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A convenient way to synthesize comb-shaped chitosan-graft-poly (N-isopropylacrylamide) copolymer

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ABSTRACT

Comb-shaped copolymers comprised of hydrophobic and hydrophilic blocks are self-assembled in aqueous solution, which results that they are suitable for delivery of hydrophobic drug molecules. Chitosan (CS) is an important biomaterial used widely in medical applications. Herein, a comb-shaped cationic copolymer composed of long biocompatible CS main chains and short PNIPAAm side chains was prepared via atom transfer radical polymerization (ATRP) by attaching an ATRP initiating group to N-phthaloyl chitosan. By subsequent removal of the protective groups on N-phthaloyl chitosan-graft-poly(*N*-isopropylacrylamide) (PHCS-g-PNIPAAm) copolymer with $N_2H_4{\cdot}H_2O$ lead to the polymer pendant amino groups, this study attempted to synthesize a pH/temperature multi-responsive material. This chitosan-graft-poly(*N*-isopropylacrylamide) (CS-g-PNIPAAm) copolymer is self-assembled in aqueous solution into stimuli-responsive core–shell micelles with hydrodynamic diameters of about 170 nm. Structural organization and solution behavior were then investigated utilizing ^{1}H NMR spectroscopy, transmission electron microscopy (TEM) and dynamic light scattering (DLS).

1. Introduction

Natural polysaccharides are ideal candidates for the design of novel biomaterials for they are renewable and nontoxic (Xu et al., 2010). The use of natural polysaccharides in different applications has attracted great interest due to increasing environmental concern. Chitosan is the linear and partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan (Muzzarelli et al., 2012). Chitosan with high biocompatibility can undergo chemical modification attributed to the presence of free amino groups (—NH_2) and hydroxyl groups (—OH), which increased the reactivity of the polymer (Li, Zhu, Liu, & Zhang, 2008) and is pH-responsive due to the protonation–deprotonation equilibrium of —NH_2 (Martins, Mano, & Alves, 2011), it is hydrophobic in its deprotonated state (pH > 6.3) and becomes water soluble in its protonated state (pH < 6.3) (Moustafine, Margulis, Sibgatullina, Kemenova, & Mooter, 2008). Because of its combination of reactivity, mechanical strength, high abundance, low cost, renewability, chitosan is an interesting natural substrate for grafting (Östmark, Harrisson, Wooley, & Malmström, 2007) and has great potential to be chemically modified to suit new application areas (Lindqvist et al., 2008). It has been reported that chitosan can be modified by graft copolymerization, via its amino (Elizabeth & Pillai, 2006) or hydroxyl groups (Liu et al., 2010) to achieve versatile molecular design.

Atom transfer radical polymerization (ATRP) (Wang & Matyjaszewski, 1995) is one of the most powerful techniques for its excellent controllability over the molecular weight and polydispersity and its facility of preparation of well-defined copolymers. The mechanism of ATRP is based on establishing a rapid dynamic equilibrium between growing radicals and dormant species and therefore keeps a relatively low concentration of radicals (Tang, Zhang, Zhu, Cheng, & Zhu, 2009; Ye & Narain, 2009). ATRP is a controlled/"living" radical polymerization technique, useful for the synthesis of functional macromolecules with controlled and complex architectures (Gao & Matyjaszewski, 2007; Lindqvist et al., 2008; Liu & Chen, 2006; Xu et al., 2009). In the past few years, the incorporation of ATRP technique has led to the preparation of many graft copolymers with well-defined structures with various architectures such as block, graft, star-like (Gorrasi, Stanzione, & Izzo, 2011), multi-armed and hyper-branched (Yang et al., 2011) polymers.

Poly(N-isopropylacrylamide) (PNIPAAm) is one of the most attractive stimuli-responsive polymer, which is water soluble at room temperature and is able to undergo a coil-to-globule transition above 32 °C (the low critical solution temperature, LCST) (Zhao et al., 2011) due to the hydrophobic (associated with

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isopropyl groups) and hydrophilic (associated with amide moiety in the pendant groups) interaction. At temperatures below LCST, the polymer is soluble in aqueous solution, preferring an expanded or hydrated coil conformation due to the formation of hydrogen bonds between the water molecules and the amide side chains, when temperature increases, the polymer becomes insoluble, preferring a folded globular structure and experiences a volume phase transition, intra-molecular hydrogen bonds are formed and precipitation of the polymer occurs due to its increased hydrophobicity at this time. It has been reported that PNIPAAm or others can be grafted onto cellulose for the preparation of stimuli responsive biomaterials (Ifuku & Kadla, 2008).

Bao, Li, Leong, and Gan (2010) synthesized a comb-shaped CS-g-PNIPAAm copolymer through "click chemistry". Amino groups were translated into alkynyl, acted as an active site for click, which may have slight effect on the pH-sensitivity of the copolymer. In this study, we planned to synthesize a comb-shaped chitosan copolymer with PNIPAAm chains grafted from chitosan as side chains. CS-g-PNIPAAm copolymer was prepared via a direct "graft-from" method, and they were characterized in detail. Additionally, the graft of short PNIPAAm chains onto chitosan enables the development of materials exhibiting both temperature and pH dependence. Meanwhile, the hydrophobicity–hydrophilicity balance of the CS-g-PNIPAAm copolymer is adjusted by the grafting copolymer composition, thus it can fabricate into core–shell micelles. It is worth noting that the natural polysaccharide chitosan used here is an environmental-friendly polymer and has already been used in bio-related researches for many years, thus the resultant comb-shaped copolymer may have great potential in drug controlled delivery systems.

