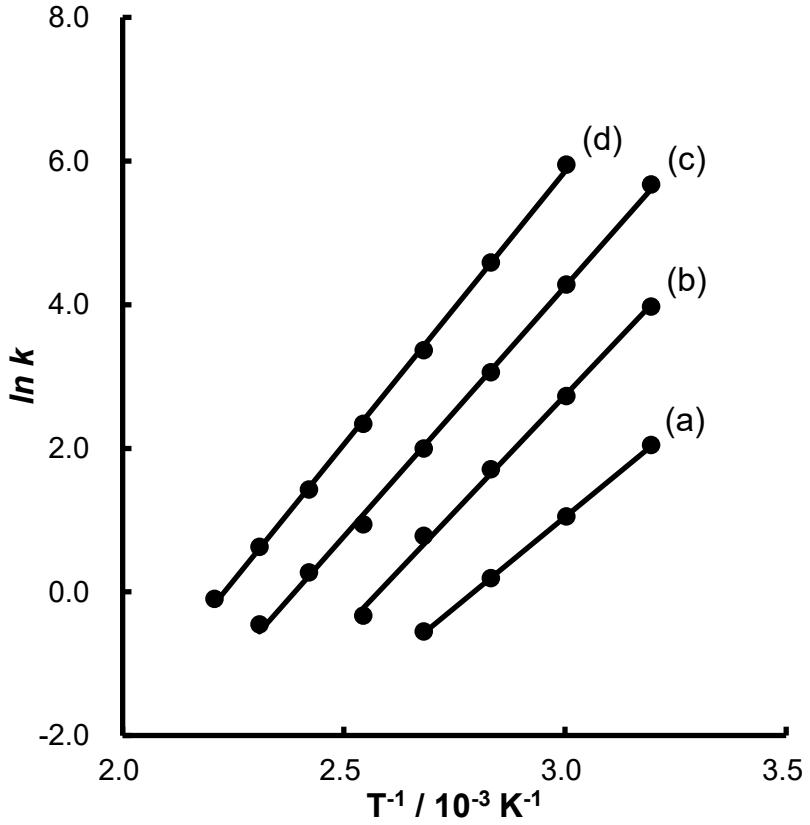

Figure 4.4 van't Hoff plots for four alkanes on the PI stationary phase. (a) decane, (b) dodecane, (c) tetradecane and (d) hexadecane. GC conditions are the same as in Figure 4.3.

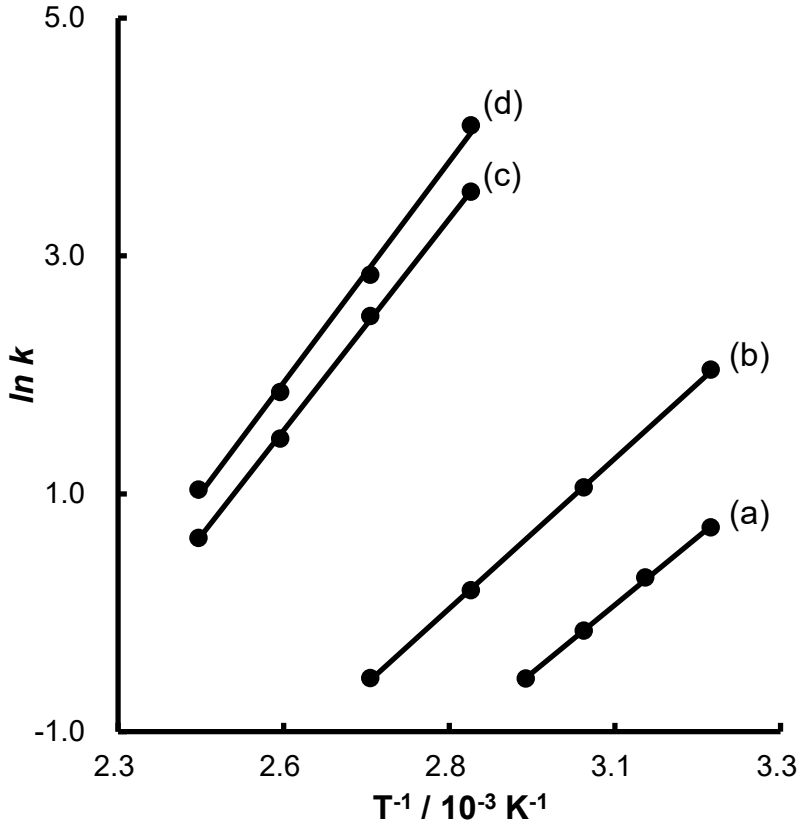

Figure 4.5 Typical van't Hoff plots for alkanes and alcohols obtained on the PI stationary phase. (a) nonane, (b) decane, (c) 1-nonanol and (d) 1-decanol. GC conditions are the same as in Figure 4.3.

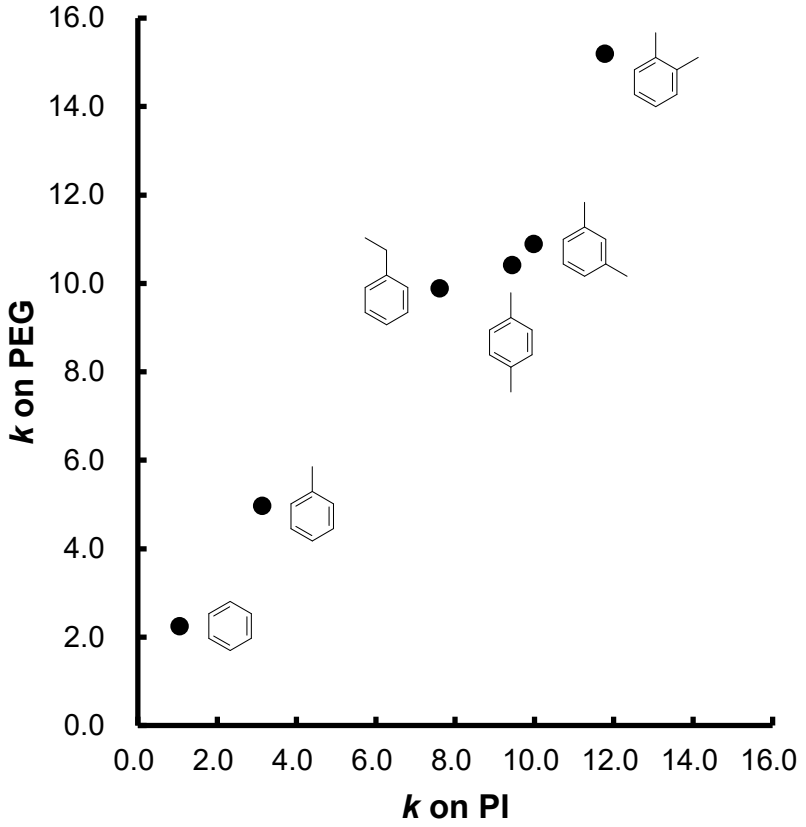

Figure 4.6 Comparison of the retentivity for BTEX on the PI and DB-WAX (PEG) phases. GC conditions for DB-WAX phase: injector and detector temperature, 200ºC; column temperature, 30ºC. Other conditions are the same as in Figure 4.3.

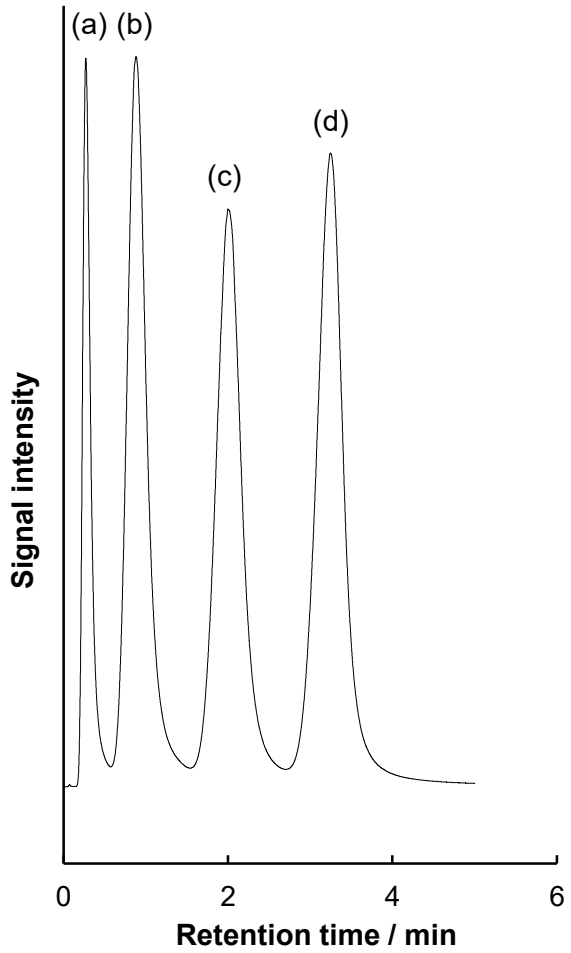

Figure 4.7 Chromatograms for the separation of a homologous alkane mixture. Conditions: temperature program, 50ºC to 150ºC at the rate of 20ºC/min. Peaks: (a) decane; (b) dodecane; (c) tetradecane and (d) hexadecane. Other conditions are the same as in Figure 4.3.

4-4. Conclusions

PI fiber was introduced as a novel extraction medium in GC and PI fiber-packed capillary column was developed. The PI column showed a good retention for alkanes and alcohols, suggesting a retention trend similar to that of conventional highly polar stationary phases such as a PEG phase. Taking advantage of a good heat-resistance of PI filaments, temperature-programmed separation was also possible. The above results indicate that the PI phase could be employed as a stationary phase in capillary GC, however the selectivity of analytes is not sufficient for retention analysis using various kinds of polar compounds as sample analytes. Therefore, a more systematic evaluation employed various types of sample analytes with different polarities and chemical structure is necessary.

