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Characteristics of Phenylalanine Imprinted Membrane Prepared by the Wet Phase Inversion Method

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Abstract−A phenylalanine imprinted membrane without a sponge layer containing macrovoids was prepared by the wet phase inversion method. The structure of the phenylalanine imprinted membrane prepared by an in-situ implanting procedure was denser than that of the membrane prepared by post implanting. Consecutive washings with distilled water and acetic acid solution removed nearly all of the template molecules from the membrane prepared by the post implanting procedure, although much of the template molecules remained in the membrane prepared by in-situ implanting. The removal of the template molecules from the membrane increased the population of the free COOH groups but reduced that of the dimerized COOH groups in the membrane. A D-Phenylalanine imprinted membrane prepared by post implanting selectively adsorbed D-phenylalanine from a racemate solution, where the adsorption selectivity reached 4.79. A D-phenylalanine imprinted membrane prepared by in-situ implanting exhibited an inverse adsorption affinity.

Key words: Molecularly Imprinted Membrane, Adsorption Selectivity, Phenylalanine

INTRODUCTION

First introduced in 1931, the concept of molecular imprinting has attracted wide interest from the beginning of the 1970s. Organic polymers with predetermined ligand selectivities were prepared independently in 1972 by Wulff and Sarhan [1972] and Klots and Takagishi [1972]. The concept of imprinting is simple. Bacterial microorganisms are entrapped in a polymer matrix after the completion of polymerization if it is carried out in a monomer solution containing microorganisms. Cavities, which remain in the polymer matrix after the microorganisms are removed from the polymer matrix by washing or extraction, are complementary to the microorganisms in shape, as shown in Fig. 1. This is similar to reports in other research [Vulfson et al., 1997]. The polymer matrix can contain recognition sites toward the bacterial microorganisms, which had been imprinted in the polymer matrix, if there are affinity ligands to the bacterial microorganisms in a cavity. Thus, the polymer matrix can selectively bind microbial cells of the same species from a solution containing various microorganisms. Although the template molecule of a practical system is much smaller than a bacterial microorganism, molecularly imprinted polymer contains recognition sites which are complementary to the template molecule in both shape and chemical functionality.

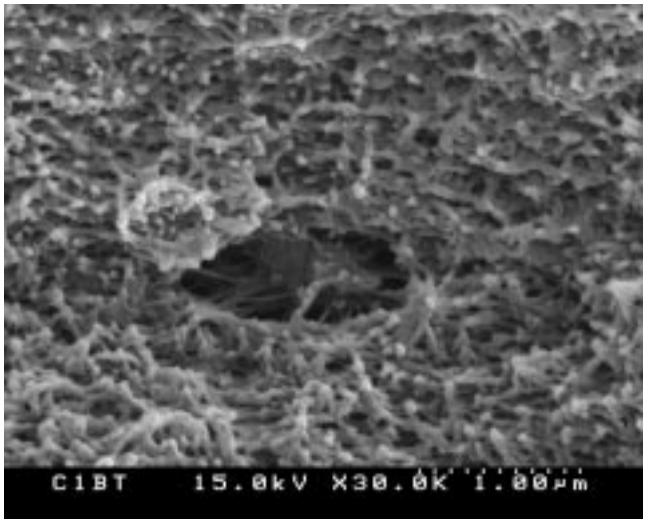

Fig. 1. Scanning electron microscopy (SEM) photograph of a cross-section of P(AN-co-AA) membrane prepared by the wet phase inversion method. Bacterial cells and the subsequent cellulose produced were included in the AN monomer solution before copolymerization. The cavity, in which a bacterial cell was entrapped, is also shown in the SEM photograph.

The conventional preparation of a molecularly imprinted polymer (MIP) is initiated by mixing template molecules with a functional monomer solution to form a template-functional monomer complex. Next, crosslinker monomers are added to this solution. Then, the copolymerization of the crosslinker with the functional monomer begins just after the addition of initiator [Wulff and Sarhan, 1972]. Template-functional monomer complexes are surrounded by a copolymer matrix. Functional monomers contribute to the composition of a copolymer, and at the same time form complexes with template molecules. The interactions between a template molecule and functional monomer may be a covalent interaction such as ester or a noncovalent interaction such as hydrogen bond and electrostatic interaction [Takeuchi et al., 1996; Cheong et al., 1998]. Imprinting a noncovalent system is easier in comparison to a covalent system. However, noncovalent bonding is not so strong and the population of the template-functional monomer complex is determined by equilibrium. Thus, an excess of functional monomer is required

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to complete template-functional monomer complexation. As a result, a large fraction of functional monomer is grafted randomly in the polymer matrix. The template molecules are removed from the polymer matrix by a simple extraction with acetic acid or methanol [Yoshikawa et al., 1999; Kobayashi et al., 1995]. Noncovalent imprinting has been applied to the preparation of polymers selective to dyes, diamines, vitamins, amino acid derivatives, peptides, theophylline, nucleotide base and naproxen [Kriz et al., 1997; Hedborg et al., 1993]. The recognition sites, which are complementary in both shape and chemical functionality to the template, are now present within the polymer matrix. A molecularly imprinted polymer has the ability to bind selectively the imprinted enantiomer from a mixture of racemates. It is also able to separate the template molecule from substrates of a similar structure. Most of the molecularly imprinted polymers prepared by this procedure were rigid gel matrices and used as solid separation media [Whitcombe et al., 1995; Mayes et al., 1996] in liquid chromatographies, capillary electrophoresis, affinity-based solid-phase extraction for separations of chiral compounds and amino acid derivatives and as artificial enzymes [Robinson and Mosbach, 1989; Ohkubo et al., 1994].

