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Gas-assisted magnetic separation for the purification of proteins in batch systems

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ABSTRACT

In this paper, gas-assisted magnetic separation (GAMS), a technique that combines magnetic separation with flotation, was investigated for the potential large-scale separation of proteins. The GAMS process includes adsorption of target proteins and magnetic separation to recover protein-loaded magnetic particles from the dilute biosuspension with the assistance of bubbles. Microsized ethylenediamine-functionalized poly(glycidyl methacrylate) superparamagnetic microspheres (MPMs) and bovine serum albumin (BSA) were used as a model system. The feasibility of GAMS for capturing BSA-loaded MPMs from an appropriate medium was shown. High recovery of BSA-loaded MPMs was obtained by simple adjustment of the initial solution pH without extra detergents and antifoaming agents. The GAMS conditions were consistent with the adsorption conditions, and no proteins were desorbed from the MPMs during this process. Under the optimal conditions, the separation rate and recovery percentage reached 410 mL/min and 98% in 0.61 min, respectively. Conformational changes of BSA during the GAMS process were investigated by fluorescence spectroscopy and circular dichroism spectrometry.

Introduction

Magnetic separation is a simple, rapid, and effective process that is widely used for the separation of proteins (Franzreb, Siemann-Herzberg, Hobley, & Thomas, 2006; Okoli, Boutonnet, Mariey, Jaras, & Rajarao, 2011; Wang et al., 2011; Horak et al., 2013) and enzymes (Altintas, Tuezmen, Candan, & Denizli, 2007; Kondo & Fukuda, 1997; Liao & Chen, 2002), wastewater treatment (Mohammed, Ketabchi, & McKay, 2014; Rubio, Souza, & Smith, 2002; Sun et al., 2014). The principle of this method is the use of functionalized superparamagnetic adsorbent particles to bind the target components via ligands to form complexes that can be removed from the bulk solutions by a magnetic field gradient. In fact, magnetic separation is a feasible method for the recovery of magnetic adsorbent particles in biological dilute solutions and other fouling materials of a similar size. Moreover, the power and efficiency of magnetic separation procedures is particularly suited for large-scale operation.

The separation technology for magnetic materials mainly includes laboratory-scale chemical analytical detection (Babinec, Krafcik, Babincova, & Rosenecker, 2010; Khajeh, 2009), and large-scale protein separation and production (Wang et al., 2014; Yavuz, Prakash, Mayo, & Colvin, 2009). However, much less attention is paid to the latter. The main limitation is that large-scale separation of magnetic particles from dilute biosuspensions still poses an enormous challenge for existing magnetic separators. Nowadays, the high-gradient magnetic separation (HGMS) is generally chosen as the preferred large-scale magnetic separation method (Fuchino et al., 2014). It is widely used because of simple equipment, easy operation, and low operating and maintenance costs. However, it still has some limitations in large-scale application, such as dramatically increasing costs with the increase of scale and capacity limits at large scale, because particles are sensitive to the distance from the magnetic force. On the one hand, magnetic separation is limited by the requirement for a short distance between particles and the source magnet. On the other hand, large-scale separation requires the use of a high gradient and high field to offset

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the decrease in magnetic force with increasing distance. These are the main limitations for the development of HGMS.

Flotation is extensively used for solid–liquid separation in biochemical and primary mineral industries, such as enzyme purifying (Linke & Berger, 2011), flotation of gold and electrum (Allan & Woodcock, 2001), and recovering unburned carbon from municipal solid (Huang, Takaoka, & Takeda, 2003). The basic principle of flotation is simple, and it is primarily based on the hydrophobic character of the solids and the low surface tension of the liquid media. The solids can attach to bubbles rising in the liquid and be removed from the liquid on the top of the liquid, attaining enrichment and separation. The technology has the advantages of easy scale-up, continuous operation, high separation efficiency, and low operation cost. If magnetic separation could be assisted by flotation, magnetic adsorbent particles could be easily carried by bubbles and these particles could be subsequently recovered from the top of the liquid by magnetic separation. With the aid of this additional external force, rapid and easily scalable separation of magnetic particles by permanent magnet would become feasible. Obviously, the force of rising bubbles on particles not only avoids the use of expensive electromagnets in large-scale separation, but overcomes the limitation of the dependence of the magnetic force on the capture distance.

Herein, we report a magnetic separation method with flotation assistance called gas-assisted magnetic separation (GAMS). Having previously established that protein-loaded magnetic nanoparticles and microspheres can be concentrated by flotation without extra detergents (Li et al., 2013a, 2013b), we show here that protein-loaded microspheres can be rapidly and remotely removed from dilute solution by GAMS. Fig. 1 shows the GAMS process. After equilibrium was achieved for protein adsorption, the target protein-loaded magnetic particles were carried onto the surface of the liquid by bubbles and recovered by a magnet suspended above the liquid surface. Adopting GAMS technology for the separation of proteins could not only increase the recovery of magnetic particles, but could greatly decrease the separation time and the required magnetic force.

