Korean J. Chem. Eng., **20**(6), 1066-1072 (2003)

Adsorption Selectivity of Phenylalanine Imprinted Polymer Prepared by the Wet Phase Inversion Method

Joong Kon Park†, Seong Joon Kim and Jeong Woo Lee

Department of Chemical Engineering, Kyungpook National University, Daegu 702-701, Korea
(*Received 20 May 2003 • accepted 30 June 2003*)

Abstract−A phenylalanine (Phe) imprinted polymer was prepared by the wet phase inversion method to investigate the optimum preparation condition which endows the polymer matrix with prominent adsorption selectivity. The addition of a large amount of porogen (dimethyl sulfoxide: DMSO) was needed to form macrovoids in the polymer matrix. For the high adsorption selectivity of the polymer matrix, a complex between functional monomers and a template molecule must be formed before the copolymerization of acrylonitrile (AN: crosslinker) and acrylic acid (AA: functional monomer). The formation of a phenyl group in the polymer matrix by the coupling reaction between D-Phe and AA seemed to cause the reverse adsorption selectivity of the polymer matrix. The adsorption selectivity of the Phe imprinted polymer was dependent on the pH of the racemate solution. The adsorption selectivity of the D-Phe imprinted polymer prepared by the post implanting method reached 11 at pH 2 and showed a reverse adsorption selectivity at pH 4 and 6.

Key words: Molecularly Imprinted Polymer, Macrovoids, Phenylalanine, pH Dependency

INTRODUCTION

A molecular imprinting polymer has recognition sites which are complementary in shape to the template molecule and which also contain functional ligands that can bind template molecules. Thus, a molecularly imprinted polymer has the ability to bind selectively the imprinted enantiomer from a racemate solution and is also able to separate target molecules from substrates of a similar structure [Park and Seo, 2002]. Molecularly imprinted polymers have been prepared by *in-situ* polymerization [Mathew-Krotz and Shea, 1996; Hong et al., 1998], dry phase imprinting [Yoshikawa et al., 1999], and the wet phase inversion method [Kobayashi et al., 1995; Wang et al., 1997]. The in-situ polymerization is also applied to the preparation of composite membranes by plasma polymerization on an alumina porous plate [Son et al., 2000; Kim and Jung, 2000; Kim et al., 2000]. Most of the molecularly imprinted polymer has been 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].

In a wet phase inversion method, a template molecule is implanted by an in-situ implanting procedure or post implanting procedure [Park and Seo, 2002]. In an *in-situ* implanting procedure, the complex of target molecule and functional monomer is formed during copolymerization, but is formed when the copolymer prepared without a template molecule is dissolved in the porogen solution containing target molecules in a post implanting process. In a previous study [Park and Seo, 2002], a dense symmetric Phe imprinted poly (AN-co-AA) membrane containing no macrovoids was produced by a wet phase inversion method. The D-Phe imprinted membrane prepared by an *in-situ* implanting procedure showed reverse adsorption selectivity, although the adsorption selectivity of the D-Phe imprinted membrane prepared by a post implanting procedure reached 4.79. Ramstrome et al. [1998] have described the pH dependency of the chromatographic resolution. When a molecularly imprinted polymer was prepared by using 2-vinylpyridine and/or methacrylic acid as a functional monomer and used as a separation media, the chromatographic resolution was dramatically influenced by the variation in pH of the aqueous mobile phase.

In this study, we tried to develop a method to form macrovoids in a molecularly imprinted polymer matrix and to determine how the macrovoids so formed affect the adsorption selectivity of the polymer matrix. We also investigated how the charge variation of the template molecules according to the pH of the racemate solution affected the adsorption selectivity of the Phe imprinted polymer. We tried to determine the reason why the adsorption selectivity of some of the D-Phe imprinted polymer was reversed, by investigating the preparation process of the molecularly imprinted polymers.

EXPERIMENTAL

1. Materials

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

2. Preparation of Phe Imprinted Polymer

The Phe imprinted polymer was prepared by the alternative wet phase inversion method using a post implanting procedure and an *in-situ* implanting procedure, which are described in detail in a previous article [Park and Seo, 2002]. The preparation of the *in-situ*

