Carbohydrate Polymers 90 (2012) 690–695

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

One-pot synthesis of poly(N-isopropylacrylamide)/chitosan composite microspheres *via* microemulsion

Yi Gong, Qing Lin Liu*, Ai Mei Zhu, Qiu Gen Zhang

Department of Chemical and Biochemical Engineering, National Engineering Laboratory for Green Chemical Productions of Alcohols, Ethers and Esters, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China

ARTICLE INFO

Article history:
Received 16 April 2012
Received in revised form 27 May 2012
Accepted 29 May 2012
Available online 6 June 2012

Keywords:
One-pot synthesis
Multisensitive microspheres
Chitosan
Poly(N-isopropylacrylamide)
Drug delivery

ABSTRACT

This work presents a new approach for the synthesis of multiresponsive composite microspheres of PNIPAM/chitosan. The resulting microspheres in a sandwich structure with PNIPAM nanoparticles embedded in the crosslinked chitosan matrix were characterized. Compared to other preparation methods, this proposed technique not only is a facile route but also endows the microspheres a desirable structure. The products undergo a temperature induced volume phase transition and exhibit an appreciable pH response. They are further tested as drug carriers to investigate potential application. The encapsulation efficiency in acidic environment (pH = 4.0) is 73.5% and much higher than that in neutral (20.3%, pH = 6.9) and alkaline (15.1%, pH = 9.2) environments. The release of the drug from the microspheres can be controlled by pH and temperature.

1. Introduction

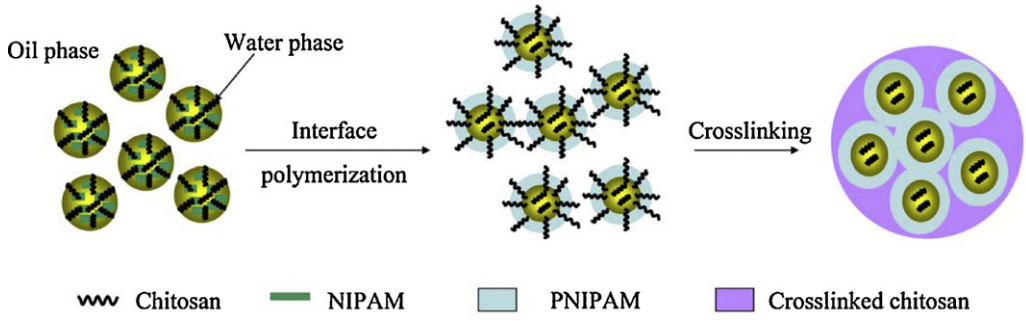
Multisensitive microspheres have currently attracted great interest for a wide variety of potential applications such as catalysis (Coutinho, Harrinauth, & Gupta, 2008), controlled drug delivery (Liu et al., 2009; Zalfen et al., 2008) and chemical separation (Chen, Shi, Xue, Chen, & Wan, 2010). They are usually composed of multicomponent where each component should play their individual role to endow the microsphere multisensitive to environmental stimuli.

Poly(N-isopropylacrylamide) (PNIPAM) is one of the most widely studied materials owing to its thermo sensitivity. It undergoes a volume phase transition at the lower critical solution temperature (LCST) around 32 °C (Housni & Narain, 2007) with temperature variation. At temperatures below the LCST, water acts as a good solvent for the polymer chains forming hydrogen bonds with the amide oxygen. When the temperature is raised above the LCST, polymer–polymer interactions become dominant expelling water (Medeiros, Santos, Fessi, & Elaissari, 2011). Chitosan has a wide variety of applications in biomedical field due to its good stability, low toxicity, and excellent biocompatibility (Muzzarelli, 2009; Muzzarelli, Boudrant, et al., 2012; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012; Nagpal, Singh, & Mishra, 2010). As a weak base, chitosan is insoluble in water and organic solvents. It is however soluble in dilute aqueous acidic solution (pH < 6.5), which can convert the glucosamine units into a soluble form of R-NH_3^+ (Sinha et al., 2004). These bring chitosan pH sensitive properties. The combination of PNIPAM and chitosan has been widely studied as a multisensitive material. A series of methods for the synthesis of multicomponent microspheres are reported and can be roughly categorized as follows.