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Chapter 5

Simultaneous Derivatization/Extraction Treatment for Volatile Amines with Fiber-Packed Sample Preparation Needle

5-1. Introduction

Monoethanolamine (MEA), 2-aminoethanol, is widely used in the chemical industry such as a buffer, chemical intermediate, coatings, plasticizer, surfactant, cosmetic emulsifier, wetting agent, and alkalizing reagent. Industrial hazards associated with the production and handling of MEA are skin and eye irritation [1,2]. The U.S. National Institute for Occupational Safety and Health (NIOSH) has set the exposure limit for MEA as a time-weighted average of 7.5 mg/m^3 (7.5 ng/mL, 3 ppm) [3], and several analytical methods for measuring MEA in air samples have been reported [4-10].

Sample preparation with derivatization for target analyte has been developed to improve sensitivity along with various derivatization reagents and corresponding derivatization reactions have been developed [6-17]. By introducing derivatization reactions to sample preparation detectability and sensitivity can be improved. Accurate quantification of the target analytes often requires sample preparation, especially for sample matrix containing complex mixtures such as biological and environmental samples. The reason was that sample preparation not only preconcentrates the target sample but also removes measurement interferents.

Recently, miniaturization for sample preparation has been focused due to allow simultaneous sample collection and preconcentration employing a small sample preparation device with extraction medium [18-34]. Based on the success of fiber-packed columns [35-40], bundles of synthetic filaments were also introduced as extraction media. The several hundreds of fine polymeric filaments were packed longitudinally into specially-designed small extraction cartridges [41-47] or needle-type devices [11-17]. Sample preparation with needle-type extraction device can be

extracted on the filaments by simply passing the gas sample into the needle. The analyte extracted on the surface of the fibrous extraction medium was desorbed by the heated injector of a gas chromatograph by carrying out similar to the injection procedure in a conventional gas chromatograph. Previous studies have shown that derivatization and extraction can be successfully performed simultaneously [11-17]. By introducing a derivatization reaction process into the sample preparation needle, the following three processes can be performed simultaneously with simple procedure: 1) sample collection, 2) derivatization of target molecules, and 3) preconcentration.

In this chapter, a simultaneous derivatization/extraction of MEA using a fiber-packed needle with derivatization reagent was investigated. The fundamental extraction performance of the fiber-packed needle was evaluated along with the optimization of several experimental parameters for the derivatization, extraction and desorption. The storage performance of the needle-type device at room temperature was investigated for on-site applications including the air environmental analysis. Furthermore, the method with fiber-packed needle was applied for other volatile amines.

5-2. Experimental

Reagents

All solvents and reagents were of analytical grade, and used without further purification. MEA, cyclohexanone, acetonitrile (ACN), *n*-butylamine and ethylenediamine were purchased either from Wako Pure Chemical, Osaka, Japan or Kishida Chemical, Osaka, Japan.

Preparation of standard gas sample

Standard gaseous MEA samples with corresponding required concentration were prepared as follows. First, a mixture of ACN and MEA (with molar ratio of 10:1, respectively) was prepared. Then, 5.2 μL of the solution was injected into a vacuum glass vessel having 1.0 L volume, and N_2 gas was also injected for dilution after the evaporation of the mixture in the vessel. A few milliliters of the above gaseous sample was injected into a gas sampling bag (1.0 L volume, Tedlar bag, GL Science, Tokyo, Japan) and diluted with pure N_2 gas to prepare a set of standard gas samples with the corresponding required concentration in the gas sampling bag. A similar procedure was repeated to prepare the standard gas samples with lower concentrations.

Preparation of extraction needle

As a heat-resistant fiber, Zylon, a poly(*p*-phenylene-2,6-benzobisoxazole), was obtained from Toyobo (Otsu, Japan). A bundle of Zylon filaments with *ca.* 11.5 μm o.d. was packed into a specially-designed extraction needle (0.5 mm i.d., 0.7 mm o.d., 85 mm length). As described previously investigation [13-17], the filaments were packed longitudinally in a section of about 30 mm length of the needle, and one end of the packed section was positioned just before the side-hole near the tip of the needle, and a total of 664 filaments was packed. In order to ensure that all filaments were aligned for parallel in the needle, following packing process was carried out [48,49].

An appropriate length of poly(vinylidene fluoride) (PVF) fishing line (64 μm o.d.) was inserted as a guide into the needle. The end of the PVF guide fiber has an extra length to form a loop the outside of the needle. Next, a second PVF fiber was inserted into the loop of the first guide fiber, and then, the first guide fiber was pulled

from the opposite end of the needle. The bundle of Zylon filaments (with a pre-cut length of 60 mm for a 30-mm packed section) to be packed was inserted into the loop of the second guide fiber, where the front-end of the bundle should be appropriately bent to make sure a smooth introduction into the needle. The second PVF guide fiber was carefully pulled from the side-hole of the needle to produce uniform introduction of the bundle into the needle. Finally, second PVF guide fiber was pulled out the needle, and fiber-packed needle was attached to the syringe connector.

GC measurements

A GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injection port and flame ionization detector (FID) was used for all GC measurements. All measurements were carried out by a split mode with a typical ratio of 100:1. Injector temperature was typically set at 270°C. N_2 was used as the carrier gas. A fused-silica capillary column coated with polydimethylsiloxane, HR-1 (0.25 mm i.d., 30 m length, 0.25 μm film thickness, Shinwa Chemical Industries, Kyoto, Japan) was used for the GC separation with an appropriate preconditioning before use. Column temperature was set at 120ºC, and the detector temperature was set at 270ºC. Other separation conditions, such as carrier gas flow-rate and column head pressure, were determined systematically based on the results of preliminary experiments. Data was collected with ChromNAV Chromatography Data Handling Software (Jasco, Tokyo, Japan) running on a personal computer.

Simultaneous derivatization/extraction with the needle

On the basis of the preliminary experiments for the derivatization treatment in

the needle-type extraction device, a derivatization reaction was carried out with cyclohexanone as the derivatization reagent as shown in **Figure 5.1**. As shown in **Figure 5.2**, the sample preparation procedure was carried out as follows: (a)-(b) before introducing the MEA gas sample, cyclohexanone was pumped into the fiber-packed needle, and (c) the remaining solution was vented by passing 20 mL of N_2 gas. Next, (d) the fiber-packed needle was attached to a commercially-available vacuum gas sampler (Komyo Rikagaku Kogyo, Tokyo, Japan).

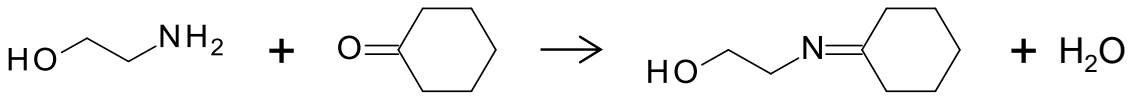

Figure 5.1 Reaction scheme of the derivatization reaction for MEA with cyclohexanone.

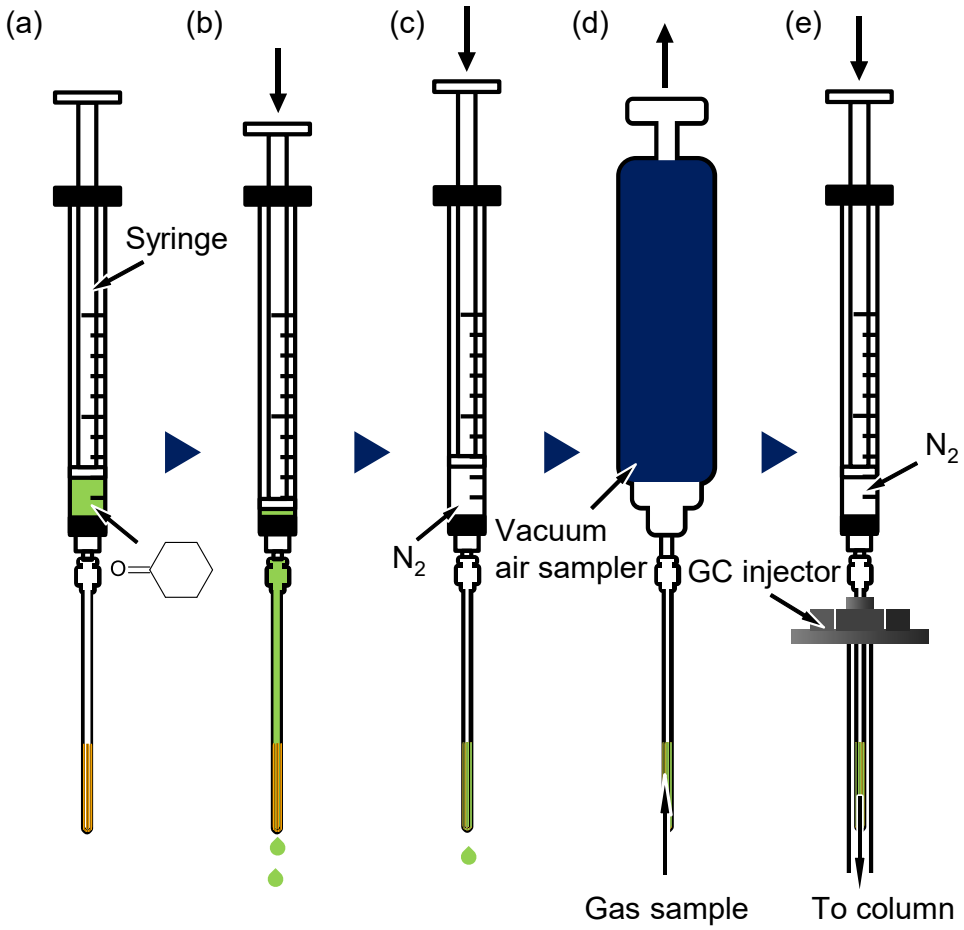

Figure 5.2 Sample preparation procedure with cyclohexanone as the derivatization reagent for volatile amines in air samples.