†To whom correspondence should be addressed.
E-mail: parkjk@knu.ac.kr

implanted membrane was initiated by stirring 7.51 g of AA containing 0.1 g of D-Phe for 2 h in order to form a complex of D-Phe and AA. This solution was mixed with 30.4 g of AN and 300 g of DMSO and was then copolymerized for 6 h in a nitrogen atmosphere with an addition of 0.22 g of 2,2'-Azo-bis-isobutyronitrile (AIBN) at 60 °C. The copolymer solution was cast on a glass plate with a thickness of 120 μm and coagulated in distilled water at 20 °C. The membrane was washed with 5% acetic acid in order to remove template molecules. The post implanting polymer was prepared by copolymerizing 30.4 g of AN with 7.51 g of AA in 100 g of DMSO for 6 h at 60 °C. The copolymer solution was coagulated in distilled water at 25 °C and dried for a week at 50 °C in a vacuum drying oven. For implantation, 10 wt% of copolymer P(AN-co-AA) was dissolved in 300 g of DMSO containing 0.02 g of D-Phe for 20 h at 50 °C. The solution was cast on a glass plate and coagulated for less than 5 min. The Phe imprinted polymer beads were prepared by dropping the polymer solution contained in a syringe which was pressed at a constant pressure (Fig. 1). The polymer drop of 2 mm fell into the water contained in a vessel and then coagulated. Beads were washed by 5% acetic acid in order to remove the template molecules from the bead polymer matrix. The structure of the polymer matrix was examined by a SEM photograph and the formation of the recognition sites in the polymer matrix was indirectly determined by measuring the peak intensity ratio using FT-IR spectra. The FT-IR spectra of the Phe imprinted polymer were measured by an FT-IR spectrometer (Galaxy 7020A, Mattson Instrument Inc., USA). The transmittance (tr) of a peak was transformed by the equation $A=-\log(tr/100)$ in order to obtain the absorbance (A) of a peak. The peak intensity ratio was defined as the ratio of peak absorbance of a functional group to that of the CN group in a polymer matrix, as represented in the literature [Wang et al., 1997].

3. Substrate Adsorption on the Phe Imprinted Polymer

The Phe imprinted polymer was immersed for 1 h in distilled water whose initial pH was adjusted to 2 by HCl or NaOH. The batch of distilled water of pH 2 was renewed at an interval of 2 h until the pH of the solution was unchanged after 1 h immersion. In order to selectively uptake the template molecules, the imprinted polymer was maintained in a 0.1 wt% Phe racemate solution for

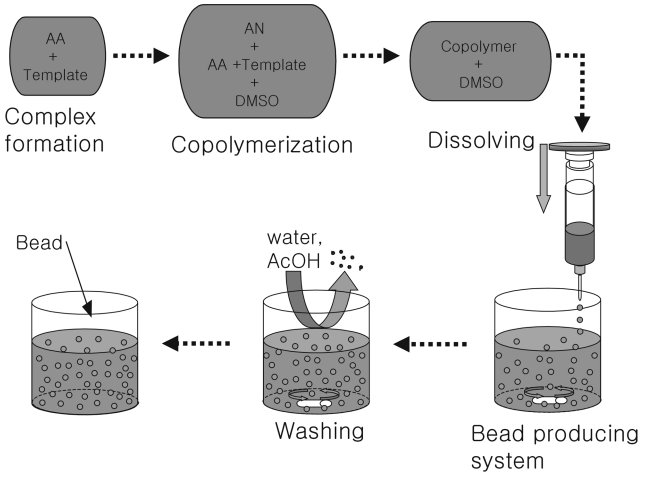

Fig. 1. Schematic presentation of the molecularly imprinted polymer bead preparation by the wet phase inversion method.