(1) *Microemulsion and solvent evaporation*. These two methods are relied on microemulsion, and the difference is only in the product separation unit. Two monomers or polymers are dissolved in the disperse phase of the microemulsion. Microspheres consisting of copolymers or crosslinked polymers are obtained when the synthesis process is completed. Poly(N-isopropylacrylamide-co-acrylic acid)/titanium dioxide (TiO_2) was prepared *via* UV-initiated free radical polymerization by Wang, Liu, and Zhu (2011) based on the microemulsion. Jiang, Qiu, and DeLuca (2003) prepared the insulin-loaded poly(acryloyl-hydroxyethyl starch)–poly(D,L-lactide-co-glycolide) composite microspheres using the solvent evaporation method. (2) *Ionotropic gelation*. This method is based on electrostatic interaction between negatively and positively charged functional groups of each polymer. Gao et al. (2006) synthesized smart polymer nanocontainers by self-assembly of polyelectrolytes consisting of chitosan and poly(N-isopropylacrylamide-co-acrylic acid). (3) *Seed polymerization*. One polymer microsphere was first obtained as seed, and the other was then tethered onto the seed microsphere surface *via* chemical bonds. As a result, core–shell structure composite microspheres were formed. Fe_3O_4/poly(N-isopropylacrylamide)/chitosan

* Corresponding author. Tel.: +86 592 2188072; fax: +86 592 2184822.
E-mail address: qlliu@xmu.edu.cn (Q.L. Liu).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.05.098



Scheme 1. Schematic of the synthesis of composite microspheres.

composite microspheres were fabricated *via* emulsion polymerization in two steps by Li, Zhu, Liu, and Zhang (2008). Multi-layer microspheres can also be prepared *via* layer-by-layer deposition in the frame of seed polymerization. Poly(ethyleneglycol dimethacrylate-co-methacrylic acid)@poly(ethyleneglycol-co-vinyl pyridine)/titania/polymer tetra-layer microspheres were synthesized by Li, Liu, Qin, and Yang (2010).

The methods mentioned above still have drawbacks. For microemulsion and solvent evaporation methods, polymers are mixed together and structure control is therefore in difficulty. Although microspheres prepared *via* ionotropic gelation and seed polymerization have certain structure, long production time and high production costs are caused by multi-process preparation. The microspheres consisting of chitosan and PNIPAM have been widely reported since they could respond to both temperature and pH (Chuang, Don, & Chiu, 2011; Duan et al., 2011; Sanoj Rejinold, Sreerekha, Chennazhi, Nair, & Jayakumar, 2011). To the best of our knowledge, however there have been rare reports on synthesis of multicomponent microspheres in desirable structure with multisensitivity *via* a one-pot polymerization approach under mild reaction conditions.

Herein, we reported a new facile one-pot route to synthesize PNIPAM/chitosan composite microspheres in a desirable structure *via* polymerization under mild reaction conditions. As shown in Scheme 1, NIPAM monomer was dissolved in chitosan solution to form a water phase, and 2,2′-azobisisobutyronitrile (AIBN) used as an initiator was dissolved in toluene to form an oil phase. Then each phase was emulsified and mixed to form water-in-oil (W/O) emulsion in the presence of a low hydrophile–lipophile balance (HLB) number surfactant. The interfacial polymerization was first triggered by heating to form PNIPAM nanoparticles, followed by cooling since the PNIPAM becomes hydrophilic at low temperature. Glutaraldehyde (GA) solution was then added into the system to crosslink the chitosan onto the surface of the PNIPAM nanoparticles. They will crosslink together to form the composite microspheres in this way.

We can thus make chitosan microspheres containing PNIPAM nanoparticles *via* this approach. To explore the potential applications, the antibiotic drug berberine hydrochloride (BBH) was loaded into these microspheres and its *in vitro* release under different conditions was investigated.

2. Materials and methods

2.1. Materials

Chitosan with deacetylation degree (DD) of 90% and molecular weight (Mw) of 200 kDa was purchased from Yuhuan Ocean Biochemical Company, China. NIPAM (Tokyo Chemical Industry Co. Ltd.) was recrystallized in hexane before use. N,N-methylenebisacrylamide (MBA, analytical grade) was from the 5th Plant of Shenyang Reagent. Glutaraldehyde (GA) was from Sinopharm Chemical Reagent Co. Ltd. The water used in this work was purified by a Milli-Q reagent grade system. Buffer solutions of various pHs of 4.0, 6.9 and 9.2 were from United Initiators (Shanghai) Co. Ltd. All other solvents and reagents of analytical grade were used without further purification.

2.2. Synthesis of PNIPAM/chitosan composite microspheres

500 mg of NIPAM and 60 mg of N,N-methylenebisacrylamide (MBA) were dissolved in chitosan solution (2 wt% acetic acid solution) to form a water phase. 1.0 g of AIBN and 10 ml of Span 80 were dissolved in 150 ml of toluene to form an oil phase. The resulting water phase and oil phase were mixed and emulsified to obtain a high quality emulsion. The emulsion was then poured into a flask equipped with a stirrer and a N_2-inlet. After bubbling with N_2 for 30 min, the flask was maintained at 60 °C for 24 h, then cooled down to 25 °C to ensure the PNIPAM nanoparticles to be hydrophilic under vigorous stirring. Afterwards, 1 ml of various concentrations of glutaraldehyde was added into the flask. After 3 h, the final product was isolated *via* centrifugation at 4000 rpm and then washed using ethanol, acetone and deionized water for several times until the oil phase was completely removed. The resulting products were freeze-dried and stored for further use.