5-3. Results and Discussion

Determination of MEA with fiber-packed needle

Gaseous MEA sample was determined by the GC employing the fiber-packed needle with cyclohexanone in the concentration range from 0.1 to 200 ppm (0.250 to 500 ng/mL). Since the amino group reacts with cyclohexanone, the detection sensitivity was significantly improved along with the peak shape due to the decrease of amino groups. The peak area of the MEA derivative was proportional to the concentration of MEA in the gaseous sample, and the correlation coefficient of the calibration curve was more than 0.99. In the determination of gaseous MEA samples using a fiber-packed needle with cyclohexanone as the derivatization reagent, all RSDs were less than 5%, and even at the lowest concentration of 0.1 ppm (0.250 ng/mL), the RSD was 4.3%, and good reproducibility was obtained in the determination of MEA with a fiber-packed needle. The limit of detection (LOD) and the limit of quantitation (LOQ) for MEA employing fiber-packed needle were 0.035 ng/mL (0.014 ppm) and 0.12 ng/mL (0.047 ppm), respectively. The exposure limit recommended by NIOSH [3] is 3.0 ppm, and the method with the needle can quantify MEA at concentrations lower than one-tenth of that value, it was suggested that the developed method can be used in practical applications.

The desorption performance of the fiber-packed needle was evaluated by the second and third desorption without further sampling after the first desorption. As shown in **Figure 5.3**, the peak area of MEA derivative at the second desorption was less than LOQ, and no peak was obtained at the third desorption. Therefore, MEA derivative more than 99.99% was desorbed by single desorption at heating GC injector.

Evaluation of storage performance

After collected MEA gas sample into the needle, it was sealed with a plug and cap made of polytetrafluoroethylene (PTFE), as shown in **Figure 5.4**, and stored at room temperature for three days. **Table 5.1** summarizes the recovery of MEA derivative in the needle after stored each period time. The recovery was calculated based on the immediate quantification after sampling of MEA gas sample. The loss of MEA derivative in the fiber-packed needle was not observed after 1 h at room temperature, even after 72 h, the loss was only 7.7%. The reason for the sample loss was thought to be leakage from a small gap between the needle and the plug or cap. However, since more than 90% of the MEA derivatives were still stored in the fiber-packed needle after 3 days, it was not necessary to carried out GC measurement immediately after sampling, suggesting that it was possible to collect the large number of on-site sampling at the same time, by sealing the needle with the plug and cap, and the needle is bring back to the laboratory, and carry out determination analysis.

Extraction capacity for the needle

The extraction capacity for MEA of fiber-packed needle was also evaluated. As shown in **Figure 5.5**, two needles were connected, and the end side of the back needle was attached to an gas sampler. Gaseous MEA sample with the concentration of 50 ppm (125 ng/mL) was vacuumed through the fiber-packed needle from the front needle, and overflow MEA from the front needle was trapped on the surface of filaments in the back needle. After sampling, the MEA collected by each needle was quantified and the extraction capacity of fiber-packed needle for MEA was evaluated.

The relationship between the amount of MEA gas sample aspirated and the

peak area of the detected MEA derivatives was shown in **Figure 5.6**. In the case of the front needle, the peak area increased until the sampling volume reached 400 mL. For the back needle, the MEA derivatives were detected when the sampling volume was more than 400 mL. The peak area of MEA derivatives detected from the front needle increased proportionally when the sampling volume was less than 350 mL. From the above results and the calibration curve in **Figure 5.6**, the maximum extraction capacity of the MEA sample was calculated to be about 357 mL. Since the above maximum extractable capacity of the gaseous MEA sample contained 45 μg of MEA, the adsorption capacity of the fiber-filled needles for MEA was estimated to be about 45 μg per needle.

Application to other amines

The method employing fiber-packed needle with cyclohexanone was also applied to the determination of other volatile amine, *n*-butylamine (NBA) and ethylenediamine (EDA). The derivatization reaction was shown in **Figure 5.7**. Gaseous samples of 200 ppm (600 ng/mL) were aspirated with a fiber-packed needle with cyclohexanone, and simultaneous derivatization/extraction of NBA and EDA was carried out under the same extraction conditions as optimized for MEA.

For the sample preparation with fiber-packed needle of NBA, no unreacted NBA was detected, confirming the significant improvement by in-needle derivatization as shown in **Figure 5.8a**. In the case of EDA, two types of products were expected, but in the separation of EDA derivatives, two peaks were observed, as shown in **Figure 5.8b**. The first peak was considered to be the reaction product of one molecule of EDA and one molecule of cyclohexanone, while the second peak was the reaction

product of one molecule of EDA and two molecules of cyclohexanone. As a result, no unreacted EDA was observed, and the sensitivity was greatly improved by using fiber-packed needles with cyclohexanone as the derivatization reagent. The result suggests that this method could be a powerful tool for the simultaneous derivatization/extraction of not only MEA but also other volatile amines.

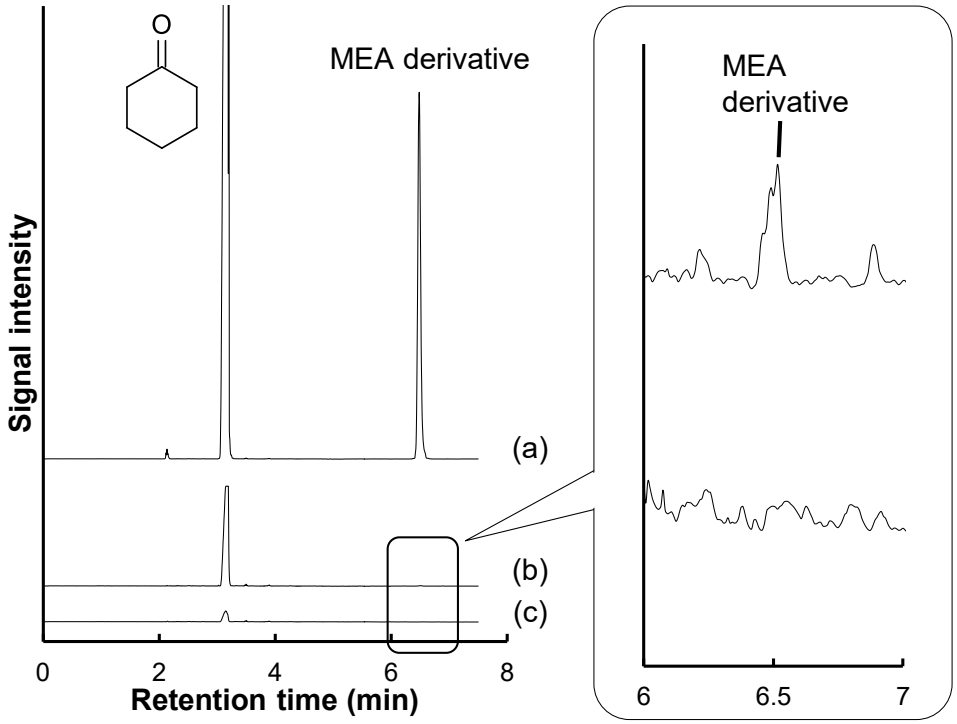

Figure 5.3 Chromatogram of a gaseous MEA sample using a continuous injection process. (a) first injection immediately after gas sampling, (b) second injection after the first injection without gas sampling, (c) third injection after the second injection without gas sampling. Extraction conditions: flow-rate, 2.5 mL/min; sampling volume, 50 mL; desorption temperature, 270ºC; analyte, MEA (200 ppm) in N_2. GC conditions: column, HR-1; column temperature, 120ºC; column head pressure, 115.5 kPa; injection mode, split; split ratio, 1:100.

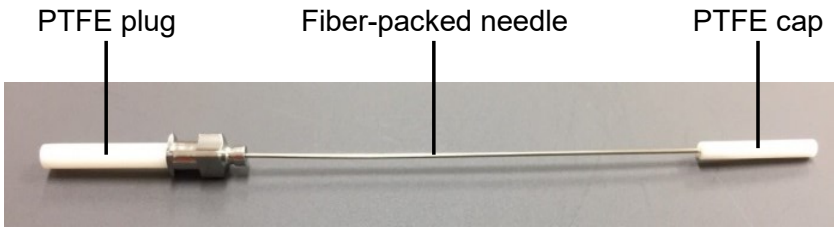

Figure 5.4 Photograph of fiber-packed needle sealed using PTFE plug and cap.

Table 1. Storage performance of the needle extraction device for MEA at room temperature.

storage time (h)	recovery (%)
0	100.0
1	100.7
6	98.4
24	95.0
72	92.3

The values are calculated as the recovery (%) based on the immediate analysis. Extraction conditions: flow-rate, 2.5 mL/min; sampling volume, 50 mL; desorption temperature, 270ºC; analyte, MEA (200 ppm) in N_2. GC conditions: GC column, HR-1; column temperature, 120ºC; column head pressure, 115.5 kPa; injection mode, split; split ratio, 1:100.

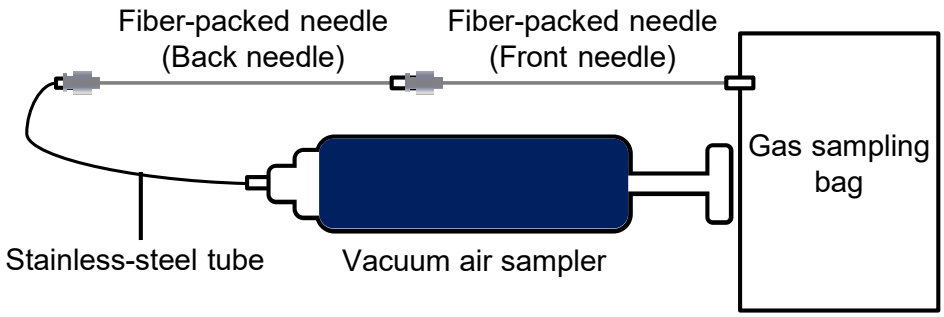

Figure 5.5 Illustration of experimental setup for the evaluation of the extraction capacity of fiber-packed needle.