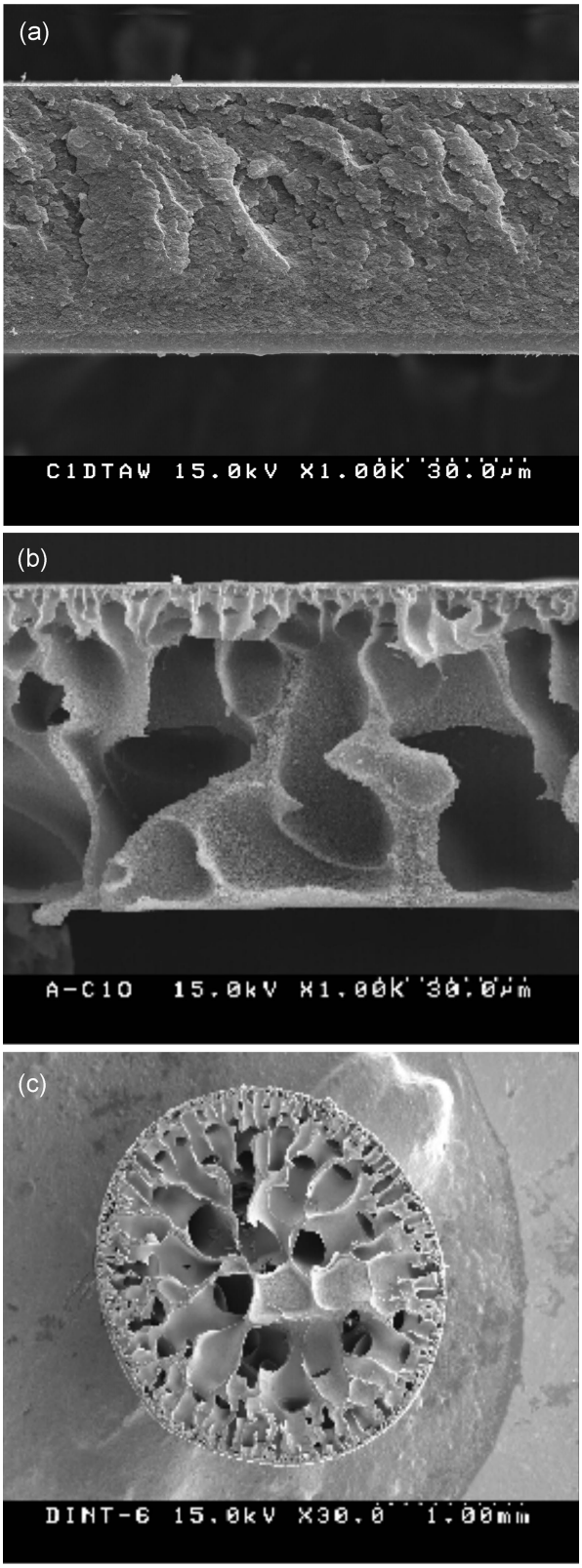

Fig. 2. SEM photograph of the cross section of the D-Phe imprinted polymer washed with acetic acid after being washed with water: (a) membrane prepared by *in-situ* implanting procedure using 100 g DMSO, (b) membrane prepared by *in-situ* implanting procedure using 300 g DMSO, (c) bead prepared by post implanting procedure using 200 g DMSO following *in-situ* implanting procedure using 100 g DMSO.

24 h at 30 °C with shaking at 150 rpm. The concentrations of D-Phe and L-Phe were analyzed by HPLC (Youngrin M930, Korea) with a TSKgel Enantio L1 column (4.6×250 mm^2). The effluent rate was 1 mL/min and the absorbance of the substrate solution was monitored by a UV detector at 254 nm. Substrate uptake was defined as the amount of Phe adsorbed by the polymer during the adsorption process. Fresh polymer was dried by a freeze dryer at −51 °C until the weight of the polymer reached constant value. The specific uptake by the polymer was the substrate uptake divided by the dry weight of the polymer. The adsorption selectivity (α) of the phenylalanine imprinted polymer was defined as α=((Phe)temp/(Phe)iso)/([Phe]temp/[Phe]iso), where (Phe)temp and (Phe)iso are the amount of Phe (template and enantiomer) adsorbed in the polymer, and [Phe]temp and [Phe]iso denote the concentrations in the solution after adsorption, respectively, as defined in the literature [Yoshikawa et al., 1998].

RESULTS AND DISCUSSION

1. Formation of Macrovoids in a D-Phe Imprinted Membrane

The symmetric Phe imprinted membrane without macrovoids prepared in a previous investigation [Park and Seo, 2002] is mechanically stronger than the asymmetric membrane with macrovoids. However, the macrovoids in the polymer matrix cause an increase in the mass transfer rate in the polymer matrix. The porogen, DMSO in the copolymer solution is exchanged with water at the coagulation step and then the copolymer is solidified. Thus, a large amount of DMSO was added to the monomer mixture before copolymerization, with the hope that the exchange of this DMSO with water at the coagulation step would form large channels and, hence, these channels would become macrovoids in the coagulated copolymer matrix. Fig. 2 shows the SEM photograph of the cross section of a D-Phe imprinted membrane prepared by an *in-situ* implanting procedure using 300 g of DMSO, three times more than the amount of DMSO used in the preparation of a symmetric membrane without macrovoids in the previous study [Park and Seo, 2002]. Macrovoids were well developed in the lower part of the membrane with a skin layer at the upper surface.

The FT-IR spectra of this asymmetric membrane were measured in order to indirectly determine the formation of recognition sites in the polymer matrix. In the previous study [Park and Seo, 2002], the FT-IR spectra of the Phe imprinted P(AN-co-AA) membrane showed well developed peaks at the wave numbers of 3,522 cm^{-1} and 3,217 cm^{-1}, although those of PAN showed no peaks and the P(AN-co-AA) membrane showed a well developed peak of dimerized COOH only at 3,217 cm^{-1} and no peak of free COOH at 3,522 cm^{-1}. It was easily explained that AA in the copolymer showed a peak of dimerized COOH at 3,217 cm^{-1} and a well developed recognition site showed a peak of free COOH at 3,522 cm^{-1}. The FT-IR spectra of the asymmetric membrane were significantly different from those of the symmetric membrane, as shown in Fig. 3. There was no peak at 3,217 cm^{-1} and 3,522 cm^{-1}. Therefore, the addition of a large amount of DMSO to the mixture of the monomer solution seemed to hinder the copolymerization of AN with AA and the formation of complex between AA and Phe molecule, and thus, recognition sites did not form in the polymer matrix.

