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Thermosensitive and control release behavior of poly(N-isopropylacrylamide-co-acrylic acid)/nano-Fe_3O_4 magnetic composite latex particle that is synthesized by a novel method

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ABSTRACT

In this study, a novel method was used to synthesize the poly(*N*-isopropylacrylamide-*co*-acrylic acid)/Fe_3O_4 (poly(NIPAAm-AA)/Fe_3O_4) magnetic composite latex. The crosslinked poly(NIPAAm-AA) polymer latex particles were first synthesized by the method of soapless emulsion polymerization, then Fe^{2+} and Fe^{3+} ions were introduced to bond with the –COOH groups of AA segments in poly(NIPAAm-AA) polymer latex particles. Further by a reaction with NH_4OH, Fe_3O_4 nanoparticles were generated in situ. The concentrations of acrylic acid (AA), crosslinking agent (*N*,*N*′-methylene bisacrylamide (MBA)), and Fe_3O_4 nanoparticles were important factors to influence the morphology and lower critical solution temperature (LCST) of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles. The poly(NIPAAm-AA)/Fe_3O_4 latex particles were used as a thermosensitive drug carrier to load caffeine. The control release of caffeine was studies. Morphology-based schematic models were proposed to explain the control release behavior of the composite particles with different compositions. Moreover, the protein (albumin, acetylated from bovine serum (BSA)) was bound on the surface of poly(NIPAAm-AA)/Fe_3O_4 composite latex particles. The effects of AA, crosslinking agent and Fe_3O_4 contents on the amount of BSA binding were investigated at different temperatures and pH values. The composition–morphology–BSA conjugation relationship was established.

1. Introduction

More and more attentions have been drawn to the preparation of different kinds of magnetic polymeric particles in the past decades due to the application of magnetism to various fields, such as cell separation [1–4], protein purification [5], targeting drug delivery [6–9], environment and food analyses [10], organic and biochemical syntheses [11], and industrial water treatment [12].

Several methodologies on the preparation of magnetic polymeric latexes have been investigated as reported [13–20]. Emulsion and emulsifier-free emulsion polymerization are the most frequently used method for the encapsulation of magnetic nanoparticles with polymers, in which the magnetic nanoparticles are firstly coated by a surfactant double layer to enhance the encapsulation. Yanase et al. [13] synthesized the magnetic latex by a batch emulsion polymerization of styrene in the presence of ferrofluid. Xie et al. [14] investigated the effects of polymerization parameters on the magnetic poly(styrene-butyl acrylate-methacrylic acid) latex via emulsifier-free emulsion polymerization. By using the similar method, Kondo et al. [21,22]

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prepared the core-shell magnetic latex particles with polystyrene in the core and poly(*N*-isopropylacrylamide) in the shell. In our previous study, poly(methyl methacrylate) latex containing magnetic nanoparticles were synthesized by using soapless dispersion polymerization, in which the lauric acid was used to coat the magnetic nanoparticles to form a surfactant double layer [4,23]. Besides, potassium persulfate and 2,2′-azobis(2-methylpropionamidine) dihydrochloride were used as initiators to synthesize Fe_3O_4/polystyrene/poly(isopropylacrylamide-*co*-methyl acrylic acid) thermosensitive magnetic composite latex, respectively. The results showed that the property of initiator significantly influenced the morphology of magnetic composite latex [24,25]. Recently, direct or inverse miniemulsion polymerization has been applied to encapsulate inorganic nanoparticles in polymer matrix. Ramirez et al. [18] encapsulated magnetic particles by polystyrene successfully by using a new three-step preparation route including two miniemulsion processes. Wormuth [15] used a W/O miniemulsion polymerization to encapsulate the magnetic nanoparticles, which was stabilized by poly(ethylene-*co*-butylene)-b-poly(ethylene oxide) (PEB-PEO) diblock copolymer, 2-hydroxyethylmethacrylate (HEMA) and methacrylic acid (MAA) in decane continuous phase. The supraparamagnetic polymeric particles thus prepared have the average diameter between 140 and 220 nm with uniform encapsulation and contain 18wt% magnetic iron oxide. However, when these magnetic polymeric particles are applied in the biological field, the small size of the latex particles could provide larger surface area for functional purposes, but the magnetic separation becomes much more difficult [21,22].

The magnetic polymeric particles can be manufactured to form the targeting drug delivery by the way of bonding the protein on the surface of magnetic polymeric particles. Most of the works pointed out that the protein requires some chemical reaction to bond on the carboxylic latexes [26–28]. Recently some authors [29–30] have indicated that the amine groups of the protein molecules could direct react with the aldehyde groups of the particle surface.

Although the methods based on emulsion or emulsifier-free emulsion polymerization have the potential to yield composite latex containing more than 20 wt% magnetic nanoparticles, they also might cause latex coagulation, or incomplete and nonuniform encapsulation depending on the composition and synthesis conditions, and the nucleation mechanism [23]. To circumvent this problem, thermoresponsive and supraparamagnetic (poly(NIPAAm-AA)/Fe_3O_4) magnetic composite latex was synthesized by a novel method in this study. This novel method prevents the disadvantage of latex coagulation, or incomplete and nonuniform encapsulation that was caused by the method of emulsion or emulsifier-free emulsion polymerization. The effects of AA content, Fe_3O_4 content and crosslinking degree on the morphology, thermosensitive property and drug release behavior of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles were studied.