2. Experimental

2.1. Materials

Chitosan (CS, deacetylation degree 83%, $M_w = 1.06 \times 10^6$, $M_w/M_n = 1.0121$) was obtained from Shiphan Chemical Co., Ltd. N-isopropylacrylamide (NIPAAm, >99%) was purchased from Tokyo Chemical Industry Co., Ltd. and recrystallized from hexane three times. Cuprous chloride (CuCl) and 2-bromoisobutyryl bromide (BIBB) were obtained from Aldrich and used without further purification. Phthalic anhydride was obtained from Jingchun Chemical Co., Ltd. Triethylamine (TEA) was received from Tianjin Chemical Company. 2,2′-Bipyridyl (bpy) was purchased from Shanghai Yuanfan Chemical Company Co., Ltd. Dimethylfomamide (DMF) was distilled under reduced pressure from magnesium sulfate and stored over molecular sieves (4 Å). Anhydrous ethanol and other reagents were of chemical pure grade or better and used without further purification. Deionized water was used for preparing all the solutions of the experiment.

2.2. Phthaloylation of chitosan

Protection by phthaloyl groups was chosen as one of the most suitable protection for the amino groups of chitosan from the point of view of our assumption (Chen, Tao, Qiu, Ren, & Hu, 2013) for solubilization of a rigid aminopolysaccharide. Phthaloylation of chitosan is performed in DMF/H_2O (95/5) with a certain amount of phthalic anhydride at 120 °C for 6.5 h (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002). Under typical reaction conditions, the reaction mixture became a clear and viscous solution after 5.5 h. The final reaction mixture was precipitated with 1000 ml deionized water. The crude product N-phthaloyl chitosan (PHCS) was extracted completely by a large amount of anhydrous ethanol for 48 h. It was obtained as a dark yellow powdery material.

2.3. Synthesis of N-phthaloyl chitosan macroinitiator (PHCS-Br)

2.0 g PHCS and 0.5 ml TEA were dissolved completely in 30 ml of anhydrous DMF with stirring and then kept in an ice bath. 240 μl BIBB dissolved in 5 ml anhydrous DMF was added dropwise into the flask through an equalizing funnel for a period of 30 min. After this addition, the flask was sealed and the reaction was allowed to proceed at room temperature for another 12 h to produce the macroinitiator PHCS-Br. The macroinitiator with a low degree of substitution of the initiating site (degree of substitution (DS) = 0.01, one initiating site per hundred glucose units) was used, by which the inter-molecular coupling reactions can be minimized between growing side chains during the reaction (Ma et al., 2010). The final reaction mixture was precipitated with 100 ml deionized water. The crude product was extracted completely by a large amount of anhydrous ethanol for 48 h. Finally, the PHCS-Br for the subsequent ATRP was dried under reduced pressure.

2.4. Synthesis of comb-shaped PHCS-g-PNIPAAm copolymer via ATRP

The comb-shaped copolymer PHCS-g-PNIPAAm was synthesized using a [NIPAAm (9.4 g, 83 mmol)]:[CuCl (82.2 mg, 0.83 mmol)]:[bpy (194.4 mg, 1.24 mmol)] molar feed ratio of 100:1:1.5 in 30 ml anhydrous DMF containing 2.5 g PHCS-Br at 70 °C. The reaction was performed in a 100 ml flask equipped with a magnetic stirrer. NIPAAm, PHCS-Br, and bpy were introduced into the flask containing 20 ml anhydrous DMF. After PHCS-Br and NIPAAm had dissolved completely, the reaction mixture was degassed by bubbling nitrogen for 30 min. Then, CuCl was added into the mixture under nitrogen atmosphere. The reaction mixture was degassed by repeated (four) freeze–pump–thaw cycles. The final reaction mixture was precipitated with 500 ml deionized water. The crude polymer was purified by reprecipitation trice in deionized water. Finally, the comb-shaped PHCS-g-PNIPAAm was dried under reduced pressure.