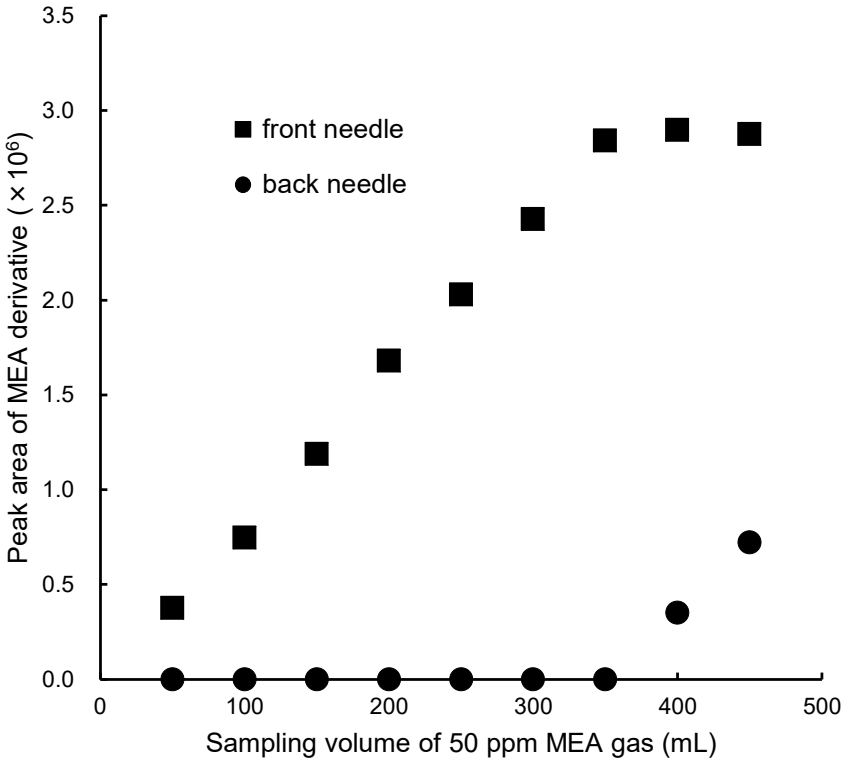

Figure 5.6 Extraction capacity of the needle extraction device for MEA. Extraction conditions: flow-rate, 2.5 mL/min; sampling volume, 50 to 450 mL; desorption temperature, 270ºC; analyte, MEA (50 ppm) in N_2. Other conditions are the same as in Figure 5.3.

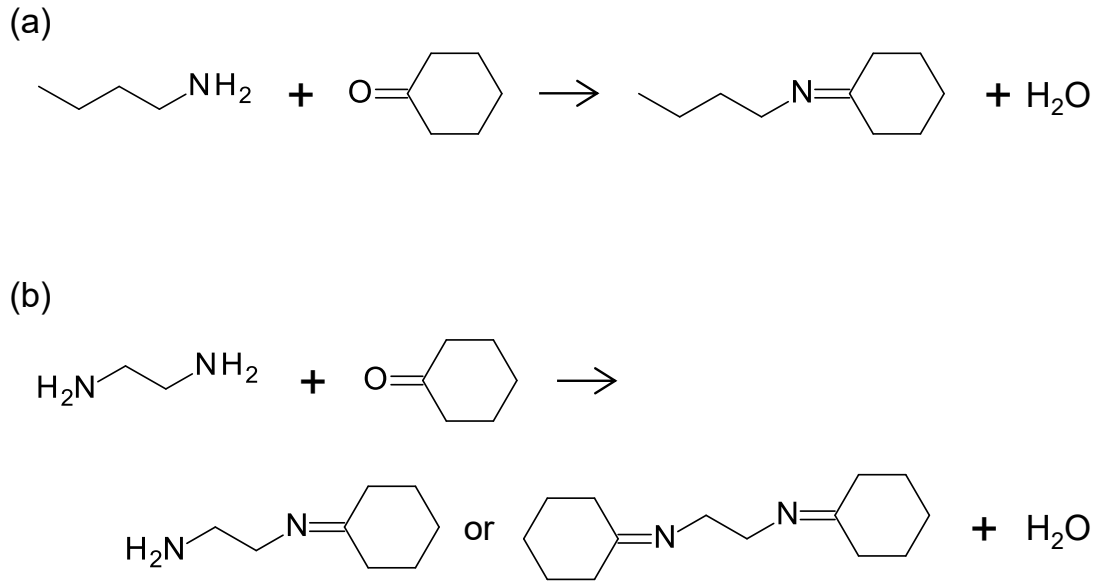

Figure 5.7 Reaction scheme of the derivatization reaction for (a) NBA and (b) EDA with cyclohexanone.

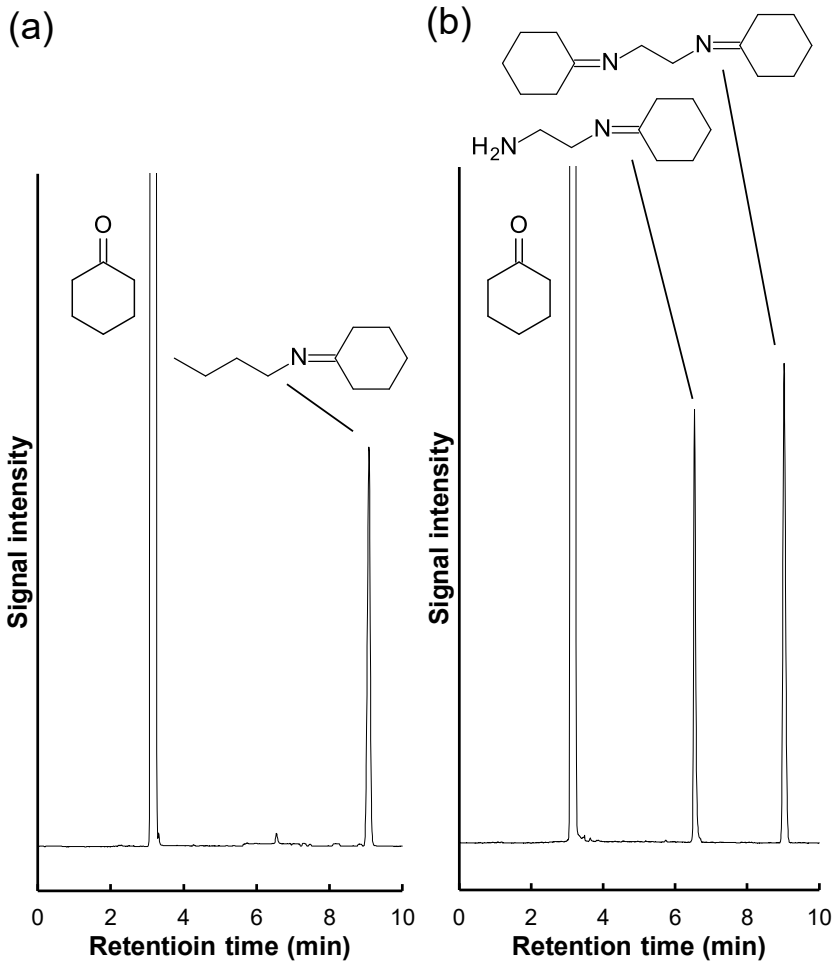

Figure 5.8 Chromatograms for the separation of (a) NBA derivative and (b) EDA derivative using the fiber-packed needle with cyclohexanone as the derivatization reagent. Extraction conditions: flow-rate, 2.5 mL/min; sampling volume, 50 mL; desorption temperature, 270ºC; analyte, (a) NBA (200 ppm) in N_2 and (b) EDA (200 ppm) in N_2. Other conditions are the same as in Figure 5.3.

5-4. Conclusions

A fiber-packed sample preparation needle with cyclohexanone, as a derivatization reagent, was introduced for GC analysis, and a sample preparation method employing the needle was developed for simultaneously derivatization/extraction of MEA in air sample. This method requires a very short processing time and the simplicity of operation by taking advantage of the needle-type extraction device. Another advantage of the needle, it allows simultaneous sampling at many sites, greatly reducing the potential risk of sample loss in tedious multi-step processing. Additionally, any desorption solvent is not required for desorbing derivatives with a heated GC injection port. The extracted samples can be measured even after storage at room temperature for at least 3 days. The excellent storage performance of the fiber-packed needle-type sample preparation device was very remarkable. In addition, the derivatization reaction with cyclohexanone is expected to improve the peak shape of amines in the subsequent GC analysis. The developed method shows a good sensitivity to gaseous MEA, suggesting the possibility of actual atmospheric sample analysis in the future.

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Chapter 6

Braided Fiber as a Novel Extraction Medium for Fiber-Packed Capillary in Microscale Sample Preparation

6-1. Introduction

Braid is one of the most popular Japanese traditional craft, and it has been employed as a part of accessories since ancient times [1]. Typical braid is consisted of bundles of fibers. Because of its excellent mechanical strength and flexibility, it has been often used in industrial applications such as ropes and flexible high pressure tubes. By changing the tension during the making of the braid, the outer diameter can be controlled reproducibly, and it allows for easy preparation of braids that could be inserted into capillaries of various sizes. One of the characteristics of braid is that it has a space in the center, and different types of fibrous materials can be successfully inserted into the central opening, suggesting the possibility of developing novel hybrid materials for sample preparation devices in separation science.