2. D-Phe Double Imprinted Beads with Macrovoids

Phe imprinted beads with macrovoids were also prepared. The copolymer solution of P(AN-co-AA) which was prepared by the *in-situ* implanting procedure using 100 g DMSO was coagulated

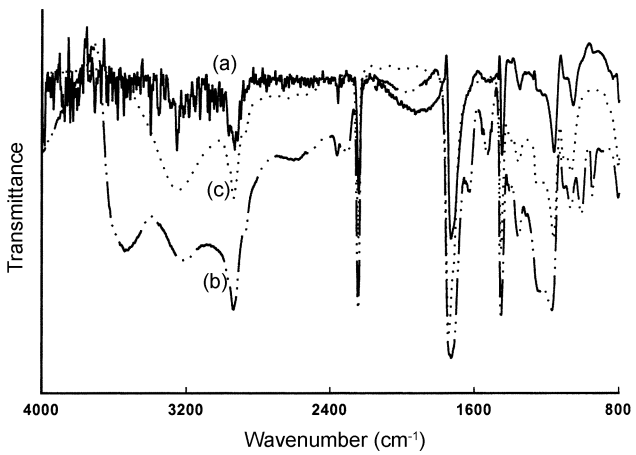

Fig. 3. FT-IR spectra of D-Phe imprinted membrane prepared by *in-situ* implanting process using (a) 300 g DMSO, (b) 100 g DMSO with individual complex formation step, (c) 100 g DMSO without individual complex formation step.

Table 1. Adsorption selectivity of D-Phe imprinted polymer. Adsorption was carried out by immersing polymer 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 three experiments. Abbreviations for the polymers include: L, L-Phe imprinted; D, D-Phe imprinted; I, in-situ implanting; and P, post implanting

Polymer	pH	mg uptaken Phe/g polymer		Selectivity α	g polymer/L solution	Remarks
		D-Phe	L-Phe			
DIDP membrane	2	0.0054	0.0048	1.17	270	Post re-implant with 100 g DMSO
	4	0.0064	0.0052	1.31		
	8	0.0061	0.0057	1.09		
DIDP bead	2	0.033	0.037	0.86	57	Post re-implant with 300 g DMSO
	4	0.033	0.035	0.91		
	8	0.042	0.059	0.43		
DI* membrane	-	0.08*	0.15*	0.42*	10*	With 100 g DMSO

* from [Park and Seo, 2002].

in distilled water. The lump of coagulated D-Phe imprinted P(AN-co-AA) polymer was dried in a vacuum oven, and then 20 g of dried polymer was dissolved in 200 g of DMSO containing 0.045 g D-Phe. The SEM photograph of the cross section of the D-Phe double imprinted P(AN-co-AA) bead (abbreviation: DIDP bead) showed many well developed macrovoids in the polymer matrix (Fig. 2). The DIDP bead selectively adsorbed the other enantiomer, L-Phe, from a 0.001 wt% racemate solution. The adsorption selectivity of the DIDP bead was 0.43 when the uptake capacity of the DIDP bead was 0.1 mg Phe/g bead at pH 8 of the racemate solution, as shown in Table 1. This adsorption selectivity was very similar to 0.42 of DI membrane (the D-Phe imprinted P(AN-co-AA) membrane prepared by an in-situ implanting procedure) measured at 0.001 wt% racemate solution when the uptake capacity of the DI membrane was 0.23 mg Phe/g membrane. The smaller uptake capacity of the DIDP bead seemed to be caused by the amount of beads, which was 6 times larger than that of the DI membrane. The decrease in the specific adsorption capacity with an increase of the adsorbent density can also be found in the biosorption of heavy metals using microbial cells. The specific copper and chrome adsorption capacity of *Aspergillus carbonarius* cells decreased 29 times and 8 times, respectively, in accordance with the biosorbent density [Kapoor and Viraraghavan, 1997]. In the literature [Puranik and Paknikar, 1999], this has been explained by the obstruction between the heavy metal combined sites.

