Adsorption/Desorption of Protein on Magnetic Particles Covered by Thermosensitive Polymers

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ABSTRACT: The adsorption and desorption behavior of protein human serum albumin (HSA) on magnetic polymer particles covered by thermosensitive polymers was investigated. The results showed that adsorption was dependent mainly on the properties of the particle surface. By increasing the temperature, particles deswelled and were susceptible to absorb larger amounts of proteins, which could be desorbed at lower temperature. The extent of adsorption was found to depend on the pH value, protein concentration, and incubation time. At higher pH, a smaller amount of proteins could be absorbed because of the electrostatic repulsive force between the protein and the surface particles. Increasing incubation time or initial protein concentration was favorable to the adsorption of proteins. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 2915–2920, 2000

Key words: thermosensitive; magnetic; particle; protein; adsorption

INTRODUCTION

The use of magnetic particles in various fields of biochemistry and medicine was described in several articles.[1,2] The area of application included diagnostics, cell separation, enzyme immunoassay, enzyme immobilization, and drug delivery.[3,4] Recently, a number of studies were carried out on poly(*N*-isopropylacrylamide) (PNIPAM) because of its thermosensitive property with a lower critical solution temperature (LCST), a property useful in studies in the biomedical or biological field, such as in drug-delivery systems and enzyme immobilization.[5–7] Crosslinked PNIPAM hydrogel or microspheres consisting of poly(NIPAM-*co*-*N*-acryloxyl succinimide) or poly(NIPAM-*co*-methylenebisacrylamide) were utilized for the separation and purification of biomacromolecules,[8,9] but PNIPAM particles have a shortcoming in their susceptibility to aggregation when they are centrifuged to separate them from the media.

Preparation of a novel multifunctional particle with thermosensitivity and a magnetic property was described in our previous article.[10] In this study, these magnetic particles were used as a support for adsorbing/desorbing human serum album (HSA) at different conditions to investigate the interactions between the protein and the particles. The results confirmed that magnetic particles covered by thermosensitive polymers were easily and rapidly separated by a magnetic field from the media without aggregates.

EXPERIMENTAL

Materials

N-Isopropylacrylamide (NIPAM, Aldrich, USA) was recrystallized from a hexane/toluene (1:1)

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mixture. Styrene (St) was treated with a 10% sodium hydrate solution to remove the inhibitor. Some other agents, including poly(ethylene glycol) (PEG, M_w = 4000), methylenebisacrylamide (MBA), potassium persulfate (KPS), and human serum album (HSA), were used without further purification.

Preparation of Particles

Fe_3O_4/P(St–NIPAM) Particles (PSN)

NIPAM (4.0 g), St (11.0 g) and PEG (6.0 g) were dissolved in a mixture of ethanol (66.0 mL) and water (39.0 mL) in a 250-mL four-necked flask after stirring 30 min using an Fe_3O_4 magnetic fluid (10.0 g) with an average size of 50 nm (which was prepared by the precipitation–oxidation method as mentioned in our previous work[10]). KPS (0.45 g) was placed into the flask and the reaction was carried out under a nitrogen atmosphere and continued for 24 h at 70°C with 300 rpm stirring. The resulting particles were purified by repetitive magnetic field separation and decantation and the above purification procedure was repeated after the particles were immersed in a 1*M* HCl solution for 48 h.

PSN/N Particles

PSN/N particles were prepared by seed polymerization of NIPAM using PSN particles as seeds: PSN particles, 2.0 g, as mentioned above, were dispersed in 110 mL distilled water in a 250-mL four-necked flask, and 4.0 g PEG, 0.5 g NIPAM, and 0.05 g MBA were added to the suspension. Nitrogen was bubbled into the mixture for 40 min under 300 rpm stirring. Polymerization was initiated by adding 0.09 g KPS at 70°C. Twenty milliliters of an aqueous solution containing 1.0 g NIPAM and 0.10 g MBA was fed into the mixture within 1.5 h and the reaction proceeded for 6 h. The resulting particles were cleaned by repetitive magnetic separation and decantation.