As mentioned in previous chapters, LC is considered powerful tools for analyzing a various complex sample matrix. In most cases, however, sample preparation is required because of significantly low concentrations of target analyte and complex sample matrix. Extraction is one of the most basic sample preparation techniques for preconcentration of target compounds in a typical complex sample matrix. Liquid-liquid extraction was the most widely used extraction method employed in the past [2-4], although this method has some disadvantages such as manual sample loss and large consumption of organic solvents. On the other hand, SPE has been recognized as an alternative method due to its low consumption of organic solvents and simply operation [5,6].

Various types of synthetic fiber materials with excellent heat resistance and solvent resistance have been introduced into separation science as one approach to miniaturized sample preparation and subsequent chromatographic separations [7-9].

The fiber materials have been employed as extraction media in sample preparation systems [10-19] and as stationary phases in chromatographic separations [20-26]. As described above, various synthetic fibers have been successfully introduced as SPE media for preconcentration and derivatization of target analytes in aqueous and aqueous matrices [27-31]. In these applications, bundles of fine filaments were packed longitudinally into capillaries prepared mini extraction cartridges for sample preparation and columns for chromatographic separation. Compared with solid-phase microextraction (SPME) and SPE using conventional particle-packed cartridges [32,33], the fine-filament-packed capillary has additional advantages such as easy on-line coupling with various chromatographic techniques.

In this chapter, braid fiber as a novel extraction medium was introduced for microscale sample preparation in LC. Zylon, poly(*p*-phenylene-2,6-benzobisoxazole) [7,9], one of the heat resistant fiber was employed as the material of the braid. Phenanthrene, a type of PACs, was employed as a sample probe to evaluate the fundamental extraction performance. For phenanthrene, the extraction and desorption conditions of the Zylon braid were optimized. Furthermore, heat-assisted desorption was carried out by applying voltage to a metal wire introduced into the central opening of the braid, and the possibility of using heat-assisted desorption as a sample preparation device was also investigated.

6-2. Experimental

Reagents and solvents

All reagents and solvents were obtained from Tokyo Kasei Industries (Tokyo, Japan), Kishida Chemical (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan). There

reagents and solvents were of analytical grade and used without any further purification. For the preparation of standard samples and mobile phases, water was prepared using by a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany). Zylon, poly(*p*-phenylene-2,6-benzobisoxazole), fiber of 166 filaments, *ca*. 11.5 μm o.d. was obtained from Toyobo (Otsu, Japan), and the chemical structure of Zylon was shown in **Figure 6.1**.

Preparation of braided fiber with metal wire

The braid was made from four bundle of Zylon fibers with stainless-steel wire. First, the each ends of the two Zylon bundles were coiled around a bobbin, and the fibers were set to cross at the center hole of a laboratory-made specially-designed device for braiding as shown in **Figure 6.2**. And then, a center weight was set on the crossed fibers. The dish-shaped support at the top of the device was made of soft polyurethane and had four cutouts (placed at 0°, 90°, 180°, and 270°) to fix each fiber bundle. Based on the results of preliminary experiments, the weight of these bobbins was set to 60 g and the weight of the center weight was changed from 100 g to 300 g, as summarized in **Table 6.1**.

As shown in **Figure 6.2**, after inserting the stainless-steel wires vertically into the center hole, the fiber bundles were knitted by the following procedure: i) a pair of bundles (**a** and **c**) placed on opposite sides of the circular plate were simultaneously moved 180° clockwise, and ii) another pair of bundles (**b** and **d**) were also simultaneously moved 180° counterclockwise. This two-step process was repeated twice, as illustrated in **Figure 6.3**, to return to the original configuration. In order to make a braided cord of about 10 cm length, the manual process from i) to iv) in **Figure**

6.3 had to be repeated about 150 times. A typical braid made in the same way was shown in **Figure 6.4**. The unbraided sections at both ends allow easy connection of an electronic wire for resistive heating. Several braid-like extraction media were prepared, as shown in **Table 6.1**. The center weight was increased and the outer diameter of the resulting braid was decreased according to the general rule of braid preparation [1]. The outer diameter of the braid was also varied depending on the diameter of the stainless-steel wire.

Preparation of braided-fiber-packed extraction device

To make fiber-packed extraction tubes, Zylon braids were packed into 0.50 mm i.d., 1.58 mm o.d., 60 mm length PEEK tubes. The fiber packing was carried out as a similar manner as described previously [34]. To pack the braid, a polyvinylidene fluoride (PVF) fishing line of 0.64 mm o.d. was inserted into the PEEK tube as a guide fiber. The tip of the guide fiber was inserted into the PEEK tube again to form a loop at the outside of the PEEK tube. As mentioned above, the braided fiber has a portion of unbraided fiber at both ends. By inserting one of those unbraided sections into the loop of the PVF guide fiber and pulling the PVF guide from the other side of the PEEK tube, we were able to insert the braid with a slightly larger outer diameter into the PEEK tube, as confirmed in our preliminary experiments. Due to the rather limited availability of the inner diameter of the PEEK tube used as the housing for the extraction capillary, a combination of a braid with an outer diameter of 0.51 mm and a PEEK tube with an inner diameter of 0.50 mm was selected for the following experiments to prepare the extraction capillary.

The fiber-packed capillary for extraction was prepared by cutting the

stainless-steel wire leaving 50 mm from both ends of the tube and also cutting the braid at both ends of the PEEK tube. As a result, the length of the packed section was 60 mm and the total length of the stainless-steel wire was 160 mm. **Figure 6.5** shows an illustration of the extraction capillary. The total amount of Zylon filament packed in the extraction capillary was about 10.2 mg, and the packing density including the stainless-steel wire was about 71.3%, calculated as the total cross-sectional area of filament and stainless-steel wire relative to the cross-section of the opening of the PEEK tube. The extra 50 mm length of metal wire at each end of the PEEK tube was specially designed for electrical connection to the power supply, as shown in **Figure 6.6**. All tubing, extraction capillaries, and electrical wiring were modified to avoid undesirable dead volume in the device and mounted on a set of PTFE tee connectors (GL Sciences).

LC measurements

LC system was consisted of an 880-PU pump (Jasco, Tokyo, Japan), a model UV-875 UV-Vis absorption detector (Jasco) and a Model 7125 injector (Rheodyne, Cotati, CA, USA) with a 40-μL loop. As a conventional ODS analytical column, Wakopak Eco-ODS (4.6 mm i.d., 150 mm length, 5 μm particle size; Wako Pure Chemical Industries, Osaka, Japan) was used. The mobile phase was prepared with a mixture of methanol and water (85:15) and the typical flow-rate was set at 1.0 mL/min. The detection was carried out using the above mentioned UV detector with the detection wavelength at 254 nm. All the LC runs were conducted at the room temperature controlled by an air conditioner at 23.5-24.5°C. For data acquisition and processing, Borwin Chromatography Software (Jasco) running on a personal computer was

employed. All the measurements were carried out at least five times, and the relative standard deviations (RSDs) were less than 4.0% for all the chromatographic analyses.

Extraction and desorption procedure

Two syringe pumps (Micro feeder MF-2, Azuma Denki Kogyo, Tokyo, Japan) equipped with gas tight syringes (MS-GAN 100, Ito Corporation, Shizuoka, Japan) were used to pump the sample solution and desorption solvent into the extraction capillary. For the extraction process, 1.0 mL of sample aqueous solution was pumped into the extraction system, and then 5.0 mL of air was passed through a manually operated micro-syringe for about 30 s to remove the remaining solution in the tube. The desorption solvent was then pumped at a flow-rate of 20 μL/min. A model 7125 injector with a 40-μL loop was placed between the syringe pump and the extraction capillary, and the two organic solvents were sequentially pumped as described below. The eluate from the extraction capillary was collected using a conventional micro-syringe (100 μL volume; MS-R 100, Ito Corporation), and the needle of the micro-syringe was inserted directly into the outlet capillary of the extraction device for analysis by LC. Conditioning was performed by passing 5.0 mL of methanol, 1.0 mL of water, and 5.0 mL of air between the run.

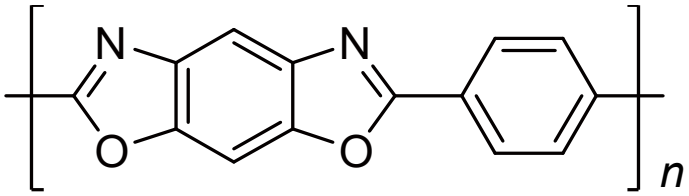

Figure 6.1 Chemical structure of Zylon as the extraction medium.