2. Experiment

2.1. Synthesis of poly(NIPAAm-AA) copolymer latex

The ingredients and reaction condition for the synthesis of poly(NIPAAm-AA) copolymer latex were listed in Table 1. Ammonium persulfate (APS) was used as an initiator. The polymerization was carried out at 70 °C for 2 h by the method of sopaless emulsion polymerization under a nitrogen atmosphere. The separation of latex particles and unreacted monomers was carried out by centrifugation at 70 °C.

2.2. Synthesis of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex

$FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ were added into the reactor to uniformly mix with the poly(NIPAAm-AA) latex for 30 min at the temperature of 25 °C. Then NH_4OH was added into the mixture to react with $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ and produce Fe_3O_4 nanoparticles. The ingredients and reaction condition for the synthesis of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex were listed in Table 2.

Table 1
Ingredients and reaction condition for the synthesis of poly(NIPAAm-AA) copolymer latex

NIPAAm (g)	2
AA (g)	0.013 (AA 1%), 0.026 (AA 2%), 0.052 (AA 4%)
MBA (g)	0.055 (MBA 2%), 0.11 (MBA 4%), 0.165 (MBA 6%)
APS (g)	0.12
Deionized water (g)	300
Reaction time (h)	2
Reaction temperature (°C)	70
Stirring rate (rpm)	300

Conversion of monomers is about 96%.
AA 1% means that mole ratio of AA/(NIPAAm + AA) = 1%.
MBA 2% means that mole ratio of MBA/(NIPAAm + AA) = 2%.

Table 2
Ingredients and reaction condition for the synthesis of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex

	Poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex with Fe_3O_4 2%	Poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex with Fe_3O_4 3%	Poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex with Fe_3O_4 4%
Poly(NIPAAm-AA) latex emulsion (g)	300	300	300
$FeCl_2{\cdot}4H_2O$ (g)	0.074	0.111	0.148
$FeCl_3{\cdot}6H_2O$ (g)	0.2	0.3	0.4
NH_4OH (ml)	3	3	3
Deionized water (g)	10	10	10
Reaction time (h)	1	1	1
Reaction temperature (°C)	25	25	25
Stirring rate (rpm)	300	300	300

The calculation of the weight of Fe_3O_4 is based on the reaction as: $Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe(OH)_2 + 2Fe(OH)_3 \rightarrow Fe_3O_4 + 4H_2O$.
Fe_3O_4 2% means that the mole ratio of Fe_3O_4/(NIPAAm + AA) = 2%.

2.3. Morphology of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles

To observe the morphology of magnetic composite latex particles, the latex solution was diluted with deionic water and dripped on a copper grid and then was observed by using a JEOL JSM-1200 EX II transmission electron microscope (TEM).

2.4. Measurement of LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles

About 0.1 g of poly(NIPAAm-AA)/Fe_3O_4 latex was poured into a buffer solution (10 g, pH 7.4) at room temperature. Afterwards the sample was taken out and measured by UV spectrophotometer. The LCST of poly(NIPAAm-AA)/Fe_3O_4 latex could be determined from the curve of relationship between UV light absorbance and temperature.

2.5. Caffeine releasing experiment

Pre-weighted dried sample, poly(NIPAAm-AA)/Fe_3O_4 latex particles, (0.3 g) was immersed in a deionized water containing caffeine (0.024 g caffeine/10 g deionization water), followed by ultrasonication for 10 min, and kept at temperature of 4 °C for 3 h to attain the swelling equilibrium. Then, the sample solution was freeze dried to obtain the caffeine incorporated copolymer powder. About 0.05 g of this powder was placed on a mold and pressed with 9 tons of force for 30 or 15 min, and the caffeine incorporated copolymer disk (tablet) with 1 cm diameter was prepared. The caffeine releasing was measured under pressed disk rather than polymer particles due to the reason that the UV–Vis spectrum of latex particles would interfere with the measurement of caffeine. The caffeine incorporated dry copolymer disk was immersed into a phosphate buffer solution (50 g, 37 °C, pH 7.4) The quantity of caffeine release from sample into buffer solution was measured using a UV–Vis spectrophotometer by detecting the characteristic absorbance of caffeine at 272 nm. The accumulative caffeine release (Wn) could be calculated as follows:

$$Wn = Cn \times (50 - 3(n-1)) + 3 \times (C_1 + \ldots + C_{n-1}) \tag{1}$$

Cn is the caffeine concentration of the nth sampling (3 g for each sampling), which was measured by UV–Vis.The caffeine release percentage % (Rc %) could be calculated as follows:

$$Rc\% = Wn/\text{amount of caffeine loaded in a sample disk} \tag{2}$$

2.6. Protein conjugation

Pre-weighed dried poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex (0.01 g) was immersed in a pH 7.4 (or pH 9)phosphate buffer solution (10 g), followed by ultrasonification for 10 min to mix the composite latex in the solution homogeneously. Then 0.004 g BSA was added into the solution, followed by re-ultrasonification for 3 min. After the solution was placed in a 25 or 37 °C thermostatic environment for 2 h, 0.004 g EDC was added into the solution for 4 h to conjugate BSA on the surfaces of the composite latex particles. For estimating the amount of BSA conjugation on the surfaces of composite latex particles, the solution was centrifuged for 20 min at 12,000 rpm. The quantities of BSA remaining in the solution, which were not conjugated on the particles, were determined using UV–Vis spectrophotometer by detecting the characteristic absorbance of BSA at 280 nm.

3. Results and discussion

3.1. Morphology of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles

The poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles containing more amount of AA would integrate with more amount of Fe_3O_4 nanoparticles as shown in Fig. 1(a) and (b). The poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles with large amount of AA would swell well in the aqueous system. Therefore, they showed a larger particle size. The concentration of crosslinking agent was the important factor to influence the morphology of poly(NIPAAm-AA)/Fe_3O_4 latex particles. Fig. 2(a) and (b) showed that Fe_3O_4 particles distributed in the core of poly(NIPAAm-AA)/Fe_3O_4 latex particles when the crosslinking degree was high. While the Fe_3O_4 particles distributed around the surface layer of poly(NIPAAm-AA)/Fe_3O_4 latex particles when the crosslinking degree was low. Moreover, the increase of crosslinking degree slightly increased the size of poly(NIPAAm-AA)/Fe_3O_4 magnetic latex particles. From the above results, it implied that high crosslinking degree confined the AA segments within the inner zone of poly(NIPAAm-AA) latex particles. On the contrary, the AA segments could move more freely toward the surface of latex particles when the crosslinking degree was low. The AA segments existing in the poly(NIPAAm-AA) latex particles were prone to attract Fe^{2+} and Fe^{3+} ions, thus produced Fe_3O_4 nanoparticles in situ to form the poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex nanoparticles distributed in the core of poly(NIPAAm-AA)/Fe_3O_4 particles with high crosslinking degree, the composite particles swelled more easily so as to increase the size of the magnetic particles. Besides, in the presence of NH_4OH, when most of Fe_3O_4 composite particles, if compared to the case that most of Fe_3O_4 nanoparticles distributed on the surface layer of the composite particles with low crosslinking degree, in which the layer of Fe_3O_4 nanoparticles restricted the swelling of composite particles.

3.2. Effect of AA content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles

Fig. 3 and Table 3 showed the effect of AA content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles. The increase of AA content increased the LCST of poly(NIPAAM-AA)/Fe_3O_4 latex particles, and the transition became broad and insignificant. The reason was due to that hydrophilic segments of AA enhanced

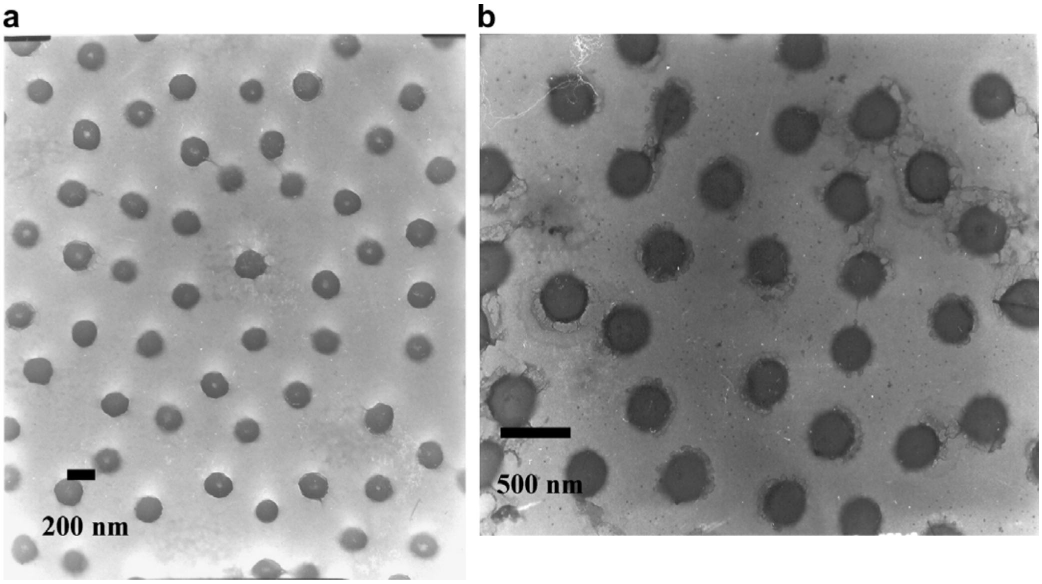


Fig. 1. TEM photographs of poly(NIPAAm-AA)/Fe_3O_4 latex particles (a) AA 2%, MBA 2%, Fe_3O_4 2% (b) AA 4%, MBA 2%, Fe_3O_4 2%.