2.3. Morphological and physico-chemical characterization

2.3.1. Morphological characterization

Transmission electron microscopy (TEM) images were recorded using a TEM (JEOL, F30, 100 kV FEI Company, Holland), and the samples for the TEM characterization were prepared by placing one drop of sample on copper grids coated with carbon. Scanning electron microscopy (SEM) characterization was carried out using LEO1530 (Germany) and XL30 (Holland). A drop of sample was put onto a silicon wafer substrate and sputter coated with a homogeneous gold layer for charging dissipation during the SEM imaging. The hydrodynamic diameters of the particles were determined by quasi-elastic light scattering (Malvern Autosizer 4700). Confocal laser scanning microscopy (CLSM) (MRC1024, Bio-Rad, UK) was used to investigate inner structure of the composite microspheres. The microspheres were suspended in distilled water. One drop of the suspension was placed directly onto glass slides for examination at the excitation wavelength of 488 nm.

2.3.2. Physico-chemical characterization

Fourier transform infrared (FTIR) spectroscopy (NICOLETFTIR-740SX, USA) spectra of the samples were recorded for structure analysis. The absorbance of the products was detected using UV–vis spectrophotometer (TU-1900, Beijing). X-ray diffraction (XRD) (Panalytical X'pert Philip, Holland) was used to determine the crystal structure of the samples.

Table 1
The recipes for preparing the composite microspheres.

	Sample code							
	MS-0	MS-1a	MS-2a	MS-3a	MS-3a-C1	MS-3a-C2	MS-4a	MS-4b
CS content (g/150 ml)	0	0.5	1	1.5	1.5	1.5	2.0	2.0
GA content (wt%)	0.25	0.25	0.25	0.25	0	0.25	0.25	0.13
NIPAM (mg)	500	500	500	500	500	0	500	500
MBA (mg)	60	60	60	60	60	60	60	60

2.3.3. Thermo and pH sensitive testing

2 mg of the product was ultrasonically dispersed in 5 ml of pH buffer solutions of various pHs of 4.0 (PBS), 6.9 (PBS) and 9.2 (borate buffer solution) to form a liquid. A certain amount of the liquid was transferred to a cuvette for absorbance measurement at the wavelength of 640 nm in a temperature range 28–37 °C. The sample for each testing should be stable for at least 2 h. The temperature and UV spectrum used for testing were strictly controlled.

2 mg of the product was dispersed into 5 ml of solutions within a pH range 1–13 adjusted using HCl and/or NaOH. Each sample was stayed still at 25 °C for 2 days before testing. The hydrodynamic diameter (D_h) of the microspheres was measured using dynamic light scattering (DLS) after ultrasonic dispersion for 3 min.

2.4. Controlled drug release testing

2.4.1. Loading

20 mg of the composite microspheres was dispersed into 20 ml of the BBH aqueous solution (0.5 mg ml^{-1}) placed in a thermostatic shaker bath at 25 °C. The BBH concentration was measured every 1 h for three days to calculate the drug loading rate. Encapsulation efficiency (*EE*) and drug loading (*DL*) can be calculated as follows:

$$EE\ (\%) = \frac{\text{weight of BBH in the feed} - \text{weight of BBH in the supernatant}}{\text{weight of BBH in the feed}} \times 100$$

$$DL\ (\%) = \frac{\text{weight of BBH in the microspheres}}{\text{weight of microspheres}} \times 100$$

The drug loaded microspheres were then isolated by standing for 24 h and then freeze-dried for drug release testing.

2.4.2. Releasing

20 mg of the BBH loaded microsphere powders was dispersed in 10 ml of buffer solution placed in a shaker at 45 °C. 2 ml of the samples was drawn at specific time intervals (2 ml of fresh buffer solution was replenished meanwhile), and the drug concentration was analyzed using a UV–vis spectrophotometer at 460 nm. When the rate of drug release from the microspheres tended to be steady, the environmental temperature was set to 4 °C to study their thermo sensitivity.

The drug release profiles under various pHs were also studied. The drug loaded MS-4a (2 mg) was dispersed into 15 ml of buffer solution placed in a shaker at 37 °C. The typical procedure is the same as that previously described. The amounts of drug loading and releasing were calculated based on the standard curve obtained from BBH in the buffer solutions.