Molecularly imprinted polymer membranes have some advantages compared to MIP particles. Some of these include a high capacity due to a large surface area, faster transport of substrate molecules and faster equilibrium of binding cavities. That is why a higher expected throughput rate has been accomplished. Molecularly imprinted polymer membranes are prepared by in-situ polymerization [Mathew-Krotz and Shea, 1996; Hong et al., 1998], dry phase imprinting [Yoshikawa et al., 1995, 1996, 1999] and the wet phase inversion method [Kobayashi et al., 1995; Wang et al., 1996, 1997]. The in-situ polymerization is also applied to the preparation of composite membranes by plasma polymerization on the alumina porous plate [Sohn et al., 2000; Kim and Jung, 2000; Kim et al., 2000]. When the wet phase inversion method is carried out, a crosslinker monomer is usually copolymerized at first with a functional monomer. Then, the copolymer is dissolved in a solvent containing template molecules. At this stage, an interaction takes place between the template and functional monomers, which are already part of a copolymer. Recognition sites are formed when the thin-casted film of the copolymer solution coagulates in water and the template molecules are deleted from the solid polymer by extraction. In this study, we surmise that the composition of a copolymer membrane will be different from that of an MIP membrane prepared by the wet phase method described above if copolymerization is carried out in a solvent solution containing crosslinker, functional monomer and template molecules. Additionally, the template-functional monomer complexation will form simultaneously when the crosslinker is copolymerized with the functional monomers. "In-situ implanting" and "post-implanting" are suggested in this study. "In-situ implanting" is to form a template-functional monomer complex during copolymerization. "Post-implanting" is to form a template-functional monomer complex in a solvent solution containing a copolymer. The membranes are prepared by the alternative post-implanting method and the conventional in-situ implanting procedure, respectively, by using template molecules as phenylalanine. Hopefully, the adsorption selectivities of the MIP membranes could elucidate why and how the membrane preparation procedure affects the compositions of the MIP membranes and their adsorption selectivities.

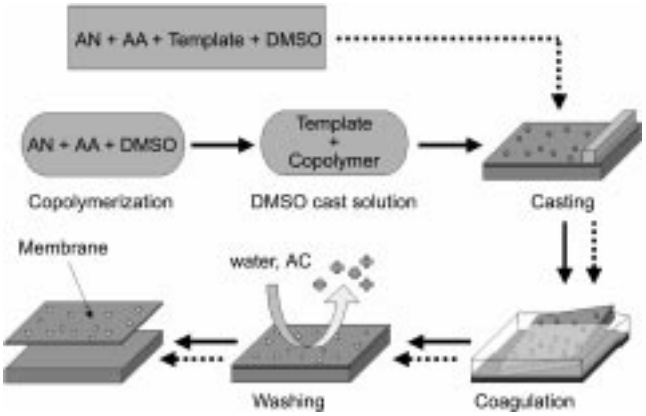

Fig. 2. Schematic presentation of the membrane preparation by the wet phase inversion method.
(a) in-situ implanting procedure (dotted line) (b) post implanting procedure (solid line).

EXPERIMENTAL

1. Materials

All reagents used in this experiment were of reagent grade. The crosslinker acrylonitrile (AN) was a product of Yakuri (Japan), the functional monomer acrylic acid (AA) was from Junsei (Japan), the porogen dimethyl sulfoxide (DMSO) was from Kanto (Japan) and L-phenylalanine (L-Phe) and D-phenylalanine (D-Phe), the template and enantiomer, were from Sigma (USA). All reagents were used without further purification.

2. Membrane Preparation by Phase Inversion

Molecularly imprinted polymer membranes were prepared by the alternative wet phase inversion method suggested by Kobayashi et al. [1995], using a "post implanting" procedure and the conventional "in-situ implanting" procedure. In-situ implanted membranes were easily prepared, as shown in Fig. 2(a), by mixing 7.51 g of AA containing 0.015 g of L-Phe, 30.4 g of AN and 100.5 g of DMSO. The mixture was then copolymerized for 20 h in a nitrogen atmosphere with an addition of 0.22 g of 2,2'-Azo-bis-isobutyronitrile (AIBN) dissolved in 10 g of DMSO at 60 °C. The copolymer solution was casted on a glass plate with a thickness of 120 μm and coagulated in distilled water at various temperatures. The post implanting membranes were prepared in two steps, as shown in Fig. 2(b). A copolymer, (P(AN-co-AA)), was prepared by using the same procedure above for 6 h, except without a template molecule. The copolymer solution was coagulated in distilled water and dried for a week at 50 °C in a vacuum drying oven. For the imprinting, 0.02 g of L-Phe was added to 100 ml of DMSO solution containing 10 wt% of copolymer P(AN-co-AA). The solution was stirred for 20 h at 50 °C. The solution was casted on a glass plate and coagulated for less than 5 min in distilled water. The template molecule, L-Phe, was removed from the gel matrix by washing it for 2 h with 5% acetic acid and rinsed with distilled water. The MIP membrane was kept in distilled water until its next use.

3. Substrate Adsorption on the MIP Membrane

The substrate molecules were uptaken by maintaining the mem-

branes in a 0.005 wt% L-Phe solution containing the same amount of D-Phe for 20 h at 30 °C with shaking at 150 rpm. The concentrations of D-Phe and L-Phe in the substrate solution were analyzed by HPLC (Youngin M910, Korea) with a TSKgel Enantio L1 column (4.6×250 cm^2). The eluent rate was 1 mL/min and the absorbance of the substrate solution was monitored by a UV detector at 254 nm. The substrate uptake was defined as the amount of phenylalanine adsorbed by the MIP membrane during the adsorption process. Fresh membranes were dried at 55 °C in a vacuum drying oven until the weight of the membranes reached a constant value. The specific substrate uptake by the MIP membrane based on the dry weight of the polymer was obtained. Selectivity of the membrane (α) was defined as α=((Phe)temp/(Phe)iso)/([Phe]temp/[Phe]iso), where (Phe)temp and (Phe)iso are the amount of phenylalanine (template and isomer) adsorbed in the membrane, and [Phe]temp and [Phe]iso denote the concentrations in the solution after equilibrium was reached, respectively, as defined in the literature [Yoshikawa et al., 1998].

