

Thermosensitive poly(*N*-isopropylacrylamide) nanocapsules with controlled permeability

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Abstract

In this paper, novel thermosensitive poly(*N*-isopropylacrylamide) (PNIPAM) nanocapsules with temperature-tunable diameter and permeability are reported. Firstly, the core-shell composite microparticles were synthesized by precipitation polymerization with isothiocyanate fluorescein (FITC) entrapped SiO₂ as core and cross-linked PNIPAM as shell. Then, the SiO₂ core was etched by hydrofluoric acid at certain condition and the pre-trapped FITC molecules remained within the inner cavity. The FITC release profile and TEM studies clearly indicate that the release behavior of FITC could be controlled effectively by the external temperature. Above the LCST of PNIPAM (32 °C), the dehydrated PNIPAM shell inhibited the release of FITC from the internal cavity while below its LCST, the fluorophore could permeate the swollen shell easily.

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1. Introduction

Environmentally responsive polymeric capsules ranging in size from nanometers to microns have attracted great attentions in recent years for their practical and potential applications in drug controlled release, heterogeneous catalysis, encapsulation and protection of guest molecules for transportation or storage and so on [1–12]. The gated pore size in the polymer wall could be controlled by external stimuli, which directly tunes the transport properties of guest molecules in and/or out of the inner cavity. Several groups have prepared such microcapsules using different methods. Meier and co-workers used vesicle-template polymerization to synthesize water-soluble, cross-linked polyelectrolyte nanocapsules that were able to undergo a reversible swelling transition upon changing the pH and/or ionic strength [4–6]. They expected the external stimuli-responsive nanocapsules could be used in drug-controlled release. Wooley et al. employed a strategy that began from

the supermolecular assembly of amphiphilic block copolymers into polymer micelles, followed by their covalent stabilization through shell cross-linking (SCK), and then core-degradation to yield a pH responsive nanocage [7–9]. In the last few years, a series of hollow polyelectrolyte multilayer capsules have been produced in Möhwald's group through Layer-by-Layer method (alternating adsorption of oppositely charged polyelectrolytes onto sacrificial template particles) [13]. The capsule wall could be triggered by pH or ionic strength to switch on and off between 'open' and 'closed' state for guest macromolecules [10–12]. All of these polymeric capsules mentioned above are sensitive to pH and/or ionic strength and/or medium polarity, however, there are few examples of submicrometer-sized temperature-sensitive capsules [3].

It is well known that thermoresponsive polymers can change their physical or chemical properties discontinuously or sharply at or around their lower critical solution temperature (LCST) [14,15]. It is expected that the microparticles made of such a material should have some temperature-related properties. As an example poly(*N*-isopropylacrylamide) (PNIPAM) microgel was found to

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exhibit outstanding capabilities as potential carriers or protective containers for biologically active molecules. Until now, many papers about PNIPAM microgel were published [16–18]. But, to our knowledge, reports about preparation of thermosensitive (PNIPAM) nanocapsules by precipitation polymerization on sacrificial template core are very few [19].

In this paper, we used a popular fluorescent probe, fluorescein isothiocyanate (FITC) as the model of guest molecules to prepare FITC-containing PNIPAM nanocapsules by the template precipitation polymerization of PNIPAM shell followed by removal of SiO₂ core. The obtained nanocapsules exhibited good spherical shape and dramatic volume phase transition near the LCST of PNIPAM (32 °C), which could control the release behavior of guest molecules from the inner cavity of PNIPAM nanocapsules. FITC was chosen as guest molecule probe in this study because its structure and size are similar to many drug compounds [20], by which we expect it could mimic the controlled permeability of guests in the confined space.

2. Experimental section

2.1. Materials

Potassium persulfate (KPS) was recrystallized twice from water. Fluorescein isothiocyanate (FITC, Fluka, 95%), tetraethoxysilane (TEOS, Shanghai Chemical Co. Ltd., China, 99%), 3-aminopropyl trimethoxysilane (APS, Witco, 98%), 3-(trimethoxysilyl)propylmethacrylate (MPS, Witco, 98%), *N*-isopropylacrylamide (NIPAM, TCI, 99%) and *N,N'*-methylene bisacrylamide (MBA, Fluka, 99%) and hydrofluoric acid (40 wt%) were all used as received without further purification. Deionized water was applied for all the experiment processes.

2.2. Synthesis of FITC-trapped silica nanoparticles

FITC-trapped silica particles were prepared with modified Stöber method [21,22]. A typical synthesis procedure was depicted as follow: Firstly, 10 mg fluorescein isothiocyanate (FITC) was reacted with 50 mg organosilicon coupling agent 3-aminopropyl trimethoxysilane (APS) in 5 g absolute ethanol by stirring for 48 h. Then this solution was mixed with 3.36 g tetraethoxysilane (TEOS) and injected into a stirring solution containing 48.2 g ethanol, 0.50 g ammonia and 7.87 g deionized water. The mixture turned turbid after 15 min and stirred for a further 48 h. The obtained particles were modified by excessive amount of 3-(trimethoxysilyl)propylmethacrylate (MPS) (0.40 g) to introduce double bond onto the surface of silica particles [23]. After that, four cycles of centrifugation and redispersion in ethanol were performed to remove the unreacted MPS completely from the suspension. The MPS-modified

FITC-trapped silica particles were then redispersed in water and diluted to 0.5 wt% before polymerization.

2.3. Synthesis of core-shell SiO₂-PNIPAM particles

Cross-linked PNIPAM shell was prepared by precipitation polymerization with the FITC-trapped silica particles as seeds. In this process, the amounts of *N*-isopropylacrylamide (NIPAM) and *N,N'*-methylene bisacrylamide (MBA) were varied. The weight ratio of NIPAM to SiO₂ was from 1 to 4 and the molar ratio of MBA to NIPAM was from 7.5 to 15%. As an example, 3 g water solution of 0.2 g NIPAM (1.77 mmol) and 0.03 g MBA (0.195 mmol) was added into 20 g 0.5 wt% silica dispersion. The temperature was elevated to 70 °C after 30 min nitrogen bubbling, then 0.005 g KPS in 2 g water was injected into the system to initiator the polymerization. The reaction mixture was mechanically stirred at 70 ± 2 °C for 4 h before being cooled down to ambient temperature. The obtained fluorescent SiO₂/PNIPAM nanoparticles were centrifuged and redispersed repeatedly in water to remove the water-soluble PNIPAM homopolymer.

2.4. Synthesis of PNIPAM nanocapsules

The whole etching process was performed at 40 °C. After immersing the core-shell particles in 5% hydrofluoric acid aqueous solution for 4 h, the SiF₄ and excessive HF were expelled out of the capsules by several centrifugation/washing cycles in water until its pH became neutral. The obtained PNIPAM nanocapsules were dialyzed against deionized water with different dialysis time at 20 and 40 °C, respectively, followed by fluorescence measurements to record the release kinetics of FITC from the capsules.

2.5. Characterizations

TEM images were obtained using a Hitachi H-600 transmission electron microscope operating at 250 kV. Samples were stained by phosphate-tungstic acid or CsOH aqueous solution. Hydrodynamic diameters of the particles or capsules were recorded on a Malvern Autosizer 4700 with a laser source of wavelength at 532 nm and analysis in CONTIN mode. Fluorescence emission spectra were measured on a Edinburgh Instruments FLS920 steady-state fluorescence spectrofluorimeter with light of 450 nm as excitation source. The optical angle was 90° and the bandwidth slits of both excitation and emission wavelengths were 5 nm. Thermal Gravimetric Analysis (TGA) was performed on Pyris 1 TGA Analyzer with elevated rate at 10 °C/min from 20 to 550 °C.

3. Results and discussion

The principle of this preparation procedure is illustrated

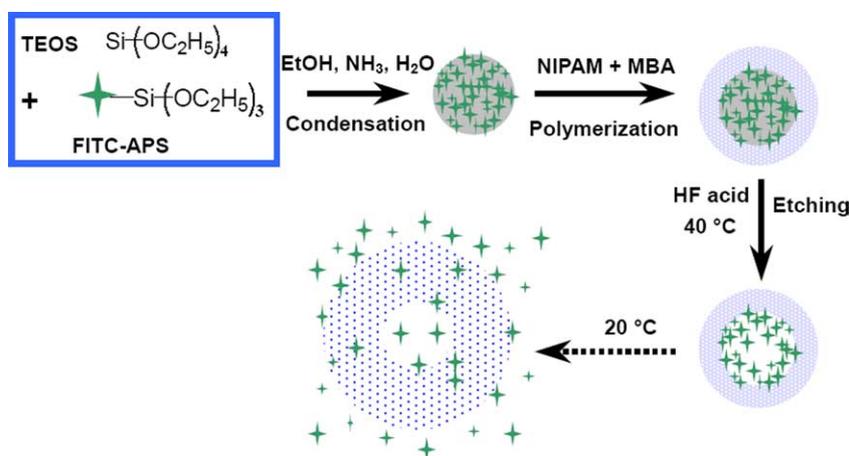


Fig. 1. Schematic representation of preparation and controlled release of the thermosensitive polymeric nanocapsules.

in Fig. 1, which has two noticeable advantages: (1) the thickness and pore size of polymer wall could be controlled easily by external temperature; (2) the cross-linked shell offers the polymer capsules long-term stability [1a]. The FITC-trapped silica particles were obtained according to the modified Stöber method with tunable diameters from tens to hundreds of nanometers and narrow size distribution (size polydispersity (μ_2/I^2) was below 0.02) [21]. The maximum fluorescence emission peak of the FITC-trapped silica particles locates at 515 nm, which is similar to that of FITC molecules in ethanol (Fig. 2). Although the shapes of the two spectra are a little different, this result suggests the successful incorporation of FITC into silica template particles. For further precipitation polymerization of cross-linked PNIPAM on SiO₂ surface, organosilicon coupling agent 3-(trimethoxysilyl)propyl methacrylate (MPS) was used to modify the surface of silica particle with double bond. The free MPS could be removed completely by

repeated centrifugation and redispersion of the silica particles in ethanol. It is worthy to note that, since our goal is to obtain silica/PNIPAM core/shell nanoparticles containing only one SiO₂ particle as core, it is essential to avoid the aggregation of the SiO₂ in the aqueous medium before polymerization. In the experiment, we find that leaving a trace of ethanol in SiO₂ aqueous dispersion is helpful for the SiO₂ seed to disperse well in water. The reason we expected is that the ethanol in water lowered the polarity of the solvent, which is a favor for the MPS-modified hydrophobic silica particles to disperse. Here, the function of MPS is to introduce double bond onto SiO₂ surface. Because the double bonds on silica surface could react with the growing PNIPAM radicals during the polymerization, the surface modification by MPS is essential to avoid the preparation of pure polymer particles containing no SiO₂ core, [23].

PNIPAM is water-insoluble at the polymerization temperature of 70 °C, so the growing polymer radical prefers precipitating onto the SiO₂ surface, when it reaches the critical length in water. The growing radical propagates further on the SiO₂ surface and cross-links into a microgel by the copolymerization of NIPAM monomer and cross-linker *N,N'*-methylene bisacrylamide (MBA). The obtained microgels are FITC-trapped SiO₂/PNIPAM core/shell nanoparticles, whose steady-state fluorescence spectrum exhibits a maximum emission at 511, 4 nm shifting to shorter wavelength comparing with the FITC-trapped SiO₂ particles in Fig. 2. TEM images clearly show the core-shell structure of the composite particles (Fig. 3), whose shell thickness and morphology could be tailored by the added amount of NIPAM and MBA (Table 1). When the weight ratio of added NIPAM monomer to SiO₂ is 1–3, the morphology of the SiO₂/PNIPAM composite particles is well-defined, which means that in most cases, the composite particles are near-monodisperse and only one SiO₂ core is embedded in one composite particle. The diameter and the corresponding shell thickness of the core/shell particles increase with the added amount of NIPAM monomer, and

