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SHORT COMMUNICATION

Separation of Phenylalanine by Ultrafiltration Using D-Phe Imprinted Polyacrylonitrile-Poly(acrylic acid)-Poly(acryl amide) Terpolymer Membrane

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Abstract−A D-phenylalanine (D-Phe) imprinted terpolymer P(AN-AA-AAm) membrane was prepared by the wet phase inversion method. Acrylamide (AAm) and acrylic acid (AA) were used as the functional monomer and acrylonitrile (AN) was used as a physical cross linker. The template molecules were removed from the terpolymer matrix by washing with a 5 percent acetic acid solution. The removal of template molecules from the membrane matrix increased the population of free COOH groups and reduced that of dimerized COOH groups in the membrane matrix, which is an indirect evidence of the formation of recognition sites. The adsorption selectivity of the D-Phe imprinted terpolymer membrane prepared by in-situ implanting method was 0.37 at pH 2 after 3 h batch adsorption using 100 mg Phe/*l* racemate solution and reached nearly 1 after 24 h. In the ultrafiltration process, the permselectivity was 0.38 at pH 2 after 2.5 min. Separation of D-Phe from the racemate solution was demonstrated by a repeated ultrafiltration batch work.

Key words: Molecularly Imprinted Polymer, Phenylalanine, Membrane, Wet Phase Inversion Method, Ultrafiltration

INTRODUCTION

Molecularly imprinted polymer (MIP) has been widely used as solid separation media [Whitcombe et al., 1995; Mayes et al., 1996] in liquid chromatographies, affinity based solid-phase extraction for separations of chiral compounds, and amino acid derivatives [Robinson and Mosbach, 1989; Ohkubo et al., 1994]. 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 selectively bind the template 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 polymer membrane has 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 can be prepared by a wet phase inversion method [Wang et al., 1996, 1997]. For the wet phase inversion method, "*in-situ* implanting" and "post-implanting" were suggested in our previous study [Park et al., 2003]. "*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.

A selective adsorption of a molecularly imprinted polymer has been found at low concentration rather than at high concentration [Chen et al., 2001; Park et al., 2003]. It is theoretically supported by the model of heterogeneous binding sites on the imprinted polymer surface, in which one is selective or high affinity and the other is nonselective or low affinity [Wang et al., 2003; Chen et al., 2001]. In our previous study [Park and Seo, 2002; Park et al., 2003], Phe imprinted copolymer, P(AN-co-AA), prepared by a wet phase inversion method showed different adsorption selectivities in a batch adsorption system depending on the implanting process. However, the selective adsorption by the Phe imprinted P(AN-co-AA) was found only at low concentration of 10 mg Phe/*l* racemate solution. At high concentrations of Phe, the Phe imprinted polymer could not preferentially adsorb any enantiomer from the Phe racemate solution. In this study, we added acryl amide to the functional monomer, acrylic acid, to supply an additional amino group in the recognition sites hoping that the membrane shows good adsorption selectivity at high concentration of racemate solution. We prepared D-Phe imprinted terpolymer, P(AN-AA-AAm), membrane using an *in-situ* implanting process. We investigated the formation of recognition sites in the polymer matrix using FT-IR spectra. Then, we tried to determine the separation characteristics of D-Phe imprinted P(AN-AA-AAm) membrane in the ultrafiltration process. We also investigated the effective recovery of D-Phe adsorbed on the membrane matrix during ultrafiltration process.

EXPERIMENTAL

1. Materials

All reagents used in the experiment were of extra pure reagent grade. Acrylronitrile (AN) as cross linker was obtained from Yakuri (Japan), acrylic acid (AA) and acryl amide (AAm) as functional monomers were from Junsei (Japan), dimethyl sulfoxide (DMSO) as porogen from Kanto (Japan), D-phenylalanine (D-Phe) as a template molecule of Sigma, L-phenylalanine (L-Phe) as an enantiomer from Sigma. All reagents were used without further purification.