The adsorption selectivity of the DIDP bead showed a reverse value at the pH range from 2 to 8 and was similar to that of DI membrane, especially at pH 8, as shown in Table 1. However, the adsorption selectivity of the DIDP membrane prepared by using 100 g DMSO at the post implanting procedure was larger than 1 at pHs ranging from 2 to 8 and was opposite to that of DI membrane. Thus, D-Phe seemed to be not effectively imprinted in the DIDP bead at the post implanting procedure which was carried out with large 300 g of DMSO. This inference is supported by the SEM photograph for the local point of the DIDP bead. As shown in Fig. 4, the microstructure of the DIDP bead was very similar to that of DI membrane which was prepared by using 300 g DMSO and far different from those of DI and DP (the D-Phe imprinted P(AN-co-AA) prepared by a post implanting procedure) membranes prepared by using 100 g DMSO. Therefore, we deduce that redissolving the Phe imprinted P(AN-co-AA), which has been prepared by an *in-situ* implanting procedure using a small amount of DMSO, in a large volume of DMSO can endow the polymer matrix with macrovoids reserving the ability of selective adsorption.

3. Complex Formation before Copolymerization

In an *in-situ* implanting procedure, functional monomers can be copolymerized in a mixture of functional monomer, cross linker, porogen, and template molecules. We investigated whether the complex formation between template molecules and functional monomers before copolymerization is essential for endowing the polymer matrix with selective adsorptivity. A D-Phe imprinted membrane was prepared by using 100 g DMSO by an *in-situ* implanting procedure without any separate complex formation steps before copolymerization. The FT-IR spectra of the membrane were analyzed and compared with those of membranes prepared by an *in-situ* procedure including a complex formation step before copolymerization and by an *in-situ* procedure using a large amount, 300 g of

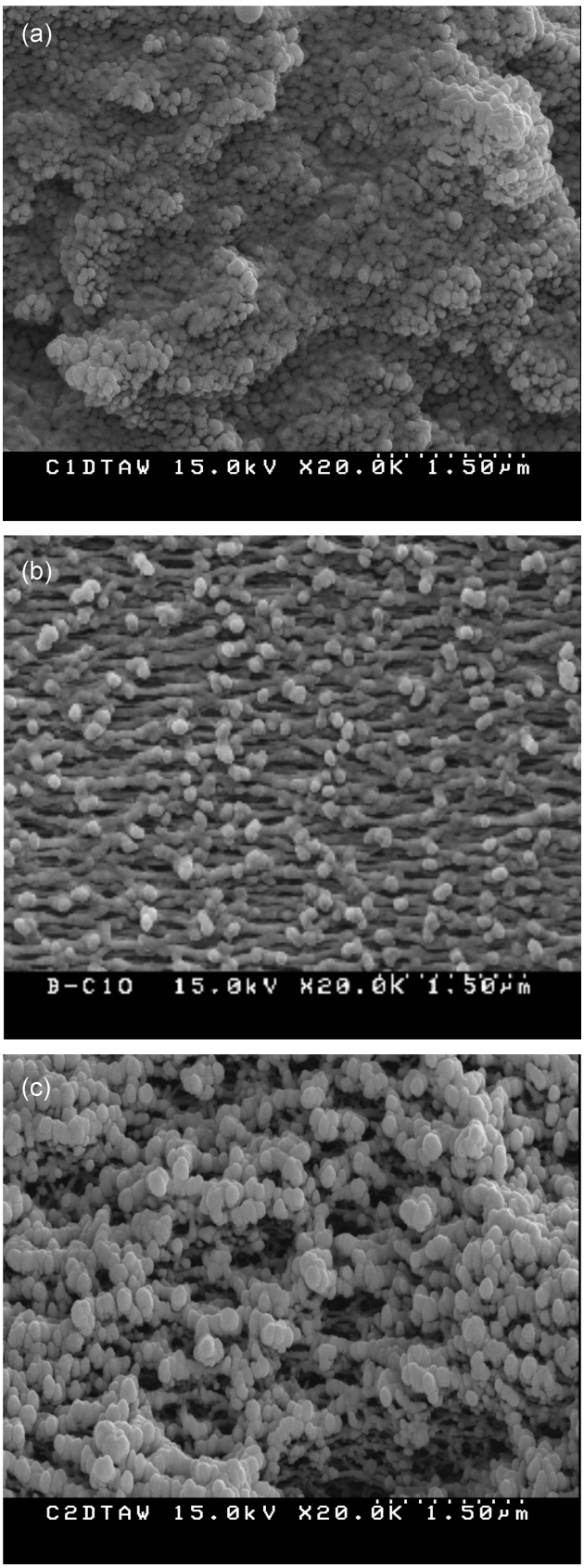

Fig. 4. SEM photograph of D-Phe imprinted membrane prepared by (a) *in-situ* implanting method using 100 g DMSO, (b) *in-situ* implanting method using 300 g DMSO, (c) post implanting method using 100 g DMSO.

