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RAPID COMMUNICATION

Preparation of D-phenylalanine imprinted microbeads by the modified suspension polymerization using a magnetic impeller

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Abstract−Molecularly imprinted polymeric microbeads (MIPMs) were prepared by using a magnetic impeller by the modified suspension polymerization method with D-phenylalanine (Phe) as the water-soluble template without derivatization, methacrylic acid as the functional monomer, ethylene glycol dimethacrylate as the cross-linker, toluene as the porogen, polyvinyl alcohol as the stabilizer, and sodium dodecyl sulfate as the surfactant. The mean diameter of MIPMs decreased from 12.45 μm to 8.86 μm by using a simple magnetic impeller instead of a conventional simple straight-blade turbine impeller during suspension polymerization. The adsorption selectivity of MIPMs prepared with a magnetic impeller was much improved as compared to the adsorption selectivity of MIPMs prepared with a conventional simple straight-blade turbine impeller. The adsorption selectivity of MIPMs changed from reversed adsorption (less than 1) to positive (higher than 1) as the washing time increased after suspension polymerization.

Key words: Molecularly Imprinted Polymeric Microbead, Phenylalanine, Modified Suspension Polymerization, Enantioselective Separation, Molecular Recognition, Impeller

INTRODUCTION

Molecular imprinting is a technique for preparing synthetic polymers that contain predetermined selective binding sites for a desired template [1,2]. However, the molecularly imprinted polymers (MIP) prepared by the conventional bulk polymerization method produce irregularly shaped particles after crushing and sieving processes [3,4]. The particles are characterized by a large variation in size along with huge amounts of fine waste [5-7].

The new modified suspension polymerization was developed to overcome the shortcomings of bulk polymerization and also to improve the adsorption characteristics of MIP microbeads (MIPMs) prepared by the conventional polymerization methods [8-14]. A novel modified suspension polymerization method utilized a surfactant in a conventional suspension polymerization mixture [15]. The D-phenylalanine (D-Phe) imprinted microbeads prepared by a novel modified suspension polymerization method without derivatization of the water-soluble template molecule using a conventional simple straight-blade turbine impeller captured successfully the template molecules in the organic phase droplets during polymerization [16]. A successful enantioseparation of D, L-Phe was also achieved for the first time using D-Phe imprinted P(MAA-co-EGDMA) microbeads as an HPLC stationary phase [16], even though many separations of amino acid were tried using D-Phe derivative imprinted microbeads [17-19].

However, the solid MIPMs converted from the liquid drops have a tendency to settle at the bottom of the reactor during suspension polymerization. This leads to bead aggregation and results in inefficient imprinting. The difficult perfect sealing of the reactor mouth, at the center of which the rotating shaft is installed, is required in order to keep the nitrogen atmosphere during polymerization in the reactor vessel. In the present study, D-Phe imprinted polymeric microbeads were prepared by the modified suspension polymerization method by using a magnetic impeller instead of a conventional simple straight-blade turbine impeller in order to maintain complete suspension and keep perfect sealing without an impeller shaft during polymerization. We hoped to prepare small MIPMs with a narrow size distribution, as well as increase the adsorption selectivity of the MIPMs.

MATERIALS AND METHODS

All experimental procedures for the preparation of MIPMs are similar to the method described in the previous report [15] except using a conventional simple straight-blade turbine impeller. The procedure is described briefly in the current study.

All chemicals used in these experiments were of reagent grade: Ethylene glycol dimethacrylate (EGDMA, Sigma), methacrylic acid (MAA, Sigma), trifluoroacetic acid (TFA, Sigma), D-phenylalanine (D-Phe, Sigma), and L-phenylalanine (L-Phe, Sigma), Polyvinyl alcohol (PVA, Yakuri, Japan), 2,2′-Azo-bis-isobutyronitrile (AIBN, Junsei, Japan), and sodium dodecyl sulfate (SDS, Fluka, Switzerland). MIPMs were prepared by the modified suspension polymerization method using four solutions. Solution-1: D-Phe (1 mmol, 0.116 g) was mixed with MAA (4 mmol, 0.34 mL), toluene (3 mL), acetic acid (0.6 mL) and TFA (0.4 mL). The mixture was stirred for 10 minutes at room temperature and then further stirred for 30 minutes at 0 °C using an ice-bath. Solution-2: AIBN (0.15 g) was dissolved in toluene (2 mL) and EGDMA (20 mmol, 3.77 mL) at room temperature for 10 minutes. Solution-3: PVA (3 g) was dissolved in hot distilled water (130 mL) at 80-85°C for 30 minutes, and then cooled to room temperature. Solution-4: 0.5 g of SDS was dissolved in distilled water (20 mL) at room temperature. Organic Solution-1 and Solution-2 were combined, while aqueous Solution-3 and Solution-4 were combined. Again each solution mixture was