4. FT-IR (Peak Intensity Ratio)

The MIP membrane was freeze-dried for 4 h at −40 °C to remove the moisture from itself. The FT-IR spectra of the MIP membrane were measured by an FT-IR spectrometer (Galaxy 7020A, Mattson Instrument Inc., USA). To obtain the absorbance (A) of a peak, the transmittance (tr) of a peak was transformed by the equation A=−log(tr/100). The peak intensity ratio was defined as the ratio of peak absorbance of a functional group to that of the CN group in a membrane as represented in the literature [Wang et al., 1997].

RESULTS AND DISCUSSION

1. Extraction of Template Molecule from the L-Phe Implanted Copolymer

A three-dimensional ball and stick model of a crosslinker, functional monomer, L-Phe and D-Phe is shown in Fig. 3. The crosslinker, AN, has no functional group able to conduct noncovalent interaction with the template molecules, L-Phe or D-Phe. A functional monomer includes in itself a carboxylic group that is composed of a C=O group and OH group, having the potential to form a hydrogen bond with the L-Phe and D-Phe molecules.

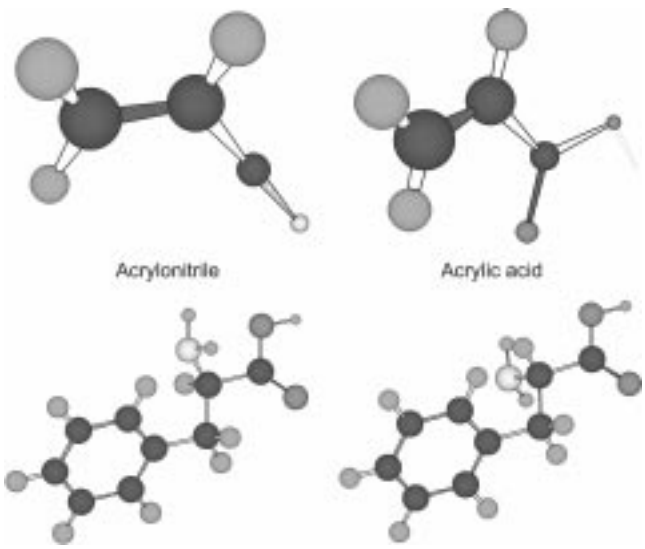

Fig. 3. Three dimensional ball-and-stick models of the template molecule (D-Phe, L-Phe), acrylonitrile, and acrylic acid.

Table 1. Template molecule removal percentage from L-Phe implanted polymer by washing with distilled water and acetic acid. 50 ml of L-Phe implanted polymer solution was poured and coagulated in 200 mL of distilled solution swirling in a beaker at 25 °C for 10 h. Washing was carried out under the same conditions. Data are the mean values of four experiments

	Washing with distilled water				Washing with 0.1% acetic acid			
	1st (coagulation)	2nd	3rd	4th	5th	6th	7th	8th
Post implanting	65.6	12.2	0	0	6.8	4.3	3.5	2.1
In-situ implanting	41.0	10.2	3.4	0	1.6	1.2	0	0

The phase inversion of a copolymer is composed of two steps: solvent exchange between DMSO and water; and coagulation of AN segments. This process is similar to the preparation of asymmetric polysulfone membranes using the dual bath method for gas separation [Lee et al., 2000]. The AN segments of the copolymer, P(AN-co-AA), coagulate in a poor solvent like water, due to the dipole interaction between CN groups [Wang et al., 1997]. After 20 h of implanting, 50 mL of copolymer solution prepared by the post implanting procedure was immersed into 200 ml of distilled water and coagulated. The amount of 65.6 wt% of the L-Phe molecule entrapped in the polymer matrix was diffused into bulk distilled water during the coagulation step. And 12.2 wt% of the template molecule was removed from the L-Phe imprinted polymer via washing with 200 mL of distilled water. These percentages are presented in Table 1. Unfortunately, a successive washing with distilled water was unable to remove additional template molecules from the L-Phe imprinted polymer. Even after four consecutive washings of each batch with 200 ml of 0.1% acetic acid solution, only another 16.7 wt% of the template molecules could be removed from the L-Phe implanted polymer. L-Phe implanted polymer prepared by using the in-situ implanting procedure coagulated and was washed like the L-Phe implanted polymer prepared by the post implanting procedure. The total amount of template molecules, however, removed by washing with distilled water and acetic acid was much less than that of the polymer prepared by post implanting. The removal of template molecules from the L-Phe implanted polymer after the first washing increased proportionally as the concentration of acetic acid increased (data not shown); the value was less than 4 wt% when washed with 10 wt% acetic acid. The L-Phe imprinted polymer was rewashed many times with acetic acid solution. However, the total removal of template molecules remained less than 10 wt% of the L-Phe added at the beginning of the copolymerization step in the in-situ implanting procedure, as shown in Table 1. In this study, even after four washings with distilled water and another four washings with acetic acid solution, more than 30% of the template molecules remained in the L-Phe implanted membrane

prepared by in-situ implanting. Nonetheless, nearly all of the template molecules were removed from the membrane prepared by using the post implanting procedure. This suggests that template molecules more effectively form the complexes with functional monomers in in-situ implanting than in post implanting. Furthermore, the structure of a copolymer matrix prepared by in-situ implanting is