In this study, microsized ethylenediamine-functionalized poly(glycidyl methacrylate) superparamagnetic microspheres (MPMs) were used for the separation of bovine serum albumin (BSA). Magnetic poly(glycidyl methacrylate) microspheres have a large number of reactive epoxy groups, which can be easily converted to a variety of functional groups. The ethylenediamine-modified magnetic poly(glycidyl methacrylate) microspheres were prepared by dispersion polymerization followed by the ring-opening reaction of epoxy groups with ethylenediamine (EDA). Once generated, the microspheres were used for the separation of protein by electrostatic attractions. In the absence of flotation agents and foam for enrichment, the consistency of the protein adsorption conditions and the operating conditions of GAMS were investigated.

Experimental

Materials

BSA was obtained from Merck (Darmstadt, Germany). Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ammonium hydroxide ($NH_3{\cdot}H_2O$, 25% w/w), polyethylene glycol 6000 (PEG-6000), and EDA were of analytical grade and purchased from Xilong Chemical Industry Co. Ltd. (Shantou, China). Glycidyl methacrylate (GMA), polyvinylpyrrolidone (PVP K-30), ethylene glycol dimethacrylate (EGDMA), and 2,2-azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich Corp. Ltd (St Louis, MO, USA).

Preparation of the MPMs

The magnetic poly(glycidyl methacrylate) microspheres were prepared according to previous work (Li et al., 2013b). First, PEG-coated magnetic nanoparticles were prepared. In brief, 5.9 g of $FeCl_3{\cdot}6H_2O$ and 2.3 g of $FeCl_2{\cdot}4H_2O$ were dissolved in 100 mL of deionized water under N_2 protection. Then, 12 mL of $NH_3{\cdot}H_2O$ was rapidly added at 80 °C and the suspension was kept at 80 °C for 30 min. The black nanoparticles were cooled to room temperature and washed several times with deionized water. Subsequently, 20 mL of deionized water containing 5 g PEG-6000 was added into the above solution, and the mixture was sonicated for 30 min so that the magnetic particles were well-coated with PEG. The magnetic poly(glycidyl methacrylate) microspheres were prepared by dispersion polymerization. In a typical experiment, 5 g of PEG-coated magnetic nanoparticle slurry and 2.5 g of PVP K-30 were dissolved in 80 mL of ethanol and sonicated for 20 min to mix well. Then, 12 mL of GMA, 100 μL of EGDMA, and 250 mg of AIBN were added as a dispersion phase. The mixture was purged with N_2 for about 30 min and shaft sealed. The polymerization was carried out at 70 °C for 24 h under continuous shaking (180 rpm). The mixture was cooled to room temperature, and the resulting particles were separated by centrifugation at 4000 rpm for 10 min two times, and then the particles were thoroughly washed with ethanol and deionized water to remove residual stabilizer and other impurities by magnetic separation.

Modification of magnetic poly(glycidyl methacrylate) microspheres with EDA was carried out by the ring-opening reaction of epoxy groups on microspheres (Ma, Guan, & Liu, 2005). In brief, 10 g of magnetic poly(glycidyl methacrylate) microspheres were dispersed in the solution composed of 75 mL EDA and 100 mL deionized water. The mixture was stirred for 12 h at 80 °C. The resulting product was then thoroughly washed several times with ethanol and water to give the MPMs.

Characterization of MPMs

The size and morphology of the MPMs were determined using a scanning electron microscope (TM3000, Hitachi, Tokyo, Japan). The magnetic property of the MPMs was measured by a vibrating sample magnetometer (Model 4 HF VSM, AED Technologies, USA).

Protein adsorption

The effects of initial BSA concentration, medium pH, and ionic strength on the BSA adsorption capacity were investigated. The adsorption experiments were carried out in batches. The initial concentration of BSA ranged from 0.2 to 2.4 mg/mL. The pH was changed from 3.8 to 8.0 using different buffer systems (0.02 M CH_3COONa–CH_3COOH for pH 4.0–5.8, 0.02 M Tris–HCl for pH 7.0–8.0). In a typical adsorption experiment, a specific amount of adsorbent and 5 mL of a specific concentration of BSA in 0.02 M buffer were mixed. The mixture was shaken at room temperature for 2 h. After magnetic separation, the supernatant was assayed for protein concentration using an UV–vis spectrophotometer (HitachiU-4100, Hitachi Limited, Hitachi, Japan) at 280 nm (Qu, Guan, Ma, & Zhang, 2009). The amount of adsorbed BSA was calculated by mass balance.

GAMS for the recovery of BSA-loaded MPMs

A specific amount of MPMs was incubated with a specific volume of protein buffer of BSA (1 mg/mL) with shaking for 2 h at room temperature to reach adsorption equilibrium. The above solution was then transferred to a flotation column. GAMS experiments were performed in the flotation column (length: 50 cm, inner diameter: