

Phenylalanine Imprinted Microbeads Prepared by Shaking Method

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ABSTRACT

Molecularly imprinted polymer (MIP) technology is an emerging tool for the separation and isolation of structurally related compounds. MIP beads are considered useful in HPLC, CC, SEC, TLC, drug delivery systems as well as in chemical and biosensors. In this work a new shaking method using baffle flask as reactor for suspension polymerization for the preparation of MIP microbeads has been developed. This attempt proved to be simple, cheap and has greatly reduced the chances of oxygen entrapment during polymerization process. Characterization of the prepared non-imprinted beads (NIBs), D-Phe imprinted beads (DIBs) and L-Phe imprinted beads (LIBs) was made with SEM and FTIR analysis. Round shape, porous structure beads with a size distribution of 1-12 μm have been synthesized. The prepared DIBs and LIBs were used for the separation of D, L-Phe racemic mixture and showed good adsorption capacity (0.109 and 0.128 mg/g respectively) and adsorption selectivity (1.271 and 1.303 respectively) compared to NIBs with adsorption capacity 0.063 mg/g and adsorption selectivity 0.969.

Keywords- *D-Phe imprinted microbeads, L-Phe imprinted microbeads, shaking method*

I. INTRODUCTION

Molecularly imprinted polymers (MIPs) often have affinity and selectivity comparable to those exhibited by poly- or mono-clonal antibodies. MIPs are less expensive and quicker to prepare than biological receptors. Furthermore, they can stand at much harsher conditions than antibodies, such as high temperature, pressure, extreme pH, and organic solvents [1]. The promising areas of application of imprinted polymers include chromatography, biosensors, catalysis, and immunoassays [2, 3].

MIPs prepared as bulk material require crushing or grinding and sieving. This process is economically not feasible. A lot of material is wasted and particles of irregular shapes are obtained which are not ideal for many chromatographic purposes [4, 5].

To get maximum yield of MIPs with efficient separation ability and useful for variety of applications the formation of microbeads is of great importance. The MIP microbeads can be used directly after synthesis and washing [5-8]. In current time, much effort has been devoted in developing novel alternative methods to prepare imprinted stationary phases, which have high efficiency and better mass transfer properties. Spherical imprinted polymers (beads) of very minute size have been prepared through several techniques, such as multistep swelling, suspension polymerization, dispersion, precipitation, emulsion and miniemulsion techniques etc [9-11]. Suspension polymerization is much better and relatively simple method used for the preparation of molecularly imprinted beads. This method does not require the common tedious mechanical grinding process. Sufficiently dilute system leads to the formation of, uniform sized beads. MIP beads using different template molecules have been prepared by the suspension polymerization in a continuous aqueous or non aqueous phase in the presence of suspension stabilizer [12-15].

Suspension polymerization methods for producing beads offer a striking alternative to bulk polymerization [15-21]. The suspension methods can produce a higher yield of particles with superior chromatographic characteristics [22]. Moral and Mayes studied the performance of imprinted polymer particles prepared by different polymerization methods. They concluded that the overall performance of suspension polymerization showed better results for propranolol rebinding in aqueous solution as well as in organic solution [23]. Different techniques such as stirring, sonication, agitation method employ suspension method for preparing imprinted microbeads. However, to further simplify the processes and to achieve good results other simpler techniques need to be developed.

In the present study an effort is made to prepare D and L-Phe imprinted microbeads by suspension polymerization employing a simple and economically feasible reactor and procedure. D and L-Phe imprinted microbeads were prepared by shaking method employing baffle flask as a reactor.

Both D and L-Phe imprinted microbeads used for the separation of respective enantiomers. The characterization was conducted by FE-SEM and FTIR analyses.