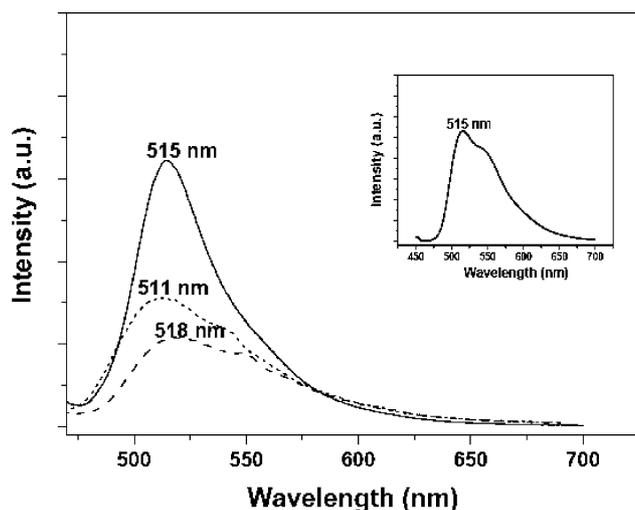


Fig. 2. Fluorescent emission spectra of FITC-trapped SiO₂ particles (solid line), SiO₂/PNIPAM particles BN2 (dot line) and PNIPAM capsules EBN2 (dash line), the inset spectrum was FITC in ethanol, all the spectra were normalized with excitation at 450 nm.

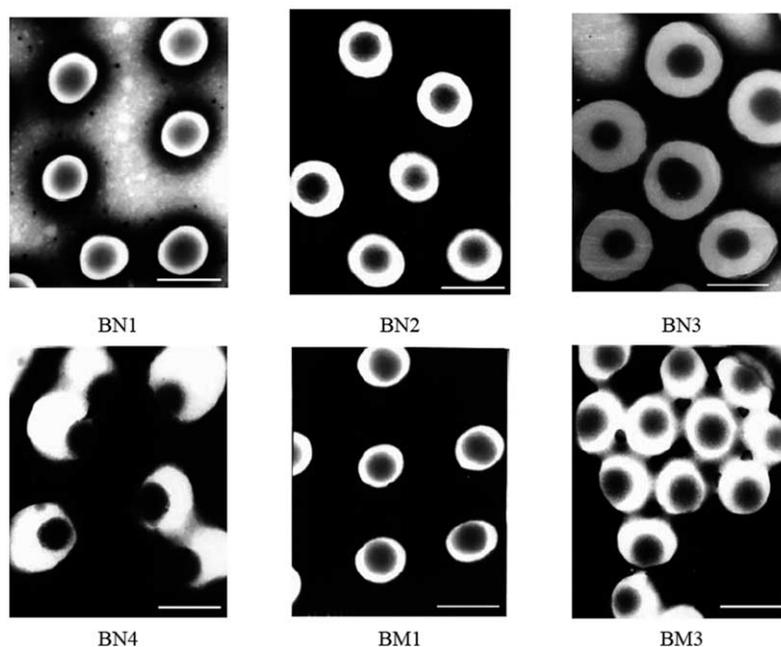


Fig. 3. TEM images of SiO₂/PNIPAM core-shell fluorescent microparticles stained by phosphate-tungstic acid, the scale bar were 200 nm.

are listed in Table 1. The results from dynamic light scattering measurement are dramatically larger than those from TEM measurement, which is attributed to the stretching PNIPAM chains in 25 °C aqueous solution and the shrinking PNIPAM chains under ultrahigh vacuum condition. When the weight ratio of added NIPAM monomer to SiO₂ is 4, considerable precipitation could be observed in the reaction bottle at the end of the polymerization and the morphology of the obtained composite particles is also less well-defined, which could be seen from the TEM photo in Fig. 3 (BN4). Besides, the amount of cross-linker MBA could also influence the morphology of the final SiO₂-PNIPAM composite particles. When the molar ratio of the added MBA to NIPAM reaches 15%, the particles become unstable (BM2).

