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SHORT COMMUNICATION

Characteristics of Selective Adsorption Using D-Phenylalanine Imprinted Terpolymer Beads

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Abstract−A D-Phenylalanine (Phe) imprinted terpolymer, Polyacrylonitrile-Poly(acrylic acid)-Poly(acryl amide) (Poly(AN-AA-AAm)) bead 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 characteristics of selective adsorption by the D-Phe imprinted terpolymer beads were investigated at high concentrations of Phe racemate solution, 1 g Phe/L, and 10 g Phe/L. The adsorption selectivity of the D-Phe imprinted terpolymer beads prepared by an *in-situ* implanting method reached 0.82 and 0.8 at 1.0 g and 10 g Phe/L racemate solution, respectively, and almost all of the adsorbed D-Phe and about 43% of the adsorbed L-Phe were desorbed by 4% acetic acid. The uptake capacities of the terpolymer beads were maintained for several repeated batches.

Key words: Molecularly Imprinted Polymer, Phenylalanine, Bead, Wet-phase Inversion Method, Repeated Batch

INTRODUCTION

A molecularly imprinted polymer (MIP) has recognition sites which are complementary in shape to the template molecule and which also contain ligands those can bind template molecules. This makes MIP bind template molecules from the racemate solution and be able to separate target molecules from substrates of similar structures [Wulff and Sarhan, 1972; Takagishi and Klotz, 1972; Wulff, 1993]. MIP has been widely used as solid separation medium in liquid chromatography and affinity based solid extraction [Ramstrom et al., 1998; Klein et al., 1997].

D-phenylalanine imprinted copolymer acrylonitrile with acrylic acid (poly(AN-co-AA)) prepared by a wet-phase inversion method showed different adsorption selectivities depending on the implanting method [Park and Seo, 2002]. D-Phe imprinted poly(AN-co-AA) beads prepared by *in-situ* implanting method showed reversed adsorption selectivity (less than 1) due to the aromatic ring from D-Phe in the backbone of the copolymer, which was formed by a coupling reaction between D-Phe and AA during copolymerization. However, the D-Phe imprinted copolymer prepared by a post implanting method adsorbed a much greater amount of D-Phe than the other enantiomer, L-Phe. The selective adsorption selectivities could be found only at low concentrations of Phe in the racemate solution because of the limit of recognition sites in the MIP [Park et al., 2004]. Dissolving a large number of template molecules in the porogen, DMSO, before copolymerization in order to endow MIP with many more recognition sites is difficult due to the limit of the solubility of D-Phe in an organic solvent.

In our previous study [Park and Kim, 2004], we added acrylamide (AAm) to the functional monomer, acrylic acid (AA), in order to supply an additional amino group in the recognition sites hoping that the MIP would show good adsorption selectivities at high concentrations of the racemate solution. AAm endows the polymer matrix with many more ligands able to form hydrogen bonds and ionic bonds with the template molecule than the well-known functional monomer, methacrylic acid [Zhou et al., 1999; Yu and Mosbach, 1997]. A D-Phe imprinted terpolymer, P(AN-AA-AAm), membrane was prepared because an ultrafiltration process has some advantages over MIP particles, such as faster transport of substrate molecule and faster equilibrium of binding sites. The concentration of D-Phe in the permeate was much higher than the other enantiomer, L-Phe, although the permselectivity was 0.38 at pH 2 after 2.5 min of ultrafiltration using 50 mg Phe/L racemate solution. However, the permselectivity of the terpolymer membrane reached 1 after 2.5 minutes of ultrafiltration using 1 g Phe/L racemate solution [Park and Kim, 2004]. Thus, in the current study, we prepared D-Phe imprinted terpolymer beads and investigated the characteristics of selective adsorption using these beads at high concentrations, 1 and 10 g Phe/L, of a racemate solution.

EXPERIMENTS

1. Materials

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

2. Terpolymer Bead Preparation by a Wet Phase Inversion

The Phe-imprinted polymer was prepared by the alternative wet-phase inversion method using an *in-situ* implanting method [Park and Seo, 2002]. The preparation of *in-situ* implanted beads was initiated by stirring the mixture of 7.51 g AA, 7.4 g AAm and 54 g DMSO containing 0.5 g D-Phe for 3 h in order to form a complex of D-Phe with AA and AAm. This solution was mixed with 34 g AN and 54 g DMSO and terpolymerization was initiated at 60 °C with the addition of 0.22 g AIBN and carried out for 6 h in a nitrogen atmosphere. The terpolymer solution dissolved in 216 g of DMSO

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was then dropped in swirling distilled water. The terpolymer beads were washed with 5% acetic acid (V/V) for 3 h and distilled water for 1 h in order to remove the template molecules from the bead matrix. The formation of recognition sites was indirectly determined by measuring the peak intensity ratio by using the FT-IR spectra. The FT-IR spectra of the D-Phe imprinted terpolymer 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 [Wang et al., 1997; Park et al., 2004].