II. MATERIALS AND METHODS

1. Chemicals and reagents

D-Phenylalanine (D-Phe), L-Phe, D,L-Phe, methacrylic acid (MAA), ethyleneglycoldimethacrylate (EGDMA), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St Louis, MO, USA); 2,2-azobisisobutyronitrile (AIBN) was obtained from Junsei Chemical Co., Ltd. (Japan); toluene was the product of Duksun Pure Chemical Co., (Korea); SDS was from Fluka (Switzerland), polyvinyl alcohol (PVA) and copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were obtained from Yakuri Pure Chemicals Co., (Osaka, Japan). All reagents used were of analytical reagent grade.

2. Polymerization process and beads preparation

The aqueous and organic solutions were prepared by the same method as reported in our previous study [8, 24]. The prepared solutions were then mixed in the baffle flask and shaken at 200 rpm at 25°C in shaking incubator for 10 mins. The mixed solution was then sonicated under N_2 supply for five minutes to remove any oxygen present in the solution. After N_2 passing the baffle flasks containing the solutions were tightly sealed with aluminum foil and were putted in the shaking incubator at 200 rpm and 60°C temperature for 24 hours for complete polymerization. This method was followed for the preparation of both D-Phe and L-Phe imprinted microbeads (DIBs and LIBs respectively). The diagrammatic representation is given

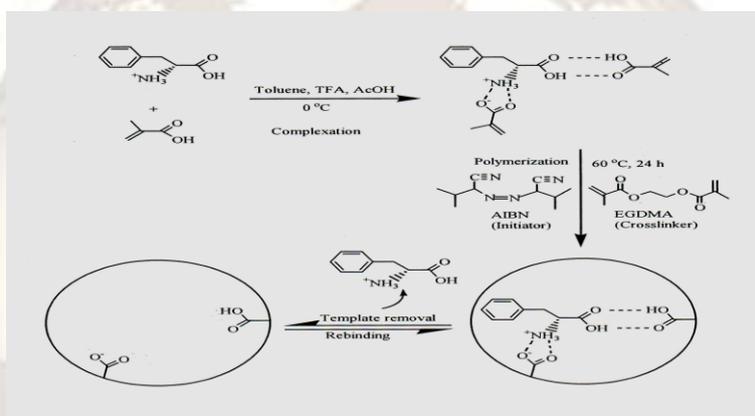


Fig 1. Illustration of recognition sites formation in the Phe imprinted P(MMA-co-EGDMA) microbeads matrix after removal of template molecules.

3. Washing and removal of template

After polymerization the prepared DIBs and LIBs were washed with 5.0% ethanol solution to remove the unreacted chemicals. Similarly, 5.0% aqueous solution of acetic acid was used for the extraction of template (D-Phe/L-Phe) molecules. This was followed by washing with distilled water until all the acetic acid was removed from the beads. The removal of acetic acid was checked by pH measurement.

4. Adsorption experiment

An amount of 0.6 g of freeze dried beads was taken and soaked in 5 mL of 100 ppm Phe recemate solution having pH 6 for 3 hours in a shaking incubator at 150 rpm and 25°C.

5. Characterization and analysis

FE-SEM micrographs were taken with HITACHI (Japan) S-4300 Field Emission Electron Microscope to study surface morphology. The size distribution ratios were determined from FE-SEM

micrographs. The analysis of the racemic solution after adsorption experiment was performed with HPLC/UV system. FTIR ART spectrometer was used for structural or functional group analysis of the prepared microbeads.

6. HPLC Analysis

The amounts of D-Phe and L-Phe in the obtained samples were determined with HPLC equipped with an M930 solvent delivery pump and M720 UV absorbance detector (Young-Lin Instruments, Anyang, Korea). A TSK gel Enantio L2 column (Tosoh, Tokyo, Japan) with dimensions of 4.6mm×250 mm was used. From HPLC results the adsorbed amount of enantiomers and adsorption selectivity of the prepared LIBs, DIBs and NIBs were calculated by the following equations [20].

