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Preparation of Polyelectrolyte Brush Layer Modified Magnetic Particles for Separation of Proteins

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Abstract. Magnetic particles are a promising tool for separation of substantial proteins by applying an external magnetic field to collect the particles after adsorption of proteins. To provide much functionality on the magnetic particles for protein separation under mild condition and by easy procedures, polyelectrolyte brush layer was constructed on the surface. Magnetic polystyrene particles were prepared by mini-emulsion polymerization. Then the particles were modified with hydrophilic polyelectrolyte brush layer with different electric charges via atom transfer radical polymerization (ATRP). The polyelectrolyte brush layer could play an important role to endow the particles with good dispersion ability and also, control protein adsorption by electrostatic interaction. That is, the proteins having electric charges under biological environment could be adsorbed onto the magnetic particles with opposite-charged polymer brush layer and quickly collected from the solution. Thus we conclude that the magnetic particles with a polymer brush layer provide excellent functions.

1. Introduction

Protein separations via conventional methods like chromatography and electrophoresis play an important role in the widespread applications ranging from diagnostics to therapeutics. However, such methods generally suffer from long separation time and complicated manipulations [1, 2], especially when dealing with massive mixture of various proteins. Therefore, a pre-separation procedure was considered to divide the proteins into several groups beforehand to facilitate the application of such column-based methods. Thereupon, magnetic field-based separations using magnetic particles that enable rapid and easy removal of functionalized magnetic particle-bound organisms from mixtures have recently drawn considerable attention. [3, 4]

Generally, mini-emulsion polymerization was a good method to prepare monodispersed polymer particles of nanoscale. By encapsulatingFe_3O_4particles in the droplet during the emulsion process, monodispersed magnetic polystyrene particles could be prepared. Moreover, it is very important for selective adsorption of proteins to design the interface between particles and proteins. Previous research showed that highly dense polyelectrolyte brush layers could adsorb proteins with opposite net charges and intensively repel those with the same net charges. [5]Atom transfer radical polymerization (ATRP) was an effective way to fabricate such polymer brush. Thus, in this research,2-(2-bromoisobutyryloxy) ethyl methacrylate (BIEM), the ATRP initiator, was synthesized and immobilized onto the surface of magnetic polystyrene particles and then different polyelectrolyte brush layers were separately fabricated on magnetic polymer particles surface via ATRP for the sake of pre-separation by charge. Typically, to reduce influence of hydrophobic interaction, hydrophilic monomers,[2-(methacryloyloxy)ethyl] trimethylammonium chloride (TMAEMA), 3-sulfopropyl methacrylate potassium (SPMA) and 2-methacryloyloxyethyl phosphorylcholine (MPC) were used to

fabricate positive, negative and neutral polyelectrolyte brush layers, respectively. Schematic representation of preparation procedure of polyelectrolyte brush layer modified magnetic particles is showed in figure 1.

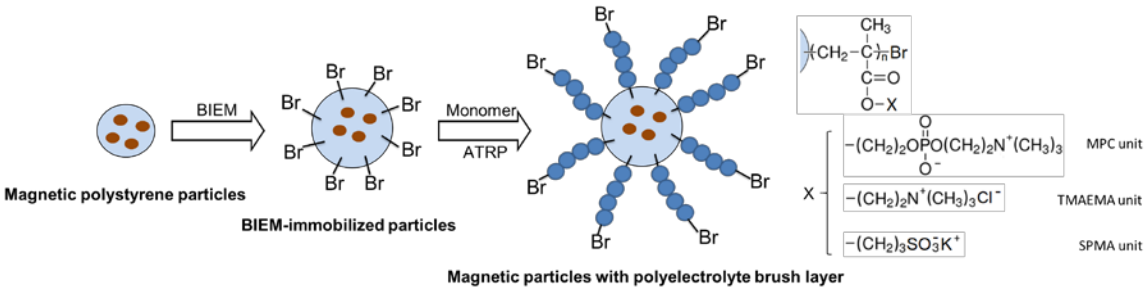

Figure 1. Scheme of preparation of polyelectrolyte brush layer on the magnetic particles

2. Experiment

2.1 Preparation of magnet particles

2.1.1 Synthesis of magnetic polystyrene particles. The magnetic polystyrene core particles were prepared by mini-emulsion polymerization. To start with, Fe_3O_4 (0.20 g) was dispersed in chloroform (4.5 g) by ultrasonic. Next styrene (3.0 g) was added into the mixture and chloroform was removed subsequently by evaporation, leaving Fe_3O_4 dispersed in styrene. Then divinylbenzene (DVB) (0.015 g) and lauroyl peroxide (LPO) (0.06 g), which served as cross-linker and initiator respectively, were added into the mixture. Afterwards, the mixture was added into aqueous solution (50 mL) of sodium dodecyl sulfate (SDS) (0.072 g), followed by ultrasonic handling for 15 min. Finally, the emulsion was transferred into a 2-neck flask and after argon gas babbling for 10 min, mini-emulsion polymerization was carried out at 70°C with mechanical stir for 20 hours. After the reaction ended, the emulsion was filtered by coarse and fine sieve successively.

2.1.2 Surface immobilization with BIEM. BIEM was synthesized as follows.2-Hydroxyethyl methacrylate (HEMA) (5.2 g) and triethylamine (TEA) (4.5 g) were diluted with dichloromethane (60 mL). Next, α-bromoisobutyryl bromide (BIBB) (9.4 g) was dissolved in 20 mL dichloromethane and added to the solution under cooling. After 24 h with stirring, the reaction finished. Then BIEM was immobilized on the particles surface by seed emulsion polymerization. [6]First, 33 mL of the filtered emulsion containing polystyrene particles and 17 mL of SDS aqueous solution (1 mg / mL) were added into a flask. Next, BIEM (purity: 99%) (560 mg), DVB (13 mg) and potassium persulfate (KPS) (28 mg) were added into the mixture. Finally, after argon gas babbling for 10 min and mechanical stir for 2 h, the reaction was conducted at 70°C with mechanical stir for 20 h. The BIEM-immobilized particles were separated from the emulsion by external magnetic field and dispersed in SDS aqueous solution.

2.1.3 Preparation of polyelectrolyte brush layer by ATRP. For the BIEM-immobilized particles, surface modification of highly dense polymer brush layers was conducted via ATRP with different monomers, MPC, TMAEMA and SPMA, respectively. Briefly, predetermined amounts of CuBr, 2, 2'-bipyridyl (Bpy) and monomer were added into a test tube and dissolved in the solution of degassed methanol and water. Then the BIEM-immobilized particles and ethyl α-bromoisobutyrate (EBIB), the free radical initiator, were synchronously added to the solution. After 10 min of argon gas babbling, polymerization was conducted with stirring at 40°C for 20 hours.

2.2 Characterization of the magnetic particles with polyelectrolyte brush layer

2.2.1 Fe_3O_4 content and magnetic response. The Fe_3O_4content of magnetic polystyrene core particles was measured by thermal gravimetric analysis (TGA). The collecting rate of the BIEM-immobilized particles was determined as follows. The particles were dispersed in water in a sample bottle and the initial absorption of light at 600 nm was determined by photometer V-560, denoted as I_i. Then a neodym magnet, with a surface magnetic flux density of 520 mT, was put next to the bottle and the absorption of light at 600 nm was measured every 10 min, denoted as I_t. Correspondingly, the collecting rate (CR)could be regarded as a function of time and calculated as equation (1):

$$CR = 1 - \frac{I_t}{I_i} \quad (1)$$

2.2.2 Surface properties of the magnetic particles with polyelectrolyte brush layer. The average size of the particles was determined by dynamic light scattering (DLS) and scanning electron microscope (SEM) image. Surface zeta-potential of the particles was determined by Malvern zetasizer. And the surface functional groups of the particles were examined by Fourier transforms infrared spectrometer (FT-IR).