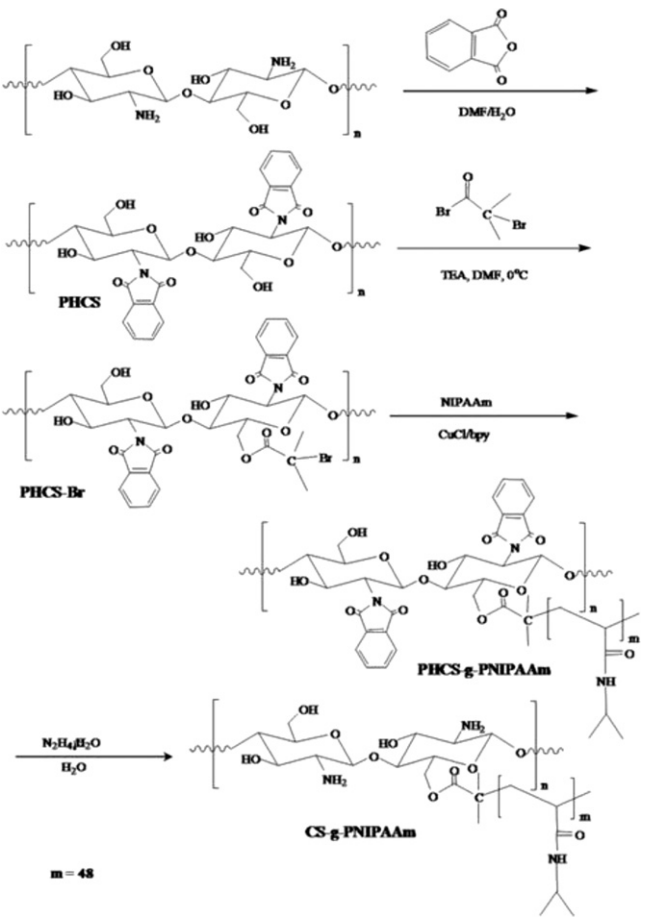
2.5. Deprotection

The phthaloyl-protected copolymer PHCS-PNIPAAm was stirred in 20 ml deionized water and heated to 100 °C under nitrogen. Hydrazine hydrate was added and the reaction was continued for 2 h to deprotect the phthaloyl group. The obtained transparent solution was dialyzed against deionized water used a dialysis membrane (molecular weight cut-off 14 KDa) to remove the impurities. The dialyzed solution was freeze-dried to get the final product, CS-g-NIPAAm comb-shaped copolymer.

3. Results and discussion

3.1. Preparation of CS-g-PNIPAAm copolymer

By attaching an ATRP initiating group to N-phthaloyl chitosan, copolymers based on natural polysaccharides can be synthesized conveniently. It is known in the literature that natural biomacromolecule/synthetic polymer hybrids can be prepared via both "graft-from" and "graft-to" strategies. In the "graft-to" (Zheng et al., 2010) method, end-functionalized polymer chains are attached to the backbone using appropriate conditions. This method is intrinsically limited to low grafting density and low molecular weight as the grafted polymer chains inhibit the diffusion of the incoming chains to the available reactive sites due to overcrowding. On the other hand, the "graft-from" (Wang et al., 2009) method is based on the in situ polymerization of the monomers from functionalized main chains. In the present work, comb-shaped



Scheme 1. Synthetic route to CS-g-PNIPAAm copolymer.

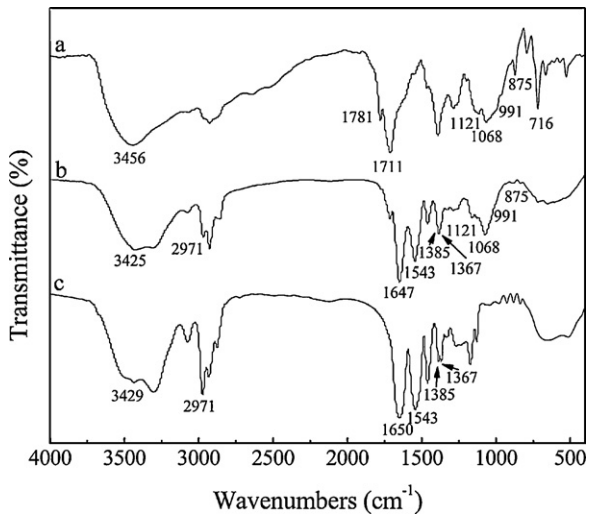


Fig. 1. FTIR spectra of (a) PHCS, (b) CS-g-PNIPAAm and (c) PNIPAAm.

copolymer of long, biocompatible chitosan and short temperature responsive PNIPAAm side chains was prepared via ATRP from the bromoisobutyryl-terminated N-phthaloyl chitosan via "graft-from" method. Grafted polymer brushes are grown from initiators with a sufficiently high grafting density such that the polymer chains are forced to stretch away or in the direction perpendicular to the backbone (Estillore, Park, & Advincula, 2010).

The detailed preparation process of CS-g-PNIPAAm copolymer has been described in the experimental part and summarized in Scheme 1. To establish a method of preparing CS-g-PNIPAAm copolymer using the ATRP method, four-step reactions involving phthaloylation of chitosan, preparation of the macroinitiator, graft copolymerization with NIPAAm monomers and deprotection to regenerate amino groups were carried out.

Chitosan is a renewable resource combining high molecular weight and reactivity. One significant drawback with using CS is, however, the difficult characterization and, in comparison to many synthetic polymers, limited solubility. To solve this problem, we prepared PHCS and chose it as a core backbone structure from which copper (I) mediated ATRP was performed. In order to prepare comb-shaped copolymers using chitosan as a backbone via ATRP, it is essential to introduce alkyl halide into CS, introduction of phthaloyl groups could reduce the inter- and/or intra-molecular hydrogen bonds, which resulted in the solubility of chitosan in organic solvents such as DMSO and DMF (Nishimura, Kohgo, & Kurita, 1991). Thus, this will facilitate the hydroxyl groups of chitosan to initiate the combination with BIBB. 2-Bromoisobutyryl ester is an excellent initiator for ATRP, and has been commonly used to prepare graft copolymers (Kim & Kadla, 2010). The hydroxyl groups on the surface of the substrate were first esterified by reaction with BIBB to yield covalently linked ATRP initiators. From an elemental analysis, the degree of polymerization is calculated to be 48 (DP = 48). The macroinitiator was then added to a reaction mixture containing monomers (NIPAAm), solvent (DMF), ligand (bpy) and was allowed to dissolve completely before the reaction was started through the addition of copper salts (CuCl) and degassing. Since the macroinitiator was of quite high molecular weight, it was important that they were allowed to dissolve thoroughly before the reaction was initiated, as polymerization from an undissolved macroinitiator would be expected to lead to uneven grafting of CS (Östmark et al., 2007). To obtain a kind of pH/temperature multi-responsive copolymer, regeneration of $-NH_2$ is an important stage, deprotection of PHCS-g-PNIPAAm is carried out as described in Section 2.5.