Characterization of Particles

The morphology, size, and size distribution of the dried particles were determined by scanning electron microscopy (AMRAY-1000), followed by statistical treatment using a computer system, and the hydrodynamic size was determined using a laser diffraction size analyzer (SALD-2001, Shimadzu, Japan). The supernatant transmittance of the particle suspension solution after being separated by a magnetic field or sedimentation at a certain time was measured using a spectrophotometer at 580 nm.

Protein Adsorption and Desorption

A 3-mL suspension solution, containing 0.05 g of PSN or PSN/N particles, was mixed with a 4 mL HSA solution. Protein adsorption/desorption was carried out at the desired pH values and temperature. The concentration of the protein remaining in the supernatant was determined by an UV spectrophotometer at 280 nm to calculate the amount of protein adsorbed/desorbed after being separated by magnetic separation in a magnetic field with an intensity of 0.42 T.

RESULTS AND DISCUSSION

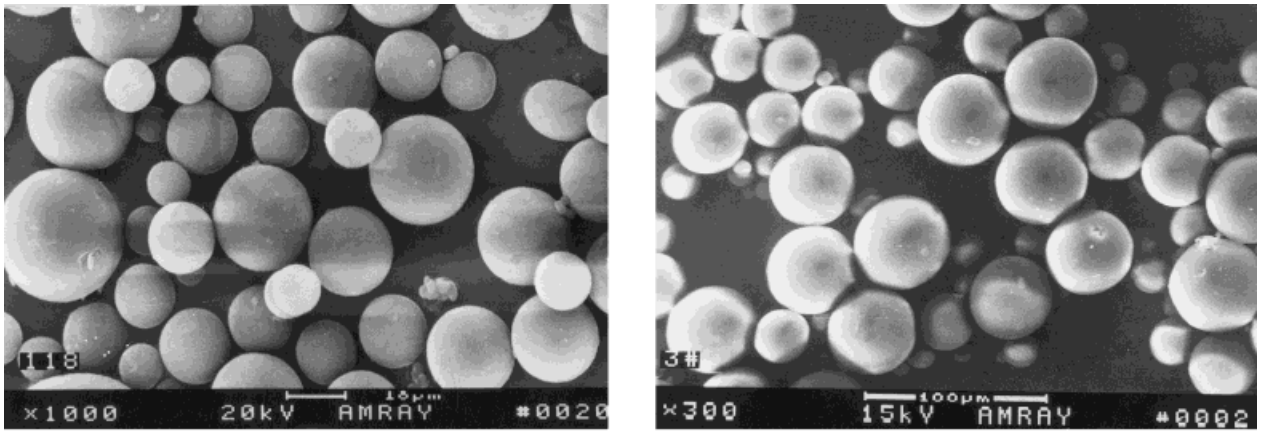
Thermosensitive Magnetic Particles

Figure 1 shows the morphology of PSN and PSN/N particles. It was observed that both PSN and PSN/N particles are polydisperse, and the statistical treatment showed that the particle-size distribution was in accord with the Gaussian distribution. The hydrodynamic size in water of both PSN and PSN/N depended on the temperature, as shown in Figure 2, which exhibited a 10–20% decrease in diameter from 25 to 40°C, while the diameter of Fe_3O_4/PSt did not display similar behavior as the temperature increased. On the contrary, it became slightly larger because of hot-swelling.

Figure 3 shows that both PSN and PSN/N particles exhibited an advantage using magnetic separation. The supernatant transmittance of the particle suspension was above 90% after being separated by a magnetic field within 5 min, compared to sedimentation which needed at least 2 h.

