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Preparation of magnetic poly(methylmethacrylate–divinylbenzene–glycidylmethacrylate) microspheres by spraying suspension polymerization and their use for protein adsorption

Chengli Yang[a,b], Huizhou Liu[a,*], Yueping Guan[a], Jianmin Xing[a], Junguo Liu[a], Guobin Shan[a]

[a]*Laboratory of Separation Science and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, P.O. Box 353, Beijing 100080, People's Republic of China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing, People's Republic of China*

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Abstract

Moderately uniform magnetic poly(methylmethacrylate–divinylbenzene–glycidylmethacrylate) microspheres (poly(-MMA–DVB–GMA) microspheres) were prepared by spraying suspension copolymerization of methyl methacrylate, divinylbenzene and glycidyl methacrylate in the presence of Fe_3O_4 magnetic fluid. A protein adsorption assay indicated that these magnetic microspheres could significantly improve the capacity of protein adsorption.

Keywords: Microspheres; Spraying suspension polymerization; Protein adsorption; Divinylbenzene; Methacrylate; Polymerization

1. Introduction

Magnetic functional polymeric microspheres have been arisen great interest in the fields of biology and medicine because they can be easily collected with the application of a magnetic field; and the coupling of appropriate ligands to such microspheres provides an effective tool to achieve rapid, simple, and specific biological separation such as cell isolation [1], enzyme immobilization [2], protein and enzyme purification [3], immunoassay [4], and guided site-specific drugs [5]. These magnetic microspheres can be prepared via monomer polymerization including conventional emulsion polymerization [6,7], dispersion polymerization [8,9], suspension polymerization [10,11], and seed polymerization [12], and activated swelling method [13]. Among these methods, suspension polymerization is simple and more suitable for massive production especially of magnetic

*Corresponding author. Tel.: +861062555005; fax: +861062554264.
E-mail address: hzliu@home.ipe.ac.cn (H. Liu).

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polymeric microspheres. Unfortunately, with a conventional mechanical stirring method, the size distribution of the prepared magnetic polymeric microspheres is likely to be quite wide [10,11], and therefore they would not be ideally suitable for applications in the field of bioseparation and biomedicine.

In the present work, moderately uniform magnetic poly(MMA–DVB–GMA) microspheres were prepared by spraying suspension polymerization (SSP). The magnetic poly(MMA–DVB–GMA) microspheres were modified by ethylene diamine (EDA) with amine groups which can be easily connected to proteins and enzymes. The morphology and magnetic properties of the magnetic poly(MMA–DVB–GMA) microspheres were examined with a scanning electron microscopy (SEM) and vibrating sample magnetometer (VSM). Furthermore, to prove that EDA modified magnetic microspheres can be conjugated to some biomolecules, a protein adsorption assay was carried out after the different bovine serum albumin (BSA) concentrations were treated with the EDA-modified magnetic microspheres. Meanwhile, the BSA adsorption and desorption capacity as a function of time was also investigated.

2. Experimental

2.1. Materials

Methyl methacrylate (MMA), divinylbenzene (DVB) and glycidyl methacrylate (GMA) were analytical grade, distilled under reduced pressure to remove inhibitors, and stored in a refrigerator prior to use. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), $NH_3 \cdot H_2O$, oleic acid, EDA and ethanol were analytical grade and used without any further purification. Polyvinyl alcohol (PVA-217, degree of polymerization 1700, degree of hydrolysis 88%) was used as a stabilizer. Benzoyl peroxide (BPO), reagent grade, was used as an initiator for polymerization. Water was purified by distillation followed by deionization using ion exchange resins. Bovine serum albumin (BSA) was purchased from Beijing Biochemical Reagent Company, Beijing, China. Other chemicals were reagent grade and used as received.

2.2. Preparation of Fe_3O_4 magnetic fluid

The Fe_3O_4 magnetic fluid with hydrophobic shell was prepared by the conventional co-precipitation method [14] with some modifications. 0.196 mol $FeCl_3 \cdot 6H_2O$ and 0.098 mol $FeCl_2 \cdot 4H_2O$ were dissolved in 600 ml deionized water in a 2.0 l beaker under nitrogen gas with vigorous stirring at 80 °C. Then, 30 ml $NH_3 \cdot H_2O$ was added to the solution, and 20 ml oleic acid was added dropwise into the suspension within 20 min. After several minutes, the resulting Fe_3O_4 magnetic fluid was isolated from the solution by a magnet and washed several times with deionized water to remove the excess oleic acid.

