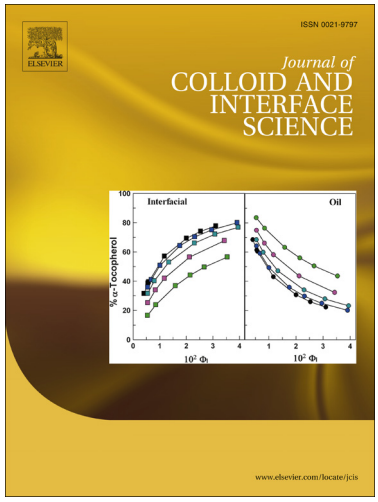

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Preparation of Thermosensitive Polymer Magnetic Particles and Their Application in Protein Separations

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ABSTRACT: This paper presents a kind of thermoresponsive polymeric magnetic particles for protein separations. The magnetofluids were directly encapsulated in hollow particles constructed by self-assembly of rod-coil poly(ethylene glycol)-poly(N-isopropylacrylamide)/α-cyclodextrin (PEG-PNIPAM/α-CD) complexes. The resulting particles showed reversible protein absorption/desorption capacity because the reversible thermo-sensitivity of PNIPAM. Above the lower critical solution temperature (LCST) of PNIPAM, these particles showed high absorptive capacities and adsorption was done at lower temperature. The protein-laden particles are readily removed from the feed solution in a magnetic field.

Key Words: self-assembly, magnetic particles, host-guest recognition, lysozyme.

1. Introduction

Adsorption and separation of proteins plays an important role in biosciences and biotechnology industry. So far, several techniques have been developed for the isolation of proteins, such as chromatography, electrophoretic, ultrafiltration, precipitation, and magnetic separation etc.[1-11] Compared to standard separation procedures like chromatography, the advantages of magnetic separation techniques were its simplicity and universality. There is no need for expensive liquid chromatography systems, centrifuges, filters or other equipment. The separation process can be performed in almost any type of bio-feedstock. Even crude samples containing suspended solid materials can be purified by magnetic separation directly. However, the relatively low absorptive capacity of most magnetic particles limits their applications.

The magnetic separation is based on the immobilization of affinity intermediates on the surface of magnetic particles, and the use of the resulting particles for the separation and concentration of biomolecules.[12-13] There are two approaches to increase the protein loading capability of magnetic particles. The first one focuses on increasing the surface area of particles by reducing their particle size. For example, Laibinis and Hatton et. al prepared a kind of colloidal magnetic nanoparticles with 8 nm, which have high absorptive capacities up to 800 mg protein/mg particles.[14] But too small size may result in that the particles can not be removed from samples efficiently by normal magnetic filtration. The second one is to increase the density of affinity ligands. Various strategies have been developed to produce magnetic particles. For the separation application, the affinity ligands are attached on the surface of magnetic core mainly by surface modification of sub-micron or nano-particles. For instance, Uddin et al. reported a series of magnetic nanoparticles covalently binded intermediates for protein adsorption. But the efficient coupling of affinity ligands is low because strongly binding ligands are not readily available for some inorganic particles and the problem of steric hindrance.

In our previous report, we have developed an approach to construct supramolecular hollow nano-particles by formation coil-rod inclusion complex between cyclodextrin (CD) and block copolymer. These hollow particles could encapsulate enzyme, gene, or super-paramagnetic Fe_3O_4 during the self-assembly process. The resulting particles have densely polymer shell and it does not require covalent interaction between the polymer shell and the entrapped component (Fe_3O_4).

In this paper, based on the previous work, we prepared a kind of thermoresponsive polymer magnetic microparticles using a simple self-assembly method and investigated their use for the protein separation. Poly(N-isopropylacrylamide) (PNIPAM) was selected as a protein trap. It has been demonstrated that the reversible thermo-sensitivity of PNIPAM lead to reversible adsorption of proteins.[15-20] Super-paramagnetic Fe_3O_4 was selected as building component because ferromagnetic materials were easy to aggregate after the removal of magnetic field.[21]

2. Materials and methods

2.1 Materials

Methoxy polyethylene glycol (mPEG, ≥99%) with molecular weights of 2000 was purchased from Aldrich Chemical Co, and dried in a vacuum oven over P_2O_5 at 40 °C for 48 h before used. The amine terminated PNIPAM with molecular weights of 2,500 and lysozyme from chiken egg white (100000 U/mg) were purchased from Sigma-Aldrich Chemical Co. 2,2,6,6-Tetramethylpiperidinooxy (TEMPO) was purchased from Aldrich Chemical Co. α-CD was purchased from Tianjin Bodi Chemical Co. Morpholinoethanesulfonic acid (MES), 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide hydrochloride (EDC), N-