Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Thermo and pH Dual-Responsive Micelles of N-phthaloylchitosan-g-Poly(N-isopropylacrylamide) and Poly(acrylic acid-co-tert-butyl acrylate) for Drug Delivery

Lixia Jia[a]; Weilan Zhong[a]; Ting Wen[a]; Fengling Bian[a]
[a] College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, P. R. China

Online publication date: 30 July 2010

To cite this Article Jia, Lixia , Zhong, Weilan , Wen, Ting and Bian, Fengling(2010) 'Thermo and pH Dual-Responsive Micelles of N-phthaloylchitosan-g-Poly(N-isopropylacrylamide) and Poly(acrylic acid-co-tert-butyl acrylate) for Drug Delivery', Journal of Macromolecular Science, Part A, 47: 9, 909 — 917
To link to this Article: DOI: 10.1080/10601325.2010.501638
URL: http://dx.doi.org/10.1080/10601325.2010.501638

Journal of Macromolecular Science, Part A: Pure and Applied Chemistry (2010) **47**, 909–917

ISSN: 1060-1325 print / 1520-5738 online
DOI: 10.1080/10601325.2010.501638

Taylor & Francis
Taylor & Francis Group

Thermo and pH Dual-Responsive Micelles of N-phthaloylchitosan-g-Poly(N-isopropylacrylamide) and Poly(acrylic acid-co-tert-butyl acrylate) for Drug Delivery

LIXIA JIA, WEILAN ZHONG, TING WEN and FENGLING BIAN*

College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, P. R. China

Received January 2010, Accepted March 2010

A novel amphiphilic copolymer N-phthaloylchitosan graft poly(N-isopropylacrylamide) and poly(acrylic acid-co-tert-butyl acrylate) (PHCS-g-PNIPAAm&P(AA-co-tBA)) was synthesized. The graft copolymer could form micelles in aqueous medium, and the critical micelle concentration (CMC) of the copolymer was 7.5×10^{-3}mg/mL. The lower critical solution temperature (LCST) of the micelles was measured to be 30°C. Transmission electron microscopy (TEM) image showed that the micelles exhibited a regular spherical shape, and the mean diameter of the micelles was 94.1 ± 0.8 nm as determined by dynamic light scattering (DLS). The potential usefulness of the micelles as drug delivery systems was investigated using anti-inflammation drug prednisone acetate as the model. The drug loading capacity of the micelles was measured to be 22.86 wt%, and the DLS results showed that the mean diameter of the drug-loaded micelles was 133.3 ± 2.4 nm. *In vitro* drug release studies indicated that the micelles exhibited thermo and pH dual-responsive release profiles.

Keywords: N-phthaloylchitosan, poly(N-isopropylacrylamide), poly(acrylic acid), polymeric micelles, double-responsive drug release

Symbols and Abbreviations:

CMC : critical micelle concentration
C(mg/L) = A/0.0231, r = 0.996, where A was absorbance; r was the correlation coefficient
DD: degree of deacetylation of chitosan
$[\eta] = KM^{\alpha}$, K=1.81×10^{-3}, $\alpha = 0.93$
I_1/I_3: fluorescence intensity ratio of the first band (375 nm) to the third band (385 nm)of pyrene emission spectra
LC : the drug loading capacity
LCST: lower critical solution temperature
LE**:** the drug loading efficiency

1 Introduction

Recently, there is much interest in developing new drug delivery systems (DDS) which could not only improve the solubility and therapeutic efficiency of hydrophobic drugs, but also reduce the severe systemic toxicities from the anti-cancer drugs. Polymeric micelles formed by amphiphilic copolymers constitute one promising candidate for DDS (1,2), and have received much attention since the pioneer work done by Ringsdorf in 1981 (3). The common nano-size polymeric micelles used as the DDS could fulfill the passive targeting drug release, but it cannot deliver the drug to the target-site to achieve the active targeting drug release. Therefore, it is highly desirable to design new polymeric micelles drug carriers which could respond to the external chemical and physical stimulus such as temperature, pH, ionic strength, light and electric field, etc. As the temperature and pH value can be easily adjusted in the human body, the thermo or pH responsive polymers (such as poly (N-isopropylacrylamide) and poly (acrylic acid)) with reversible phase transition characteristics are particularly attractive as drug release systems(4,5).