Protein Adsorption

HSA protein adsorption was carried out on PSN and PSN/N particles at different temperatures between 30 and 45°C. The amount of HSA absorbed on the particles is shown in Figure 4. It was observed that a larger amount of protein was absorbed at higher temperature. Two effects would be caused by increasing temperature: On one hand, particle volume and surface area would be decreased, thus resulting in a smaller amount of HSA that could be absorbed. On the other hand, the affinity for the protein adsorption tends to increase, because the particle surface changes from hydrophilic to hydrophobic as the tempera-



a PSN b PSNN

Figure 1 Scanning electron micrographs of magnetic particles covered by thermosensitive polymers.

ture increases, resulting in a larger amount of HSA being absorbed. The experimental results indicate that temperature-dependent adsorption of HSA on PSN or PSNN/N particles is attributed mainly to the changes in the properties of the particles' surface. Figure 4 shows also that the amount of protein absorbed on PSN was larger than that on PSN/N at the same incubation temperature, and the adsorption behavior was different between them. The cause of this phenomenon probably is that the hydrophilicity of the outer shell is different between PSN and PSN/N.

The results of the protein absorbed at different pH values are shown in Figure 5. The amount of HSA absorbed decreased dramatically as the pH value increased. Because PSN or PSN/N particles

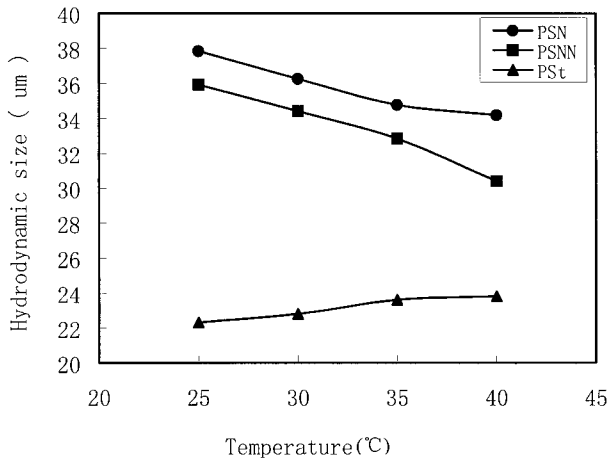
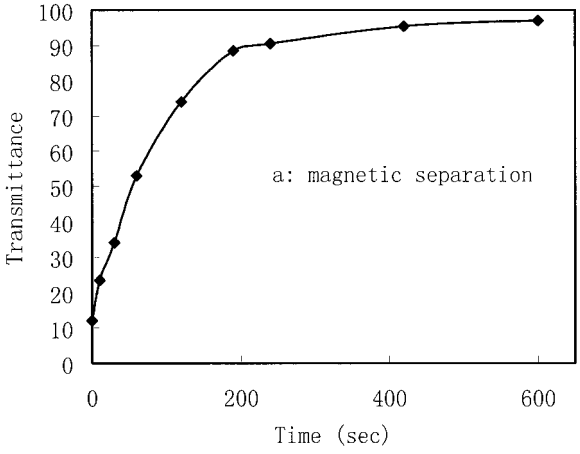


Figure 2 Particle hydrodynamic sizes in distilled water at different temperatures.



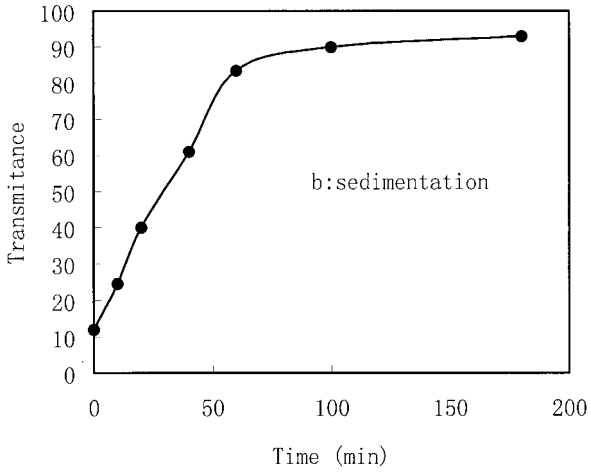


Figure 3 Transmittance of magnetic particle suspensions separated by a magnetic field or sedimentation at different times.