III. EQUATIONS

1. Amount of Phe adsorbed

$$Q = (C_i - C_e) \times V / W \quad (1)$$

Where Q is the amount of D- or L-Phe adsorbed on

the beads (mg g^{-1}), while C_i and C_e are the initial concentration (mg L^{-1}) in feed solution and concentration (mg L^{-1}) after adsorption respectively. V is the volume in liter and W is the dry weight in grams.

2. Adsorption selectivity

$$\alpha_{ads} = \frac{\{(Q_1)/(Q_2)\}}{\{[Template]/[Counter enantiomer]\}} \quad (2)$$

Where α_{ads} is the adsorption selectivity, (Q_1) and (Q_2) are the amounts (mg) of template and the counter enantiomer adsorbed per 1 g of dry beads, respectively and $[Template]/[Counter enantiomer]$ is the ratio of concentration (mg L^{-1}) of template and counter enantiomer.

IV. RESULTS AND DISCUSSION

The use of baffle flask as a reactor and the shaking method maintained uniform mixing of chemicals for proper polymerization. This method also reduced the radical scavenging by the atmospheric oxygen which is a big problem in other techniques. The creation of the inert medium at the start of polymerization and then sealing of the reactor was enough for complete and safe polymerization. Contrary to the stirring method here no separate heating system and special reactor were required. This newly introduced shaking method may also be helpful for conducting other polymerization process involving the use of free radical polymerization. In this method the prior sonication process caused micro size droplets which acted a micro reaction chambers and resulted in micron sized MIP beads.

The SEM micrographs represented as **Fig. 2** indicate that the present method resulted in round shape beads with porous surfaces. Beads size distribution ratio indicates that most of the beads are in the range 0.10 to 12 μm . **Fig. 3** shows that the ratio of the beads in the range of 3 to 6 μm is the highest. Hence these beads are suitable for use as stationary phases in chromatographic techniques and other useful applications. Contrary to the conventional stirring method where breaking down of beads occur as a result of mechanical stress applied during stirring the beads remained safe in

the present study. The prepared DIBs and LIBs are suitable for using in various chromatographic techniques and can also be applicable in preparing biochemical sensors.

From **Table 1** it is clear that both DIBs and LIBs showed selective adsorption for template molecules. The adsorption capacity of imprinted microbeads (DIBs and LIBs) is ≥ 1.3 times higher than NIBs. Similarly, it is also evident that the adsorption capacity and the adsorption selectivity of the LIBs is relatively high compared to the DIBS [25, 26].

FTIR spectra shown in **Fig. 4 (a), (b) and (c)** illustrate the functional groups present in the prepared Phe imprinted beads. The spectra revealed that compared to non-imprinted beads the Phe imprinted beads exhibited broad and intense peaks for -CO- and OH groups which are mainly responsible for template bonding. Thus the FTIR spectra act as a clue for active imprinting achieved in the imprinted polymers compared to non-imprinted polymers.

The FT-IR spectra of the prepared beads are given in **Fig. 4 (a), (b) and (c)** in which the peak at $\sim 3600 \text{ cm}^{-1}$ represents OH stretching of free carboxylic group derived from MAA (functional monomer), while the peak at $\sim 1,730 \text{ cm}^{-1}$ stand for C=O stretching. The peaks at $\sim 1,240$ and $\sim 1,145 \text{ cm}^{-1}$ were assigned to the CO bending of ester group and CO stretching of COOH and ester groups. The small absorption bands at ~ 951 , ~ 806 , and $\sim 753 \text{ cm}^{-1}$ were attributed to C-CH₃ rocking, CH bending (vinyl out of plane), and CH₂ rocking, respectively. Similar results were also obtained in previously reported studies [25, 27] related to the imprinted Poly(MAA-co-EGDMA) polymers. From these spectra, it is clear that the hydroxyl peak at $\sim 3,600 \text{ cm}^{-1}$ for LIBs and DIBs is visible than that for NIBs. Similarly, the intensities of other absorption peaks at $\sim 1,730$, $\sim 1,240$ and $\sim 1,145 \text{ cm}^{-1}$ which correspond to C=O and CO groups, respectively, were also found to be higher in case of LIBs and DIBs compared to NIBs. This indirectly confirms the formation of recognition sites in the LIBs and DIBs because of the existence of a large population of free carboxyl groups after template removal [25, 27].