3. Results and discussion

3.1. Preparation of PNIPAM/chitosan microspheres

The microspheres were prepared using microemulsion method in one pot. The water phase was the mixture of chitosan solution, NIPAM and MBA. The initiator AIBN and surfactant Span 80 were dissolved in toluene to form the oil phase. The process for the preparation of the composite microspheres includes two stages: the synthesis of PNIPAM nanoparticles and chitosan crosslinking.

3.1.1. Synthesis of PNIPAM nanoparticles

The reaction was triggered by the AIBN when the system temperature reached 60 °C. The polymerization was taken place on the oil and water interface since the initiator and monomer were in the continuous phase and dispersed phase respectively. PNIPAM wall was formed when the reaction was completed. Stirring speed is found to be a crucial factor in this process. No product can be received under violent stirring. This is because the polymer wall formed on the surface is too weak to suffer from the shear force of stirring.

3.1.2. Chitosan crosslinking

GA was added to crosslink chitosan for tethering the PNIPAM nanoparticles together when the system was cooling down. Chitosan will be grafted onto PNIAM since the amino groups of chitosan are well-known chain transfer agents. In this case, the PNIPAM nanoparticles were agglomerated to form big microspheres since chitosan on the nanoparticles surface was crosslinked with each other or the chitosan in the unreacted drops under violent stirring. The PNIPAM/chitosan composite microspheres were obtained after centrifugation. The effect of chitosan concentration on the synthesis of microspheres was studied. The synthesis details are shown in Table 1.

As shown in Fig. 1, the microspheres were almost collapsed at a low chitosan concentration. This is because low mechanical strength cannot stand their morphology. The products tend to have stronger mechanical strength, better morphology and smoother surface with increasing chitosan concentration.

3.2. Morphology of PNIPAM nanoparticles

To describe the microsphere formation process and mechanism more clearly, we first characterized the pristine PNIPAM nanoparticles (MS-0). The SEM image of the pristine PNIPAM nanoparticles of approximately 100 nm is shown in Fig. S1(a) (Supplementary data). The size distribution of the nanoparticles is narrow and different from the conventional emulsion polymerization in which size ranges from nano to micron meter (Fundueanu, Constantin, & Ascenzi, 2010; Wang et al., 2011). There are two possible reasons for this phenomenon. First, NIPAM is a hydrophilic monomer dissolved in the water phase, initiator AIBN is however dissolved in the oil phase. The polymerization only takes place when the water drops capture the initiator from the continuous oil phase. Small drops have large specific surface area and thus get more chance to form polymer wall. Second, the polymer wall on the big droplet interface will suffer from larger mechanical shear force and is thus hard to form. Nanoparticles can also be observed in the TEM image of MS-3a-C1 without GA crosslinking (Fig. S1(b) in Supplementary data).

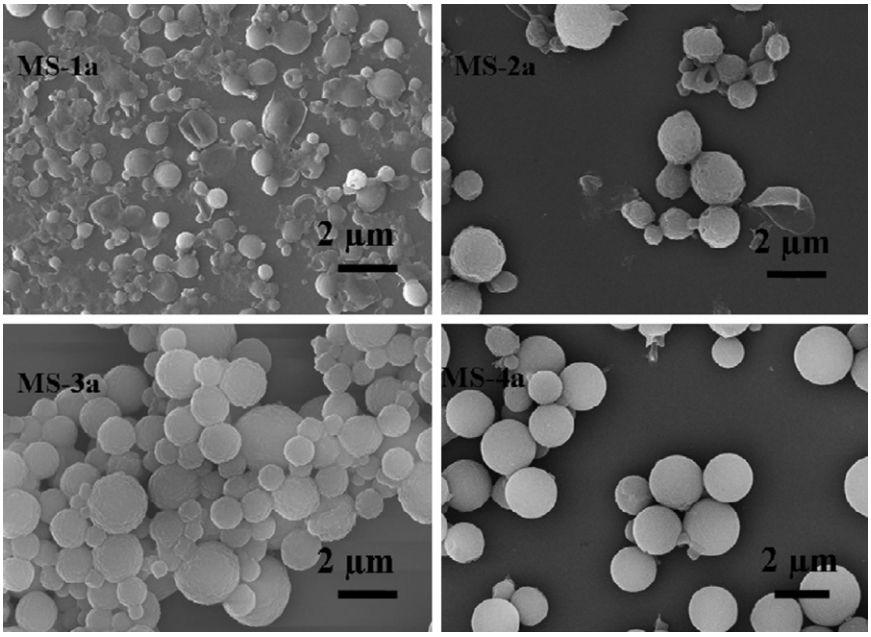


Fig. 1. SEM images of the microspheres of MS-1a, MS-2a, MS-3a and MS-4a.

This indicates that nanoparticles were also formed when the chitosan was added into the dispersed phase.