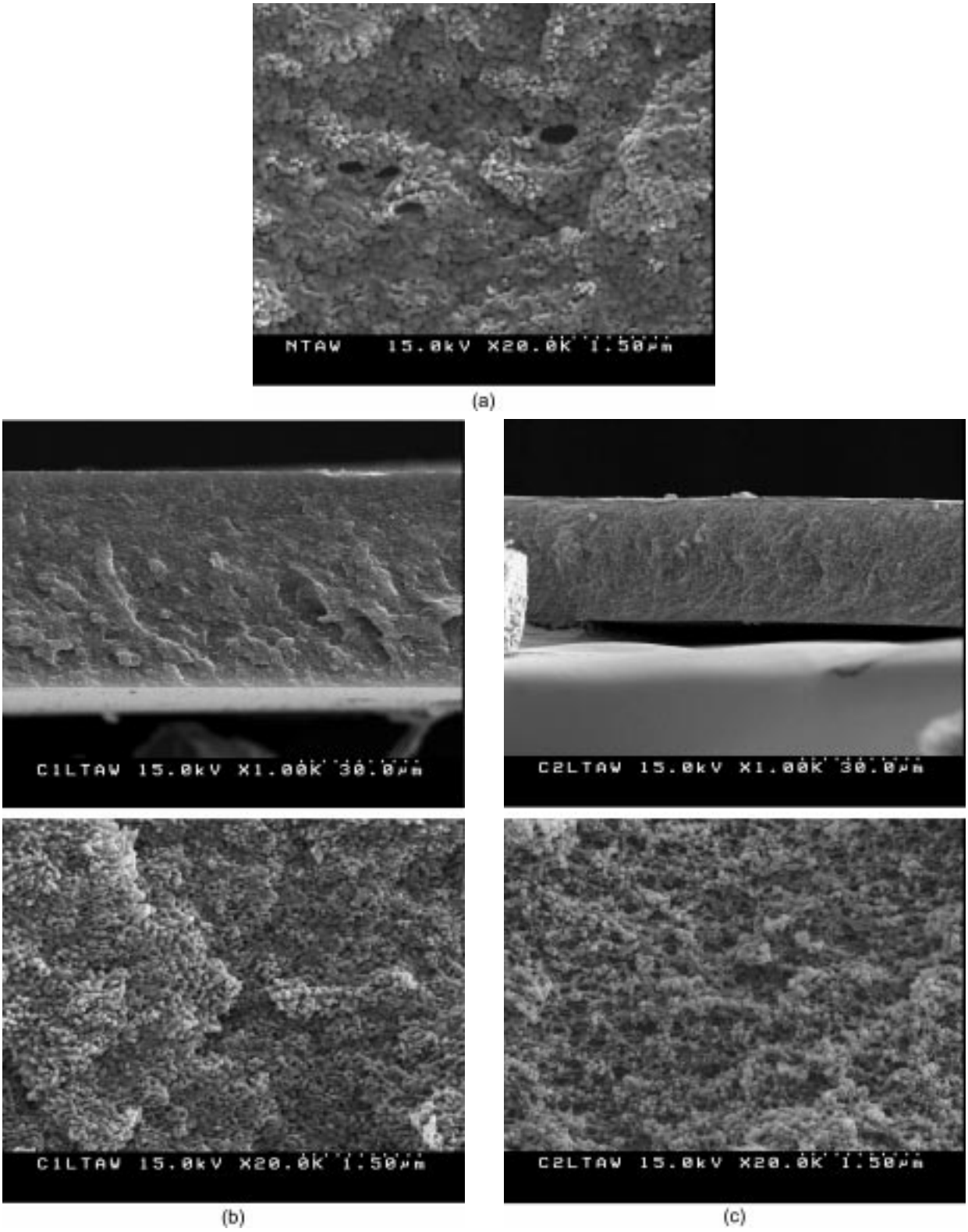

Fig. 4. SEM photographs of a cross-section of the P(AN-co-AA) membrane prepared by the wet phase inversion method.
(a) prepared without a template (b) prepared by in-situ implanting with L-Phe (c) prepared by post implanting with L-Phe (d) prepared by in-situ implanting with D-Phe (e) prepared by post implanting with D-Phe.