2.3 Protein adsorption of the surface modified particles
Bovine serum albumin (BSA) and lysozyme were used for the adsorption experiment. The polyelectrolyte brush layer modified particles (0.2 mg) were added separately to the BSA and lysozyme proteins solutions (pH = 7.4, 1 mL). Then the solutions were incubated at 37°C for 2 h. Afterwards the particles were collected from the solutions by external magnetic field for 30 min. Then μBCA assay was conducted to determine the remaining proteins concentration and the amounts of adsorbed proteins were calculated.

3. Results and discussion

3.1 Preparation of the particles
The IR spectra of the particles at different stages were showed in figure 2 below. The two peaks at 1500 cm^{-1} and 1600 cm^{-1} belonged to the phenyl group of styrene unit; and the peak at 1730 cm^{-1} belonged to the carbonyl groupof BIEM. Therefore, it could be known that magnetic polystyrene core particles were successfully prepared by mini-emulsion polymerization and BIEM groups were immobilized on the surface. Besides, peaks at 970 cm^{-1}, 1070 $cm^{-1}$and 1250 cm^{-1} belonged to the PTMAEMA, PSPMA and PMPC, respectively. It indicated that the polymer brush layer was fabricated onto the particles surface via ATRP.

3.2 Characterizations of the different particles
The Fe_3O_4 content of the magnetic polystyrene core particles was 30% and the magnetic response of the BIEM-immobilized particles was showed in figure 3. The magnetic polymer particles can be collected fast by external magnetic field (80% within 30 min) and can quickly disperse in water again by ultrasonic. It should be noted that an increase in Fe_3O_4 content could accelerate the collection of particles. Moreover, the average size and surface zeta-potential of the different particles were summarized in table 1 below. The PTMAEMA, PSPMA and PMPC modified particles had similar sizes, about 500 nm but they showed different zeta-potentials, 40 mV, -37 mV and -2 mV, respectively. The SEM image of the PTMAEMA brush layer modified particles was showed in figure 4 below. From the image, most of the particles were sphere and the average diameter was about 500 nm, which accorded with the results of DLS.