Poly(N-isopropylacrylamide) (PNIPAAm) is a well-known thermo-responsive polymer which undergoes a sharp coil-globule transition and phase separation at its lower critical solution temperature (LCST) in water around 32°C(6). However, despite this attractive property, PNIPAAm is not biodegradable. This problem could be solved by grafting biodegradable polymers onto PNIPAAm segments(7,8), and thus formed graft copolymers possess

*Address correspondence to: Fengling Bian, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China. Tel: +86 931 8912387; Fax: +86 931 8912582; E-mail: bianfl@lzu.edu.cn
Contract/grant sponsors: The Talent Training Fund National Natural Science Foundation of China (NSFC). Contract/grant number: J0730425

the same thermo-responsive properties as PNIPAAm, and are biodegradable.

Chitosan is a non-toxic, biodegradable and biocompatible nature-occurring polymer. Graft copolymers of N-isopropylacrylamide (NIPAAm) and chitosan have been prepared, and their drug-carrying behaviors are of current academic interest. Most research in this area are focused on the thermo-responsive behaviors of DDS (9–11). Recently, there are some reports on chitosan-based drug carriers which are pH-responsive. Li et al. (12) have prepared negatively charged micelles formed from prednisone(PNS)-loaded poly(tert-butylacrylate-co-ethylacrylate-co-methacrylicacid), and found that the release rate and the release amount of PNS increased with the increase in pH value. In another study, paclitaxel-loaded polyNIPAAm/chitosan nanoparticles were prepared, and the drug-loaded nanoparticles were found to exhibit pH-responsiveness to tumor pH (13).

Yuan et al. (14) recently reported a novel magnetic nanoparticle drug carrier, the Fe_3O_4/chitosan-g-poly (N-isopropylacrylamide-co-N,N-dimethylacrylamide). The drug-releasing behavior of the magnetic nanoparticles was investigated using doxorubicin as the drug model. The results showed that the doxorubicin-loaded nanoparticles were both thermo- and pH-responsive. This double responsiveness of drug-carriers is very interesting, and might be of practical value. However, there are some drawbacks in using magnetic nanoparticles as drug-carriers. As the model drug doxorubicin was linked to the hydrophilic Fe_3O_4 nanoparticles via acid-labile hydrazone bond, the drug-loading procedure is complicated. Besides, drugs that could be carried are rather limited. Another disadvantage of this magnetic nanoparticle drug-carrier lies in its relatively low drug loading capacity. As the magnetite content in drug carrier was about 70 wt%, only 10 wt% of the drug could be carried.

In this work, we prepared a new type of thermo and pH dual stimuli-responsive micelles based on *N*-phthaloylchitosan graft poly(N-isopropylacrylamide) and poly(acrylic acid-co-tert-butyl acrylate). Hydrophobic drugs were incorporated into the micelles carrier by a simple dialysis method. The drug release behaviors of micelles were explored on the alterations of pH or temperature.

2 Experimental

2.1 Materials

Chitosan (CS) was purchased from Zhejiang Yuhuan Ocean Biochemical Co. Ltd. The degree of deacetylation (DD) of CS was determined by linear potentiometric titration (15) to be 96%. The viscosity average molecular weight of CS was 18000, as determined in 0.1 M acetate acid/0.2 M NaCl aqueous solution at $25 \pm 0.5^{\circ}$C by means of Ubbelohde Viscometer, according to the Mark-Houwink equation, $[\eta] = KM^{\alpha}$, K=1.81×10^{-3}, $\alpha = 0.93$ (16).

N-isopropylacrylamide (NIPAAm) was purchased from J&K Chemical Ltd., and was recrystallized from hexane before use (17). tert-Butyl acrylate (tBA, 98%) was obtained from the J&K Chemical Ltd., and distilled under reduced pressure(18). 3-Mercaptopropionic acid (MPA) was provided by Jinan Chenghui-Shuangda Chemical Co. Ltd. and was used after distilled under reduced pressure. *N*, *N*-azobisisobutyronitrile (AIBN) was provided by Shanghai Chemical Reagent Co. Ltd. and was used after recrystallization with methanol. *N*, *N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled under reduced pressure from calcium hydride. All other reagents and solvents were used without further purification.

2.2 Synthesis of Carboxy-Terminated PNIPAAm (PNIPAAm-COOH)

The carboxy-terminated PNIPAAm (PNIPAAm-COOH) was prepared by radical polymerization using MPA as a chain transfer agent. After dissolving NIPAAm, MPA and AIBN in ethanol, the solution was degassed by bubbling with nitrogen for 30 min. Polymerization was carried out at 70°C for 24 h. After the reaction, the mixture was evaporated under reduced pressure, and the residual was dissolved in acetone, and the product precipitated by addition of a large volume of hexane. The precipitated product was collected and dried under vacuum. Then, the product was purified twice by repeated cycle of dissolution (in acetone) and precipitation (in hexane).