3. Batch Adsorption and Desorption

The D-Phe imprinted terpolymer beads were immersed for 2 h in distilled water whose initial pH was adjusted to 2 by HCl or NaOH as previously described [Park et al., 2003]. The batch of distilled water was renewed until the pH of the solution did not change. 22 g of terpolymer beads were maintained in 1 L of 1% (w/w) Phe racemate solution at 25 °C with shaking at 150 rpm in order to selectively uptake the template molecules. The Phe adsorbed on the terpolymer matrix was desorbed by shaking the beads for 3 h at 150 rpm in 1 L of 4% acetic acid solution followed by shaking 1 h at 150 rpm in distilled water. The concentrations of D-Phe and L-Phe were analyzed by HPLC (Youngin M910, Korea) with TSKgel Enantio L1 column (4.6 cm×250 cm). The effluent rate was 1 ml/min and the absorbance of the substrate was measured by a UV detector at 254 nm. The adsorption selectivity, α of the D-Phe imprinted terpolymer was defined as ((D-Phe)/(L-Phe))/([D-Phe]/[L-Phe]), where (D-Phe) and (L-Phe) are the amount of Phe adsorbed in the polymer, and [D-Phe] and [L-Phe] denote the concentrations in the solution after adsorption [Yoshikawa et al., 1998].

RESULTS

1. Formation of Recognition Sites

The FT-IR diagram of the D-Phe imprinted terpolymer bead shown in Fig. 1 is similar to that of D-Phe imprinted terpolymer membrane described in our previous report [Park and Kim, 2004]. The formation of recognition sites in terpolymer bead matrix was indirectly determined by measuring the peak intensity of the free carboxyl group at 3,450 cm^{-} in the FT-IR spectra [Wang et al., 1997; Park and Kim, 2004]. Recognition sites seemed to be well-formed in the terpolymer bead matrix. The peak intensity of free carboxyl

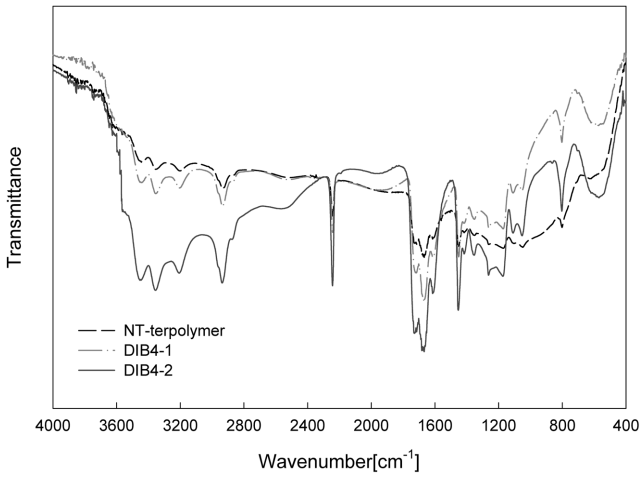

Fig. 1. FT-IR spectra of a D-Phe imprinted P(AN-AA-AAm) bead prepared *in-situ* implanting process. NT-terpolymer; P(AN-AA-AAm) prepared without template, DIB4-1; P(AN-AA-AAm) bead before template D-Phe was removed, and DIB4-2; P(AN-AA-AAm) bead after template was removed by washing the terpolymer bead with 5% AcOH and water.

group increased after the template molecules were removed from the polymer matrix by washing with an acetic acid solution, as shown in Table 1. However, the FT-IR diagram of the terpolymer bead prepared by using 324 g DMSO, 1.5 times more than the proper amount (216 g), did not show a remarkable increase in the peak intensity of free carboxyl group (data not shown). The addition of a large amount of DMSO in the dissolving step before dropping the terpolymer solution seemed to hinder the formation of recognition sites during solidification of the terpolymer solution as mentioned in the preparation of copolymer beads [Park et al., 2003].