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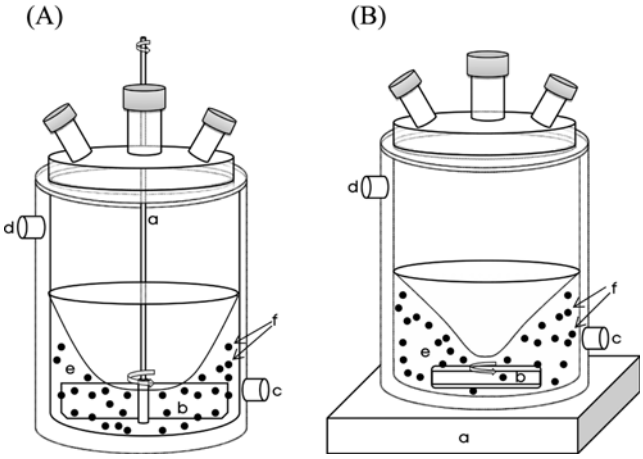

Fig. 1. Schematic diagrams of reactors for copolymerization with (A) a conventional simple straight-blade turbine impeller: a, shaft; b, impeller; c, inlet of circulating water of constant temperature; d, outlet of circulating water; e, aqueous medium; f, liquid droplets and MIP microbeads, and (B) a magnetic impeller: a, magnetic stirring plate; b, magnetic impeller; c, inlet of circulating water of constant temperature; d, outlet of circulating water; e, aqueous; f, liquid droplets and MIP microbeads.

stirred separately for 2 hours at room temperature before polymerization. Finally, all two solutions were combined in a three-neck double jacket glass vessel, purged with N_2 for 5 minutes, and stirred with a magnetic impeller of 50 mm at 750 rpm (or with a conventional simple straight-blade turbine impeller of 75 mm diameter at 600 rpm) for 60 minutes at room temperature. Polymerization was carried out at 60 °C for 24 hours with continuous stirring under N_2 atmosphere with simple sealing of the reactor mouth.

Unreacted monomers and template molecules (D-Phe) were removed from the MIPMs matrix by washing with a 5% (v/v) acetic acid solution for 3 hours followed by a washing with distilled water for 1 hour. These alternating washings were repeated several times. Micrographs of the platinum-coated polymer samples were taken with a field-emission scanning electron microscope (FE-SEM) (S-4300; Hitachi Co., Japan) to determine their morphology, surface topography, and particle size.

The MIPMs (0.6 g) were added to 5 mL of 100 mg/L Phe racemate solution and shaken for 1 hour at 30 °C and 200 rpm. After adsorption, the sample was centrifuged at 13,000 rpm for 3 minutes and filtered through a nylon membrane (0.45 μm, Alltech). The D-Phe and L-Phe concentrations of the sample solution were determined by HPLC (Youngrin M930, Korea) with a TSKgel Enantio L1 column (4.6 mm ID×250 cm). Copper sulfate ($CuSO_4 \cdot 5H_2O$, 0.2 g/L) was used as the mobile phase at a flow rate of 1 mL/min. The absorbance of the substrate solution was monitored at 254 nm by a UV detector. The adsorption selectivity (α) of the D-Phe imprinted polymeric microbeads was obtained with the following formula: (α)={(D-Phe)/(L-Phe)}/([D-Phe]/[L-Phe]). Where (D-Phe) and (L-Phe) are the amounts of Phe adsorbed on the polymer, and [D-Phe] and [L-Phe] denote the concentrations in the solution after adsorption [12-14].

RESULTS AND DISCUSSION

1. Particle Size Reduction with a Magnetic Impeller

The irregular shape of MIPMs and a wide size distribution of MIPMs lead to a decrease in the separation efficiency of HPLC column packed with MIPMs [20]. Thus, it is required to produce MIPMs of homogeneous size distribution with high adsorption selectivity. In this study, MIPMs produced by the modified suspension polymerization using a magnetic impeller were compared with MIPMs prepared by the same method using a conventional simple straight-blade turbine impeller.

In suspension polymerization for the preparation of MIPMs, an impeller is used for multipurposes: principally to mix the liquids, form organic micro droplets and suspend them in the bulk solution. However, it is very difficult to fix the impeller shaft vertically in the polymerization vessel. An inclined shaft leads to vibration and, then, it becomes impossible to polymerize at the nitrogen atmosphere under perfect sealing. There may be some side reactions with oxygen. During copolymerization with a conventional simple straight-blade turbine impeller, bubbles are generated on the surface of the cavity and penetrate into the solution. This may lead to inefficiency in imprinting and the production of beads of non-homogeneous size distribution. A propeller-type impeller is considered to be more efficient in suspension than a turbine impeller because the propeller-type impeller produces a strong vertical fluid flow. Even in a propeller-type impeller system, there exists a stagnant region at the periphery of the bottom of the reactor. Thus, a magnetic impeller was tried in the current study to suspend particles even at the periphery of the bottom.