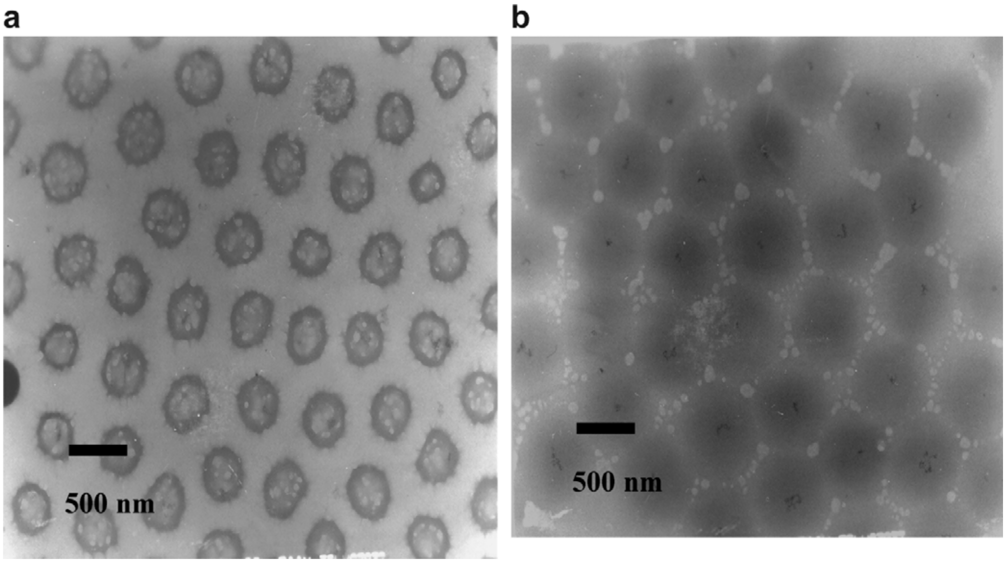


Fig. 2. TEM photographs of poly(NIPAAm-AA)/Fe_3O_4 latex particles (a) AA3%,MBA 2%, Fe_3O_4 2% (b) AA 3%, MBA 4%, Fe_3O_4 2%.

the hydrogen bonding between poly(NIPAAm-AA) copolymer and H_2O, so as to increase the LCST of poly(NIPAAM-AA)/Fe_3O_4 particles.

3.3. Effect of crosslinking structure on the LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles

Fig. 4 showed the curve of relationship between LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles and concentration of crosslinking agent (MBA). The increase of MBA concentration increased the LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particles. Out of expectation an opposite trend was observed. It was due to the morphology difference as discussed above. Table 3 showed the data of LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles. For the case of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particles with low crosslinking degree, most of the AA segments, which bonded with Fe_3O_4 nanoparticles, located at the surface layer of magnetic composite particles. The Fe_3O_4 nanoparticles on the surface layer hindered H_2O from diffusing into the particles so as to lower the hydrogen bonding between poly(NIPAAm) and H_2O, and lower the LCST of the magnetic composite particles. On the other hand, for the case of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particles with high crosslinking degree, most of Fe_3O_4

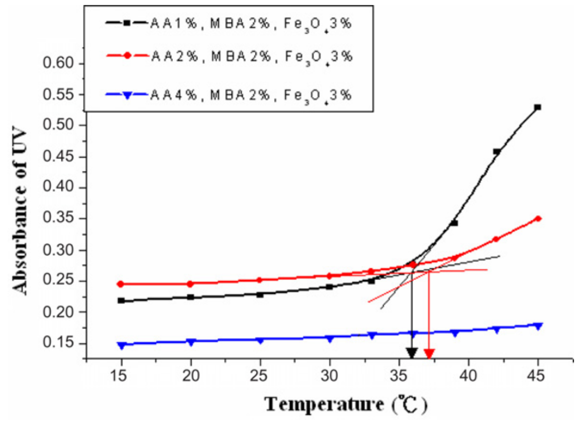


Fig. 3. Effect of AA content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 latex particles.

Table 3
The effect of AA content, crosslinking agent content and Fe_3O_4 content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particles

Composition of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particle	LCST of latex particles (°C)
Effect of AA content	
AA 1% MBA 2% Fe_3O_4 3%	36.1 ± 0.2
AA 2% MBA 2% Fe_3O_4 3%	37.0 ± 0.2
AA 4% MBA 2% Fe_3O_4 3%	>45.0 ± 0.2
Effect of crosslinking agent content	
AA 1% MBA 2% Fe_3O_4 2%	36.7 ± 0.2
AA 1% MBA 4% Fe_3O_4 2%	37.1 ± 0.2
AA 1% MBA 6% Fe_3O_4 2%	37.7 ± 0.2
Effect Fe_3O_4 content	
AA 1% MBA 2% Fe_3O_4 2%	36.7 ± 0.2
AA 1% MBA 2% Fe_3O_4 3%	36.1 ± 0.2
AA 1% MBA 2% Fe_3O_4 4%	35.9 ± 0.2

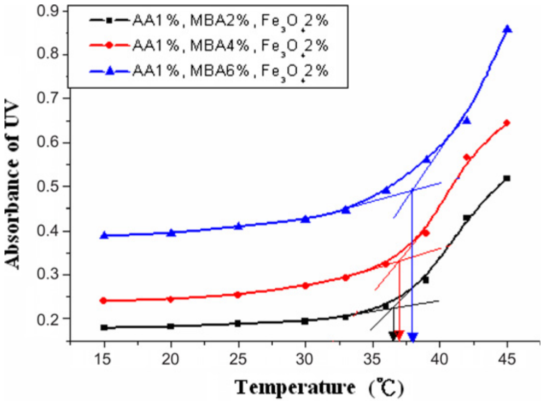


Fig. 4. Effect of crosslinking agent content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 latex particles.

nanoparticles were restricted within the core of magnetic composite particles, and the better swelling of composite particles resulted in a higher LCST of poly(NIPAAm-AA)/Fe_3O_4 composite particles.

