Available online at www.sciencedirect.com
ScienceDirect
Separation and Purification Technology 53 (2007) 164–170
Separation Purification Technology
www.elsevier.com/locate/seppur

Thermosensitive polymer coated nanomagnetic particles for separation of bio-molecules

N. Shamim, L. Hong, K. Hidajat, M.S. Uddin *

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore

Abstract

Core–shell type thermosensitive magnetic particles were prepared via seed polymerization process. Double layer surfactant coated magnetic particles were first synthesized and then a rich poly-(*N*-isopropylacrylamide) (PNIPAM) shell layer was attached using double layer magnetic particles as the seeds. Thiodiglycolic acid was used as the primary surfactant and 4-vinylaniline as the secondary surfactant. Carboxylated thermosensitive microspheres were prepared by adding methacrylic acid (MAA) in the polymerization process. PNIPAM has a lower critical solution temperature (LCST) of 32 °C in water, and changes from hydrophilic below the LCST to hydrophobic above it. The size of these thermosensitive polymer coated magnetic particles was measured by using transmission electron microscopy (TEM). TEM results show that magnetic particles were nanosized and the calculated mean diameter of the particles was about 12 nm. Bovine serum albumin (BSA) was selected as a model protein for the separation study. Adsorption of BSA on the thermosensitive magnetic particles was mainly dependent on the properties of the particles' surface. By increasing the temperature above the LCST of PNIPAM, the particles shrank and were able to adsorb larger quantity of proteins, which was subsequently desorbed at lower temperature. It was believed that carboxylated thermosensitive particles adsorb proteins through hydrogen bonding. When the two extremes of hydrophobic interaction and hydrogen bonding were compared, it was found that more proteins are adsorbed using the later interaction.

Keywords: Double layer magnetic particles; Thermosensitive magnetic particles; Poly-(*N*-isopropylacrylamide); Lower critical solution temperature (LCST); Methacrylic acid (MAA); Bovine serum albumin (BSA); Adsorption

1. Introduction

During the past decade, much work has been done on nanostructured magnetic iron oxide particles because of its unique and novel physicochemical features, such as large surface area and high mobility and quick response [1]. Therefore, such magnetic particles have many potential applications for the magnetic separation of various chemical compounds, such as metal ions [2]; biologically active compounds, such as biochemical [3], cells [4], nucleic acid [5] and specific drug delivery [6]. The principle of this method is to bind magnetic particles with the target molecule via some intermediates to form a complex, which can be subsequently separated from the bulk solution by gradient magnetic field. Compared to conventional separation processes, the advantage of magnetic separation is attributed to its speed, accuracy and simplicity.

In recent years, interest has been focused on the design of the smart and intelligent polymeric materials for technological applications and fundamental studies. These materials can respond in shape and volume changes to small external stimuli, such as temperature, pH, ionic strength, magnetic field, etc. Among these intelligent polymeric materials, *N*-isopropylacrylamide (NIPAM) is the most widely studied thermosensitive polymer. Poly-(NIPAM) has a lower critical solution temperature (LCST) of 32 °C in water, which is very close to room temperature. It changes from hydrophilic below the LCST to hydrophobic above it, due to the reversible formation and cleavage of hydrogen bond between the amide groups and the surrounding water molecules [7]. This reversible thermosensitivity is used in biomedical or biological fields, such as enzyme immobilization, cell sorting [8–11], protein adsorption and purification [12,13] and drug delivery [14].

Adsorption is a conventional but very important separation process. It has been widely used in the chemical, biological, analytical and environmental fields. Like adsorption, desorption is also important for the recovery of target molecules. Ding et al. [15] and Elaissari and Bourrel [16] prepared submicron sized

* Corresponding author. Tel.: +65 6874 2886; fax: +65 6779 1936.
E-mail address: cheshahb@nus.edu.sg (M.S. Uddin).

1383-5866/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.seppur.2006.06.021

styrene and thermosensitive poly-(NIPAM) coated magnetic particles and showed reversible adsorption and desorption of human serum albumin (HSA) sensitive to temperature. However, Ding et al. [15] found that HSA absorbed at higher temperature (40 °C) could be desorbed from the particles at lower temperature (25 °C) but a smaller fraction. Recently, Duracher et al. [17] studied the separation of protein using aminated thermosensitive core–shell latexes and suggested hydrophobic interaction as well as electrostatic interaction is influencing the adsorption. Moreover, they investigated desorption below lower critical solution temperature of the polymer was mainly controlled by the incubation time during the adsorption step. Thus, desorption depends on various parameters. Yoshida and Kataoka [18] noted that desorption of BSA from cross-linked chitosan could be done by an alkaline buffer solution. Honda et al. [19] used alkaline condition (pH 10–13) for desorption of recombinant *Escherichia coli* from chitosan-conjugated magnetite. Quantitative effects of surface functionalized group and interaction forces on highly carboxylated microspheres was studied by Yoon et al. [20]. In addition to this they investigated the adsorption behaviors of BSA on these microspheres. However, they did not report any desorption results from the carboxylated thermosensitive nanomagnetic particles.