There was a strong peak of the amide group at 1,668 cm^{-}. The peak intensity of the amide group in the D-Phe imprinted terpolymer bead was higher than that of terpolymer bead without imprinting, as shown in Table 1, because this amide group was attributed to the coupling reaction between D-Phe amino group and the functional monomer carboxyl group occurring during the *in-situ* implanting process [Park and Seo, 2002]. This indicates that the phenyl group from D-Phe, which was combined with the polymer matrix by the coupling reaction during polymerization and placed in the recognition site, hinders the approach of template molecules in a racemate solution like the chiral stationary phase (CSP) of chroma-

Table 1. Peak intensity ratio of FT-IR spectra of D-Phe imprinted P(AN-AA-AAm) terpolymer bead prepared by the *in-situ* implanting procedure. NT-terpolymer; P(AN-AA-AAm) prepared without template, DIB4-1; P(AN-AA-AAm) bead before template D-Phe was removed, and DIB4-2; P(AN-AA-AAm) bead after template was removed by washing the terpolymer bead with 5% AcOH and water

Polymer	Peak intensity ratio									
	Free COOH 3,453 cm^{-} 3,445 cm^{-}	NH stretching 3,355 cm^{-} 3,354 cm^{-}	Dimerized COOH 3,207 cm^{-} 3,203 cm^{-}	Dimerized COOH 2,563 cm^{-} 2,516 cm^{-}	C=O stretching 1,728 cm^{-} 1,717 cm^{-}	C=O Amide 1,668 cm^{-}	NH Amide 1,615 cm^{-}	CH_2 bonding 1,455 cm^{-} 1,451 cm^{-}	C-O stretching 1,174 cm^{-} 1,171 cm^{-}	CH_2 rocking 803 cm^{-} 802 cm^{-}
NT-terpoly	0.74	0.77	0.78	0.81	1.16	1.25	1.13	1.18	1.19	1.06
DIB4-1	0.76	0.81	0.82	0.75	1.18	1.48	1.15	1.14	0.96	0.61
DIB4-2	0.97	1.03	0.88	0.67	1.35	1.55	1.05	1.18	0.97	0.66

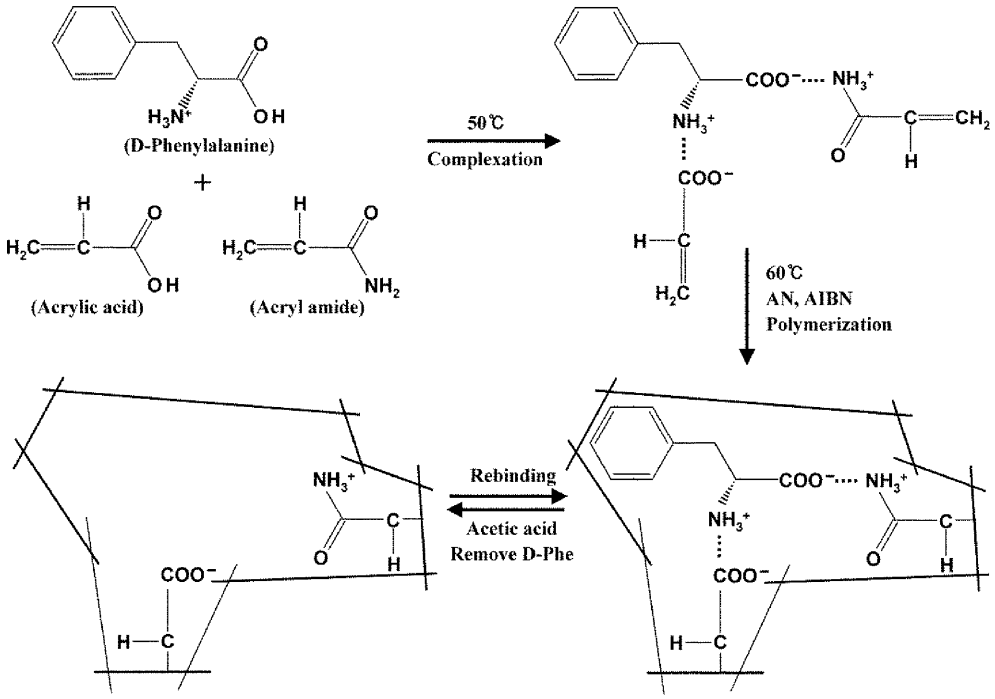

Fig. 2. Schematic illustration of D-phenylalanine imprinting in P(AN-AA-AAm) terpolymer by wet phase inversion method.

tography [Jin, 1999].