3.2. Characterizations

3.2.1. FTIR

FTIR spectra of PHCS, CS-g-PNIPAAm and PNIPAAm are given in Fig. 1. From Fig. 1a, the strong broad absorption at 3456 cm^{-1} is corresponding to the N—H stretching vibration of $-NH_2$ group and O—H stretching vibration of —OH groups in the chitosan main chain. Peaks at 1781 and 1711 cm^{-1} (imide C=O) are characteristic absorption bands of phthalimido groups (Kurita et al., 2002; Nishimura et al., 1991). The skeletal vibration of pyranose in chitosan (C—O—H) appeared at 1121, 1068, 991 and 875 cm^{-1} (Tapia et al., 2004; Ying, Xiong, Wang, Sun, & Liu, 2011). A strong absorption band at 716 cm^{-1} is assigned to the C—H bending vibration of phenyl ring, whose appearance indicates the introduction of phthanoyl groups onto chitosan.

Fig. 1b and c shows the FTIR spectra of CS-g-PNIPAAm and PNIPAAm, respectively. Characteristic peaks of PNIPAAm appeared at 1647–1650 cm^{-1} (C=O stretching amide I), 1543 cm^{-1} (N—H bending, amide II), 3425–3429 cm^{-1} (N—H stretching), 2971 cm^{-1} ($-CH_3$ asymmetric stretching) (Estillore et al., 2010) and 1367, 1385 cm^{-1} ($-CH_2$ bending, two methyl group in isopropyl group). Fig. 1b also shows that the characteristic peaks of phthanoyl group (1781, 1711 cm^{-1}, imide C=O) and phenyl ring (716 cm^{-1}) have disappeared, whereas peaks at 1647, 1543 cm^{-1} (amide I, II of PNIPAAm) and 1121, 1068, 991, 875 cm^{-1} (pyranose in chitosan) still exist in the final grafted product, whose appearance indicated the introduction of the PNIPAAm chains onto the chitosan. This fact indicates that the N-phthanoyl groups have been removed during

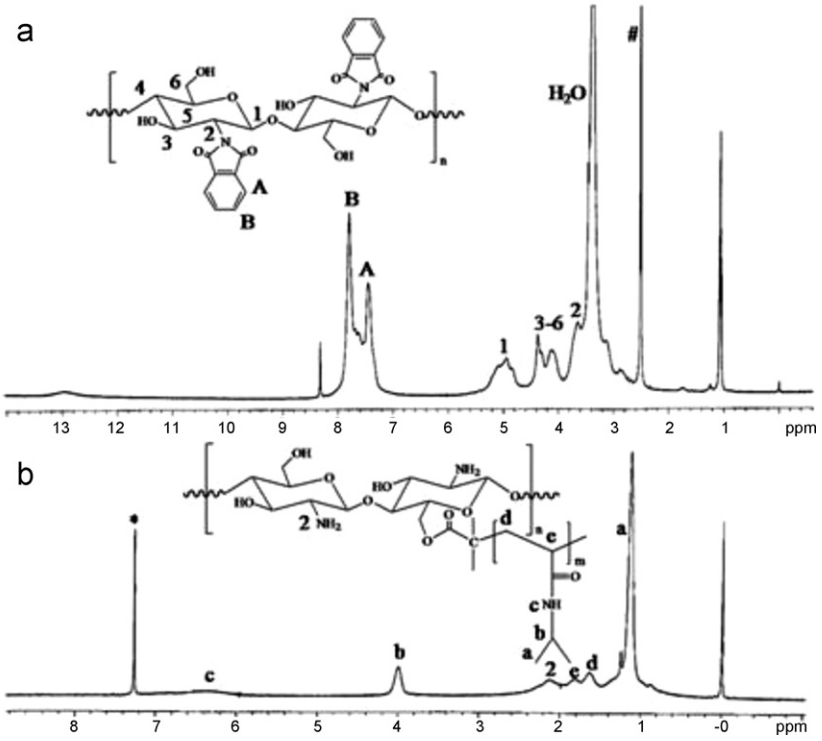


Fig. 2. ^{1}H NMR spectra of (a) PHCS in DMSO-d_6 and (b) CS-g-PNIPAAm in $CDCl_3$ (# DMSO-d_6 and * $CDCl_3$).

the deprotection reaction, and all peak assignments are consistent with previously studied PNIPAAm systems (Estillore et al., 2010).

3.2.2. ^{1}H NMR

^{1}H NMR spectra of PHCS is shown in Fig. 2a, peaks at 3.3, 3.9–4.5 and 4.9 ppm are assigned to H2, H3–6 and H1 of pyranose repeat units in CS. Aromatic phthalimido peaks of PHCS appear at 7.2–8.0 ppm. In Fig. 2b, signals of H-4, H-5, H-6 of chitosan is rather weak and cannot be fully resolved due to overlapping with those of PNIPAAm except for a typical peak of the proton on the carbon bearing the amino group (H2, 3.3 ppm) with a shift to the right side (2.4 ppm), which can also be found in the spectrum of PHCS. The graft copolymer not only show the original signals of chitosan, but also has new peaks of PNIPAAm side chains, peaks at 1.2, 1.6, 1.8, 4.0 and 6.4 ppm were assigned to Ha, Hd, He, Hb and Hc of PNIPAAm side chains (Duan et al., 2010).