3.3. Morphology of PNIPAM/chitosan composite microspheres

The size distribution of the microspheres was measured using dynamic light scattering. Fig. S2(a) (in Supplementary data) shows the average diameter of 1.77 μm in a size range 1.5–2.0 μm. This is suitable to be administered intravenously because the diameter of the smallest blood capillary is approximately 4 μm (Müller, 1991).

Wei et al. (2007) reported that the chitosan microspheres crosslinked with GA were found to be autofluorescent. This conclusion was verified in the present work. Fluorescence signal was detected from the PNIPAM/chitosan microspheres (Fig. 2). Meanwhile, some areas in the microspheres without florescence signal were also observed. This indicates that both fluorescence and non-fluorescence areas coexist in the microspheres which are related to the crosslinked chitosan and PNIPAM nanoparticles respectively. This suggests the PNIPAM nanoparticles being embedded in the chitosan microspheres.

Fig. S2(b) (in Supplementary data) shows the TEM image of the crosslinked PNIPAM/chitosan microspheres. The nanoscale sphere shadows with size matching PNIPAM nanoparticles were observed in the microspheres. The products in the present work have a sandwich structure, as noted by Elodie (2004). However, if microspheres were synthesized without addition of PNIPAM, the products present a homogeneous contrast grade (Fig. S2(c) in Supplementary data). This is adminicular evidence for PNIPAM nanoparticles being embedded in the chitosan microspheres.

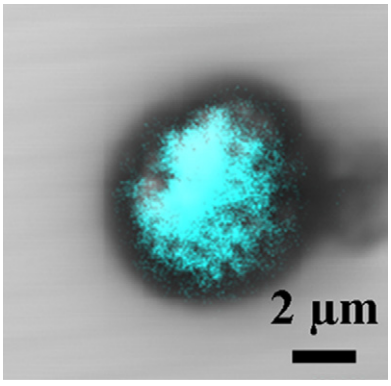


Fig. 2. CLSM image of the sample of MS-4a.

3.4. Structure of PNIPAM/chitosan composite microspheres

Fig. 3 shows the FTIR spectra of the pristine chitosan, NIPAM monomer and PNIPAM/chitosan composite microspheres. The peak around 3400 cm^{-1} observed in curves (a) and (b) is related to the —OH group. For the IR spectrum of chitosan, the peak of 1384 cm^{-1} is associated with —C—O stretching of the primary alcoholic group in chitosan. This peak can also be observed in the composite microspheres. Two peaks at 1657 and 1556 cm^{-1} can be observed in both (b) and (c). These are associated with the C=O stretching and the secondary amide N—H deformation vibration of NIPAM. The results suggest that the microspheres consist of chitosan and PNIPAM.

XRD patterns of the composite microspheres prepared with various chitosan concentrations are shown in Fig. S3 (Supplementary data). The characteristic chitosan peak is around $2\theta = 20\,^{\circ}C$ (Silva, Silva, Coutinho, Mano, & Reis, 2004), and the peak

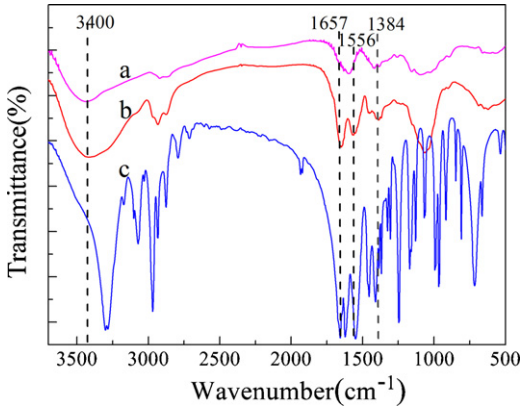


Fig. 3. FTIR spectra of chitosan (a), PNIPAM/chitosan composite microspheres (b) and NIPAM (c).

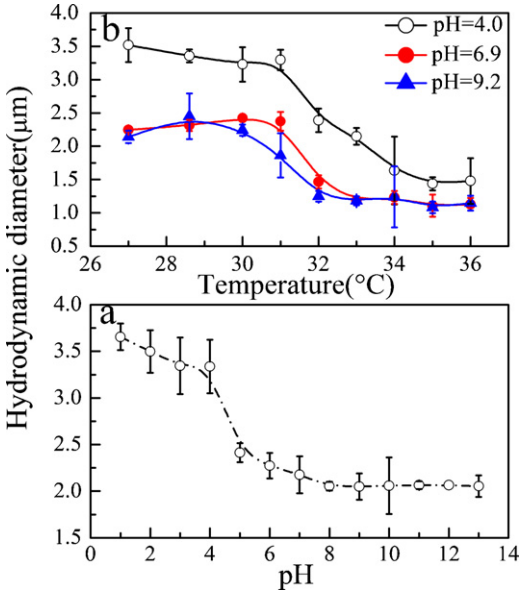


Fig. 4. Relationship between the hydrodynamic diameter of the composite microspheres with pH at 25 °C (a) and temperature at various pHs of 4.0, 6.9 and 9.2 (b).