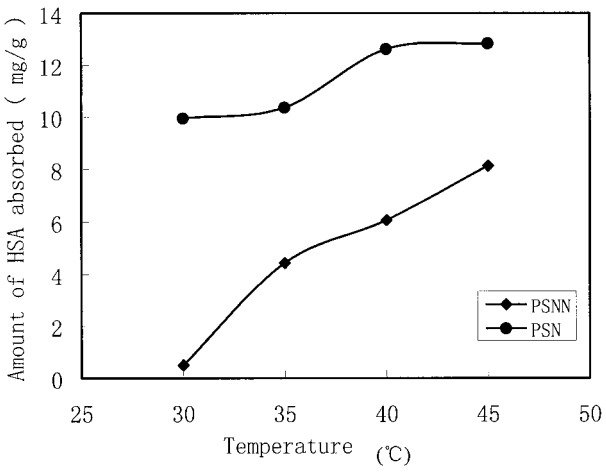


Figure 4 Amount of HSA adsorbed on PSN and PSN/N particles at different temperatures: pH 4; incubated for 30 min; HAS = 1.0 mg/mL.

have an anionic initiator residue (SO_2^{-2} group) at the polymeric chain ends and the HSA molecule has a negative charge at pH values above its isoelectric point (~4.6), there is an electrostatic repulsive force between the HAS molecules and the PSN(N) particles at higher pH values. These results imply that the electrostatic repulsive force seems to be one of the most dominant factors, like temperature, affecting the adsorption.

The effect of incubation time on protein adsorption was also investigated. These results are shown in Figure 6. It was observed that a protracted incubation time is beneficial to the adsorption. The amount of adsorption did not increase linearly with the incubation time, but increased rapidly within 15 min with a lower increase when the incubation time was longer than 15 min.

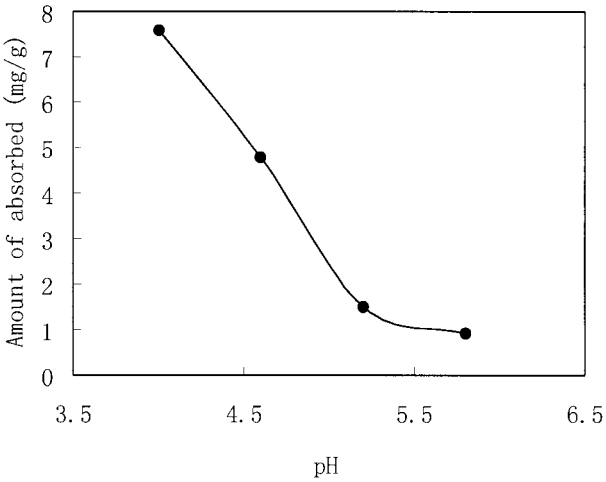
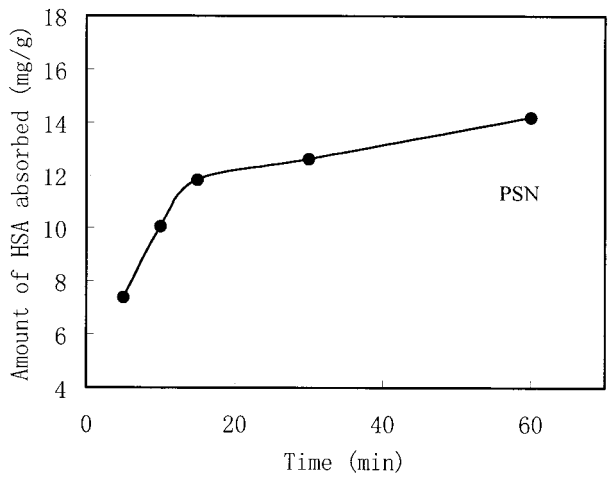


Figure 5 Effect of pH on adsorption of HSA on PSN/N particles incubated at 40°C for 30 min; HSA = 1.0 mg/mL.