Table 2. Peak intensity ratio of FT-IR spectra of D-phenylalanine imprinted membrane without macrovoids prepared by in-situ implanting. Peak intensity ratio is defined as the ratio of peak intensity of functional ligand to that of CN in each membrane. Abbreviation DI means a D-phenylalanine imprinted P(AN-co-AA) prepared by in-situ implanting

Membrane	OH stretching: free COOH	OH stretching: dimerized COOH	OH stretching: dimerized COOH	C=O stretching	C-O stretching
	3,522 cm^{-1}	3,217 cm^{-1}	2,604 cm^{-1}	1,728 cm^{-1}	1,248 cm^{-1}
Polyacrylonitrile	0.081	0.022	0.022	0.019	0.119
P(AN-co-AA)	0.11	0.87	0.14	1.67	0.76
DI with independent complexation	0.75	0.85	0.21	1.30	0.97
DI without independent complexation	0.017	0.563	0.131	1.987	0.537

DMSO, as shown in Fig. 3. There was a peak of dimerized COOH at 3,217 cm^{-1}, which was clearly higher than that of DI membrane prepared by using 300 g DMSO. As shown in Table 2, the intensities of peaks at 3,522 cm^{-1} and 2,604 cm^{-1} which came from AA segments in the copolymer chain were somewhat smaller than those of P(AN-co-AA) and DI prepared with independent complex formation. However, there was no peak of unoccupied free COOH at 3,522 cm^{-1} in the FT-IR spectra of the DI membrane prepared without separate complex formation before copolymerization. Thus, Phe could not be effectively implanted in the polymer matrix without a separate complex formation before copolymerization and a small amount of AA was also not copolymerized with the cross linker, AN. As expected, the adsorption selectivity of the DI membrane prepared without complex formation before copolymerization was approximately 1, indicating that the membrane could not selectively adsorb the template molecule from the Phe racemate solution.

4. The Adsorption Selectivity Dependent on the pH of the Racemate Solution

Although the mechanism of selective adsorption by a chiral stationary phase of a chromatography is not clear and no perfect model has yet been formulated, a three-point interaction model is widely cited [Jin, 1999]. Three ligands on the chiral stationary phase bind the corresponding ligands of the target molecule, respectively. The enantiomer also binds at one or two corresponding ligands with a geometric hindrance by the side chain of the polymer matrix. The recognition site of a molecularly imprinted polymer supplies one or two attractive ligands in a cavity which is complementary in shape to the template molecule. Chen et al. [2001] have reported that the functional groups of phenylalanine anilide imprinted polymer bind an analyte with hydrogen binding and/or ion exchange and the saturation capacity of the chiral sites was nearly twice larger at pH= 5.8 than at pH=3.0 [Chen et al., 2001]. The recognition site in the Phe imprinted polymer also contains carboxyl groups which can bind the template molecule using a hydrogen or electrostatic interaction. We investigated the variation of the adsorption selectivity of the D-Phe imprinted membrane, which was prepared by the post implanting procedure (DP membrane) without a coupling reaction, using 100 g DMSO, with respect to pH of the 0.01 wt% racemate solution. The DP membrane selectively adsorbed the template molecule, D-Phe at pH 2 of the racemate solution with 11.6 of adsorption selectivity. However, the adsorption selectivity of the DP membrane decreased with the increase in pH of the solution and reached 0.09 at pH 6. The DP membrane selectively adsorbed the enantiomer, L-Phe, at pH 8 and the uptake capacity was 0.09 g Phe/g membrane, which was much smaller than 0.14 g Phe/g membrane at pH 2.

Given the decrease in uptake capacity with an increase in pH, we investigated why the adsorption selectivity decreased with pH and reversed at high pH. If a template-functional monomer complex was formed mainly by the hydrogen bond at the beginning of copolymerization, the carboxyl group (COOH) remained in the recognition site after extraction of the template molecule from the polymer matrix. Thus, the template molecule fits into the recognition site which is complementary in shape and binds to the polymer matrix better than the enantiomer at a low pH. However, the carboxyl group (COOH) of the template molecule converts to COO^- with an increase in pH of the solution, and the COO^- fraction calculated by the Henderson-Hasselbach equation increases from 21% at pH 2 and reaches 99.9% at pH 6. Nevertheless, the amino group of the template molecule remains as NH_3^+ even at pH 6. The carboxyl group (COOH) in the recognition site also converts to COO^- as the pH of the solution increases. At a high pH, the template molecule with a shape complementary to the recognition site cannot bind to the polymer matrix because of a repulsive force caused by the same charges of carboxyl groups (COO^-) of the template molecule and