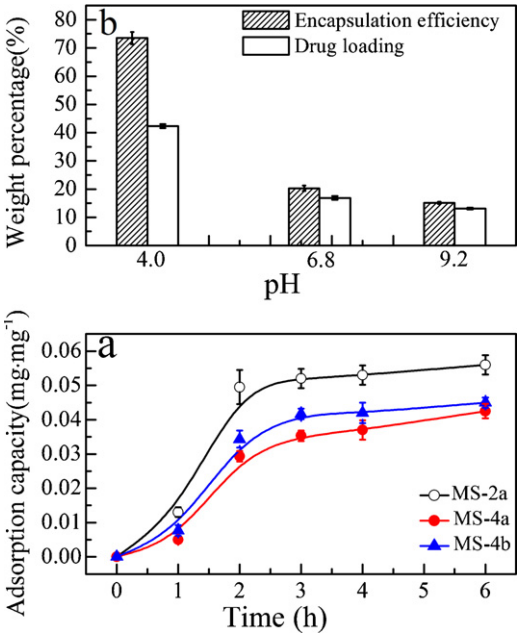


Fig. 5. Drug loading curves of MS-2a, MS-4a and MS-4b at 25 °C (a) and encapsulation efficiency and drug loading of MS-4a in different pH environments at 25 °C after adsorption for 3 days (b).

intensity tends to weaken with decreasing chitosan concentration. This indicates that the crystallinity is in a decreasing order from MS-4a to MS-2a. One knows that there are crystal region and amorphous region in crystal polymers. Compared to the amorphous region, the molecular chains in the crystal region are in an orderly arrangement and thus have a compact structure and high density. Therefore, the polymer structure is in a decreasing order in compactness from MS-4a to MS-2a. These conclusions can also be confirmed in the drug loading and releasing testing.

3.5. Thermo and pH sensitivity

The sensitivity of the microspheres toward the environmental stimuli is essential for their application in controlled release. The thermo and pH sensitive properties of the products are therefore characterized. The pH sensitivity of the product is tested by DLS (Fig. 4). The hydrodynamic diameter of the microspheres decreased with increasing pH value. In an acidic medium, the $-NH_2$ group of chitosan is protonated and the polymer chain stretches so the diameter of the microspheres increases accordingly. As shown in Fig. 4(a), the diameter decreased sharply when approaching pH 5. This suggests a good pH sensitivity of the microspheres.

The influence of temperature on the hydrodynamic diameter of the composite microspheres in various pH media was studied. The thermo-induced dimensional variations of the microspheres around 32 °C were observed (Fig. 4(b)). This indicates that the PNIPAM in the composite microspheres swells or shrinks when the temperature is below or above the LCST. The thermo sensitivity can also be demonstrated in different pH environments.

3.6. Controlled drug release

Considering its unique properties, BBH was used as a model drug to investigate the potential applications of the microspheres in controlled drug release. The drug loading is found mainly to be dependent on chitosan concentration. Fig. 5(a) shows that the amount and the rate of drug loading decreased with increasing chitosan concentration. The polymer structure is loose at a lower chitosan concentration and thus drug is easy to permeate across it. The relaxed structure can also enhance the drug loading because there is more space in the polymer for the drug to reside. The rate of drug loading slowed down after 2 h.

The encapsulation efficiency and drug loading of the composite microspheres are found to be pH-dependent. The drug is easily adsorbed into the microspheres when pH decreases. As shown in Fig. 5(b), the BBH encapsulation efficiency at pHs of 4.0, 6.9 and 9.2 was 73.5, 20.3 and 15.1% respectively. This is another evidence of pH sensitivity of the composite microspheres. The pH sensitivity makes the microsphere volume increase when pH decreases. The microspheres became swollen and loosened at low pH condition. It is thus easy for BBH to load in. The drug loading content is in a similar trend to the encapsulation efficiency for the same reason.