To fabricate the FITC-trapped polymeric nanocapsules, the last step was to remove the silica core by hydrofluoric acid. The product of etching reaction was SiF₄, which is

small enough to pass through the porous polymer wall easily. In order to prevent the larger fluorescent molecules FITC from leaking out of the nanocapsules during the etching process, we set 40 °C as the working temperature for the whole process of silica etching, because at this temperature, the dehydrated PNIPAM shell is expected to keep most of the entrapped FITC in the capsules. The reaction product SiF₄ and excessive HF could be removed by several centrifugation and washing cycles in deionized water. Fig. 4 shows the FTIR spectrum of PNIPAM nanocapsule EBN2 after 4 h etching by 5% HF solution at 40 °C. Compared with the FTIR spectrum of BN2 before etching, the sharp decrease of SiO₂ adsorption intensity at 1100 cm⁻¹ suggests that most of the SiO₂ core has been etched by HF. While the FITC trapped in the capsules after HF etching gives the maximum fluorescence emission at 518, 7 nm red shift compared with the SiO₂-PNIPAM composite particles (Fig. 2). In order to quantify the amount

Table 1
Recipe of various SiO₂/poly(NIPAM) fluorescent microparticles

Sample ^a	SiO ₂ /NIPAM (weight ratio) ^b	MBA/NIPAM (molar ratio) (%)	Diameter (nm)		Shell Thickness (nm)	
			TEM	DLS (25 °C)	TEM	DLS (25 °C)
BN1	1/1	11	165	320	33	91
BN2 ^c	1/2	11	190	398	45	129
BN3	1/3	11	250	462	75	162
BN4	1/4	11	280	733	90	297
BM1	1/2	7.5	180	419	40	140
BM2	1/2	15	175	411	37	136

^a The diameter of FITC-trapped SiO₂ is 100 nm (TEM).

^b All the SiO₂ solution was diluted to 0.5 wt% before polymerization.

^c The nanocapsules of BN2 after HF etching was designated as EBN2.

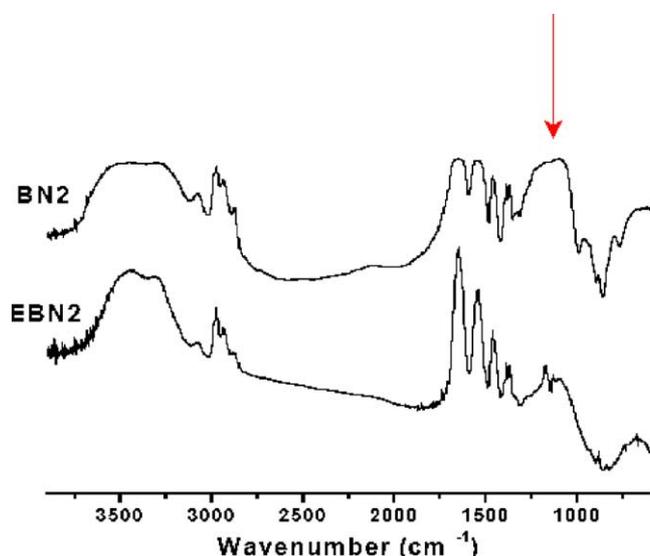


Fig. 4. FTIR spectra of SiO₂/PNIPAM microparticles BN2 and capsules EBN2.

of removed SiO₂ core, thermogravimetric analysis (TGA) was used to measure the weight percentage of SiO₂ of the SiO₂-PNIPAM composite particles before and after HF etching. The results are 37.4 and 4.56 wt% respectively, which indicate that 92% of SiO₂ has been removed through the HF etching.

The temperature-stimuli volume transitions of the PNIPAM nanocapsules EBN2 and silica/PNIPAM nanoparticles BN2 were recorded by dynamic light scattering (DLS) measurement. Although the volume phase transition temperatures (T_{VPT}) of the particles and capsules are both near 32 °C, their volumetric contractions are different. The hydrodynamic diameter (D_h) of the BN2 composite nanoparticles decreases from 418 to 277 nm when environmental temperature increases from 20 to 50 °C (Fig. 5(a)). In