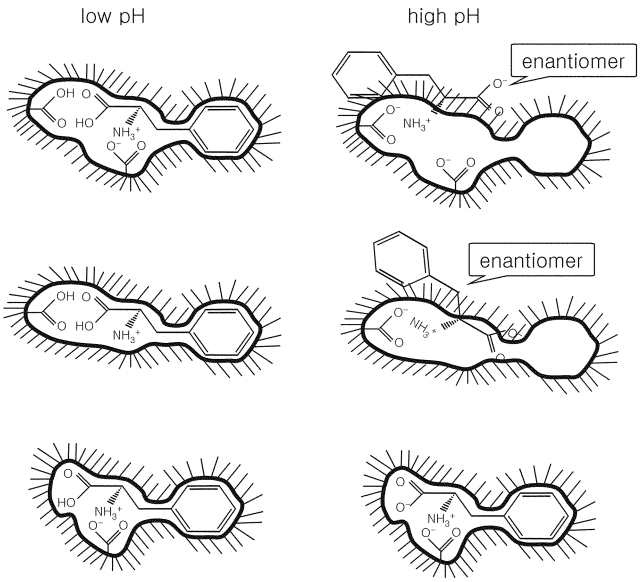

Fig. 5. Schematic representation of a molecule of Phe adsorbed on imprinted recognition sites of the polymer matrix at different pH values.

Table 3. Adsorption selectivity of D-Phe imprinted membrane without macrovoids prepared by post implanting procedure (abbreviation; DP) with and without coupling reaction. Adsorption was carried out by immersing polymer 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

Membrane	pH	mg uptaken Phe/g polymer		Selectivity, α	g membrane /L solution
		D-Phe	L-Phe		
DP with coupling reaction	2	0.003	0.004	0.72	300
	6	0.003	0.005	0.58	
	8	0.007	0.013	0.4	
DP without coupling reaction	2	0.13	0.012	11.6	16
	6	0.008	0.085	0.09	
	8	0.020	0.069	0.29	
	-*	0.21*	0.05*	4.79*	10*

*from [Park and Seo, 2002].

polymer matrix. However, the amino group (NH_3^+) of the enantiomer, which is not complementary to the recognition site in shape, binds to the carboxyl group (COO^-) in the cavity with a lower probability, as shown in Fig. 5. Hence, this results in the reverse adsorption selectivity of the DP membrane at high pH with a small uptake capacity.

5. Coupling Reaction in Post Implanting

In the previous work [Park and Seo, 2002], the D-Phe imprinted membrane prepared by an *in-situ* implanting procedure (DI membrane) showed a reverse adsorption selectivity, although the D-Phe imprinted membrane by a post implanting procedure (DP membrane) selectively adsorbed D-Phe from a racemate solution with adsorption selectivity, 4.79. The key difference in the two FT-IR spectra of DI and DP membranes is the existence of a peak at 1,650 cm^{-1}, which indicates an amide group. This amide group arose from the coupling reaction between D-Phe and the functional monomer occurring during the *in-situ* implanting process. In the current investigation, we facilitated the coupling reaction by putting the copolymer solution in a vacuum oven for 20 h at 50 °C after the post implanting procedure. As shown in Table 3, the D-Phe imprinted membrane prepared by a post implanting procedure with coupling reaction showed reverse adsorption selectivity regardless of the pH of the Phe racemate solution. This seems to be caused by the phenyl group from D-Phe which was combined with the polymer matrix by the coupling reaction after the implanting procedure and which was placed in the recognition site, which hinders the approaching of template molecule like the chiral stationary phase (CSP) of chromatography [Jin, 1999]. The reverse selectivity of DIDP bead, in which part of the D-Phe was coupled with acrylic acid in the polymer chain at the *in-situ* implanting and D-Phe was not effectively implanted at the post implanting step, was found at pH from 2 to 8, as shown in Table 1. Accordingly, we deduce that the phenyl group in the polymer matrix hindered the selective adsorption of D-Phe on the D-Phe imprinted polymer by an unclear mechanism.

CONCLUSION

A Phe imprinted polymer with macrovoids can be produced by using a large amount of porogen, DMSO, at the copolymerization step. However, adding a large amount of porogen in the mixture of a functional monomer and crosslinker seemed to hinder both the copolymerization of AA with AN and the formation of a complex between the template molecule and AA. The preparation of MIP by two steps is recommended to provide macrovoids in the polymer matrix preserving the capability of selectively binding a target molecule from a racemate solution. In a two step method, template molecules should be implanted by using a small amount of DMSO, and then dissolved in a large amount of porogen. The complex formation between a template molecule and functional monomers before copolymerization is also required for a polymer matrix to be capable of selectively adsorbing a target molecule from a racemate solution. The adsorption selectivity varied sensitively depending on the pH of the solution and selectivity was reversed at a certain range of pH because the charge of an affinity ligand in a recognition site changes with pH. However, the D-Phe imprinted polymer prepared with a coupling reaction between D-Phe and AA showed reverse adsorption selectivity regardless of the pH of the solution.

ACKNOWLEDGMENT

The authors are indebted to the Center for Advanced Bioseparation Technology for supporting this research.