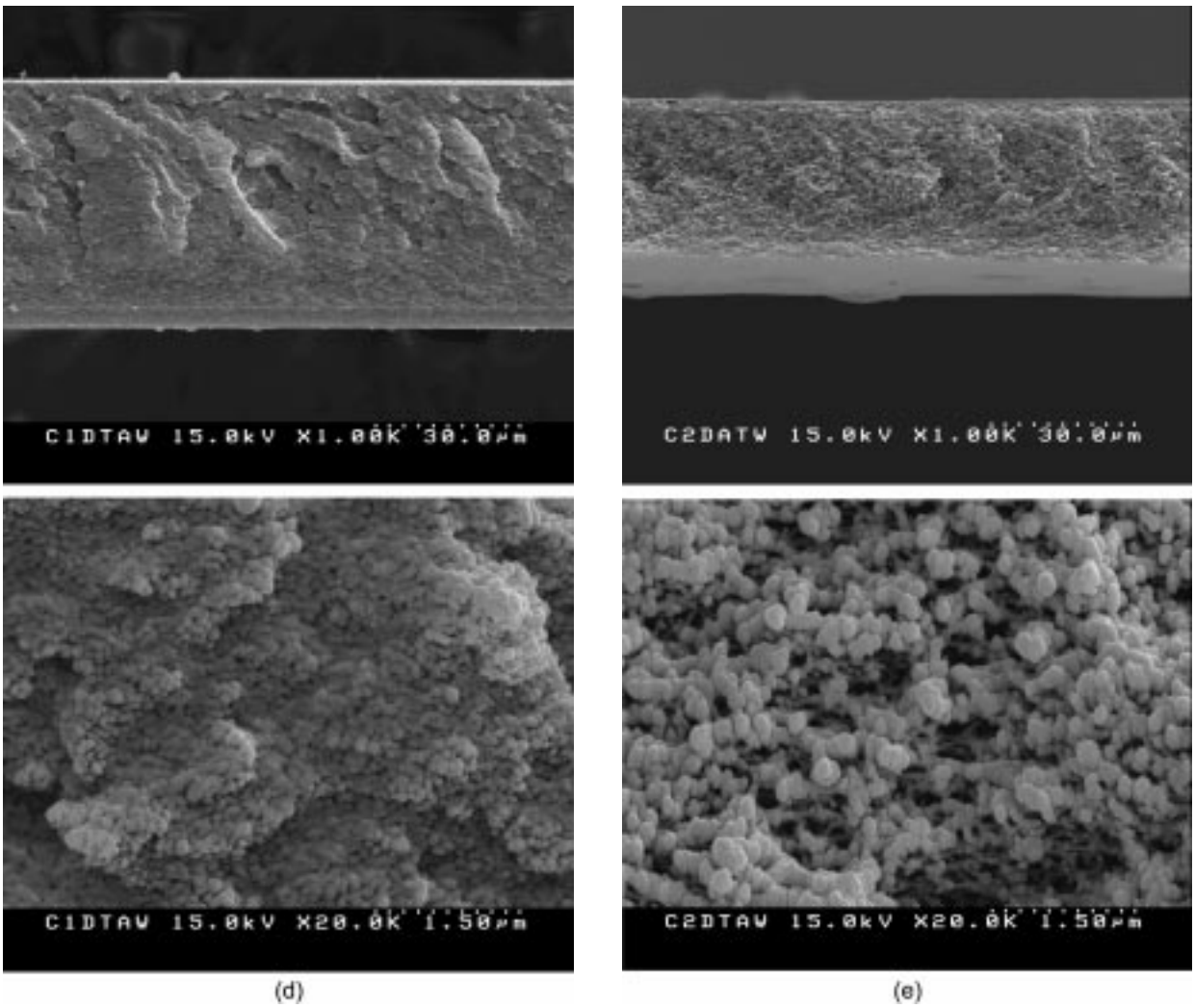

Fig. 4. Continued.

denser than that prepared by post implanting.

Fig. 4 shows scanning electron microscopy (SEM) photographs of the cross-section of an L-Phe imprinted polymer. The photographs only exhibit a dense layer without finger-like macrovoids, although the theophylline (THO) imprinted P(AN-co-AA) membrane [Wang et al., 1997] prepared by a similar procedure showed a thick sponge-like matrix under a dense skin layer. In this study, a glass plate was shaken slowly in the water during the coagulation step. The polymer membrane was shrunk quickly and the membrane came off the glass plate in 2-5 min.

The thickness of the L-phenylalanine imprinted membrane prepared by post implanting was only 30 µm, although the thickness of the THO imprinted membrane prepared by Wang et al. [1997], by post implanting, was 60 µm. The thickness of the L-Phe imprinted membrane prepared by in-situ implanting procedure was 50 µm, however, much thicker than that of the post implanted membrane. This might be because the total amount of AA and AN that is added to the DMSO solution in in-situ implanting is much greater than that of P(AN-co-AA) copolymer dissolved in the DMSO solution in post implanting. Additionally, there is a much larger fraction of the microvoids, which are distributed evenly in the post implanted membrane. Moreover, the size of the microparticle grains in the membrane matrix prepared by post implanting is smaller than that of the membrane prepared by in-situ implanting.

2. Functional Groups in the P(AN-co-AA) Membrane

The development of noncovalent interaction in the MIP membrane was investigated by measuring the peak intensity of the FT-IR spectra of the MIP membrane. FT-IR spectra of the polyacrylonitrile membrane, P(AN-co-AA) membrane and L-Phe imprinted P(AN-co-AA) membrane prepared by in-situ implanting are in Fig. 5. For the polyacrylonitrile membrane, there is no peak for unoccupied OH stretching of free COOH (wave number 3,522 cm^{-}), OH stretching of dimerized COOH (2,604, 3,217 cm^{-}), C=O stretching (1,728 cm^{-}) and C-O stretching (1,248 cm^{-}). All of these come from the functional groups of AA. Both the P(AN-co-AA) membrane and L-Phe imprinted P(AN-co-AA) membrane prepared by in-situ implanting have the peaks mentioned above. The peak intensity ratios were calculated and are displayed in Table 2. There are peaks for functional groups, which come from AA segments, in the P(AN-co-AA) membrane. In addition, the peak intensity ratios of C=O and C-O are a little smaller than those of the L-Phe imprinted P(AN-co-AA) membrane. Additionally, as shown in Fig. 5, there is the

Table 2. Peak intensity ratios of the FT-IR spectra of polyacrylonitrile, P(AN-co-AA) and L-Phe imprinted P(AN-co-AA) membranes prepared by in-situ implanting. The peak intensity ratio is defined as the ratio of peak intensity of functional ligand to that of CN in each membrane

Membrane	OH stretching: free COOH	OH stretching: dimerized COOH	OH stretching: dimerized COOH	C=O stretching	C-O stretching
	3,522 cm^-	3,217 cm^-	2,604 cm^-	1,728 cm^-	1,248 cm^-
Polyacrylonitrile	0.08	0.02	0.02	0.02	0.12
P(AN-co-AA)	0.11	0.87	0.14	1.67	0.76
L-Phe imprinted P(AN-co-AA)	0.27	0.80	0.18	1.84	0.81