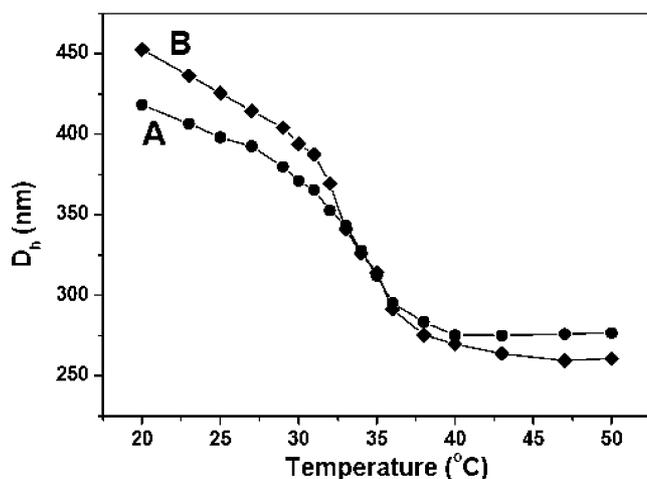


Fig. 5. The hydrodynamic diameter (D_h) of SiO₂/PNIPAM nanoparticles BN2 (A) and EBN2 after HF etching (B) as function of temperature.

comparison, the PNIPAM nanocapsules EBN2 after SiO₂ core removal show a larger D_h (437 nm, 20 °C) below T_{VPT} and a smaller D_h (261 nm, 50 °C) above T_{VPT} (Fig. 5(b)). This phenomenon is caused by removing the restriction of the covalently bound SiO₂ core inside, and is similar to the D_h expansion of core-shell micelle after degradation of core polymer [8,9,24].

Fig. 6(a) shows the TEM image of the hollow nanocapsule EBN2 after HF etching. It is interesting that most of the nanocapsules retain their spherical morphology due to the cross-linked PNIPAM wall. Taking a close look at Fig. 6(a), some dark shadow within the hollow chambers of PNIPAM nanocapsules could be found, which represents the trapped FITC and 8 wt% unetched SiO₂.

Since FITC still exists within the capsules after etching, could this temperature-tunable volume-transition of the nanocapsules control effectively its release? To explore the controlled release property, TEM images could give us some assistance. Because Cs⁺ is an effective marker of the carboxylic acid groups of FITC molecules, the staining by CsOH renders FITC visible under electron microscopy [25, 26]. We dialyzed the PNIPAM nanocapsules against deionized water for three days at two different temperatures (20 and 40 °C), and then stained them by CsOH. Fig. 6(b) shows the image of the nanocapsules after extensive dialysis at 40 °C, in which, we could still observe a black core that suggested that the FITC was trapped within the hollow chamber of the cross-linked PNIPAM capsules. But for the nanocapsules dialyzed against cold water (20 °C), there are no longer any dark centers (Fig. 6(c)), which suggests that all the trapped FITC molecules have leaked out of the swollen capsules during dialysis. This result indicates that changing the external temperature could control the release behavior of FITC from the PNIPAM capsules.

To elucidate the relation of FITC-release kinetics with the dialyzing time in more detail, we used the fluorescence emission spectra to measure the FITC loading amount in the nanocapsules dialyzed after different time at 20 and 40 °C. Inspection of Fig. 7 indicates that the kinetics of FITC release strongly depends on the temperature of the release solution. For the nanocapsules dialyzed at 20 °C, 31.1% of FITC was left in the capsules after 48 h, while for the nanocapsules dialyzed at 40 °C, 66.3% of FITC remained after 48 h. Such a difference on FITC releasing suggests strongly that temperature is an important factor to control the FITC releasing rate and capacity. It is noteworthy that there is still a considerable release of FITC from the nanocapsules even when they are exposed in a solution with temperature above the T_{VPT} of PNIPAM capsules (32 °C). However, it may be possible to minimize this behavior by thickening the polymer shell, so as to provide a thicker barrier towards FITC release. The result of Fig. 7 indicates that the external temperature-stimuli could effectively change the release behavior of the model guest molecules FITC. Above T_{VPT} , the PNIPAM wall is dehydrated and the FITC within the inner cavity permeates more slowly. While