2. Membrane Preparation by a Phase Inversion

A molecularly imprinted membrane was prepared by the wet phase inversion method. An in-situ implanting MIP membrane was easily prepared by mixing 0.5 g of D-Phe solved in a mixture of 7.51 g

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of AA, 7.4 g of AAm and 50 g of DMSO, and adding 30.4 g of AN, and then polymerizing with an addition of 0.22 g of AIBN solved in 50.5 g of DMSO for 6 h at 60 °C under nitrogen atmosphere. The polymer solution was cast on a glass plate with a thickness of 120 m and then immersed in distilled water at 25 °C to coagulate the polymer film. The template molecule, D-Phe, was removed from the gel matrix by washing it for 2 h with 5% acetic acid at 25 °C in a shaking incubator stirring at 150 rpm and rinsed with distilled water. The MIP membrane was kept in distilled water until its next use.

The formation of recognition sites in the membrane matrix was indirectly determined by comparing the peak intensities of the FT-IR spectra of the MIP membrane. 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 of a peak. The peak intensity of a certain group was the absorbance divided by the absorbance of the cyanide group from the acrylonitrile segments in the terpolymer matrix, as presented in the literature [Wang et al., 1997].

3. Ultrafiltration and Batch Adsorption

The separating ability of the Phe imprinted membrane was determined by passing 50 ml of 50 mg Phe/*l* solution through the membrane at 1 bar gauge pressure in an ultrafiltration kit (Amicon Inc., USA). The substrate concentrations were analyzed by HPLC (Younggin M910, Korea) with a TSKgel Enantio L1 column. The eluent rate was 1 ml/min and the absorbance of the substrate solution was monitored at 254 nm with a UV detector. The membrane was dried at –50 °C in a freeze dryer until the weight of the membrane reached constant value. The specific substrate uptake by the D-Phe imprinted membrane based on the dry weight of the polymer was obtained by dividing the amount of adsorbed Phe with the dry weight of the terpolymer membrane. The permselectivity of the membrane was defined as $\alpha=S_D/S_L$ as used by Wang et al. [1997], where S_D and S_L are the amounts of template and isomer molecules adsorbed by the membrane during ultrafiltration. The membrane, which had been used for ultrafiltration, was eluted with 50 ml of a 5% acetic acid solution at 1 bar gauge pressure using the same ultrafiltration kit in order to remove the adsorbed Phe molecules from the membrane matrix and the amount of substrate in the permeate was measured.

The uptake capacity of the Phe imprinted terpolymer was also measured by carrying out the batch adsorption in a Phe racemate solution. The substrate molecules were uptaken by 4.2 g dry wt of the membrane in a 300 ml of a 100 mg Phe/*l* solution for 24 h at 25 °C with shaking at 130 rpm. The adsorption selectivity of the terpolymer membrane was defined as $\alpha=(S_D/S_L)/(C_D/C_L)$, where S_D and S_L are the amounts of template and isomer molecules adsorbed by the membrane, and C_D and C_L denote the concentrations of template and isomer molecules in the remaining solution after adsorption process, respectively.

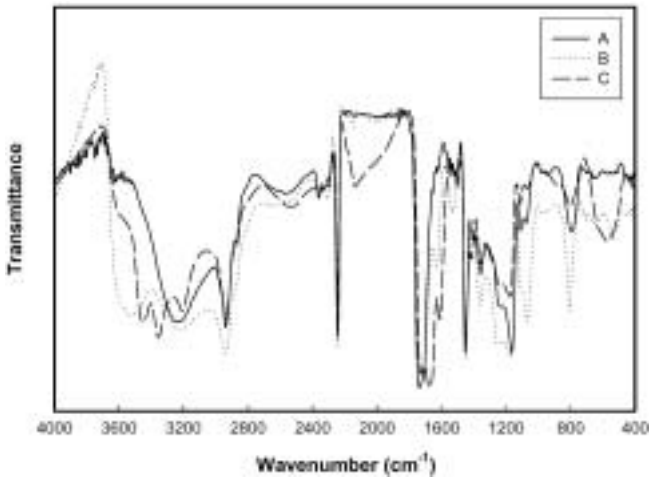

Fig. 1. FT-IR spectra of the P(AN-co-AA) (A), D-Phe imprinted P(AN-co-AA) (B), and D-Phe imprinted P(AN-AA-AAm) (C) membranes prepared by the *in-situ* implanting process.

RESULTS AND DISCUSSION

1. Formation of Recognition Sites

The formation of recognition sites in P(AN-co-AA) membrane matrix was indirectly determined by measuring the peak intensity of the free carboxyl group in the FT-IR spectra in the literatures [Wang et al., 1996; Park and Seo, 2002]. The free carboxyl group that remained in the polymer matrix after the template molecules were removed from the polymer matrix by washing with an acetic acid solution.