3.4. Effect of Fe_3O_4 content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles

With the increase of Fe_3O_4 particles, the LCST of poly(NIPAAM-AA)/Fe_3O_4 composite latex particle decreased as shown in Fig. 5 and Table 3. The Fe_3O_4 nanoparticles hindered the swelling and motion of the copolymer, poly(NIPAAm-AA), so as to decrease the LCST of poly(NIPAAm-AA)/Fe_3O_4 composite latex particles.

3.5. Effect of AA content on the control release of caffeine

Scheme 1(a) and (b) were proposed to interpret the caffeine release from poly(NIPAAm-AA)/Fe_3O_4 latex particles containing small and large amount of AA, respectively. According to the results of our previous study [31], the morphology of the poly(NIPAAm-AA) particles was core-shell structure with a poly(NIPAAm) rich core and poly(AA) rich shell. After the process of caffeine loading, caffeine was not only trapped in the latex particles, in both the core zone of poly(NIPAAm-AA) and the shell zone of AA segment, but also located in the region outside latex particles. In this study, the caffeine drug was loaded to composite particles at 4 °C. At this temperature, all composite particles were in swollen state. The copolymer latex particles with large content of AA exhibited a thick layer of AA segment so that a large amount of caffeine was trapped in both the core and shell zones and a small amount of caffeine located in the region outside latex particles. On the contrary, the copolymer latex particles with small amount of AA exhibited a thin layer of AA segments so that a relatively small amount of caffeine was trapped in both the core and shell zones and a relatively large amount of caffeine located in the region outside the latex particles. When in the release experiment, caffeine existing in the region outside latex particles diffused out quickly, followed by the gradual diffusion from the interior of latex particles. Fig. 6 showed the effect of AA content on the control release of caffeine at temperature of 37 °C. The increase of AA content decreased the release rate of caffeine initially, but increased the release rate of caffeine in the final stage of release. The copolymer latex particles with small content of AA exhibited a lower LCST (<37 °C). The latex

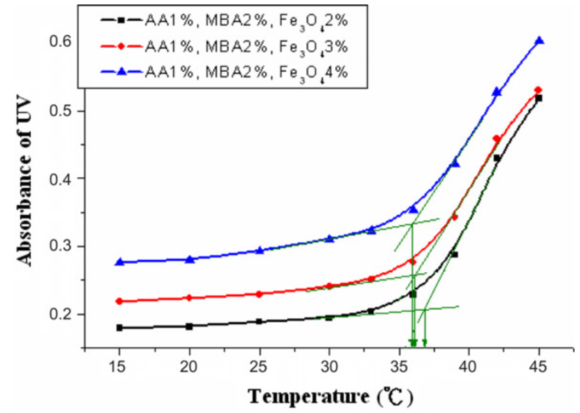
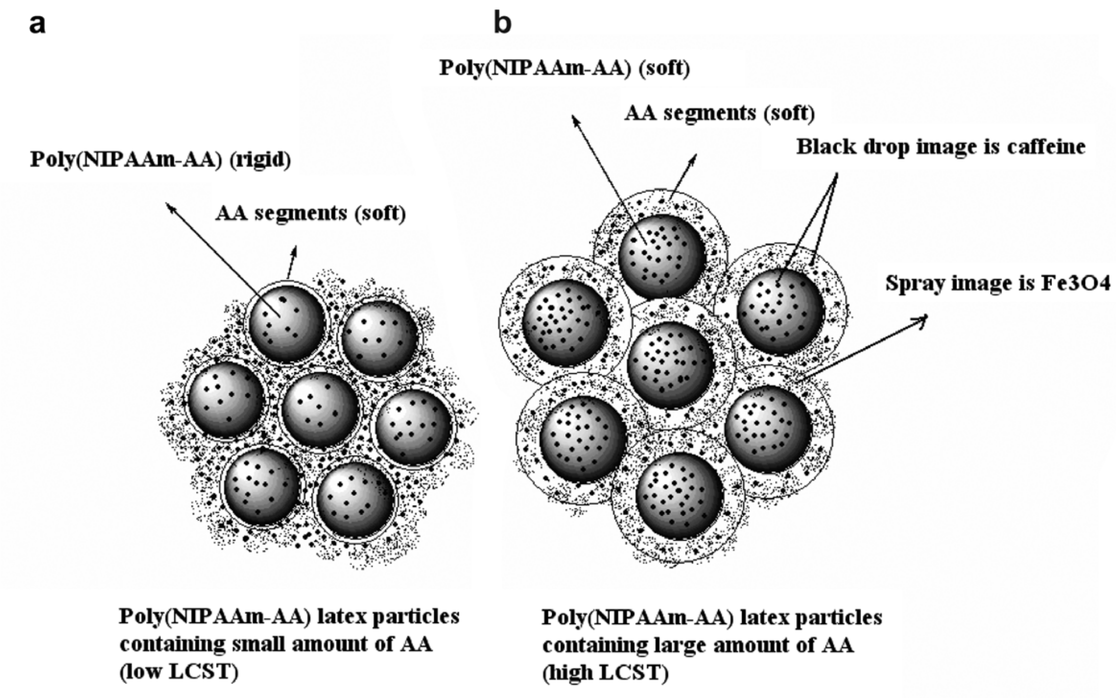


Fig. 5. Effect of Fe_3O_4 content on the LCST of poly(NIPAAm-AA)/Fe_3O_4 latex particles.