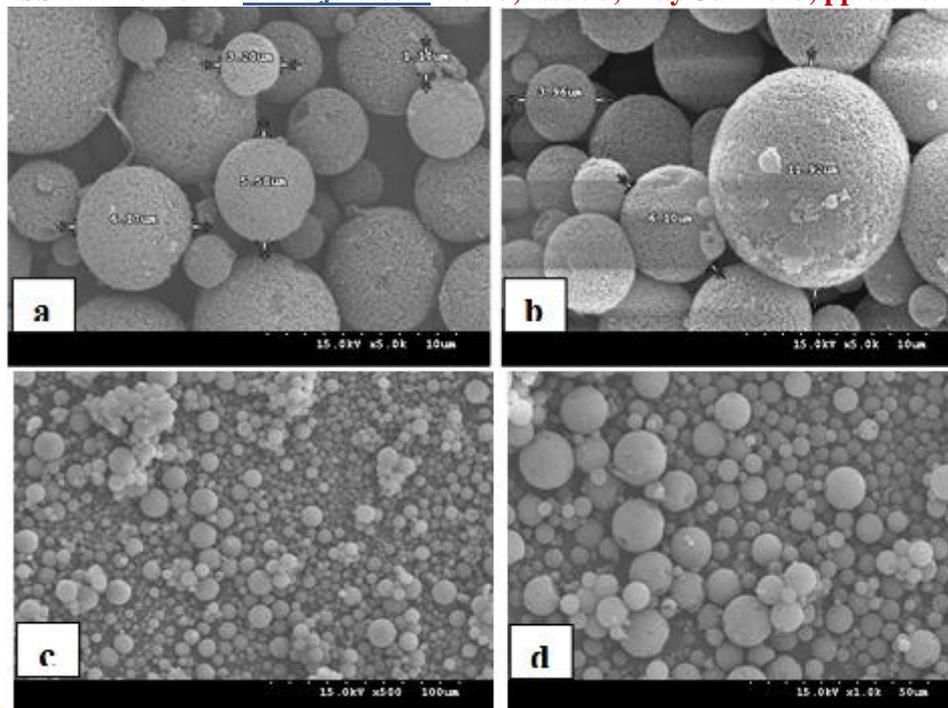


Fig. 2. (a) to (d). FE-SEM images for the size and morphological study of the beads prepared by shaking baffle flask method.