The FT-IR spectra of a Phe imprinted P(AN-AA-AAm) is much different from that of a Phe imprinted P(AN-co-AA), as shown in Fig. 1. A peak of a free carboxyl group is also found at the wave number of 3,522 cm^{-1} in the FT-IR spectra of a Phe imprinted ter-

Table 1. Peak intensity ratio of FT-IR spectra of the D-Phe imprinted membrane. NT: P(AN-co-AA) without imprinting, DI: D-Phe imprinted P(AN-co-AA) prepared by in-situ implanting procedure, NT1: P(AN-AA-AAm) without imprinting, DIM1: D-Phe imprinted P(AN-AA-AAm) prepared by in-situ implanting procedure and the ratio of AAm to AA is 1

Membrane	Peak intensity ratio						
	OH stretching: free COOH 3,522 cm^{-1}, 3,500 cm^{-1}	OH stretching: free COOH 3,450 cm^{-1}	OH stretching: dimerized COOH 3,206 cm^{-1}, 3,217 cm^{-1}	OH stretching: dimerized COOH 2,604 cm^{-1}, 2,534 cm^{-1}	C=O stretching: 1,727 cm^{-1}	C=O stretching: (amide I) 1,675 cm^{-1}	NH stretching: (amide II) 1,611 cm^{-1}, 1,626 cm^{-1}
NT	0.11		0.87	0.14	1.67		
DI	0.75		0.85	0.21	1.30		0.45
NT1		0.85	0.83	0.31	1.86	2.43	0.88
DIM1	0.67	1.21	1.08	0.35	2.01	2.55	1.17

polymer and another distinct peak of a free carboxyl group is also found at 3,450 cm^{-1}. Recognition sites seem to be well formed in the terpolymer matrix. The peak intensity of the stretching of C=O from COOH found at 1,728 cm^{-1} is much greater than that of P(AN-co-AA) or Phe imprinted P(AN-co-AA), as shown in Table 1 considering the molecular structure of the template [Park and Seo, 2002]; thus the ratio of the AA segment to the AN segment of the terpolymer is much larger than that of the copolymer. A large fraction of the functional monomer, AA, segment in the terpolymer chain may increase the population of recognition sites in the polymer matrix.

2. Batch Adsorption

It is well known that a molecularly imprinted polymer can selectively adsorb at low concentration rather than at high concentration [Chen et al., 2001]. The adsorption selectivity of an amino acid derivative imprinted polymer bead prepared by surface imprinting technique and used in a batch adsorption system was reported to be very low as 1.2 for N-benzyloxycarbonyl-glutamic acid (Z-Glu), 0.94 for Z-Asp, and 0.97 for Z-gln even though the racemate concentration is less than 50 mg/L [Araki et al., 2002]. In order to investigate the effect of the addition of AAm on the adsorption selectivity of the D-Phe imprinted terpolymer membrane, a batch adsorption experiment was performed.

The DIM1 (terpolymer, AAm/AA ratio is 1) membrane was inserted in 100 mg Phe/*l* racemate solution and the amount of Phe adsorbed was measured according to the process time. It showed a very interesting time profile of Phe adsorption in the Phe racemate solution, as shown in Fig. 2. At the beginning of adsorption, the adsorption rate of L-Phe by D-Phe imprinted terpolymer was much faster than that of D-Phe, and the amount of adsorbed L-Phe during 3 h of adsorption was more than 2.5 times than that of adsorbed D-Phe. The adsorption of L-Phe ceased at 3 h, but the amount of adsorbed D-Phe increased continuously. The adsorption selectivity of DIM1 membrane varied from 0.37 to 0.91, with adsorption time from 3 h to 24 h. Thus, in the P(AN-AA-AAm) membrane, the amide group containing aromatic rings from D-Phe reverses the adsorption affinity and the adsorption rate of L-Phe becomes faster.