MIPMs were produced by using a conventional simple straight-blade turbine impeller of 75 mm diameter rotating at 600 rpm in the previous study [15]. In the current study, a magnetic impeller of 50 mm length was used. The cavity was also formed in the magnetic impeller system and the cavity height increased as the rotating speed of the magnetic impeller increased and, at 750 rpm, reached the same cavity height formed with a conventional simple straight-blade turbine impeller. In other words, the same cavity height was obtained

Table 1. Size distribution (%) of D-Phe imprinted P(MAA-co-EGDMA) microbeads prepared by a modified suspension polymerization method with a conventional simple straight-blade turbine impeller and a magnetic impeller

	Size distribution (%)						Average diameter (μm)	Variance	Standard deviation (μm)
Diameter (μm)	3-6	6-9	9-12	12-15	15-18	>18			
Beads prepared with turbine impeller	9	21	21	22	16	11	12.5	24.4	4.9
Beads prepared with magnetic impeller	32	32	14	12	10	-	8.9	15.5	3.9

at the same angular acceleration velocity of both impeller systems.

The mean diameter of MIPMs prepared by the modified suspension method with a magnetic impeller was 8.86 μm with a variance of 3.93 as shown in Table 1. MIPMs prepared using a conventional simple straight-blade turbine impeller were 12.45 μm with variance of 4.94. A magnetic impeller was superior to a conventional simple straight-blade turbine impeller in preparing smaller beads of uniform size.

2. Complete Washing Requirement for Adsorption Selectivity

There are many template molecules combined with functional monomers in the core of the microbeads just after polymerization. These template molecules should be removed from the polymer matrix in order to build up recognizing sites. Thus, the microbeads should be washed sufficiently to detach and take out template molecules from the matrix even in the core of the polymer beads.

The adsorption selectivity of the MIPMs produced by the magnetic impeller was measured in a batch adsorption experiment. MIPMs prepared by washing 40 hrs after polymerization showed reversed adsorption selectivity, 0.68. This reversed adsorption selectivity was sometimes found in the previous report concerning the batch adsorption [12]. However, the MIPMs prepared by washing more than 100 hrs showed positive adsorption selectivity to the template molecule, 1.81. Insufficient washing after polymerization results in the absence of template molecules in the core of the MIPMs. During adsorption in a batch, templates in the core seem to hinder the adsorption of the D-Phe molecules on the recognizing sites and then lead to the reversed adsorption selectivity. Thus, sufficient washing time seems to be essential for the preparation of effective MIPMs.

3. Adsorption Selectivity Improvement of MIPMs

The uptake capacity and adsorption selectivity of the MIPMs prepared with a magnetic impeller were compared with MIPMs prepared with a conventional simple straight-blade turbine impeller by washing 140 hrs after polymerization. The uptake capacity of MIPMs prepared with a conventional simple straight-blade turbine impeller was 0.54 mg Phe/g MIPMs and higher than 0.38 mg Phe/g MIPMs prepared with a magnetic impeller. However, the adsorption selectivity of MIPMs prepared with a magnetic impeller is 1.89 and much higher than 1.28 for MIPMs prepared with a conventional simple straight-blade turbine impeller as shown in Table 2. The reason for the improvement in adsorption selectivity of MIPMs prepared by magnetic stirrer is speculated to be based on the observed phenomena during polymerization; in a conventional simple straight-blade turbine impeller system, gas bubbles and foams were formed at the interface between the solution and gas phases and bubbles broke into the solution. Bubbles formed by agitation with a conventional simple straight-blade turbine impeller reduced the stability of the complex of functional monomer with template molecules during polymerization. This led to the reduction in the population of the recognizing sites originating from the complex of functional monomer with the template molecules. However, a huge amount of single functional monomers distributed randomly in the polymer matrix of MIPMs adsorb Phe molecules without adsorption selectivity. For the selective adsorption, more than one functional monomer is required for each template molecule because there are one or multiple functional monomers in a recognizing site, which results in a small uptake capacity of MIPMs. MIPMs prepared with a magnetic impeller retained more recognizing sites in the polymer matrix and showed higher adsorption selectivity with a little smaller uptake capacity.

Table 2. Effect of the type of impeller used during copolymerization on the uptake capacity and adsorption selectivity of D-Phe imprinted P(MAA-co-EGDMA) microbeads. 0.6 g of MIPMs were added to 5 mL of 100 mg/L Phe racemate solution and shaken for 1 hour at 30 °C and 200 rpm

	Uptake capacity (mg Phe/g dry bead)		Adsorption selectivity (α)
	D-Phe	L-Phe	
Beads prepared with turbine impeller	0.30	0.24	1.28
Beads prepared with magnetic impeller	0.25	0.13	1.89

CONCLUSIONS

In the current study, we have described an improvement in the uniform size distribution of MIPMs, increase in adsorption selectivity and a decrease in the uptake capacity of MIPMs prepared by the modified suspension polymerization with a magnetic impeller instead of a conventional simple straight-blade turbine impeller. In building up recognizing sites in the MIPMs matrix, designing the reactor for the polymerization seems to be as important as selecting excellent functional monomer and cross linker molecules. The advantages of this proposed simple method include spherical-shaped polymers and smaller particle sizes, microbeads with narrow size distributions and improvement in adsorption selectivity. This method is anticipated to be useful in a broad range of applications including future developments in molecular imprinting.

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