However, in our knowledge no work has been published to compare interacting forces on thermosensitive and carboxylated thermosensitive polymer coated nanomagnetic particles. In this work, we prepared both thermosensitive (NIPAM) and carboxylated thermosensitive (MAA-NIPAM) polymer coated magnetic particles and studied their adsorption behavior and interaction forces for adsorption of bovine serum albumin (BSA). Adsorption isotherms were obtained as a function of temperature. Throughout this paper thermosensitive and carboxylated thermosensitive polymer coated nanomagnetic particles are defines as PNIPAM and MAA-PNIPAM coated nanomagnetic particles, respectively. Desorption of BSA from the surface modified PNIPAM and MAA-PNIPAM magnetic nanoparticles were also studied.

2. Materials and method

2.1. Chemicals

The following chemicals were used in the study: Iron(II) chloride tetrahydrate (99%): Fisher (USA); Iron(III) chloride hexahydrate (98%): Nacalai Tesque (Japan); ammonium hydroxide (25%): Merck (USA); thiodiglycolic acid (98%), 4-vinylaniline (97%), *N*-isopropylacrylamide (97%), *N*,*N*-methylene bis acrylamide, potassium persulfate (99%), 2-mercaptoethanol (98%), methacrylic acid (99%) and bovine serum albumin: Sigma–Aldrich (USA). All these chemicals were used as received without further treatment.

2.2. Methods

2.2.1. Preparation of surface modified nanomagnetic particles

Magnetic particles were prepared by chemical co-precipitation method under inert environment. A complete precipitation of Fe_3O_4 was achieved under alkaline condition and the molar ratio was maintained at Fe^{2+}:Fe^{3+} = 1:2. A typical synthesis to obtain 1 g of Fe_3O_4 precipitate, 0.86 g of $FeCl_2{\cdot}4H_2O$ and 2.35 g of $FeCl_3{\cdot}6H_2O$ were dissolved under N_2 atmosphere in 40 ml of deaerated Mili-Q water with vigorous stirring at a speed of 1000 rpm. As the solution was heated to 80 °C, 100 mg of thiodiglycolic acid (TDGA) was added, followed by addition of 5 ml NH_4OH. Further, TDGA was added to the suspension in five 0.2 g amounts over 5 min. Therefore, in total 1.1 g of TDGA was added in the suspension. The experiment was continued for 30 min at 80 °C. About 0.2 ml of 4-vinylaniline was added to the fresh precipitate and the mixture was heated to about 50 °C under vigorous stirring. The experiment was carried out for 30 min. The stable water based suspension was then cooled at room temperature and washed using Mili-Q water. The particles were isolated from the solvent by magnetic decantation. The washing–decantation procedure was repeated five times to eliminate free electrolyte and remove the excess surfactants used for the coating. The primary surfactant thiodiglycolic acid is soluble in water and the solubility increases the interaction between the individual molecule of acid and the iron oxide crystal, which is important for preventing agglomeration during the early stage of crystal growth. Moreover, sulfur has great affinity for iron, thus it not only makes the orientation of the surfactant at the particle surfactant interface favorable but also some chemisorption occurs. On the other hand carboxyl group of thidiglycolic acid couples with the $–NH_2$ functional group of 4-vinyl aniline to form the double surfactant coated nanomagnetic particles.

These double surfactant coated nanomagnetic particles were then used for seed polymerization using 0.3 g *N*-isopropylacrylamide as the main monomer, 0.03 g *N*,*N*-methylene bis acrylamide (MBA) as the cross linker and 0.006 g potassium persulfate (KPS) as the initiator. 2-Mercaptoethanol was used as the chain transfer reagent. MBA was also used to increase the mechanical strength of the particles. To introduce carboxyl group in the NIPAM chain 0.18 ml MAA was added to the mixture. The reaction was allowed to proceed for 6 h at 70 °C under continuous mechanical stirring at (150–180) rpm. The reaction was conducted at a higher temperature than the LCST of the polymer in order to promote the precipitation of oligomers formed in aqueous solution. The particles were washed at least three times before adsorption experiments were carried out. The reaction procedures of thermosensitive nanomagnetic particles are shown step by step in Fig. 1.

2.2.2. Characterization of surface modified magnetic particles

A bright field TEM (Model JEM 2010) was used for the size measurement. TEM samples were prepared by coating a copper grid (200 mesh and covered with formvar/carbon) with a thin layer of dilute particle suspension. The copper grid was then dried at room temperature for 24 h before the measurement.

2.2.3. Adsorption/desorption of BSA on surface modified magnetic particles

Adsorption of BSA on both PNIPAM and MAA-PNIPAM coated nanomagnetic particles were carried out by mixing 5 ml