The outstanding reversed adsorption selectivity of the D-Phe imprinted terpolymer at the beginning of batch adsorption can be partly explained by the coupling reaction during terpolymerization, as suggested in our previous study [Park and Seo, 2002]. An amide peak was found at 1,611 cm^{-1} in the FT-IR spectra of the D-Phe imprinted P(AN-AA-AAm) membrane, as shown in Fig. 1. A certain fraction of the amide group found at 1,611 cm^{-1} was determined to come from the coupling reaction between D-Phe and AA during the *in-situ* implanting process by the comparison of two FT-IR spectra in which the peak intensity ratio of the amide group in the D-Phe imprinted P(AN-AA-AAm) was higher than that of P(AN-AA-AAm) without imprinting, as shown in Table 1. The reversed adsorption selectivity seems to be caused by the phenyl group from D-Phe which was combined with the polymer matrix by the coupling reaction during polymerization 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].

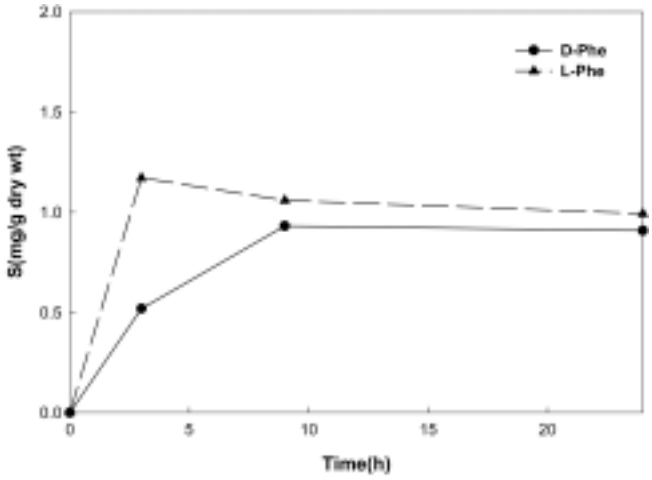

Fig. 2. The adsorption profile of DIM1 (D-Phe imprinted P(AN-AA-AAm), AAm/AA ratio is 1) membrane prepared by *in-situ* implanting procedure. 3.34 g of the membrane was immersed in 300 ml of the Phe racemate solution of 100 mg/*l*.

3. Ultrafiltration

The operation time of the ultrafiltration process is generally shorter than that of batch adsorption because the penetration speed of a racemate solution in the membrane matrix will increase by a driving force like an external pressure. The flux (permeation velocity) of a racemate solution through the D-Phe imprinted P(AN-co-AA) membrane without macropore prepared by the *in-situ* implanting process is 0.9×10^{-5} m/s·bar, which is much smaller than 6.4×10^{-5} m/

Table 2. The flux and permselectivity (α) of D-Phe imprinted membrane, DIM1, DIM2, and DIM3 prepared by *in-situ* implanting process. 50 ml of 50 mg Phe/*l* racemate solution was permeated through the membrane at 1 atm gauge pressure. DIM1, DIM2, DIM3: the ratio of AAm to AA based on mass is 1, 0.5, 0.25, respectively

pH	DIM1					DIM2					DIM3				
	UF time (min)	Flux ($m/sec\times10^{-5}$)	mg uptaken Phe/g memb.		α	UF time (min)	Flux ($m/sec\times10^{-5}$)	mg uptaken Phe/g memb.		α	UF time (min)	Flux ($m/sec\times10^{-5}$)	mg uptaken Phe/g memb.		α
			D-Phe	L-Phe				D-Phe	L-Phe				D-Phe	L-Phe	
2	1	5.3	0.18	0.31	0.57	2	1.1	0.17	0.25	0.70	2	1.5	0.41	0.50	0.82
	2.5	4.6	0.15	0.40	0.38	5	1.1	0.33	0.42	0.79					
4	1	5.3	0.25	0.42	0.60	2	1.7	0.24	0.31	0.77	2	0.63	0.09	0.18	0.52
	2.5	5.6	0.26	0.54	0.49	5	1.8	0.36	0.53	0.69	5	0.63	0.11	0.22	0.49
6	1	4.4	0.25	0.50	0.50	2	1.6	0.09	0.21	0.44	2	1.0	0.11	0.17	0.61
	2.5	4.3	0.38	0.94	0.41	5	1.7	0.15	0.44	0.34	5	1.0	0.13	0.28	0.47