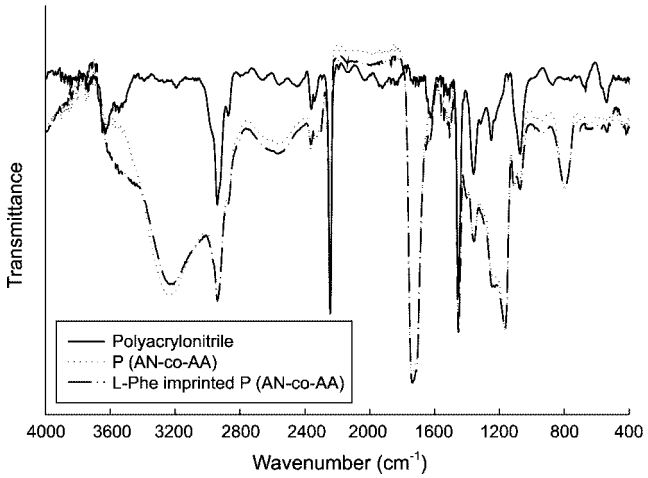

Fig. 5. FT-IR spectra of the PAN, P(AN-co-AA) membrane and L-Phe imprinted P(AN-co-AA) membrane prepared by in-situ implanting.

remarkable existence of a peak for unoccupied OH of free COOH (3,522 cm^-) in the L-Phe imprinted P(AN-co-AA) membrane. Usually, COOH groups of AA segments of the P(AN-co-AA) copolymer exist as dimers [Wang et al., 1996, 1997]. The template molecule, L-Phe, added to the AN solution at the beginning of the copolymerization step, seemed to interact with the functional group, COOH of AA segments. Some of the COOH groups were left free after the L-Phe was removed from the implanted copolymer matrix at the coagulation step. The increase in the intensity ratio of the unoccupied OH group implies the possibility of recognition site formation in the L-Phe implanted copolymer membrane. The increases in other peak intensity ratios may come from some remaining template molecules in the membrane matrix after the coagulation step or from the change of AA segments in the P(AN-co-AA) chain. These might be caused by the existence of L-Phe in the monomer solution during the copolymerization step.

3. Recognition Site Formation in the Phenylalanine Imprinted Membrane

On the consideration of the Ac-D-Trp imprinted membrane, which was prepared by the dry phase imprinting method involving tetrapeptide derivatives as chiral-recognition sites, nothing has selectivity toward amino acids although the Ac-L-Trp imprinted membrane adsorbed selectively the target amino acid [Yoshikawa et al., 1998]. Thus, the three-dimensional structures of D-Phe and L-Phe need to be investigated to determine why and how the molecularly imprinted membrane contains effective recognition sites. Both D-Phe and L-Phe have an amino group that can establish a hydrogen bond and electrostatic interaction with the carboxyl group of a functional monomer, depending on the pH of the bulk substrate solution, as shown in Fig. 3. They also have a carboxylic group that can build a hydrogen bond with the functional monomer, AA. The instantaneous three-dimensional structure of the functional group of D-Phe is slightly different from that of L-Phe. The amino group of D-Phe is placed near the carboxyl group, but the amino group of L-Phe seems to be hidden by CH and placed separately from the carboxyl group because they are isomers. This structural difference may lead to a different yield of the template-functional monomer complex formation in the MIP matrix. As a result, it may show different template molecule specificity, depending on whether L-Phe or D-Phe is used as the template molecule. Template-functional monomer complexes can be formed during copolymerization (in-situ implanting) or during implantation after copolymerization (post implanting). Existence of L-Phe in the monomer solution during the copolymerization step may change the composition of the copolymer, such as the arrangement order and length of the AA and AN segments in the copolymer chain, compared to that of the P(AN-co-AA) copolymer, which is prepared without a template molecule.

FT-IR spectra of L-Phe and D-Phe imprinted membranes are shown in Figs. 6 and 7. The peak intensity ratios calculated from these FT-IR spectra are written in Table 3. Wang et al. [1996] reported that the fraction of AA segments in the P(AN-co-AA) copolymer can be obtained by measuring the peak intensity ratio of

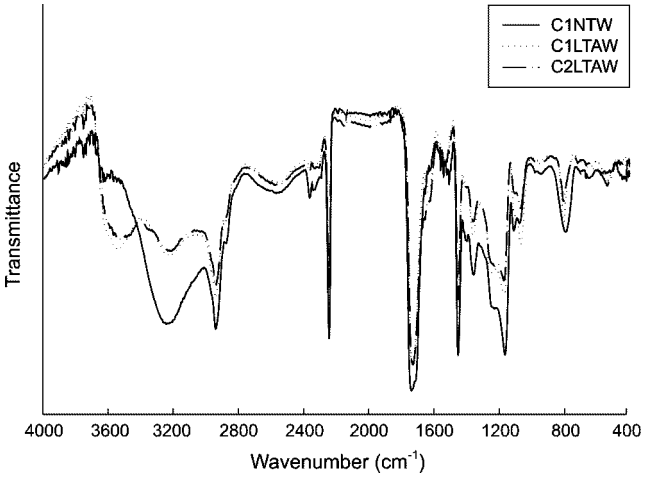

Fig. 6. FT-IR spectra of the L-Phe imprinted membrane. (a) prepared without template (b) prepared by in-situ implanting procedure (c) prepared by post implanting procedure.

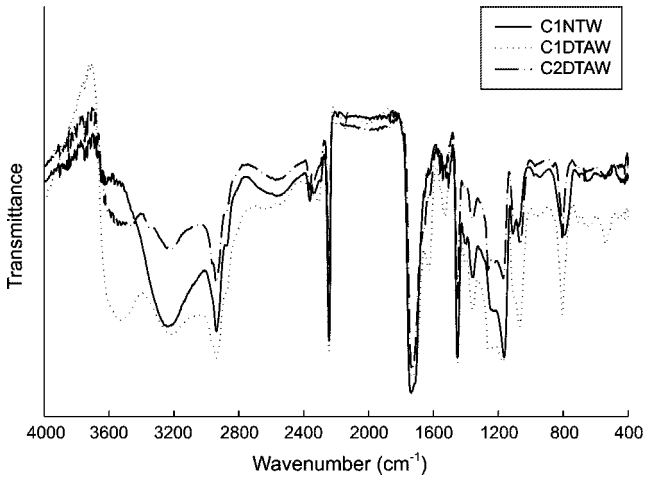

Fig. 7. FT-IR spectra of the D-Phe imprinted membrane.
(a) prepared without template (b) prepared by in-situ implanting procedure (c) prepared by post implanting procedure.