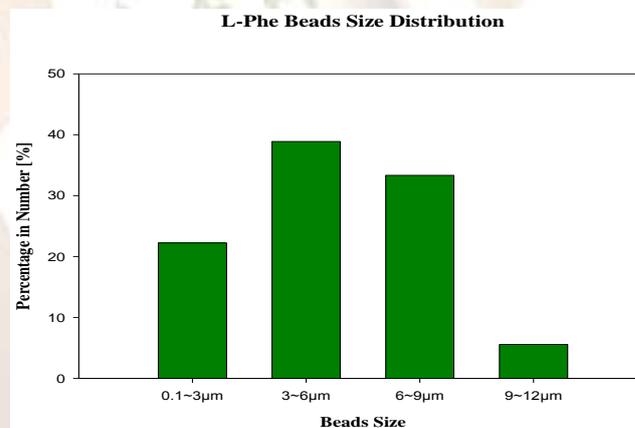
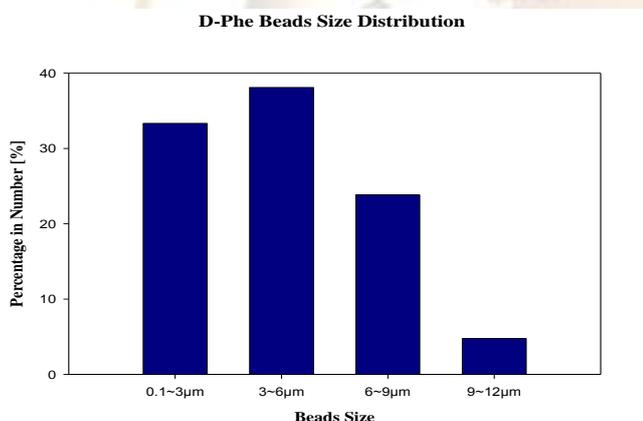
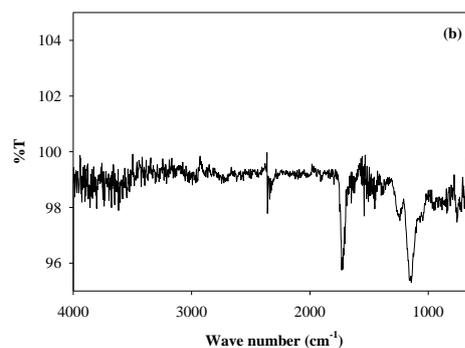
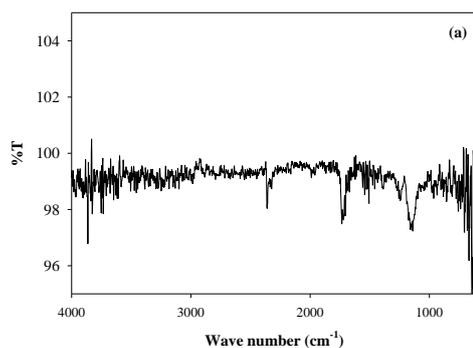


Fig. 3. Size distribution of MIP microbeads prepared by shaking method of suspension polymerization where (a) DIBs (b) LIBs



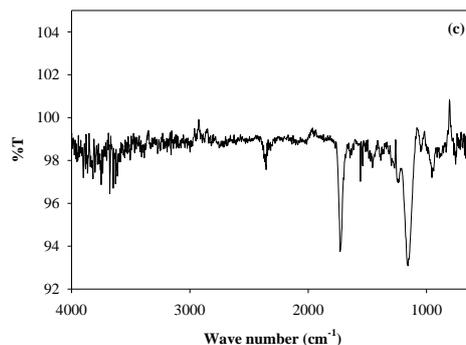


Fig. 4. FTIR spectra of (a) NIBs (b) DIBs and (c) LIBs prepared by shaking flask method.

Table 1. Adsorption capacity and selectivity of NIBs, DIBs and LIBs prepared by suspension polymerization employing shaking method at 200 rpm, 60 °C for 24 h.

Microbeads	Phe enantiomer	Adsorption capacity Q(mg/g dry wt)	Adsorption selectivity (α)
NIBs	D-Phe	0.031	0.969
	L-Phe	0.032	
DIBs	D-Phe	0.061	1.271
	L-Phe	0.048	
LIBs	D-Phe	0.055	1.303
	L-Phe	0.073	

V. CONCLUSION

An economically feasible and simple shaking method for suspension polymerization was successfully employed for the preparation of DIBs and LIBs. The wastage of product was small and size distribution ratio was favorable. Both DIBs and LIBs showed selective adsorption for their respective template molecules. The adsorption capacity and the adsorption selectivity of L-Phe imprinted beads was relatively high compared to the D-Phe imprinted beads. FTIR analysis supported the formation of specific functional groups in the prepared imprinted polymer microbeads. Besides synthesizing imprinted polymers this technique will also be feasible for other polymerization techniques involving risk of radical scavenging.

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