s·bar of THO imprinted P(AN-co-AA) membrane previously reported by other researchers [Wang et al., 1996], because the thickness of the D-Phe imprinted membranes without macropore is 50 μm and the skin layer of the THO imprinted membrane is 20 μm. As shown in Table 2, the permeation velocity through the D-Phe imprinted membrane was increased according to the amount of AAm added to the reactant monomer mixture at the beginning of polymerization. Permeation velocity through DIM1 (terpolymer, AAm/AA ratio is 1) is 4.3×10^{-5} m/s·bar, and that through DIM3 (terpolymer, AAm/AA ratio is 0.25) is 1.0×10^{-5} m/s·bar, which is somewhat greater than 0.9×10^{-5} m/s·bar of the D-Phe imprinted P(AN-co-AA) membrane. The increase in the permeation velocity accompanying the addition of AAm seems to be due to the decrease in the AN fraction per dry weight of the membrane because the AN segment causes coagulation of the terpolymer by the dipole interaction of the CN group in water.

The permselectivity of DIM1 during 2.5 min of ultrafiltration was 0.38 at pH 2 of the racemate solution, as shown in Table 2. Saturation of the Phe imprinted membrane in a batch adsorption was obtained after 10 h of operation and the saturated adsorption selectivity was nearly 1 at the 100 mg Phe/L racemate solution in the previous study [Park et al., 2003]. Phe uptake by DIM1 increased with ultrafiltration time, and permselectivity decreased and reached 0.41 at 2.5 min of ultrafiltration at pH 6 of racemate solution. Phe uptake by DIM2 (terpolymer, AAm/AA ratio is 0.5) increased monotonically during 5 min of ultrafiltration at each pH of racemate solution, 2, 4, and 6. As shown in Table 2, the D-Phe imprinted terpolymer membrane showed reversed permselectivity regardless of the pH of the Phe racemate solution. Thus, we can speculate that a high Phe uptake capacity and outstanding permselectivity can be achieved in a short time of ultrafiltration of the time magnitude of min because nearly all of selective adsorption sites in a polymer matrix take part in adsorption in the ultrafiltration process.

4. Repeated Ultrafiltration

A repeated ultrafiltration batch job using a D-Phe imprinted P(AN-AA-AAm) membrane, DIM1 and DIM2 was applied to purify D-Phe from a Phe racemate solution. Each ultrafiltration batch was processed for 10 min at 1 bar, as shown in Fig. 3. The permeate collected after 10 min of the first ultrafiltration using a DIM2 membrane shows 4.2 percent difference in the composition of D and L-Phe, as shown in Table 3. The fractional difference between D and L-Phe in the permeate increases with the repeated batch number of ultrafiltration and the Phe concentration in the permeate decreases with batch number. After the fifth batch of ultrafiltration, the amount of D-Phe in permeate was 2.3 times larger than L-Phe. However, the loss of D-Phe during five batches of ultrafiltration was about 90 percent of the original racemate solution when all membranes used in ultrafiltration were discarded.

Thus, the used membrane was washed with 5 percent acetic acid solution in the ultrafiltration cartridge at a pressure of 1 bar. At the first batch of washing, all of the adsorbed D-Phe was removed, although only 32 percent of adsorbed L-Phe was deleted from the membrane matrix. However, the fraction of D-Phe removed in the washing step after ultrafiltration decreased with repeated ultrafiltration, as shown in Table 3. The adsorption and desorption profile of D and L-Phe during ultrafiltration and washing steps using DIM1 membrane shows a similar pattern to that using a DIM2 membrane. However, the fraction of D-Phe removed during washing after ultra-