C=O stretching to CN stretching. The peak intensity ratio of C=O stretching in the L-Phe implanted membrane prepared by in-situ implanting is 1.67. This value is a little higher than the 1.36 for the P(AN-co-AA) membrane prepared without a THO template. It is also higher than the 1.41 for the THO imprinted P(AN-co-AA) membrane [Wang et al., 1996]. In the previous section, it was explained that washing the L-Phe implanted membrane with acetic acid and water reduces the population of the dimerized COOH groups, but increases that of the free COOH groups. This is verified by the FT-IR spectra in Fig. 6. However, Table 3 shows that all of the peak intensity ratios of OH stretching (3,522, 3,217, 2,604 cm^{-}), C=O stretching (1,728 cm^{-}) and CO stretching (1,248 cm^{-}), which come from the AA carboxyl group in the L-Phe imprinted membrane prepared by the post implanting procedure, are higher than those in the L-Phe imprinted membrane prepared by the in-situ implanting procedure. This may be caused by the coupling reaction [Lee, 2000] between the L-Phe amino group and AA carboxyl group in the P(AN-co-AA) copolymer that occurred during implantation in the DMSO solution containing the 10 wt% P(AN-co-AA) and template molecule, L-Phe. In Fig. 6, the C=O (amide) group peak formed by the coupling reaction is at 1,650 cm^{-} in the FT-IR spectra of the L-Phe imprinted membrane prepared by post implanting. However, there is a weak C=O (amide) group peak in the FT-IR spectra of the L-Phe imprinted membrane prepared by in-situ implanting.

As shown in Fig. 7, the FT-IR spectra of the D-Phe imprinted membrane prepared by post implanting also have a C=O (amide) group peak at 1,650 cm^{-}. Intensity ratios of all peaks for the D-Phe imprinted membrane prepared by post implanting are larger than those of the L-Phe imprinted membrane prepared by in-situ implanting, as shown in Table 3. The FT-IR spectra of the D-Phe imprinted membrane prepared by in-situ implanting, however, is much different from that of the D-Phe imprinted membrane prepared by post implanting. The dimerized COOH group and free COOH group exhibit strong intensities in the FT-IR spectra of the D-Phe imprinted membrane prepared by in-situ implanting. This can be explained by the strong coupling reaction between the D-Phe amino group and AA carboxyl group, which occurred during the copolymerization step. A strong peak of the C=O (amide) group formed by the coupling reaction appears at 1,650 cm^{-} in the FT-IR spectra of the D-Phe imprinted membrane prepared by in-situ implanting. The intensity ratios of all peaks except that of C=O (from AA) are much higher than those of the L-Phe imprinted polymer prepared by in-situ implanting. The weak intensity ratio of C=O could be the unknown complex interaction between COOH groups.

The difference in implanting order of the wet phase inversion method used to prepare the phenylalanine imprinted membrane changed the specific dry weight of the membrane, as shown in Table 4. The specific dry weights of the L-Phe and D-Phe imprinted membranes prepared by in-situ implanting were heavier than those of the post implanting procedure. The D-Phe imprinted membrane prepared by in-situ implanting also had a much heavier weight than that of the L-Phe imprinted membrane. This depends on the membrane structure, as shown in Fig. 4. The thicknesses of the membranes prepared by in-situ implanting were 50 μm, while the membranes prepared by post implanting were 30 μm, regardless of the kind of template. For the post implanting procedure, the D-Phe imprinted membrane had a much larger void fraction in the polymer matrix than the L-Phe imprinted membrane, although the particle size in the D-Phe imprinted membrane was larger than that of the L-Phe imprinted membrane. In terms of gas separation, the ranking of permeability coefficients correlates well with fractional free volume [Kim et al., 1999]. In addition, porous inorganic membranes are made by impregnating metals, so a palladium impregnated porous membrane exhibits excellent hydrogen permeability [So et al., 1999; Jung et al., 1999]. For in-situ implanting, the D-Phe imprinted membrane is denser than the L-Phe imprinted membrane. This might be because of the strong coupling reaction between D-Phe and AA during copolymerization of AN with AA.

Table 3. Template implanting influence on the peak intensity ratio of functional ligands in the MIP membrane washed for 2 h with acetic acid and for 10 h with distilled water. Abbreviations for the membranes include: L; L-Phe imprinted membrane, D; D-Phe imprinted membrane, I; in-situ implanting and P; post implanting

Membrane	Peak intensity ratio				
	OH stretching: free COOH 3,522 cm^{-}	OH stretching: dimerized COOH 3,217 cm^{-}	OH stretching: dimerized COOH 2,604 cm^{-}	C=O stretching 1,728 cm^{-}	C-O stretching 1,248 cm^{-}
LI	0.53	0.57	0.19	1.67	0.69
LP	0.61	0.70	0.24	2.13	0.83
DI	0.75	0.85	0.21	1.30	0.97
DP	0.52	0.71	0.21	2.34	0.83

Table 4. Specific dry weight of the phenylalanine imprinted membrane. Copolymer solution was casted by a film applicator with a slit of 120 μm in height. Data are the mean values of three experiments. Abbreviations for the membranes include: L; L-Phe imprinted membrane, D; D-Phe imprinted membrane, I; in-situ implanting and P; post implanting