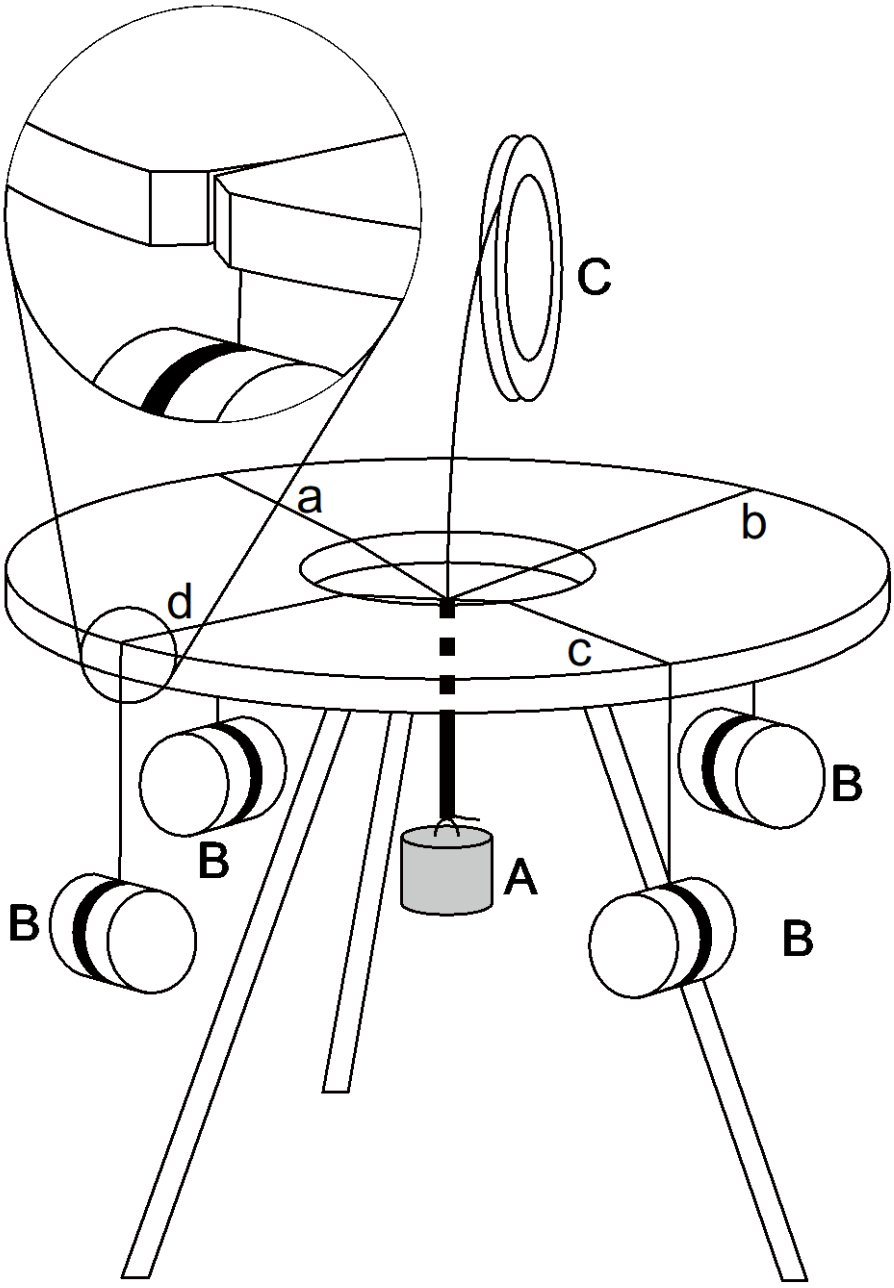

Figure 6.2 A laboratory-made set-up for the preparation of braided fiber. (A) Center weight; (B) bobbins; (C) stainless-steel wire.

Table 6.1 Various types of braids and the corresponding extraction capillaries prepared in this work.

entry	braid			extraction capillary	
	o.d. of stainless-steel wire (mm)	center weight (g)	o.d. of braid (mm)	void volume (μL)	packing density (%)
1	0.2	100	0.51	3.38	71.3
2	0.2	200	0.48	4.85	58.9
3	0.1	100	0.47	5.64	52.1
4	0.1	200	0.43	6.32	46.3
5	0.1	300	0.39	6.78	42.5

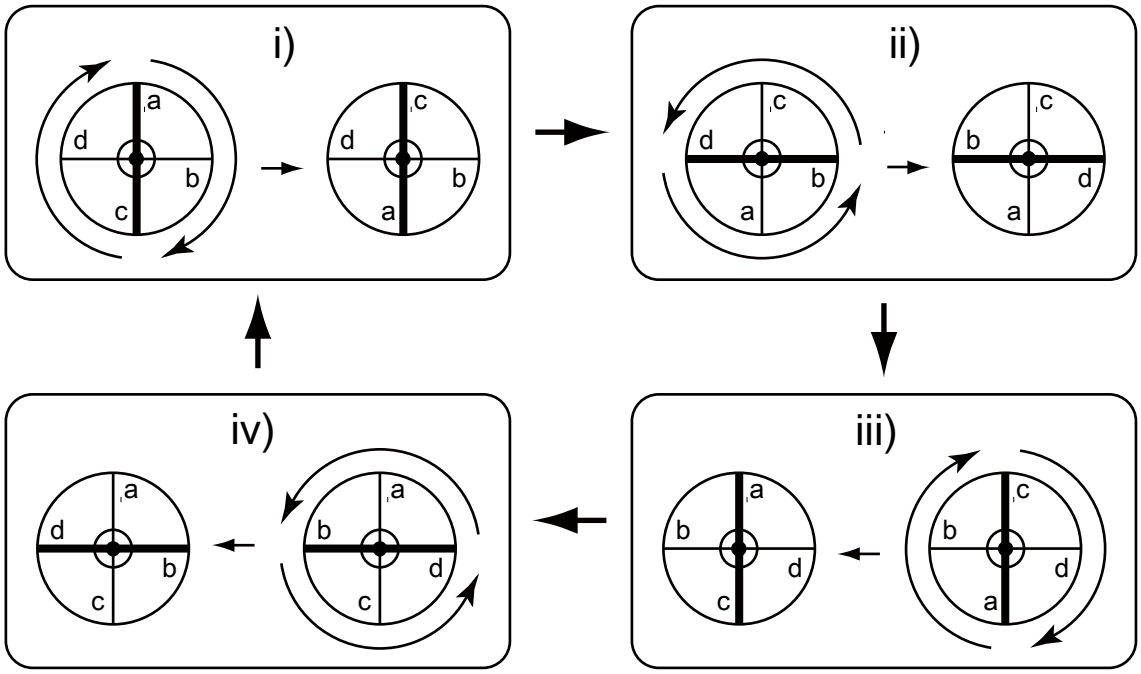

Figure 6.3 Preparation of braid-type extraction medium. Bobbin positions (a)-(d) correspond that in Figure 6.2. First, i) a pair of bundles placed at the opposite of the circular plate (a and c) were simultaneously moved clockwise 180°, and then, ii) another pair of bundles (b and d) were also simultaneously moved counterclockwise 180°. This two-step process was repeated once more as shown in iii) and iv), to return to the original configuration i).

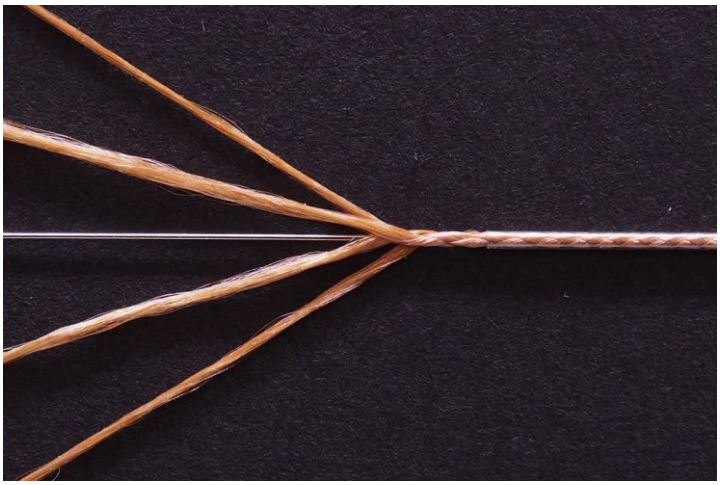

Figure 6.4 Typical photograph of a braided fiber prepared in this work.

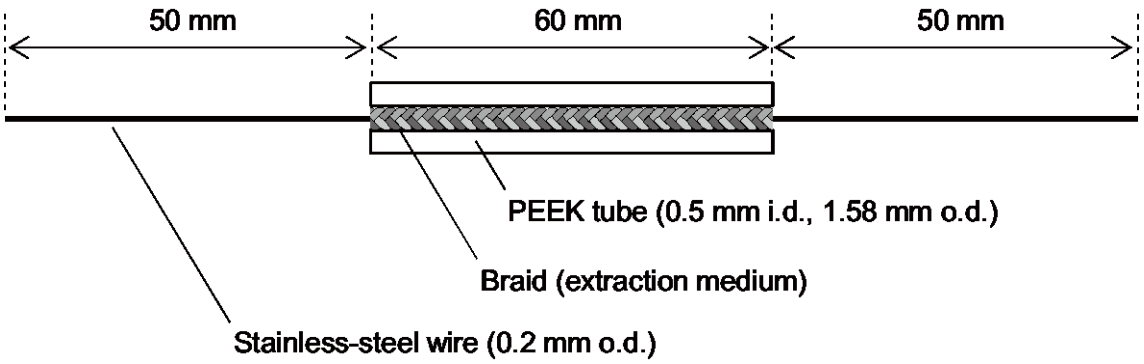

Figure 6.5 Schematic of the braid-packed extraction capillary with a stainless-steel wire therein.

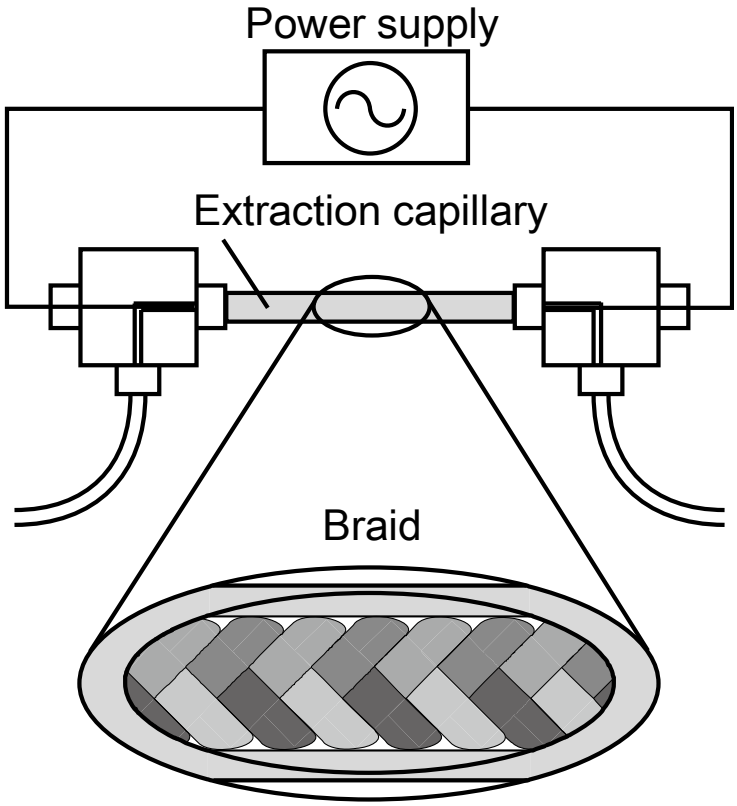

Figure 6.6 Illustration of the miniaturized SPE device connected with an electric power supply developed in this work.