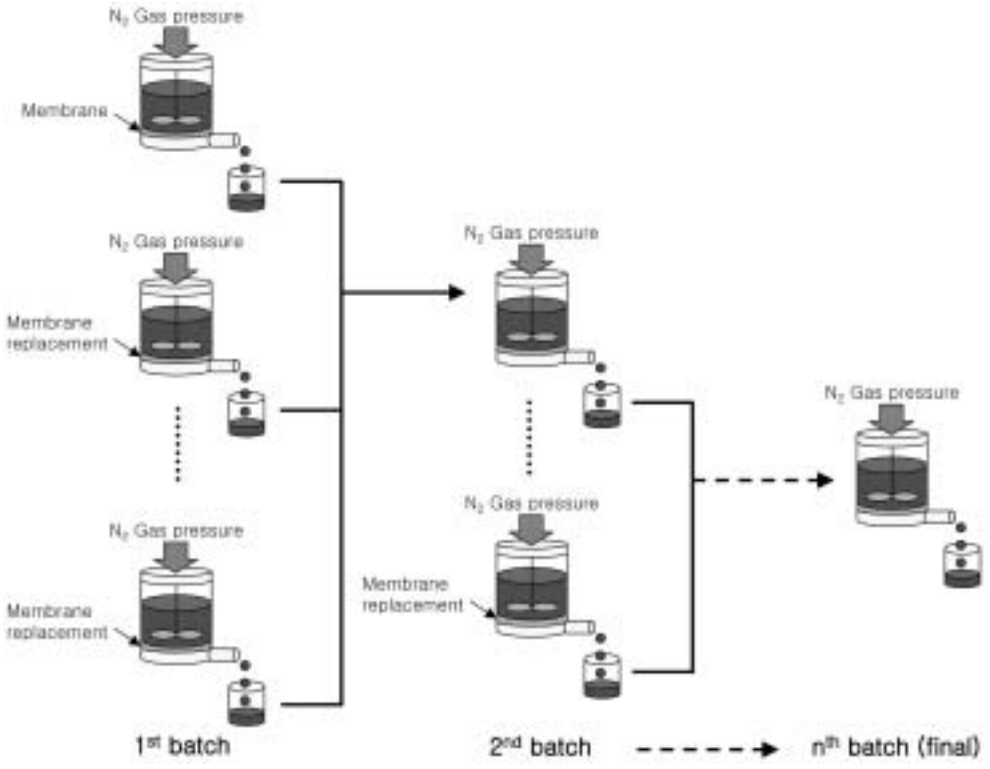

Fig. 3. Schematic illustration of the consecutive UF batch process. Each ultrafiltration batch is operated for 10 min at 1 bar. The permeate collected at 1st batches is used as the feed stream of the 2nd batches.

Table 3. Batch profiles of the amounts of adsorbed (desorbed) D,L-Phe, volume flux measured by a repeated UF process using the D-Phe imprinted membrane, DIM1 and DIM2. Five pieces of membranes were packed at each batch operation. Initial D,L-Phe conc.: each 25 mg Phe/*l*, pH 6, working volume: 50 ml

Batch	DIM1							DIM2						
	Phe in permeate (mg/*l*)		Perm. vol. (ml)	Phe uptaken by membrane (mg)		Phe desorbed by washing (mg)		Phe in permeate (mg/*l*)		Perm. vol. (ml)	Phe uptaken by membrane (mg)		Phe desorbed by washing (mg)	
	D	L		D	L	D	L	D	L		D	L	D	L
1	16.4	14.2	11.9	0.103	0.128	0.0948	0.040	15.9	14.6	10.2	0.093	0.105	0.092	0.034
2	11.0	8.6	6.9	0.036	0.039	0.034	0.018	10.5	9.0	6.0	0.032	0.033	0.030	0.018
3	7.9	5.2	3.2	0.010	0.011	0.009	0.001	5.9	4.5	3.0	0.014	0.014	0.0019	0.0016
4	4.8	2.6	1.7	0.005	0.005	0.004	0.001	3.2	1.8	1.9	0.005	0.005	0.0007	0.0007
5	2.9	1.2	0.8	0.001	0.001	0.001	-	2.3	1.0	0.9	0.001	0.001	0.0004	-

filtration was much higher than that of L-Phe even at the fifth batch of ultrafiltration. This may be caused by the unknown role of AAm in the polymer matrix and it requires future research.

CONCLUSION

Using two functional monomers of AAm and AA seems to endow the terpolymer P(AN-AA-AAm) matrix with many recognition sites and amide groups created by coupling reaction during polymerization. The D-Phe imprinted terpolymer shows a faster adsorption velocity of an isomer, L-Phe, than the template molecule. The adsorption selectivity to L-Phe is remarkable at the beginning of batch adsorption although it decreases with adsorption time. Thus, D-Phe imprinted P(AN-AA-AAm) may be used to separate Phe racemate solution with the favorable adsorption selectivity at the beginning of adsorption in a batch system by controlling the adsorption time. The operation time can be shortened to a time magnitude of minutes, reserving high affinity to L-Phe and high specific uptake capacity by applying ultrafiltration with DIM membrane. The fraction of D-Phe in the permeate increases with the number of repeated batches. However, we suggest the recovery of D-Phe from the DIM membrane by washing after ultrafiltration to improve the recovery for future studies.

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