3.2.3. X-ray diffraction

The X-ray diffraction pattern of CS, PHCS and CS-g-PNIPAAm are shown in Fig. 3. The diffraction pattern exhibited two broad diffraction peaks with different intensities at 20° and a relatively weak reflection centering at 10° associated with the most ordered region involving the acetamide groups and its intensity reflects the hydrated crystal content (Kumar, Varadaraj, & Tharanathan, 2007), which reveals that chitosan has some ordered regions inside its structure (Fajardo, Lopes, Rubira, & Muniz, 2012). Chitosan showed a strong reflection at 20° and a relatively weak reflection centering at 10° associated with the most ordered region involving the acetamide groups and its intensity reflects the hydrated crystal content (Kumar et al., 2007), which are associated with the crystalline regions and have been used to estimate crystallinity (Kolhe & Kannan, 2003). PHCS prepared in DMF/H_2O showed certain crystallinity as shown in Fig. 3b, with a weak reflection at 7°, 13° and 20°. The 10° reflection for chitosan is absent and the strong reflection at 20° is diminished and exists as a broad peak in PHCS. This indicates that there is a decrease in chitosan crystallization upon blending.

In Fig. 3c, CS-g-PNIPAAm does not have the peak $2\theta = 10°$, and the intensity of the peak $2\theta = 20°$ decreased drastically in comparison with chitosan, this is because of the introduction of the long bulk PNIPAAm side chains (Kurita et al., 2002). On the other hand, the copolymer does not show any well-defined CS crystalline peak, this suggests that there may be a significant reduction in the crystallinity of the chitosan in the copolymer.

A comparison of the XRD profiles of CS and PNIPAAm graft copolymer indicated that the grafting has destroyed the original crystallinity of chitosan. There is a decrease in chitosan crystallization upon grafting, and the grafting of phthaloyl groups or PNIPAAm onto chitosan chain takes place along the chain, giving

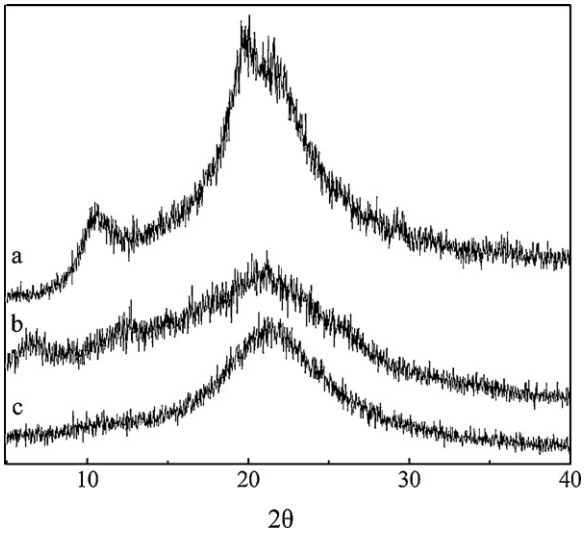


Fig. 3. X-ray diffraction pattern of (a) chitosan, (b) PHCS and (c) CS-g-NIPAAm.

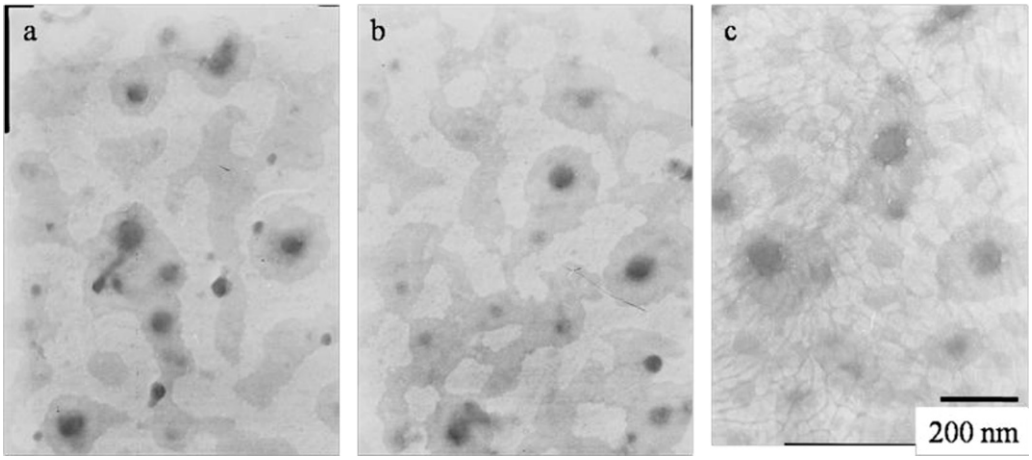


Fig. 4. TEM images of polymeric micelles of CS-g-PNIPAAm obtained for 2.5×10^{-4} g/ml aqueous solutions (pH 6.3) at 25 °C.

rise to a random copolymer (Elizabeth & Pillai, 2006). The addition of phthaloyl group destroyed the hydrogen bonds of chitosan matrix, PHCS became amorphous, and the graft copolymerization further decreased the crystallinity of PHCS due to the introduction of bulky pendant chains of grafted PNIPAAm.

The above characterizations showed that CS-g-PNIPAAm hybrid amphiphile was synthesized via a convenient "graft-from" method. On the basis of the above results, we can confirm the synthesis of graft CS copolymer with a well-defined molecular structure. In the subsequent section, the thermo-responsive association behavior of the copolymer in aqueous solutions will be investigated by TEM, DLS and UV–vis, respectively.