6-3. Results and Discussion

Optimization of extraction flow-rates

1.0 mL of aqueous phenanthrene solution as a model sample was extracted on the braid, and the optimum extraction flow-rate was investigated. The results are summarized in **Table 6.2**. At all the flow-rate investigated, more than 90% of phenanthrene can be extracted, and it was confirmed that the extraction efficiency slightly increased with a slower flow-rate. In the case of flow-rate slower than 40 μL/min, the extraction efficiency was slightly improved, although a longer time was required for each extraction operation. Therefore, 40 μL/min was chosen as the optimum extraction flow-rate considering the total analysis time required for sample preparation and LC measurement.

Optimization of desorption solvent

Extracted phenanthrene on the braid fiber was desorbed by the typical organic solvents as desorption solvent such as *n*-hexane, toluene, dichloromethane, acetone and methanol, and better desorption efficiency was obtained when acetone was employed. However, for the desorption of phenanthrene from surface of Zylon filaments, it has been reported from the results of a previous investigation [28] that the desorption efficiency of non-polar organic solvents was higher than that of polar organic solvents due to the characteristics of the surface. Since the braided capillary consists of a bundle of fine filaments, its composition was considered to be quite complex compared with the conventional fiber-packed extraction capillaries [12,13]. Therefore, it was considered that some water remained between the thin filaments even if air was passed to remove the remaining water inside the extraction capillary after extracted sample

solution. It could be assumed that the remaining water interferes with the efficient desorption of aromatic compounds when only non-polar desorption solvents are used in the desorption of aromatic compounds.

In this study, a sequential pumping procedure was introduced into the desorption step, first pumping polar solvent and then non-polar solvent, allowing the effective removal of water from the extraction capillary by polar solvent and the effective desorption of the extracted analytes by non-polar solvent. The sequential pumping procedure in the desorption process was shown in **Figure 6.7**. The loop of the 6-port valve was filled with a non-polar solvent (second desorption solvent) and then partially replaced by a polar solvent (first desorption solvent). The resulting series of desorption solvents, acetone and *n*-hexane, were pumped into the extraction capillary in this order. **Table 6.3** summarizes the desorption efficiency obtained with the sequential pumping procedure of the two solvents, with different ratios of polar and non-polar solvents. The introducing sequential pumping of the two organic solvents, acetone and *n*-hexane, the desorption efficiency was significantly increased. This result suggests that a small amount of water in the extraction capillary was effectively washed away by acetone in advance of the desorption of phenanthrene by *n*-hexane. Based on these results, the sequential pumping of acetone and *n*-hexane, 5 μL and 35 μL, respectively, was employed in the following experiments.

Heat-assisted desorption

Taking advantage of the braid which was inserted a different material inside, heat-assisted desorption was investigated. As described in the previous section, the extraction capillary with braided fiber has a specially-designed configuration to be

compatible with resistive heating during the desorption process. The surface temperature of the extraction device during the thermal desorption process was observed with a thermal imaging camera (FLIR i7, FLIR Systems, Wilsonville, OR, USA). A programmable AC voltage application was carried out using a laboratory-made power supply system. The voltage application program was set to "3 s of voltage application followed by 27 s of no voltage application" based on a systematic study in preliminary experiments. To desorption of the extracted analytes from the extraction capillary, the above voltage application program was repeated four times while the desorption solvent flowed at 20 μL/min for 2 min, and 40 μL of desorption solvent was supplied to the extraction capillary with the temperature raised by resistive heating.

Figure 6.8 shows a typical thermographic photograph during the voltage application, and **Table 6.4** summarizes the desorption efficiency with different applied voltages. Compared to the case where no voltage was applied, desorption efficiency was significantly improved by applying voltage to the stainless-steel wire in the braided fiber, and completely desorption was achieved only when a low voltage of 2 or 3 V was applied. **Figure 6.9** compares the chromatograms of phenanthrene obtained with and without the sample preparation process developed in this study. Under the optimized conditions, the limit of quantification (LOQ) and limit of detection (LOD) of phenanthrene were 0.048 μg/mL and 0.014 μg/mL, respectively, with an overall recovery of 96.6% and an RSD of less than 3.0% (n=5). Furthermore, even after more than 100 analyses, there were no major problems such as degradation of extraction efficiency, indicating that the miniaturized sample preparation device with braid fiber developed in this study has a good stability for repeatable use.

Table 6.2 Comparison of extraction flow-rates.

extraction flow-rate (μL/min)	extraction efficiency (%)
40	96.0
60	94.2
80	90.8
120	90.1

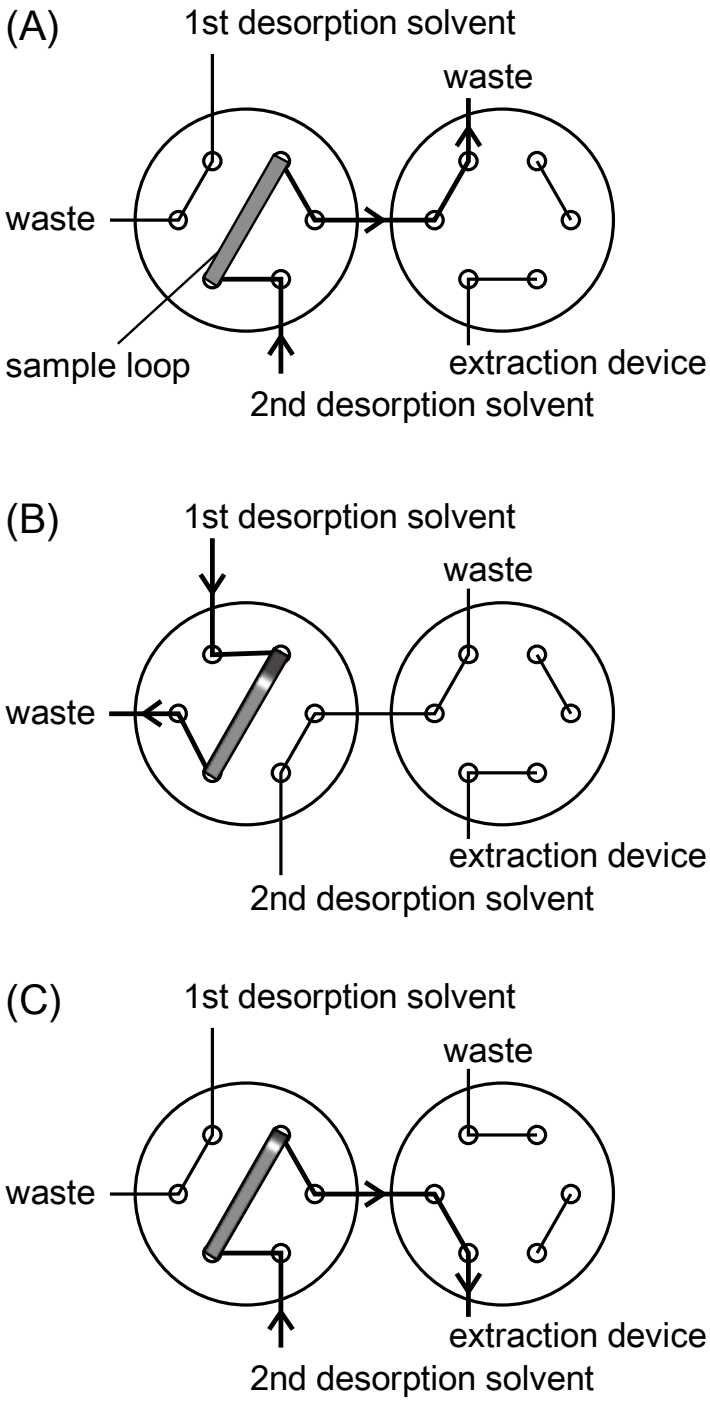

Figure 6.7 Scheme of the sequential pumping procedure with two types of organic solvents. (A) Fill the sample loop of the injection valve with the 2nd desorption solvent, (B) switch the injection valve, and inject certain volume of 1st desorption solvent, where a small volume of air was inserted between section of the 2nd and 1st desorption solvents, (C) switch the valve again, and start the sequential pumping of two desorption solvents.