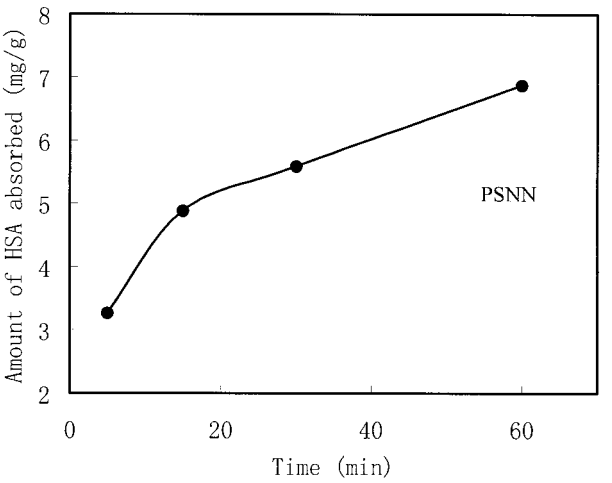


Figure 6 Adsorption of HSA on PSN and PSN/N particles at different incubation times: pH 4.0; HAS = 1.0 mg/mL.

Experiments were also performed with different HSA concentrations in order to investigate the effect of the initial concentration of HSA on the adsorption. These results are shown in Figure 7. As the initial concentration of HSA increased, the amount of HSA absorbed also increased. The adsorption behaviors are similar to those of shown in Figure 6, namely, an increasing initial concentration of HSA leads to similar results as those of a protracted incubation time.

Protein Desorption

Figure 8 shows that the protein absorbed on the particles at higher temperature could be desorbed

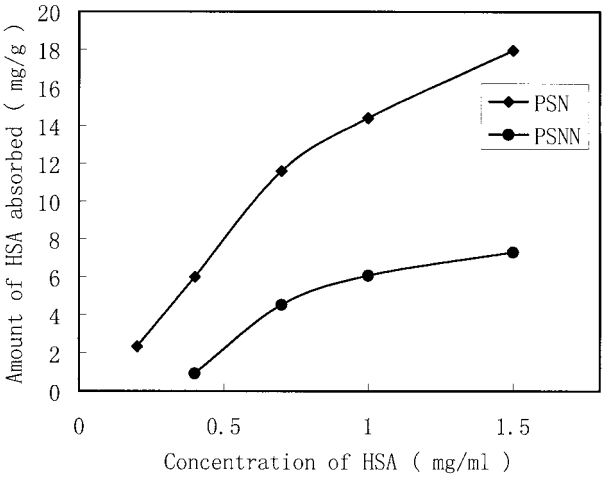


Figure 7 Effect of HSA concentration on adsorption of HSA on magnetic particles.

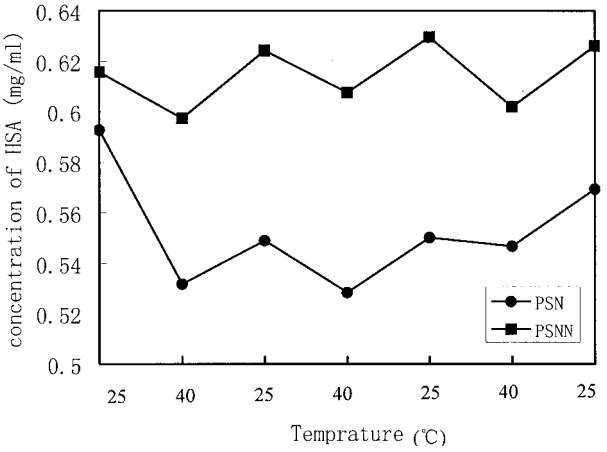


Figure 9 Variations of the concentration of HSA in solutions of PSN/N and HAS mixture, measured alternately at 40°C (incubated for 30 min) and 25°C (incubated for 60 min) at pH 4.0 and HAS = 1.0 mg/mL.

at lower temperature (25°C). The affinity of the adsorption between the protein and the particle could be changed, along with the hydrophilicity of the particle surface, which could be changed reversibly from the hydrophilic state to the hydrophobic state by changing the temperature alternately between lower (25°C) and higher temperature. This is because of the reversible formation and cleavage of the hydrogen bond between the amide group and the surrounding water molecules with changing temperature.[9] The amount of HSA absorbed on the particles increased with increasing temperature, but the smaller fraction of HSA absorbed at higher temperature could be desorbed from the particles at 25°C. It seems that the absorbed protein molecules can be easily deformed by the interactions between the protein molecules and the polymeric chains or the electrostatic force at higher temperature; therefore, only a small fraction of undeformed HSA could be desorbed from the particles.