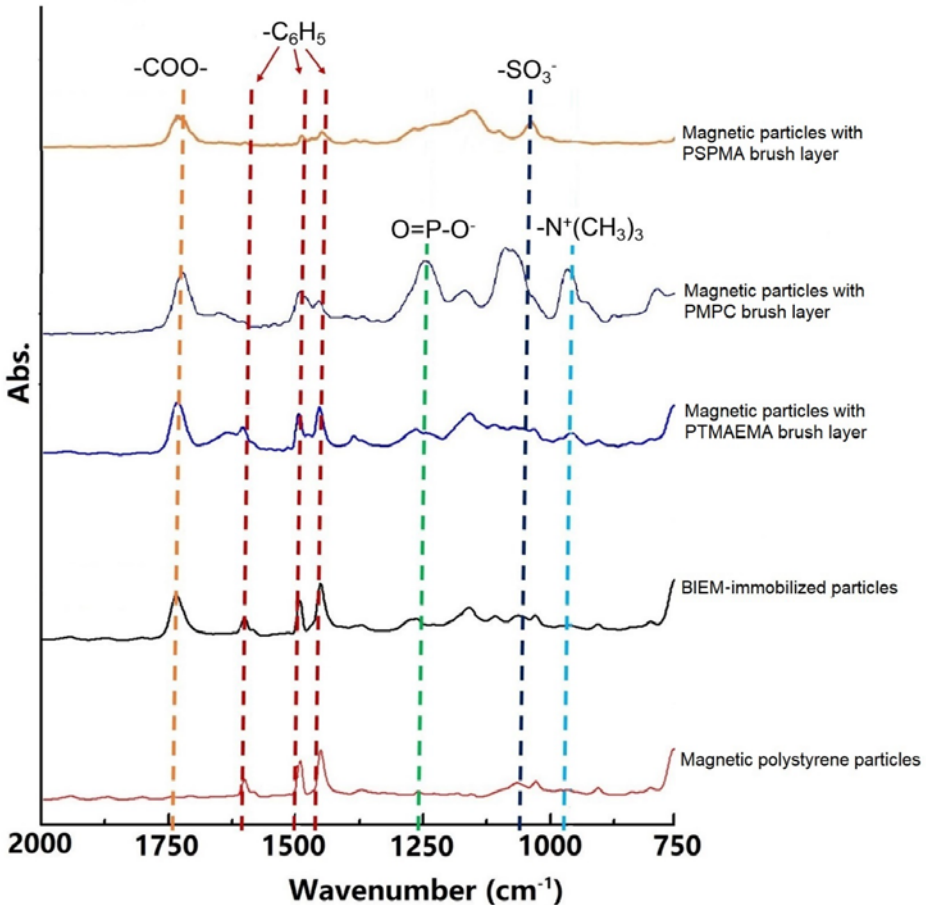

Figure 2. IR spectra of the magnetic particles without/with polyelectrolyte brush layer

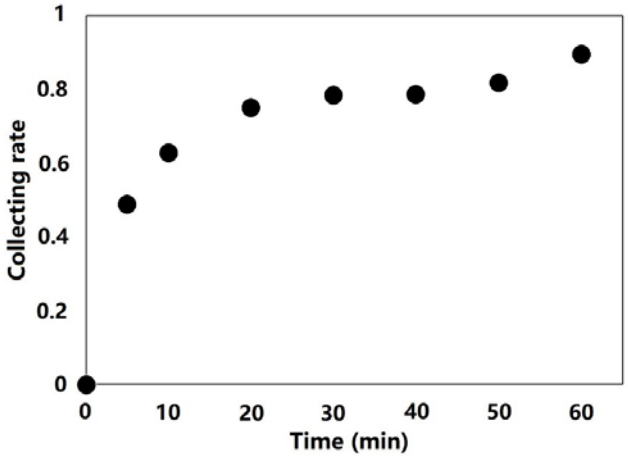

Figure 3. Magnetic response of the BIEM-immobilized particles in water

Table 1. Characteristics of magnetic nanoparticles with various polyelectrolyte brush layers

Particles with different brush layer	PTMAEMA	PSPMA	PMPC
Size (d. nm)	480 ± 15	520 ± 20	550 ± 10
Zeta-potential	40 mV	-37 mV	-2 mV