Table 6.3 Comparison of desorption solvent.

desorption solvent volume (acetone + *n*-hexane)	desorption efficiency (%)	RSD (%)
40 μL + 0 μL	68.4	2.5
20 μL + 20 μL	78.0	3.7
10 μL + 30 μL	83.7	2.7
5 μL + 35 μL	87.5	0.6
0 μL + 40 μL	22.7	3.5

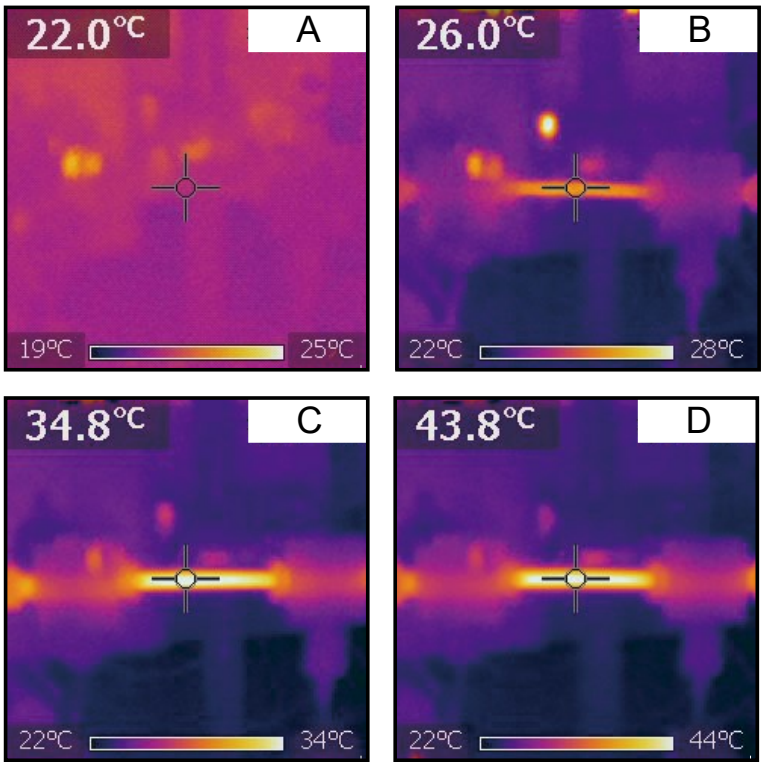

Figure 6.8 Thermographic observation of the surface temperature of the extraction capillary during a resistive heating process. Applied voltage: (A) 0V, (B) 1V, (C), 2V and (D) 3V. Other conditions are in the text.

Table 6.4 Surface temperature of the extraction capillary and the desorption efficiency with thermal desorption at different applied voltages.

applied voltage (V)	surface temperature of capillary tube (ºC)	desorption efficiency (%)	RSD (%)
0	22	87.5	0.6
1	26	95.0	1.2
2	35	100.6	1.3
3	44	99.4	2.1

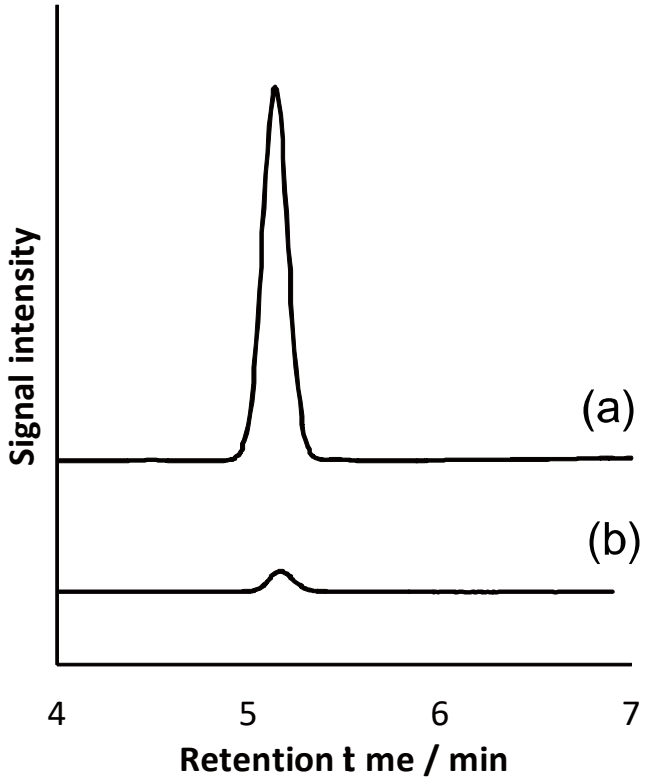

Figure 6.9 Comparison of chromatograms observed for (a) the sample solution preconcentrated by the miniaturized SPE device, and (b) that without preconcentration. Extraction conditions: extraction flow-rate, 40 μL/min; extraction volume, 1.0 mL; concentration of phenanthrene standard, 1.0 μg/mL. Desorption conditions: desorption flow-rate, 20 μL/min; desorption solvent volume, 40 μL; desorption solvent and volume, acetone 5 μL + *n*-hexane 35 μL. Thermal desorption condition: applied voltage, 2V; voltage was applied continuously while desorption process. LC conditions: mobile phase, methanol/water = (85/15); flow-rate, 1.0 mL/min; detection, UV at 254 nm.

6-4. Conclusions

Introducing a braided-fiber-packed capillary, as a extraction medium, a novel miniaturized sample preparation devise was developed. The extraction conditions were systematically optimized, and an efficient desorption was confirmed by sequential pumping of two organic solvents under the optimized conditions. Experimentally complete desorption was achieved for a target analyte in water samples by applying a low voltage to the metal wire in the braided fiber as a extraction medium. Although more systematic investigations are needed in the future, the unique configuration of the extraction cartridge is considered to be one of the most promising techniques in miniaturized sample preparation and is expected to have a wide range of applications in the near future.

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Chapter 7

General Conclusions

General Conclusions

In this thesis, several types of synthetic polymers as extraction and separation media have been introduced in chromatographic analysis to enhance the analytical performance. Two types of novel stationary phase, P4VP and PBT, showed retention behaviors significantly different from that of conventional ODS. In the P4VP stationary phase, a good linear relationship between logarithmic retention factor and *F* number of the group of PACs was observed along with a good selectivity for planarity for PACs of similar molecular sizes. Furthermore, the P4VP phase showed unique selectivity for planer PACs, where "square-like" analyte molecules were retained more strongly than "rod-like" analyte molecules. However, only a small amount of alkylbenzene was retained on the P4VP phase compared with the conventional stationary phases. For the structural isomers of disubstituted benzenes, the P4VP phase showed a unique molecular shape selectivity, suggesting that contribution of a dipole-dipole interaction between the solute molecule with an electron withdrawing substituent and the nitrogen atoms of the P4VP ligands. On the other hand, the PBT stationary phase showed the similar retention tendency to the P4VP stationary phase, and the PBT phase retained "rod-like" analyte molecules more strongly than "square-like" analyte molecules. The results suggests that separation mechanism on the PBT phase was different from that on the P4VP phase, and it can be interpreted that the retention of analytes on the PBT was based on following interaction between analyte and PBT stationary phase: hydrophobic interaction, π-π interaction between and polar interaction.

In addition, fibrous polymer material as extraction and separation media showed excellent performance by packing them into a capillary column in GC.

Polyimide filament packed into a fused-silica capillary was able to separate homologous alkanes quickly, and taking advantage of heat resistance of the fiber, temperature-programmed separation was successfully carried out. With the fiber-packed extraction needle, simultaneous derivatization/extraction was possible to improve signal intensity for the detection of volatile amines. By putting on plug and cap to the needle, collected sample could be stored for several days at room temperature, suggesting that it is suitable for a large number of on-site sampling at the same time. As further application of synthetic filaments, braided fiber with metal wire was introduced as a novel extraction medium for microscale sample preparation in LC. The device with a braid as the extraction medium and was able to extract aromatic compounds from water samples, and heat-assisted desorption was also possible using resistive heating generated when voltage was applied to the metal wire inside of the braid.

By introducing several types of polymer as separation media, a unique retention behavior was observed different from that on conventional stationary phases in LC. The application of synthetic polymers as stationary phases is also possible in GC as a novel stationary phase that enables rapid temperature-programmed separations. In addition, polymeric media can be applied to not only separation media but also extraction media, and extraction of organic compounds in gaseous samples was successfully carried out using a needle-type sample preparation device. Furthermore, a novel braided fiber as extraction media was developed. Several successful applications reported in this thesis suggest that the usefulness of synthetic polymer materials as extraction and separation media along with further possibilities in separation science.

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Publications

Original research papers related to this dissertation

1. "Simultaneous Derivatization and Extraction of Volatile Amines with Fiber-Packed Needle and Subsequent Analysis in Gas Chromatography"
   K. Nakagami, T. Tazawa, O. Sumiya, I. Ueta, Y. Saito
   Chromatography, **39**, 75-81 (2018).

2. "Polyimide Filaments as a Novel Stationary Phase in Packed-Capillary Gas Chromatography"
   K. Nakagami, O. Sumiya, T. Tazawa, T. Monobe, M. Watanabe, I. Ueta, Y. Saito
   Chromatography, **39**, 91-96 (2018).

3 "Braid Configuration Designed for Fiber-Packed Capillary in Microscale Sample Preparation"
   K. Nakagami, T. Monobe, O. Sumiya, K. Takashima, I. Ueta, Y. Saito
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4 "Retention Behavior of Various Aromatic Compounds on Poly(butylene terephthalate) Stationary Phase in Liquid Chromatography"
   K. Nakagami, M. Amiya, K. Shimizu, O. Sumiya, R. Koike, I. Ueta, Y. Saito
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5 “Molecular Shape Selectivity for Polycyclic Aromatic Compounds on a Poly(4-vinylpyridine) Stationary Phase in Liquid Chromatography”

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1. "Retention Behavior of Polycyclic Aromatic Compounds in a Novel Polymer-Based Stationary Phase Liquid Chromatography"
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Books

1. "Sample Preparation for the Analysis of Drugs in Biological Fluids (Chapter 1)" in G. Hempel (Ed.), *Methods of Therapeutic Drug Monitoring including Pharmacogenetics*, Elsevier Science, Amsterdam, The Netherlands, October 2019, pp. 1-13, ISBN: 9780444640666.
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2. "Fullerenes and Polycyclic Aromatic Hydrocarbons in Separation Science (Chapter 12)" in P. K. Zarzycki (Ed.), *Pure and Functionalized Carbon Based Nanomaterials: Analytical, Biomedical, Civil and Environmental Engineering Applications*, Science Publishers, Enfield, NH, USA, July 2020, pp. 273-297, ISBN: 9781138491694.
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