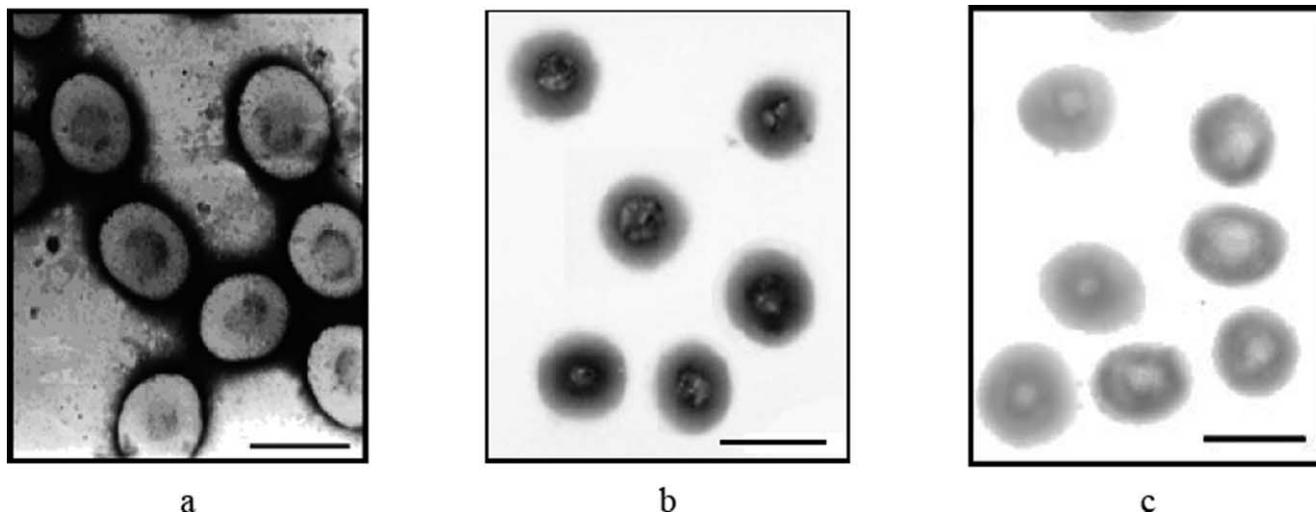


Fig. 6. TEM images of FITC-trapped nanocapsules EBN2 after HF etching at 40 °C (a), stained by phosphate-tungstic acid and further after 3-day dialysis at 40 °C (b), 20 °C (c), both stained by CsOH aqueous solution. The scale bars were all 200 nm.

below T_{VPT} , the inner FITC could pass through the swollen PNIPAM wall easily.

4. Conclusion

We have developed a convenient method to prepare novel thermosensitive PNIPAM nanocapsules through SiO_2 -template precipitation polymerization. The model guest molecules FITC were entrapped within the capsule core before the formation of cross-linked polymer shell. After removal of SiO_2 core by HF etching at 40 °C, the pre-trapped FITC molecules still remained within the inner cavity and their release could be controlled by the external temperature change. Further experiments to study the

release rate and reversible loading of FITC in the capsules are under way.

Acknowledgements

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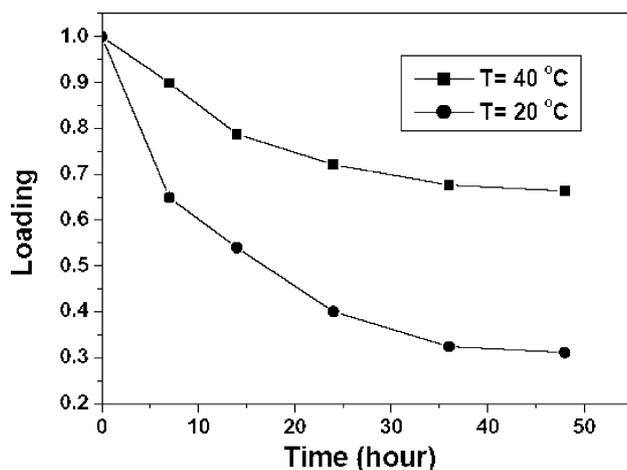


Fig. 7. Release profiles of the PNIPAM nanocapsules EBN2 at different temperatures. Data were obtained using fluorescence emission intensity of the nanocapsules at 518 nm, all the spectra were normalized with excitation at 450 nm.

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