Membrane	LI	LP	DI	DP
mg dry wt/cm^2	19.0	9.3	23.8	8.8

4. Adsorption Selectivity of the Phenylalanine Imprinted Membrane

The addition of a template molecule, either L-Phe or D-Phe, into the DMSO solution containing the P(AN-co-AA) copolymer induced a coupling reaction between the phenylalanine amino group and the AA segment carboxyl group in the P(AN-co-AA). Thus, an amide group, which can build a hydrogen bond with a template molecule, was formed in the phenylalanine imprinted membrane without a reduction in the number of carboxyl groups in the copolymer chain. Furthermore, a strong coupling reaction occurred between D-Phe and AA during copolymerization, which formed many more amide groups in the D-Phe imprinted membrane prepared by in-situ implanting. However, only a weak coupling reaction occurred between L-Phe and AA during copolymerization. We cannot confirm from this information that the recognition sites are well formed.

The D-Phe imprinted membrane had no ability to adsorb selectively D-Phe from the isomer L-Phe after being washed only with distilled water. However, a successive washing with acetic acid enabled the imprinted membrane to selectively adsorb the template molecule from the racemate solution. As shown in Table 5, the adsorption selectivity of the D-Phe imprinted membrane prepared by post implanting is 4.79, much higher than any other amino acid imprinted membrane. This adsorption selectivity is very high compared to those of the amino acid derivative imprinted membrane containing peptide derivatives as recognition sites prepared by the dry phase imprinting method [Yoshikawa et al., 1995, 1996, 1997, 1998, 1999]. Wang et al. [1997] prepared a THO imprinted membrane by the phase inversion method. The adsorption selectivity of the THO imprinted membrane toward the template molecule was only 3.9 in the THO and 2-hydroxyethyltheophylline (HETHO) mixture, although the adsorption selectivity toward THO was 52 in a THO and caffeine (CAF) mixture. HETHO contains a hydroxyethyl group and CAF has no group capable of hydrogen bonding [Wang et al., 1997]. The interesting point is that the adsorption selectivity of the D-Phe imprinted membrane prepared by in-situ implanting is only 0.42. This value, less than 1, indicates that a much greater amount of the other isomer, L-Phe, was adsorbed into the membrane instead of the template molecule, D-Phe. This phenomenon is explained partly by the fact that much of D-Phe, which was added to the monomer solution hoping that all of it would be imprinted in the copolymer of P(AN-co-AA), was coupled to the polymer chain during the copolymerization step. The phenylalanine imprinted membrane prepared by in-situ implanting had consistent results with inverse adsorption selectivity. However, what causes this appearance still remains a mystery. For it to be fully understood, detailed research must be verified. The adsorption selectivity value of the L-Phe imprinted membrane is smaller than that of the D-Phe imprinted membrane. The higher adsorption selectivity of the D-Phe imprinted membrane may be caused by the three-dimensional structure of D-Phe, which is different from the isomer, L-Phe. The functional groups in the recognition sites of the D-Phe imprinted polymer interact more strongly with the template molecule in the solution compared to the L-Phe imprinted membrane. The L-Phe imprinted membrane prepared by in-situ implanting exhibited an inverse affinity; that is, selectivity was toward the other isomer even though the adsorption selectivity is close to 1. This result partially coincides with a report that (CH_3)COCO-L-tryptophane (Boc-L-Trp) imprinted membrane prepared by the dry phase imprinting method has no permselectivity toward L-Trp [Yoshikawa et al., 1995].

Table 5. Adsorption selectivity of the phenylalanine imprinted membrane. Adsorption was carried out by immersing 1 g of membrane in a 100 mL solution containing 0.5 mg L-Phe and 0.5 mg D-Phe and maintaining it for 24 h at 30 °C with shaking at 150 rpm. Data are the mean values of four experiments. Abbreviations for the membranes include; L; L-Phe imprinted membrane, D; D-Phe imprinted membrane, I; in-situ implanting and P; post implanting

Membrane	mg uptaken phenylalanine /g membrane		Selectivity, α
	L-Phe	D-Phe	
LI	0.98	1.05	0.93
LP	1.03	0.94	1.09
DI*	0.15	0.08	0.42
DP*	0.05	0.21	4.79

*initial concentration of L-Phe, D-Phe: 5 mg/L each.

CONCLUSION

A dense symmetric phenylalanine-imprinted membrane without finger-like macrovoids can be produced by using the wet phase inversion method. The structure of the membrane prepared by in-situ implanting is more compact than that prepared by post implanting. There is also a much larger fraction of the evenly distributed microvoids in the post implanted membrane. In addition, the size of the microparticle grains in the membrane matrix prepared by post implanting is smaller than that of the membrane prepared by in-situ implanting. The difference in membrane structure seems to affect the degree of template removal from the membrane matrix during the washing step. Nearly all of the template molecules are removed from the membrane prepared by the post implanting procedure after consecutive washings with distilled water and acetic acid solution. However, many of the template molecules remain in the membrane prepared by in-situ implanting even after many washings. The removal of template molecules from the membrane by washing with acetic acid solution reduces the population of the dimerized COOH group, but increases that of the free COOH group in the membrane matrix, which is responsible for the adsorption of the template molecules. The adsorption selectivity of the D-Phe imprinted membrane prepared by post implanting is much higher than that of the D-Phe or L-Phe implanted membrane prepared by in-situ im-

planting. The D-Phe implanted membrane prepared by in-situ implanting contains several amide groups formed by a strong coupling reaction between D-Phe and AA during copolymerization. The D-Phe imprinted membrane prepared by in-situ implanting shows consistent inverse adsorption selectivity. More detailed research is now underway in our laboratory to explain clearly this inverse selectivity.

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