Scheme 1. The caffeine release from poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles with different contents of AA (MBA 2%).

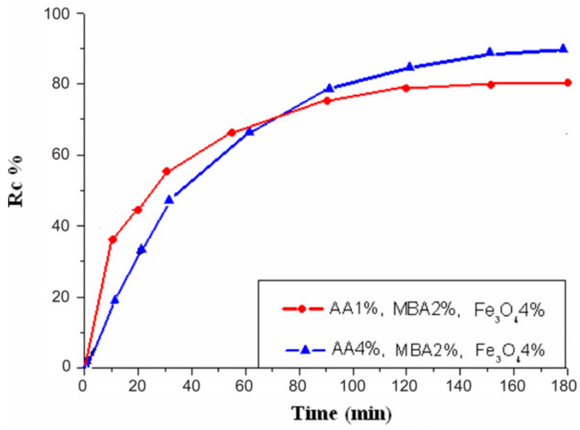
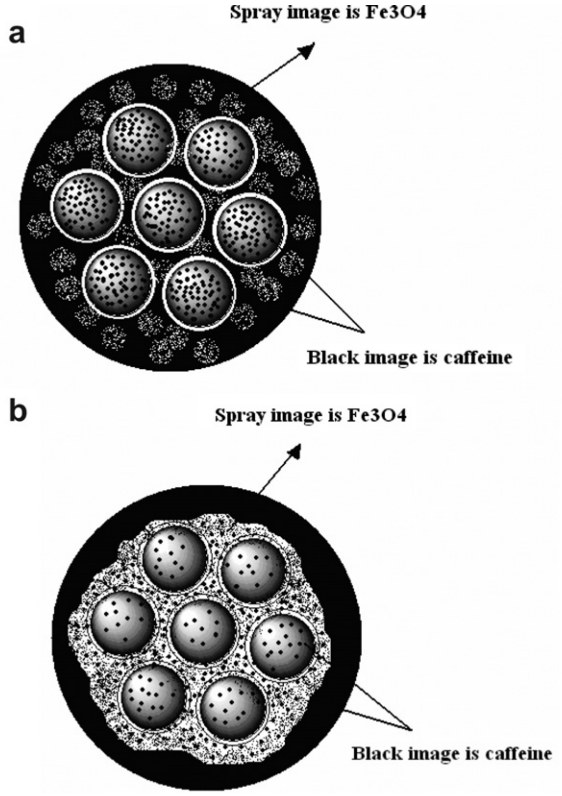


Fig. 6. Effect of AA content on the control release of caffeine at 37 °C.

particles were hydrophobic (rigid in H_2O) at 37 °C, and the hydrophilic zone of AA segments over the shell region of particles was thin. As seen in Scheme 1(a), relatively large portion of caffeine would stay in the region outside particles. On the other hand, in the case of large content of AA, the LCST of latex particles could be higher than 37 °C. The latex particles were hydrophilic (soft in H_2O) at 37 °C and the hydrophilic shell of AA segments was thick. Therefore, caffeine would mostly stay inside particles as seen in Scheme 1(b). The results of release experiments in Fig. 6 could be explained by the proposed Scheme 1. With small content of AA, the initial release rate was faster because large portion of caffeine existed in the region outside particles, but the release rate gradually decreased when the caffeine trapped inside particles started to diffuse out from the rigid poly(NIPAA-AA) zone. With large content of AA, at 37 °C, the latex particles could swell and most caffeine was trapped in the soft particles, so that the release rate was slower initially but faster in the latter stage of release experiment.

3.6. *Effect of Fe_3O_4 content on the control release of caffeine*

Scheme 2(a) and (b) were proposed to interpret the caffeine release from poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particles containing small and large amount of Fe_3O_4, respectively. After the process of caffeine loading, caffeine was not only trapped inside the latex particles but also located in the region outside latex particles. Fig. 7 showed the effect of Fe_3O_4 content on the control release of caffeine. In the experiment of caffeine release, the caffeine release from the latex particles without Fe_3O_4 was the fastest. Besides, the caffeine release from the poly(NIPAAm-AA)/Fe_3O_4 composite latex particles containing 4% of Fe_3O_4 was faster than that from the magnetic composite latex particles containing 2% of Fe_3O_4. The Fe_3O_4 nanoparticles were the barrier for caffeine loading into the latex particles. In the case of 4% Fe_3O_4, a large amount of caffeine located in the region outside latex particles rather than loaded into the inner region of latex particles due to the barriers of Fe_3O_4 nanoparticles. On the contrary, caffeine was easier to load into the latex particles with 2% Fe_3O_4. When in the release experiment, caffeine that existing in the region outside latex particles diffused out quickly, followed by the gradual diffusion from the inner region of latex particles. Therefore, the caffeine release from the poly(NIPAAm-AA)/Fe_3O_4 composite



Scheme 2. The caffeine release from poly(NIPAAm-AA)/Fe_3O_4 magnetic composite latex particles with different contents of Fe_3O_4. (a) The magnetic composite latex particles with lower amount of Fe_3O_4. (b) The magnetic composite latex particles with larger amount of Fe_3O_4.