2.3. Preparation of magnetic poly(MMA–DVB–GMA) microspheres

A schematic diagram of SSP is shown in Fig. 1. A spraying nozzle with pore size of 100 μm was installed over the polymerization reactor. The oil phase was stored in a storage tank, which was

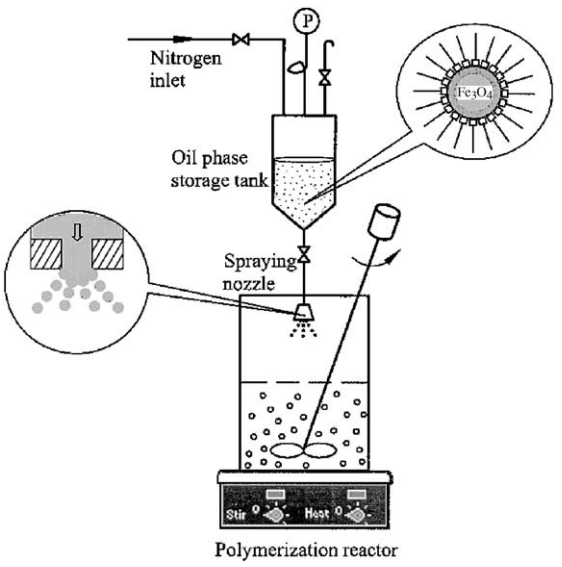

Fig. 1. Schematic diagram of spraying suspension polymerization (SSP).

connected to a nitrogen gas inlet. The aqueous phase was stored in the polymerization reactor and stirred gently with a mechanical stirrer to prevent the coalescence of droplets.

The oil phase was composed of 20 g Fe_3O_4 magnetic fluid, 60 ml MMA, 30 ml GMA, 10 ml DVB and 6 g BPO. Twenty-gram PVA was dissolved in 1000 ml deionized water at 80 °C, and was used as an aqueous phase. After the pressure of the oil phase storage tank was controlled at 0.15 MPa, the oil phase was sprayed through the spraying nozzle into the aqueous phase to form uniform droplets, which were polymerized rapidly in 1 h with gentle stirring. The resulting magnetic poly(MMA–DVB–GMA) microspheres were separated by a HGMS system and washed several times with deionized water and ethanol.

2.4. Modification of magnetic microspheres

The modification of magnetic poly(MMA–DVB–GMA) microspheres with EDA was carried out by the ring-opening reaction of epoxide groups on microspheres. Briefly, 10.0 g dry magnetic microspheres was mixed with a 10-fold excess of EDA at pH 8.5 under very gentle stirring and the mixture was kept at 80 °C for 8 h. The modified samples were washed thoroughly with ethanol, water and finally 0.1 M phosphate buffered saline (PBS) (pH 5.0) prior to protein adsorption.

2.5. Protein adsorption and desorption

BSA was used as a model protein to test the adsorption characteristics of the modified magnetic microspheres. The modified magnetic microspheres were dispersed in PBS (0.1 M, pH 5.0) with concentrations of 5 g l^{-1}. Different BSA concentrations were added into 10 ml solutions of magnetic microspheres, respectively. The mixtures were incubated at 25 °C for 2 h. At the end of adsorption, the solid phase was magnetically separated and the supernatant was analyzed for residual protein concentration. Adsorption capacity of BSA was calculated by mass balance.

In a typical kinetic experiment, capped glass tubes each containing 0.04 g magnetic microspheres and 6.0 ml of BSA solution with a definite concentration were shaken end-to-end in a shaking incubator at 25 °C. The tubes were taken out successively for supernatant protein concentration measurement. By this procedure, the decrease of BSA concentration with time was determined. In addition, 1.0 M NaCl solution was employed as a dissociation agent to test the desorption characteristics of BSA.

2.6. Analysis and measurement

The diameter and surface features of magnetic poly(MMA–DVB–GMA) microspheres were investigated by SEM (JSM-6700F, JEOL. Japan). The magnetization curve of the samples was measured at room temperature with a VSM (model-155, Digital Measurement System, Inc.). The concentrations of BSA were determined from the absorbance at 280 nm by using a DU-640 spectrophotometer (Beckman, Fullerton, CA, USA).

3. Results and discussion

3.1. Properties of magnetic microspheres

Magnetic poly(MMA–DVB–GMA) microspheres were prepared by SSP. In our present work, PVA was employed as a stabilizer in the aqueous solution to stabilize the droplets. The Fe_3O_4 magnetic fluid with hydrophobic shell could be easily mixed with organic mixture of MMA, DVB, GMA and BPO to form a uniform oil phase. Under a particular pressure of nitrogen gas, the oil phase was sprayed through the spraying nozzle into the aqueous phase to form uniform droplets, which were stabilized by PVA dissolved in the aqueous phase. The temperature of the aqueous solution in polymerization reactor was maintained at around 80 °C, so the droplets were polymerized rapidly after they enter into the aqueous solution, while the conglomeration between droplets was avoided. After 1 h, the resulting magnetic microspheres were separated quickly by a HGMS system, and the aqueous solution was recycled to the polymerization reactor. In this SSP, the

droplets were dispersed uniformly by spraying dispersion instead of conventional mechanical stirring. Therefore, the microspheres prepared were moderately uniform.