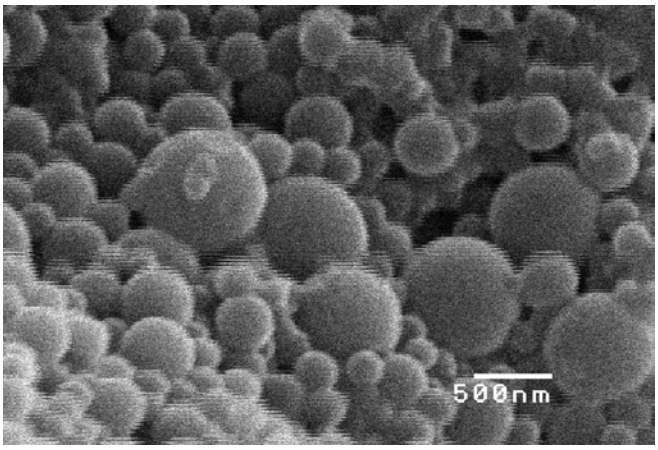

Figure 4. SEM image of the particles with PTMAEMA brush layer

3.3 Proteins adsorptions

Such three kinds of particles exhibited totally different proteins adsorption behaviours. The results were showed in figure5 and figure6 below. In detail, particles with PTMAEMA brush layer displayed good efficiency of adsorbing negative charged BSA proteins (600 ng/cm^2) while hardly adsorbed lysozyme that was positive charged. However, the particles with PSPMA brush layer (1000 ng/cm^2 for lysozyme) were in complete antithesis to PTMAEMA ones. And neither BSA nor lysozyme was much adsorbed by particles with PMPC brush layer. Actually, proteins adsorption and desorption simultaneously existed on the particles surface. While the proteins adsorption rate was almost the same for the three polymer brush layers, the electrostatic interaction between brush layers and proteins with opposite charges could significantly decrease the desorption rate, which finally caused the proteins adsorbed onto the surface. So the adsorption mass of proteins corresponded to the electrostatic interaction forces on the surfaces and their charge properties. [5] Moreover, there existed a maximum amount of adsorbed proteins for a certain number of particles.

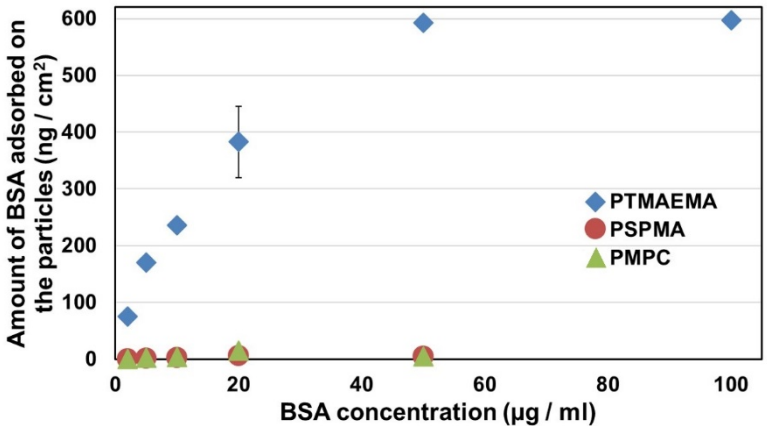

Figure 5. Amount of BSA adsorbed on the magnetic particles with polyelectrolyte brush layer

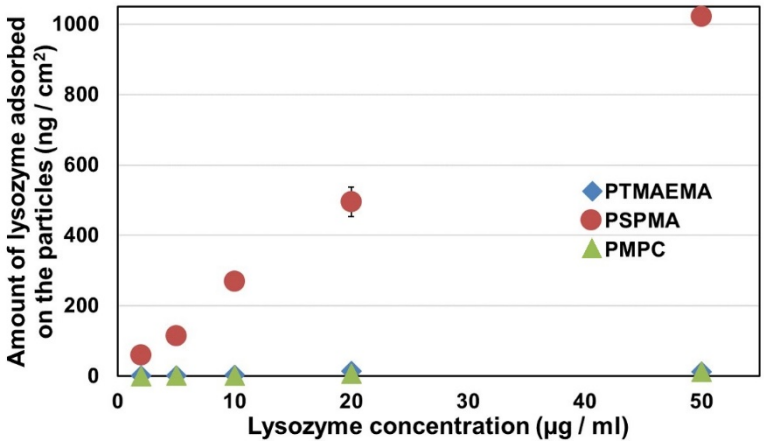

Figure 6. Amount of lysozyme adsorbed on the magnetic particles with polyelectrolyte brush layer

4. Conclusion

Magnetic polystyrene particles were successfully prepared by mini-emulsion polymerization; and the ATRP initiator BIEM could be immobilized onto the surface of the particles via seed emulsion polymerization.

Moreover, the surface of particles could be modified with PTMAEMA, SPMA and PMPC brush layer via ATRP, respectively. The modified particles had similar sizes but different zeta-potentials, which had much influence on their proteins adsorption behaviours.

Hydrophilic polyelectrolyte brush layer modified magnetic particles could control the proteins adsorption by electrostatic interaction and realize a rapid and easy collection by external magnetic field. Thus, we conclude the magnetic particles with a polymer brush layer provide excellent functions.

5. References

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