The pH of the mixture of AA, AAm, and D-Phe prepared to form the complex between template molecules and functional monomers was 6, whereas that of the terpolymer solution was approximately 6. According to the Henderson-Hasselbach equation, more than 99% of the carboxyl and amino groups of D-Phe remain as COO^- and NH_3^+ at pH 6. All of the amino group of AAm remained as NH_3^+ and 98% of the carboxyl group of AA remained as COO^- at pH 6. Thus, we can deduce that the predominant shape of the recognition site resulted from the ionic bondings between the template molecule and functional monomers, as shown in Fig. 2.

2. Adsorption Selectivity

D-Phe imprinted terpolymer beads selectively adsorbed D-Phe from the Phe racemate solution for 1 h at pH 6. However, these beads adsorbed more L-Phe than D-Phe from that time and the adsorption selectivity reached 0.81 at 3 h of adsorption, as shown in Table 2. This reverse selective adsorption can be explained with the phenyl group from D-Phe and the excess amount of functional monomer on the surface of the terpolymer matrix. The complex of D-Phe with functional monomers was formed by noncovalent interaction during the complexation step before terpolymerization. An excess of functional monomer was required to complete template-functional monomer complexation because noncovalent interaction was not so strong and the population of the template-functional monomers was determined by equilibrium [Park and Seo, 2002]. As a result, a large fraction of the functional monomer was grafted randomly to the polymer matrix as described in the literature [Mosbach and Haupt, 1998] because the mole ratio of functional monomers to template molecule was 71 in this study. The recognition sites were filled with template molecules at the beginning of adsorption because the adsorption on the selective binding site was stronger than that on non-selective binding sites [Park et al., 2004]. Then, both the D-Phe and L-Phe were adsorbed by the ligands of the randomly grafted functional monomers, although the approach of D-Phe was hindered by the phenyl group from D-Phe generated by the coupling reaction as observed in the previous studies [Park et al., 2003; Park and Kim, 2004]. At pH 2 and 4, more L-Phe was adsorbed from the beginning of the adsorption, as shown in Table 2. Thus, it is concluded that the ligands in the recognition site were not sufficient for ionic bonding with template molecules at a pH different from 6 and that they could not selectively bind template molecules.

3. Repeated Use

The Phe adsorbed on the terpolymer matrix was desorbed by 4% acetic acid solution. Nearly all of the adsorbed D-Phe was removed from the terpolymer bead matrix, but less than 45% of L-Phe adsorbed at pH 2 and 6 was desorbed, whereas 65% of L-Phe adsorbed at pH 4 was desorbed, as shown in Table 2. The adsorption capacities for D-Phe and L-Phe remained constant after repeated use, although all of adsorbed L-Phe was not deleted from the terpolymer bead. It was much different from the repeated use of D-Phe imprinted polymer prepared by the sol-gel transition method [Park et al., 2004]. The Phe adsorption capacity of sol-gel beads decreased with repeated use and reached a constant value after two repeated batches when all of the adsorbed D-Phe and L-Phe were desorbed by the acetic solution. The sol-gel beads were prepared through swelling and shrinking by the HCl and NaOH solution for long time, more than 3 days, at a high D-Phe concentration, 20 g D-Phe/L, in an aqueous solution. Thus, a huge number of recognition sites were effectively prepared, reducing the hindering effect of the phenyl group from the D-Phe formed by the coupling reaction. However, the recognition sites in terpolymer beads prepared with the limit of solubility of D-Phe in an organic solvent were formed in a short time during solidification. As a result, the D-Phe imprinted terpolymer showed reversed selectivity. In addition, the high L-Phe uptake capacity of

Table 2. Batch profiles of the amounts of adsorbed (desorbed) D, L-Phe on the D-Phe imprinted P(AN-AA-AAm) bead. Adsorption; initial concentration 1.0 g Phe/l, 25 °C with shaking at 150 rpm. Desorption; used beads were washed with 4% acetic acid solution. Adsorption (%); Phe uptaken by bead/ initial Phe in racemate solution. Desorption (%); Phe desorbed by washing/Phe uptaken by bead. All values are mean of triple experiments.