The release of BBH from the microspheres was also investigated. The influence of chitosan concentration, amount of crosslinker and temperature on drug release was taken into account in this work. There are two steps in drug release. Testing was first performed at 45 °C until drug release achieved equilibrium. Then the system temperature was adjusted to 4 °C to demonstrate thermo sensitivity of the microspheres. We choose 4 °C because the thermo sensitivity is more remarkable within a larger temperature drop (4–45 °C). Fig. 6(a) shows the *in vitro* release profiles of BBH from MS-2a, MS-4a and MS-4b in phosphate buffer saline (PBS, pH 7.4). The result indicates that low chitosan concentration leads to more and faster drug release in the first stage. One may conclude that the microspheres prepared with low chitosan concentration have a loose structure and the drug is easy to permeate in them. Lower crosslinker concentration can also lead to faster drug release. This is because polymer interchain spacing increases with decreasing the crosslinking degree. This is beneficial for drug diffusion. Some drug is found to release out at the environmental temperature below 4 °C. This is because PNIPAM changes into hydrophilic and is in a swollen state in this case. The drug in the PNIPAM nanoparticles can release out since PNIPAM network size tends to expand due to its hydrophilic interaction. The change of the temperature from 45 to 4 °C could in fact alter the solubility of the drug. The slow release of the drug from the microspheres could also arise from the drug dissolution and not from the drug diffusion through the network of the microspheres. As such, the release of free drug (blank

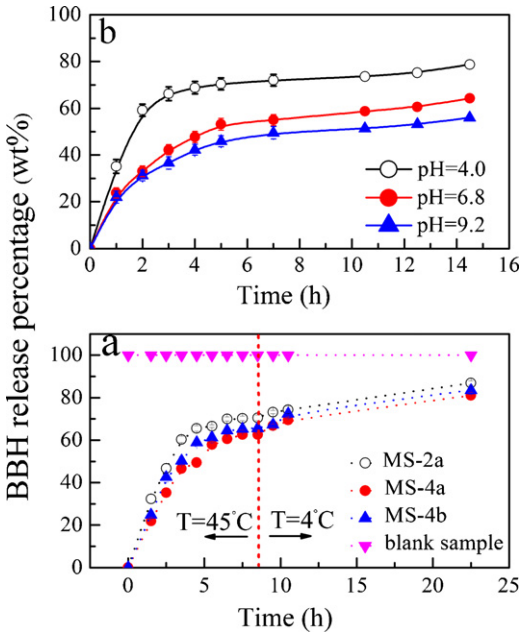


Fig. 6. BBH *in vitro* release curves of MS-2a, MS-4a, MS-4b and blank sample in phosphate buffer saline (PBS, pH 7.4) in two temperature steps (a) and drug release profiles from MS-4a at 37 °C under different pHs (b).

sample) was investigated at different temperatures to preclude the influence of the solubility of BBH on the drug release.

Fig. 6(b) shows drug release from MS-4a at 37 °C (physiological temperature) in different pH environments. The rate of drug release slowed down after 7 h at pHs of 6.9 and 9.2 with 60.4 and 50.9% drug lost respectively. But the release rate slowed down after 5 h at pH of 4.0 with 72.3% drug lost. One may thus conclude that the drug can release more and faster in an acidic medium. This is in accordance with the pH sensitivity mentioned above.

4. Conclusions

One-pot synthesis of the composite microspheres with PNIPAM nanoparticles embedded in chitosan microspheres was introduced in this work. The outer chitosan matrixes guarantee pH sensitivity and biocompatibility. The inner PNIPAM nanoparticles endow the composite microspheres with special thermosensitivity. The size of the microspheres can be controlled around 1.77 μm within a narrow size distribution. They could be used as drug carriers in the controlled drug release. The drug loading and releasing were carefully investigated.

Acknowledgments

Financial support from National Nature Science Foundation of China Grant Nos. 20976145, 21076170 and 21107089, Nature Science Foundation of Fujian Province of China Grant Nos. 2009J01040 and 2011J01055, and the research fund for the Doctoral Program of Higher Education (Nos. 20090121110031 and 20110121120041) in preparation of this article is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.05.098.