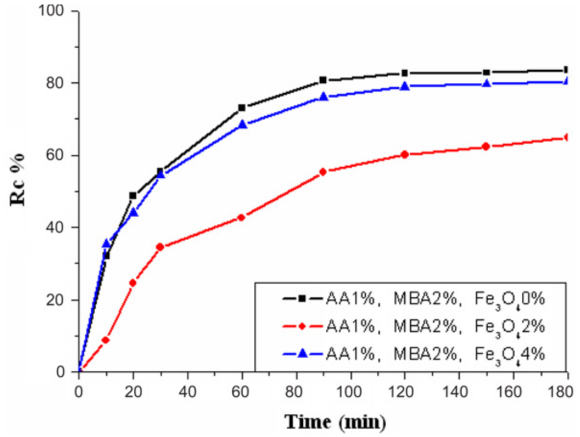


Fig. 7. Effect of Fe_3O_4 content on the control release of caffeine at 37 °C.

latex particles with 4% Fe_3O_4 was faster than that from the magnetic composite latex particles with 2% Fe_3O_4. Also, it was understandable that caffeine was not only easy to load into the latex particles but also easy to release from the latex particles, if without the barrier of Fe_3O_4 nanoparticles, so that the caffeine release from the latex particles without Fe_3O_4 was the fastest. It is notable that in the sample with 4% Fe_3O_4, large amount of caffeine stayed outside latex particles, so the release rate was much faster than the sample with 2% Fe_3O_4, but if compared with the sample without Fe_3O_4, the Fe_3O_4 nanoparticles hindered the diffusion of caffeine to some extent, therefore, the release rate was slightly smaller than the sample without Fe_3O_4.

3.7. Effect of crosslinking agent content on the control release of caffeine

Fig. 8 showed the effect of MBA content on the control release of caffeine at 37 °C. The increase of MBA content insignificantly influenced the release rate of caffeine at initial stage, but increased the release rate of caffeine in the final stage of release. In the caffeine release experiment, caffeine existing in the region outside latex particles diffused out quickly at initial stage, followed by the gradual diffusion from the interior of latex particles. The morphology of poly(NIPAAm-AA)/Fe_3O_4 latex particles influenced the release rate of caffeine significantly. In the case of low crosslinking degree, Fe_3O_4 nanoparticles distributed around the surface layer of poly(NIPAAm-AA)/Fe_3O_4 latex particles, so that the diffusion of caffeine from the inner zone of latex particles was restricted by the surface layer's Fe_3O_4 nanoparticles. In the case of high crosslinking degree, Fe_3O_4 particles distributed in the core of poly(NIPAAm-AA)/Fe_3O_4 latex particles. The caffeine diffusion from the inner zone of latex particles was not so restricted by the Fe_3O_4 nanoparticles. Therefore, the caffeine diffusion from the poly(NIPAAm-AA)/Fe_3O_4 latex particles with high crosslinking degree was faster than that from the latex particles with low crosslinking degree at the final stage of release. In Fig. 8, the samples were undergone a different process of mold compression for tablet formation. The compression was held with 9 tons of force for 15 min. But the compression was held for 30 min on samples in Figs. 6 and 7. The tablets in Fig. 8 were not as compact as the tablets in Figs. 6 and 7. Therefore, the release rate of tablets in Fig. 8 was faster than those in Figs. 6 and 7.

3.8. Amount of BSA binding

The amount of BSA conjugation on the surface of poly(NIPAAm-AA)/Fe_3O_4 particles was influenced by many factors such as the size of swollen particles, the hydrophobic

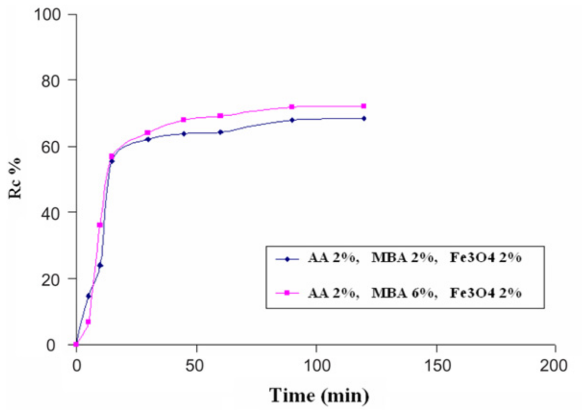


Fig. 8. Effect of crosslinking agent (MBA) content on the control release of caffeine at 37 °C.

properties of particles, the concentration of COOH groups on the particles and pH values. The larger swollen particles would reduce the steric hindrance of protein (BSA) conjugation, the large amount of COOH group would increase the binding of protein on the surface of latex particles, the hydrophobic property of particles would increase the adsorption of protein on the surface of particles, and pH value would change the interaction between BSA and COOH groups.