Fig. 2 shows the SEM micrograph of the overall appearance of magnetic poly(MMA–DVB–GMA) microspheres prepared by SSP. Obviously, Fig. 2 shows that the microspheres are moderately uniform with average diameter of around 10 μm. The results verified that the SSP method used in this work was suitable for preparation of moderately uniform magnetic microspheres.

The magnetization curve for the magnetic poly(MMA–DVB–GMA) microspheres was measured with VSM [15] as shown in Fig. 3. There is no hysteresis in the magnetization with both remanence and coercivity being zero, which means that such magnetic microspheres are superparamagnetic. The saturation magnetization was found to be 17.0 emu g^{-1}, which is higher than those of other similar works reported in the Refs. [10,11]. With such high saturation magnetization, the magnetic microspheres responded very rapidly to a magnetic field, typically being attracted to an NdFeB permanent magnet from a suspension in about 10 s, leaving a clear supernatant, which could readily be removed by aspiration or decantation.

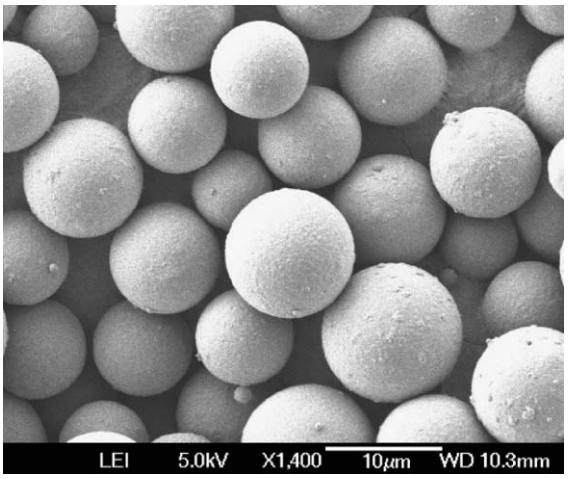

Fig. 2. SEM micrograph of magnetic poly(MMA–DVB–GMA) microspheres.

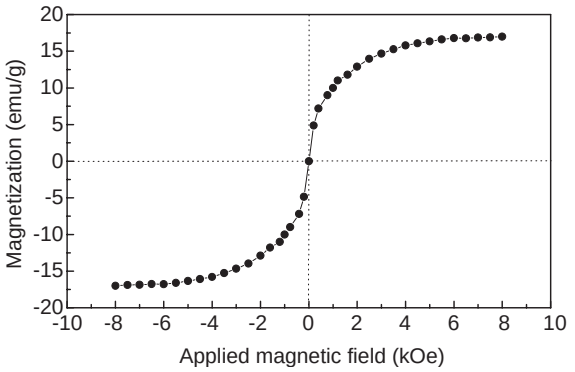

Fig. 3. Magnetization curve of magnetic poly(MMA–DVB–GMA) microspheres obtained by VSM.

3.2. Adsorption and desorption equilibrium

In the present work, the functional epoxy groups containing magnetic poly(MMA–DVB–GMA) microspheres were prepared by SSP, and the epoxy groups on the magnetic poly(MMA–DVB–GMA) microspheres were modified with EDA as shown in Fig. 4.

The adsorption capacity of protein on the magnetic microspheres is usually maximal at around their isoelectric point [16]. The pH 5.0 was chosen for the adsorption pH because the isoelectric point of BSA is 4.7. Fig. 5 shows the BSA adsorption and desorption capacity as a function of time for magnetic microspheres. The adsorption equilibrium of BSA was obtained quickly in about 60 min because the adsorption between the BSA and magnetic microsphere was carried out on the surface microspheres and the rate of mass transfer through the liquid film is very fast. BSA desorption was also carried out in high NaCl concentration solution. About 80% BSA was desorbed from the magnetic microspheres by adding 1.0 M NaCl solution in about 60 min as shown in Fig. 5. It can be explained that increasing the NaCl concentration in the liquid phase can reduce the electrostatic interaction between BSA and the amine groups on the magnetic microspheres. The results verified that the adsorption and desorption between BSA and magnetic microspheres are a reversible process, which shows fast adsorption and desorption kinetic characteristics.