3.2.4. *Morphological properties of the self-assembled micelles*

As we all know, CS is insoluble in deionized water, after modification by PNIPAAm, the CS-g-PNIPAAm comb-shaped copolymer is soluble in deionized with a solubility of about 0.8 mg/ml (<LCST). This indicates that the PNIPAAm chains not only changed the hydrophobicity–hydrophilicity balance of the CS-g-PNIPAAm amphiphiles, but also probably reduced the inter- and/or intramolecular hydrogen bonds of the CS (Feng & Dong, 2006). Stimuli-responsive copolymers soluble in water have attracted growing attention due to their diverse self-assembly behavior in response to multiple stimuli, such as pH, temperature, and salt (Wan, Jiang, & Zhang, 2007). Amphiphilic polymers have generated increasing interest especially in the biomedical and biochemical fields in recent years due to their ability to form supermolecular structures in the aqueous environment. This kind of particles possesses some attractive features: (1) the hydrophilic shells not only provide different surface functional groups but also allow for further chemical modifications and bioconjugations on the particle surfaces. (2) The particle cores can provide appropriate mechanical properties required for desired application (Ho, Li, Wong, & Li, 2010; Thompson et al., 2008). Pure chitosan has an apparent pK_a of 6.3, as measured by potentiometric titration and the solubility of chitosan is poor when pH value is above 6.3. Specifically, at pH 6.3, amino groups became deprotonated so that chitosan became hydrophobic and then formed the insoluble core, while PNIPAAm chains kept soluble and surrounded the core to form the shell, thus a PNIPAAm shell, chitosan core micelle was formed.

Morphological properties of this self-assembled micelle is investigated by TEM and AFM, as shown in Figs. 4 and 5, It can be recognized that the microgels have a smooth spherical surface structure and exhibit well monodisperse spheres in distilled water. In addition, the images clearly reveal a two-layered spherical structure, the dark spherical area presents the CS core and the light corona around the core shows the PNIPAAm shell. TEM observation demonstrates that the typical size of this micelle is about 170 nm.

PNIPAAm bears both hydrophilic (amide) and hydrophobic (isopropyl and backbone) groups, so water molecules from cage-like structures to surround the hydrophobic moieties of PNIPAAm at low temperatures. While at pH 5.0, amino groups became protonated and chitosan was hydrophilic, the comb-shaped copolymer would be a fully hydrophilic chain at this time. An increase in

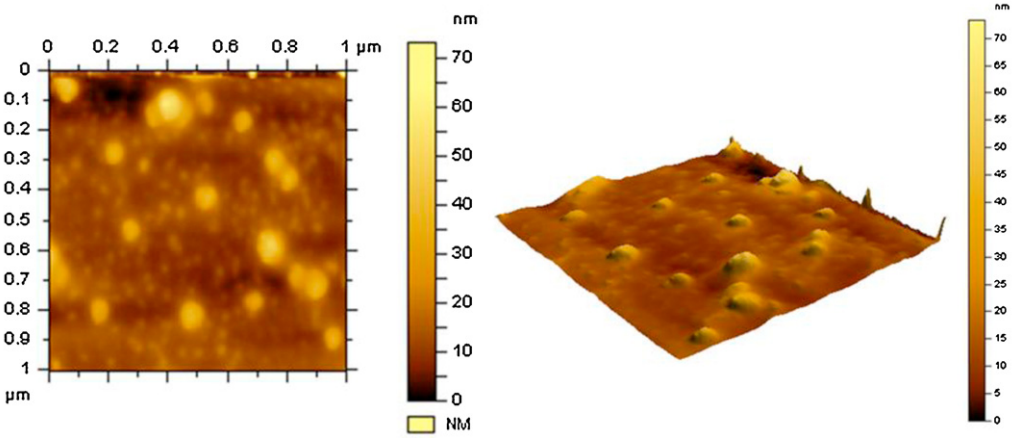


Fig. 5. AFM images of polymeric micelles of CS-g-PNIPAAm obtained for 2.5×10^{-4} g/ml aqueous solutions (pH 6.3) at 25 °C.

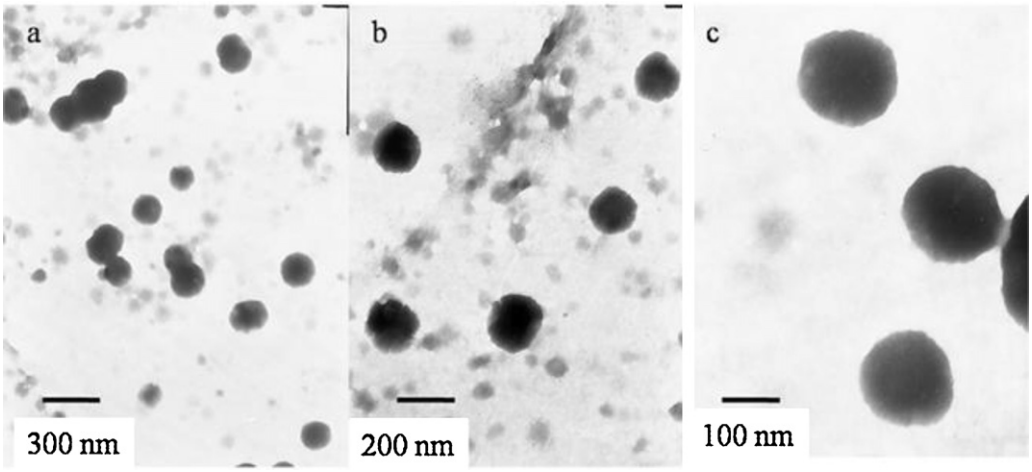


Fig. 6. TEM images of polymeric micelles of CS-g-PNIPAAm obtained for 2.5×10^{-4} g/ml aqueous solutions (pH 5.0) at 40 °C.