References

Elodie, B. L. (2004). Organic/inorganic nanocomposite colloids. *Encyclopedia of Nanoscience and Nanotechnology*, *8*(1), 305–332.
Chen, F., Shi, R. B., Xue, Y., Chen, L., & Wan, Q. H. (2010). Templated synthesis of monodisperse mesoporous maghemite/silica microspheres for magnetic separation of genomic DNA. *Journal of Magnetism and Magnetic Materials*, *322*(16), 2439–2445.
Chuang, C. Y., Don, T. M., & Chiu, W. Y. (2011). Preparation of environmental-responsive chitosan-based nanoparticles by self-assembly method. *Carbohydrate Polymers*, *84*(2), 765–769.
Coutinho, C. A., Harrinauth, R. K., & Gupta, V. K. (2008). Settling characteristics of composites of PNIPAM microgels and TiO_2 nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *318*(1–3), 111–121.
Duan, C., Zhang, D., Wang, F., Zheng, D., Jia, L., Feng, F., et al. (2011). Chitosan-g-poly(N-isopropylacrylamide) based nanogels for tumor extracellular targeting. *International Journal of Pharmaceutics*, *409*(1–2), 252–259.
Fundueanu, G., Constantin, M., & Ascenzi, P. (2010). Poly(vinyl alcohol) microspheres with pH- and thermosensitive properties as temperature-controlled drug delivery. *Acta Biomaterialia*, *6*(10), 3899–3907.
Gao, Y. Y., Chen, X. R., Liao, B., Ding, X. B., Zheng, Z. H., Cheng, X., et al. (2006). Polyelectrolyte self-assembly approach to smart nanocontainers. *Polymer Bulletin*, *56*(4–5), 305–311.
Housni, A., & Narain, R. (2007). Aqueous solution behavior of p(N-isopropyl acrylamide) in the presence of water-soluble macromolecular species. *European Polymer Journal*, *43*(10), 4344–4354.
Jiang, G., Qiu, W., & DeLuca, P. P. (2003). Preparation and in vitro/in vivo evaluation of insulin-loaded poly(acryloyl-hydroxyethyl starch)–PLGA composite microspheres. *Pharmaceutical Research*, *20*(3), 452–459.
Li, L. Y., Liu, G. Y., Qin, D. B., & Yang, X. L. (2010). Synthesis of P(EGDMA-co-MAA)/P(EGDMA-co-VPy)/titania/polymer tetra-layer microspheres. *Polymer Chemistry*, *1*(5), 650–657.
Li, P., Zhu, A. M., Liu, Q. L., & Zhang, Q. G. (2008). Fe_3O_4/poly(N-isopropylacrylamide)/chitosan composite microspheres with multiresponsive properties. *Industrial & Engineering Chemistry Research*, *47*(20), 7700–7706.
Liu, C. Y., Guo, J., Yang, W. L., Hu, J. H., Wang, C. C., & Fu, S. K. (2009). Magnetic mesoporous silica microspheres with thermo-sensitive polymer shell for controlled drug release. *Journal of Materials Chemistry*, *19*(27), 4764–4770.
Medeiros, S., Santos, A., Fessi, H., & Elaissari, A. (2011). Stimuli-responsive magnetic particles for biomedical applications. *International Journal of Pharmaceutics*, *403*(1), 139–161.
Muzzarelli, R. A. A. (2009). Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. *Carbohydrate Polymers*, *76*(2), 167–182.
Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*(2), 995–1012.
Muzzarelli, R. A. A., Greco, F., Busilacchi, A., Sollazzo, V., & Gigante, A. (2012). Chitosan, hyaluronan and chondroitin sulfate in tissue engineering for cartilage regeneration: A review. *Carbohydrate Polymers*, *89*(3), 723–739.
Müller, R. H. (1991). *Colloidal carriers for controlled drug delivery and targeting: Modification, characterization and in vivo distribution*. CRC Press.
Nagpal, K., Singh, S. K., & Mishra, D. N. (2010). Chitosan nanoparticles: A promising system in novel drug delivery. *Chemical & Pharmaceutical Bulletin*, *58*(11), 1423–1430.
Sanoj Rejinold, N., Sreerekha, P. R., Chennazhi, K. P., Nair, S. V., & Jayakumar, R. (2011). Biocompatible, biodegradable and thermo-sensitive chitosan-g-poly (N-isopropylacrylamide) nanocarrier for curcumin drug delivery. *International Journal of Biological Macromolecules*, *49*(2), 161–172.
Silva, R. M., Silva, G. A., Coutinho, O. P., Mano, J. F., & Reis, R. L. (2004). Preparation and characterisation in simulated body conditions of glutaraldehyde crosslinked chitosan membranes. *Journal of Materials Science: Materials in Medicine*, *15*(10), 1105–1112.
Sinha, V. R., Singla, A. K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., et al. (2004). Chitosan microspheres as a potential carrier for drugs. *International Journal of Pharmaceutics*, *274*(1–2), 1–33.
Wang, S. Q., Liu, Q. L., & Zhu, A. M. (2011). Preparation of multisensitive poly (N-isopropylacrylamide-co-acrylic acid)/TiO_2 composites for degradation of methyl orange. *European Polymer Journal*, *47*(5), 1168–1175.
Wei, W., Wang, L. Y., Yuan, L., Wei, Q., Yang, X. D., Su, Z. G., et al. (2007). Preparation and application of novel microspheres possessing autofluorescent properties. *Advanced Functional Materials*, *17*(16), 3153–3158.
Zalfen, A. M., Nizet, D., Jerome, C., Frankenne, F., Foidart, J. M., Maquet, V., et al. (2008). Controlled release of drugs from multi-component biomaterials. *Acta Biomaterialia*, *4*(6), 1788–1796.