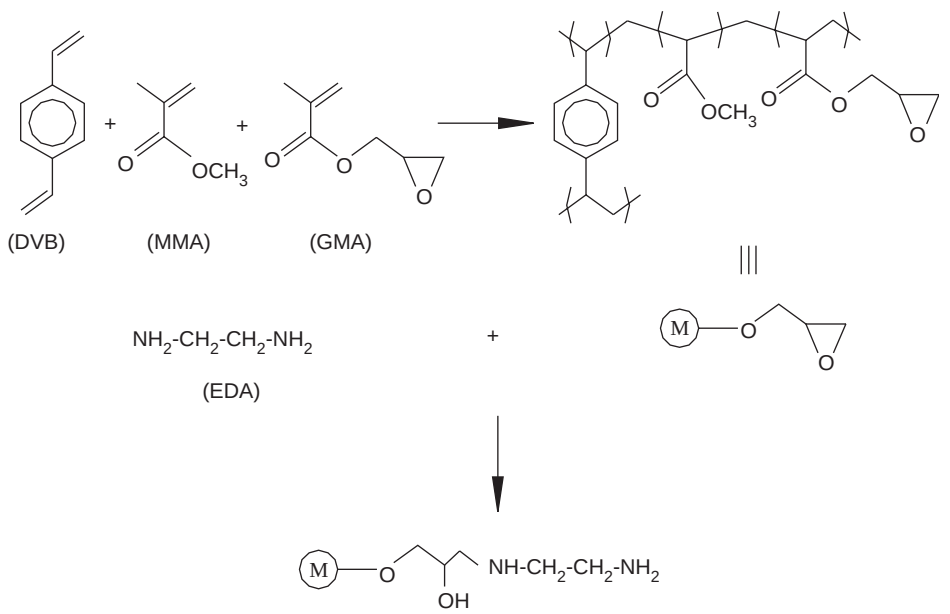

Fig. 4. Chemical structure for surface of magnetic poly(MMA–DVB–GMA) microspheres.

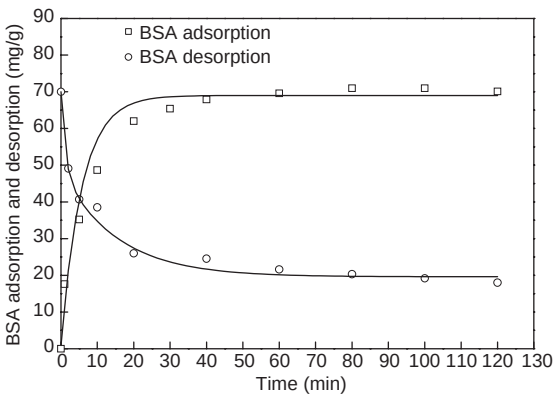

Fig. 5. BSA adsorption and desorption capacity as a function of time for magnetic poly(MMA–DVB–GMA) microspheres.

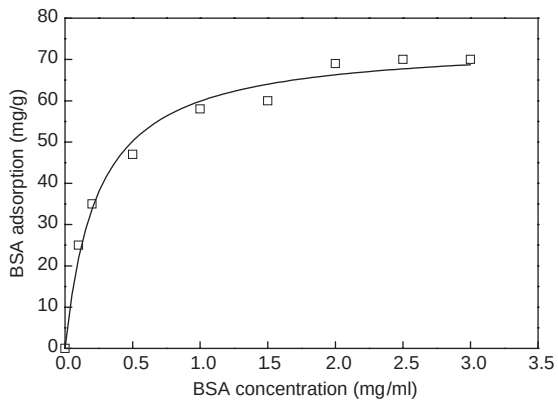

Fig. 6. *Langmuir* isotherm of BSA adsorption onto magnetic poly(MMA–DVB–GMA) microspheres.

Fig. 6 shows the adsorption isotherms of BSA to magnetic microspheres. The solid lines are calculated from the *Langmuir* model as follows:

$$q = \frac{q_m C}{K_d + C}. \quad (1)$$

This model can be regarded as either rigorous or empirical, depending on whether the adsorption obeys its premises or not, and has been widely used in characterizing the adsorption of BSA to ion exchanger [17,18] and affinity adsorbents [19]. The values of q_m and K_d were estimated by least-squares regression. Obviously, the adsorption capacity of BSA (q_m) is about $70\,mg\,g^{-1}$, and the equilibrium dissociation constant (K_d) is $0.12\,mg\,ml^{-1}$. The high capacity of protein adsorption is attributed to the

contribution of modified magnetic microspheres whose active amine groups can be conjugated to BSA by adsorption.

Acknowledgments

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