temperature will cause the destruction of the "water cage" and the exposure of the hydrophobic groups, leading to the formation of hydrophobic aggregates of PNIPAAm. When temperature increased above LCST, PNIPAAm chains became hydrophobic and formed the core, then chitosan formed the hydrophilic shell. Morphological properties of this self-assembled micelle were also investigated by TEM with a hydrodynamic diameter of about 160 nm as shown in Fig. 6.

Taking the above experimental results into consideration, the following mechanism for pH/temperature responsive self-assembly of CS-g-PNIPAAm copolymer is proposed as illustrated in Scheme 2.

As shown in Scheme 2, graft copolymer CS-g-PNIPAAm comprising natural material chitosan as the backbone and a kind of complementary polymer as the grafts is self-assembled in water and aggregate spontaneously upon appropriate temperature changes, it can dissolve molecularly in acidic aqueous solutions, and become self-assembled as temperature increased above LCST.

3.2.5. Determination of LCST of the copolymer

As described earlier, LCST is defined as the temperature at which the macromolecules undergo a coil-to-globule transition. The factors that can disrupt the critical balance between the hydrophilic and hydrophobic interactions can affect the LCST. Fig. 7 shows the temperature dependence of optical transmittance at 500 nm obtained for aqueous solution of the CS-g-PNIPAAm copolymer at different pH values. It was observed that, the final product obtained was soluble in water at lower temperatures, showing a transparent liquid appearance, when temperature was increased above LCST, solution changed to a white opaque aspect (Fig. 7).

At pH 6.3, the optical transmittance exhibits no obvious changes in the range of 25–33 °C. In this temperature range, the outer zone of PNIPAAm brushes still remains well-solvated and aggregation between particles does not occur (Wu, Zhang, Wang, & Liu, 2008). Above 33 °C, transmittance decreases abruptly from 78% to about 42% in the temperature range 33–36 °C due to the aggregation of hybrid particles. Hybrid nanoparticles with already collapsed PNIPAAm brushes tend to collide with each other at this temperature. This type of collision will surely contribute to the aggregation between different hybrid nanoparticles since the PNIPAAm corona becomes "sticky" above the critical phase transition temperature (Wu et al., 2008) (Scheme 2). In Fig. 7, at pH 6.3, the light transmittance fell sharply in a narrow temperature range until reached 42%, then the light transmittance increased a little, at this point, micelles aggregated and formed clusters, these large size clusters (>2500 nm, Fig. 8) tended to precipitate and thus the light transmittance increased.

At pH 5.0, amino groups are protonated and thus CS-g-PNIPAAm is a fully hydrophilic copolymer below LCST (Scheme 2). The LCST of the copolymer at pH 5.0 shifts to a higher temperature than that in pH 6.3. With the increase of the hydrophilic character of the protonated amino groups, the LCST increased, because the incorporation of the hydropholic block prevents the chain aggregation (Qiao, Niu, Wang, & Cao, 2010). As temperature increased, PNIPAAm side chains lost its solubility and formed the core, protonated chitosan formed the shell. Due to the positive charge on chitosan, this micelle tend to keep away from each other and the transmittance decreased slowly as temperature increased, as shown in Fig. 7.

3.2.6. DLS

Dynamic light scattering (DLS) characterization was carried out to study the effect of temperature on the size of the micelle. DLS experiment was performed at 25, 35, 40 and 45 °C, respectively.

We can see from Fig. 8 that, changes in the surface charge properties of the particles played a dominating role for pH-responsive behaviors. In contrast, the surface charge properties of this pH-responsive micelle change simply from an electrically charged state to an uncharged state when the pH monotonically changed