REFERENCES

Chen, Y., Kele, M., Quinones, I., Sellergren, B. and Guiochon, G., "Influence of the pH on the Behavior of an Imprinted Polymeric Stationary Phase-Supporting Evidence for a Binding Site Model," *J. Chromatg. A*, **927**, 1 (2001).

Hong, J. M., Andersson, P. E., Qian, J. and Martin, C. R., "Selectively-Permeable Ultrathin Film Composite Membranes Based on Molecularly-Imprinted Polymers," *Chem. Mater.*, **10**(4), 1029 (1998).

Jin, J. S., "Preparation of HPLC Chiral Stationary Phases and Resolution of Enantiomers," PhD Thesis, Pusan National University, Korea (1999).

Kapoor, A. and Viraraghavan, T., "Heavy Metal Biosorption Sites in *Aspergillus niger*," *Bioresource Technol*., **61**, 221 (1997).

Kim, K. S. and Jung, I. H., "The Permeability Characteristics of Non-Porous Membrane by $C_7H_5F_3/SiH_4$ Plasma Polymeric Membrane," *Korean J. Chem. Eng*., **17**, 149 (2000).

Kim, K. S., Jeon, B. J. and Jung, I. H., "Preparation of CHF3 Plasma Polymeric Composite Membrane and Characteristics of Surface Modification," *Korean J. Chem. Eng*., **17**, 33 (2000).

Kobayashi, T., Wang, H. Y. and Fujii, N., "Molecular Imprinting of Theophylline in Acrylonitrile-Acrylic Acid Copolymer Membrane," *Chem. Lett*., **10**, 927 (1995).

Mathew-Krotz, J. and Shea, K. J., "Imprinted Polymer Membranes for the Selective Transport of Targeted Neutral Molecules," *J. Am. Chem. Soc*., **118**, 8154 (1996).

Mayes, A. G. and Mosbach, K., "Molecularly Imprinted Polymer Beads: Suspension Polymerization using a Liquid Perfluorocarbon as the Dispersing Phase," *Anal. Chem.*, **68**(21), 3769 (1996).

Ohkubo, K., Urata, Y., Hirota, S., Honda, Y. and Sagawa, T., "Homogenous and Heterogenous Esterolytic Catalyses of Imidazole-contain-

ing Polymers Prepared by Molecular Imprinting of a Transition-state Analogue," *J. Mol. Catal*., **87**, L21 (1994).

Park, J. K. and Seo, J. I., "Characteristics of Phenylalanine Imprinted Membrane Prepared by the Wet Phase Inversion Method," *Korean J. Chem. Eng.*, **19**, 940 (2002).

Puranik, P. R. and Paknikar, K. M., "Biosorption of Lead, Cadmium, and Zinc by *Citrobactor* Strain MCM B-181: Characterization Studies," *Biotechnol. Prog*., **15**, 228 (1999).

Ramstrom, O., Ye, L. and Gustavsson, P.-E., "Chiral Recognition by Molecularly Imprinted Polymers in Aqueous Media," *Chromatographia*, **48**, 197 (1998).

Robinson, D. K. and Mosbach, K., "Imprinting of a Transition State Analogue Leads to a Polymer Exhibiting Esterolytic Activity," *J. Chem. Soc., Chem. Commun*., **14**, 969 (1989).

Sohn, W. I., Koo, J. K. and Oh, S. J., "Permeation of Single and Mixed Gases through Composite Membranes Prepared by Plasma Polymerization of Fluorocarbon Compounds," *Korean J. Chem. Eng*., **17**, 678 (2000).

Wang, H. Y., Kobayashi, T., Fukaya, T. and Fujii, N., "Molecular Imprint Membranes Prepared by the Phase Inversion Technique. 2. Influence of Coagulation Temperature in the Phase Inversion Process on the Encoding in Polymeric Membranes," *Langmuir*, **13**, 5396 (1997).

Whitcombe, M. J., Rodriquez, M. E., Villar, P. and Vulfson, E. N., "A New Method for the Introduction of Recognition Site Functionality into Polymers Prepared by Molecular Imprinting: Synthesis and Characterization of Polymeric Receptors for Cholesterol," *J. Am. Chem. Soc*., **117**, 7105 (1995).

Yoshikawa, M., Fujisawa, T. and Izumi, J., "Molecularly Imprinted Polymeric Membranes Having EFF Derivatives as a Chiral Recognition Site," *Macromol. Chem. Phys*., **200**, 1458 (1999).

Yoshikawa, M., Fujisawa, T., Izumi, J., Kitao, T. and Sakamoto, S., "Molecularly Imprinted Polymeric Membranes Involving Tetrapeptide EQKL Derivatives as Chiral-recognition Sites Toward Amino Acids," *Anal. Chim. Acta*, **365**, 59 (1998).