Batch trial	pH	Time [hr]	Adsorption		Selectivity (α)	Desorption	
			mg Phe/g bead (%)			mg Phe/g bead (%)	
			D-Phe	L-Phe		D-Phe	L-Phe
1st	2	3	2.26(12.3)	2.69(14.7)	0.82	1.96(86.7)	1.22(54.0)
	4	0.5	3.32(18.1)	3.92(21.4)	0.81	2.68(85.1)	2.41(65.8)
		1	3.10(16.9)	3.66(20.0)	0.81		
		2	3.03(16.6)	3.61(19.7)	0.81		
		3	3.15(17.2)	3.66(20.0)	0.83		
	6	0.5	2.20(12.0)	1.89(10.3)	1.19	3.20(94.4)	1.75(43.4)
		1	2.92(16.0)	2.92(16.0)	1.00		
		2	3.30(18.0)	3.99(21.8)	0.83		
		3	3.39(18.5)	4.03(22.0)	0.81		
2nd	2	0.5	2.38(13.0)	3.02(16.5)	0.76	3.04(99.3)	1.51(42.9)
		1	2.64(14.4)	3.06(16.7)	0.84		
		2	2.87(15.7)	3.35(18.3)	0.83		
		3	3.06(16.7)	3.52(19.2)	0.84		
	4	0.5	3.83(20.9)	4.25(23.2)	0.88	2.77(90.8)	2.38(65.6)
		1	2.85(15.6)	3.66(20.0)	0.74		
		2	3.05(16.7)	3.58(19.6)	0.81		
		3	3.05(16.7)	3.63(19.8)	0.81		
	6	0.5	2.22(12.1)	2.71(14.8)	0.80	3.19(90.9)	1.77(42.4)
		1	2.89(15.8)	3.42(18.7)	0.81		
		2	3.39(18.5)	3.95(21.6)	0.83		
		3	3.51(19.2)	4.17(22.8)	0.80		
3rd	2	0.5	2.76(15.1)	3.24(17.7)	0.83	2.74(94.5)	1.46(42.9)
		1	3.08(16.8)	3.50(19.1)	0.86		
		2	3.33(18.2)	3.85(21.0)	0.84		
		3	2.90(15.8)	3.40(18.6)	0.83		
	4	0.5	2.14(11.7)	2.84(15.5)	0.72	2.76(88.2)	2.40(66.5)
		1	2.36(12.9)	2.97(16.2)	0.76		
		2	2.55(13.9)	3.09(16.9)	0.80		
		3	3.13(17.1)	3.61(19.7)	0.84		
	6	0.5	2.42(13.2)	2.80(15.3)	0.85	3.30(86.6)	1.74(39.2)
		1	3.14(17.2)	3.51(19.2)	0.88		
		2	3.78(20.7)	4.32(23.6)	0.84		
		3	3.81(20.8)	4.44(24.3)	0.82		

D-Phe imprinted terpolymer bead was caused by another unclear mechanism than the simple adsorption on the randomly grafted ligands from functional monomers at high concentration of Phe. A great deal of L-Phe may accumulate by precipitation after the adsorption on the ligands from functional monomers, as found at high concentration in the adsorption/desorption process at an industrial amino acid recovery. This might be partially supported by our experimental observation that the high uptake capacity was retained at high racemate concentrations with the same adsorption selectivity because the adsorption selectivity usually approaches 1 as the concentration of the racemate solution increases and the affinity of conventional molecularly imprinted polymers decreases with the template concentration in a solution [Chen et al., 2001]. The uptake capacities of terpolymer beads were 8.3 g Phe/g bead with an adsorption selectivity of 0.82 at 1 g Phe/L racemate solution and 81.4 g Phe/g bead with an adsorption selectivity of 0.8 at 10 g Phe/L racemate solution.

Thus, the uptake of D-Phe imprinted terpolymers is much different from the common mechanism of adsorption by MIP polymers, which requires much more future research.

CONCLUSIONS

The D-Phe imprinted terpolymer bead contains an aromatic ring formed by the coupling reaction between the template molecule and functional monomers. These phenyl groups from D-Phe hinder the

approach of D-Phe in the racemate solution as observed in the previous studies. The specific uptake capacity of Phe was maintained at concentrations of the racemate solution, from 1 g Phe/L to 10 g Phe/L, with nearly the same adsorption selectivity of 0.8. The constant uptake capacity during repeated uses with different desorption yields, more than 95% of D-Phe and about 43% of L-Phe, might be applied to the separation of a racemate solution hoping that the remainder of L-Phe will be desorbed at a higher temperature or at a different acetic acid concentration after first desorption, although the mechanism of adsorption-desorption on terpolymer beads is still unclear at this stage.

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