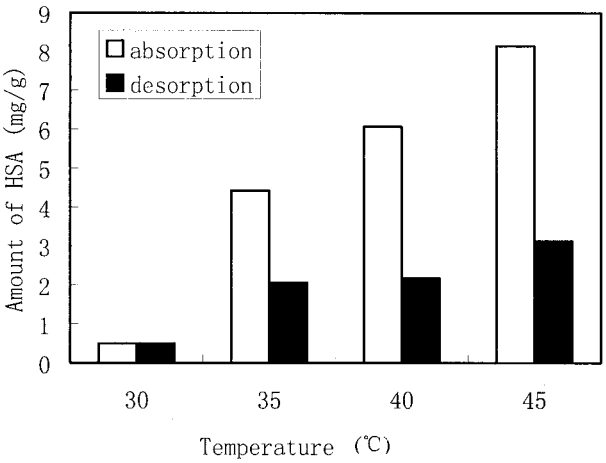


Figure 8 Desorption of HSA absorbed on PSN/N at higher temperatures for 30 min by lowering the temperature to 25°C. (Total bar) Amount of HSA adsorbed at 40°C for 30 min; (solid part of bar) amount of HSA desorbed at 25°C for 60 min.

Figure 9 shows the change of the HSA concentration in the mixture with the temperature changed alternately between 40 and 25°C. It was observed that, in both PSN and PSN/N particles, HSA concentrations at 25°C were higher than those at 40°C; in other words, a large amount of HSA could be absorbed on PSN or PSN/N particles at 40°C and some of them could be desorbed at 25°C. This behavior was reversible between 40 and 25°C. The results of cycling experiments confirmed that the adsorption exhibited good reproducibility and the particles did not form aggregates with each other when they were separated from the media by a magnetic field (Fig. 10). From the above-mentioned results, we can conclude that the reversible thermosensitivity of PSN or PSN/N particles and their easy magnetic separation property would be considered useful in some fields, such as in the purification and separation of protein or the recovery of enzymes.

CONCLUSIONS

Our investigation confirmed that the adsorption/desorption of protein on thermosensitive magnetic particles had a remarkable temperature

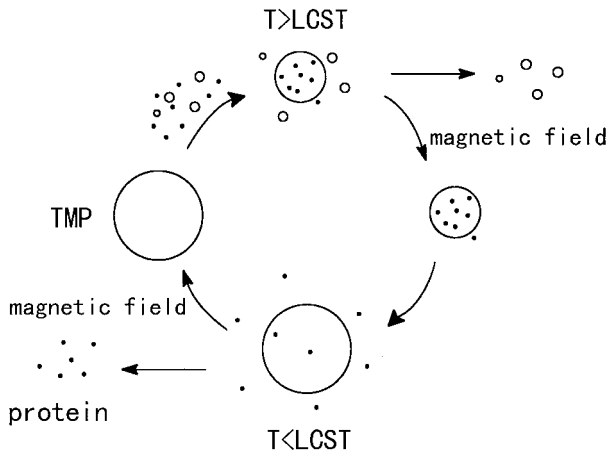


Figure 10 Protein separation scheme for thermosensitive magnetic particles.

sensitivity. The extent of adsorption was influenced by the pH values, the incubation time, and, also, the initial concentration of the protein. The results of the adsorption–desorption cycles implied that magnetic particles covered by thermosensitive polymers have the potential to be used in the separation of some biomacromolecules such as protein and enzymes.

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