The amount of BSA binding was significantly influenced by AA content and Fe_3O_4 content as shown in Table 4. With the increase of AA content, the amount of COOH groups increased so as to increase the BSA conjugation on particles. On the contrary, with the increase of Fe_3O_4 content, the amount of BSA conjugation decreased on particles. It was due to the fact that in the case of low crosslinking degree (2% MBA), Fe_3O_4 particles located at the surface layer of poly(NIPAAm-AA)/Fe_3O_4 composite latex particles. Therefore, Fe_3O_4 nanoparticles hindered the binding between BSA and COOH groups. It was interesting to compare the results of the amount of BSA conjugation on particles at 37 and at 25 °C. The effect of temperature on the amount of BSA binding was shown as Table 4. The temperature of 37 °C was better for the binding of BSA with poly(NIPAAm-AA)/Fe_3O_4 composite latex particles. Although the particles swelled better at 25 than at 37 °C, the Fe_3O_4 nanoparticles which located at the surfaces of composite particles hindered the swelling of the composite particles. Therefore, the difference in the particle size between 37 and 25 °C was not large that the factor of steric hindrance didn't dominantly influence the amount of protein (BSA) conjugation. On the other hand the composite particles showed more hydrophobic nature at 37 than at 25 °C. Therefore, the protein exhibited more binding with the composite particles at the temperature of 37 °C. Table 4 showed the effect of crosslinking agent content on the amount of BSA conjugation. With the increase of crosslinking agent content the amount of BSA conjugation increased. It was due to the different location of Fe_3O_4 nanoparticles on the poly(NIPAAm-AA)/Fe_3O_4 latex particles as changing the degree of crosslinking. As mentioned above, the Fe_3O_4 nanoparticles distributed in the core of latex particles when the crosslinking degree was high while the Fe_3O_4 nanoparticles distributed around the surface layer of latex particles when the crosslinking degree was low. The Fe_3O_4 nanoparticles around the surface layer of latex particles would hinder the binding between BSA and COOH groups so much as to lower the amount of BSA conjugation. However, the Fe_3O_4 nanoparticles distributed in the core of latex particles didn't hinder the binding between BSA and COOH groups. Therefore, the amount of BSA conjugation increased with increasing the crosslinking degree in the latex particles. The factor of pH value significantly influenced the amount of BSA conjugation. Table 4 showed that the amount of BSA conjugation at pH 7.4 was larger than that at pH 9. The isoelectric point of BSA was at pH 4.7 so that BSA was negatively charged at the pH value larger than 4.7. With the increase of pH value, the degree of ionization of COOH groups increased on the latex particles. Therefore, the repulsive force between BSA and COO^- groups at the condition of pH 9 was larger than that at pH 7.4. The BSA conjugation of latex particles at pH 7.4 was larger than that at pH 9.

Table 4
The effect various variable on the amount of BSA conjugation on the surfaces of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particles

Composition of poly(NIPAAm-AA)/Fe_3O_4 magnetic composite particle	Amount of BSA conjugation (%)
Effect of AA content (25 °C, pH 7.4)	
AA 1% MBA 2% Fe_3O_4 2%	40.7
AA 4% MBA 2% Fe_3O_4 2%	46.9
Effect of crosslinking agent content (25 °C,pH 7.4)	
AA 4% MBA 2% Fe_3O_4 2%	46.9
AA 4% MBA 6% Fe_3O_4 2%	54.2
Effect of Fe_3O_4 content (25 °C, pH 7.4)	
AA 1% MBA 2% Fe_3O_4 2%	40.7
AA 1% MBA 2% Fe_3O_4 4%	39.4
Effect of temperature	
AA 1% MBA 2% Fe_3O_4 2% (25 °C, pH 7.4)	40.7
AA 1% MBA 2% Fe_3O_4 2% (37 °C, pH 7.4)	42.1
Effect of pH	
AA 1% MBA 2% Fe_3O_4 2% (25 °C, pH 7.4)	40.7
AA 1% MBA 2% Fe_3O_4 2% (25 °C, pH 9)	24.2

4. Conclusion

In this work, poly(NIPAAm-AA)/Fe_3O_4 composite latex particles were synthesized by a novel method. The concentration of AA, the concentration of crosslinking agent and the Fe_3O_4 nanoparticles were important factors to influence the morphology and LCST of poly(NIPAAm-AA)/Fe_3O_4 composite latex particles. Either increasing the AA amount or increasing the concentration of crosslinking agent would increase the LCST of poly(NIPAAm-AA)/Fe_3O_4 latex particles. But the increase of Fe_3O_4 content would decrease the LCST of the composite particles. The poly(NIPAAm-AA)/Fe_3O_4 composite latex particles could be used as carriers to load caffeine. The content of AA, the degree of crosslinking and the amount of Fe_3O_4 influenced the control release of caffeine significantly. The caffeine release from the poly(NIPAAm-AA)/Fe_3O_4 composite latex particles containing 4% Fe_3O_4 was faster than that from the magnetic composite latex particles containing 2% Fe_3O_4. Morphology-based schematic models were proposed to explain the control release behavior of the composite particles. The BSA conjugation on the composite latex particles could be increased under the condition of higher content of AA, higher degree of crosslinking, higher temperature, lower pH value and lower amount of Fe_3O_4. The amount of BSA conjugation was found increased at temperatures higher than LCST, because the more hydrophobic nature of particles favored the reaction of protein conjugation.

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