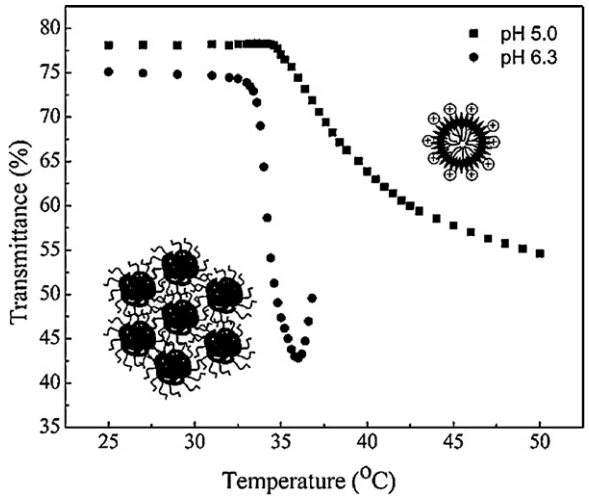
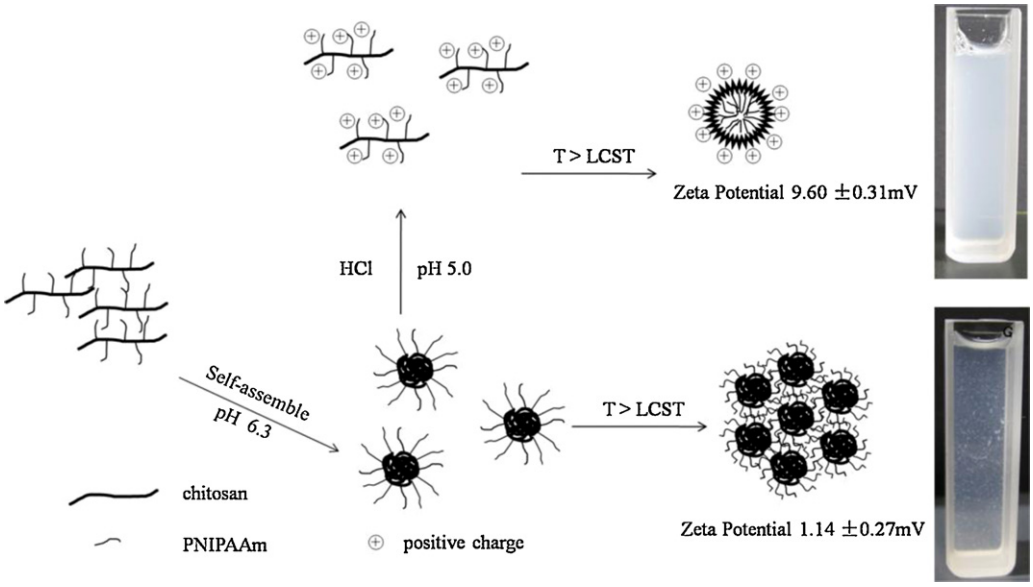


Fig. 7. Temperature-dependent optical transmittance at 500 nm obtained for 2.5×10^{-4} g/ml aqueous solution of the CS-g-PNIPAAm copolymer at different pH values.



Scheme 2. Mechanism for the pH responsive self-assembly of CS-g-PNIPAAm particles.

as shown in Scheme 2. At pH 6.3 (pK_a), part of NH_2 is deprotonated, there was little positive charge on the particle surface and zeta potential of the micelles is 1.14 ± 0.27 mV, hydrodynamic diameters measured by DLS indicated that the self-assembled micelles increased as temperature increased because the micelles aggregated to form clusters with the absence of electrostatic repulsion (Yang et al., 2010). While at pH 5.0, CS-g-PNIPAAm is a fully hydrophilic copolymer below LCST, as temperature increased, intra-molecular hydrogen bonds are formed, and PNIPAAm side chains became hydrophobic to form the core, protonated chitosan formed the shell, a protonated amino groups provided positive charges on the surface, zeta potential of the micelles was 9.60 ± 0.31 mV, electrostatic repulsion made the micelles not to aggregate and hydrodynamic diameters decreased as temperature increased. It is to be noted here that chitosan (pK_a 6.3) will be deprotonated in a neutral aqueous solution, which leads to the increase in the electrostatic repulsive force and the copolymer become more hydrophilic. As a result, the balance between electrostatic repulsion and hydrophobic attraction is perturbed, and the increased hydrophilic association leads to phase separation occurring at higher temperature (Bao et al., 2010), as shown in Fig. 7.

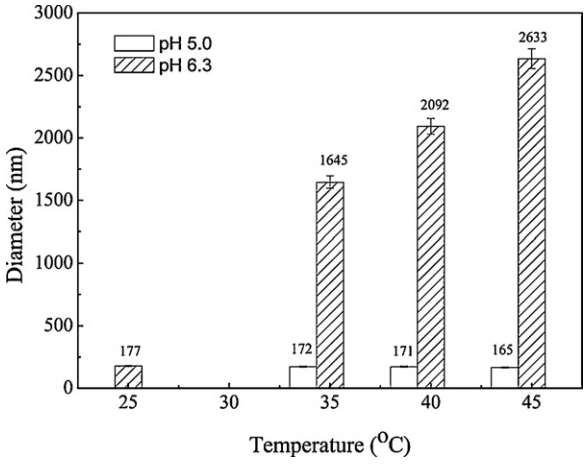


Fig. 8. Size distribution of CS-g-PNIPAAm micelles at different temperatures obtained for 2.5×10^{-4} g/ml aqueous solution at different pH values (□ pH 5.0 and ▨ pH 6.3, $n = 3$, means ± SD).

4. Conclusions

pH/Temperature multiple stimuli-responsive comb-shaped CS-g-PNIPAAm copolymer was synthesized via ATRP from the bromoisobutyryl-terminated CS and used PNIPAAm as side chains. LCST of this CS-g-PNIPAAm aqueous solution was calculated to be 33 °C at pH 6.3 and 35 °C at pH 5.0, which indicated that, this copolymer was pH-responsive due to the $-NH_2$ groups on chitosan. Due to hydrophobicity–hydrophilicity balance of $-NH_2/-NH_3^+$ and isopropyl/backbone groups, this copolymer self-assembled into micelles in aqueous solution with chitosan core, PNIPAAm shell of a hydrodynamic diameter of about 170 nm at room temperature. As a drug carrier, chitosan helps overcome certain adverse characteristics of drugs such as insolubility and hydrophobicity, more and more researches are looking forward to synthesize chitosan carriers which can respond to various stimuli to suit new application areas. The experimental results indicate that chitosan can be conveniently modified conveniently by attaching an ATRP initiating group to N-phthaloyl chitosan.

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