The molecular weight and molecular weight distribution of the PNIPAAm-COOH were determined using GPC equipment (Alliance GPCV2000, Waters Co., America). THF was used as eluent and the calibration was carried out with polystyrene monodisperse standard. The molecular weight and molecular weight distribution index of the sample are measured to be 3872 and 2.05, respectively.

2.3 Synthesis of Carboxy-Terminated PtBA (PtBA-COOH)

The carboxyl terminated PtBA (PtBA-COOH) was prepared by radical polymerization using MPA as chain transfer agent. After dissolving tBA, MPA and AIBN in THF, the solution was degassed by bubbling with nitrogen for 30 min. Polymerization was carried out at 70°C for 24 h. After the reaction, the product was precipitated in the mixed solution of methanol/distilled water (v/v, 4/1). The precipitated product was collected and dried under vacuum. Then the product was purified twice by a repeated cycle of dissolution and precipitation. The average molecular weight of PtBA-COOH polymer was 575 as measured by non-aqueous potentiometric titration. A definite volume of copolymer solution was titrated by 0.01 M sodium

methoxide in a mixture of methanol/dioxane (1/9, v/v) under nitrogen atmosphere (19).

2.4 Preparation of N-phthaloylchitosan (PHCS)

N-phthaloylchitosan was synthesized as previously reported (19). The degree of substitution of phthaloyl groups within PHCS was determined to be about 1.12, as calculated by elemental analysis.

2.5 Synthesis of PHCS-g-PNIPAAm&PtBA Copolymers

The grafting procedure of PHCS-g-PNIPAAm&PtBA copolymer is shown in Scheme 1. PHCS(0.0040 mol pyranose) was stirred with PNIPAAm-COOH (0.006 mol), PtBA-COOH (0.004 mol) in a 30 mL DMF solution, into which 1-Hydroxybenzotrizole (HOBt) (0.03 mol) was added as catalyst and the mixture was stirred at room temperature until fully dissolved. Then, diisopropylcarbodiimide (DIC, 0.03 mol) was added. After stirring the reaction mixture for 48 h at room temperature, the product was precipitated in ethanol, and the precipitate was collected and dried in vacuum at room temperature overnight to obtain white powder. The submitted degree of PNIPAAm and PtBA was 44.8% and 33%, respectively.

2.6 Hydrolyzing of Graft Copolymers

The graft copolymer PHCS-g-PNIPAAm&P(AA-co-tBA) was synthesized by hydrolyzation of PHCS-g-PNIPAAm& PtBA graft copolymers. The synthesis route of the graft copolymer was shown in Scheme.1. PHCS-g-PNIPAAm&PtBA (0.174 mol tBA repeat units) copolymer was added into DMF, and the mixture was stirred until the copolymer completely dissolved. Then, TFA (8.7×10^{-1} mol) was added, and the reaction was allowed to proceed for 24 h at 25°C. The final solution was vacuum stripped to remove solvent and TFA, and the remaining solid was washed overnight with excess deionized water, and dried under vacuum.

2.7 Micelle Formation

Dialysis method was used to prepare the self-assembled micelles of PHCS-g-PNIPAAm&P(AA-co-tBA) graft copolymer as previously reported (19). First, 10 mg of graft copolymer was dissolved in 3 mL of dried DMF, and then distilled water was slowly added (1 drop min^{-1}) into the copolymer solution under vigorous stirring until the solution was slightly turbid. Finally, the mixed solution was put into a dialysis bag (MWCO=14000) and dialyzed against distilled water for 3 days to remove DMF. The distilled water was replaced every 8 h. The micelle solution (1mg/mL) was purified by ultrafiltration using a filtration membrane of 0.45 μm.

2.8 Measurement of the Critical Micelle Concentration

The critical micelle concentration (CMC) of the PHCS-g-PNIPAAm&P(AA-co-tBA) graft copolymer was determined by fluorescence spectroscopy (19). Pyrene was used as a hydrophobic fluorescent prober. Aliquots of pyrene solution (4.8×10^{-4}M in diethyl ether, 5 μL) were added into tubes and after the diethyl ether was evaporated, Aliquots of 4 mL aqueous solution of the copolymer at different concentrations (1 to 1×10^{-7}mg/mL) was added to each tube containing the pyrene residue. It should be noted that all the aqueous solution of the sample contained excess pyrene residue at the same concentration of 6×10^{-7}M. The solution was sonicated for 30 min and stored overnight at room temperature to reach the dissolution equilibrium of pyrene in the aqueous phase. An excitation spectrum was measured at 336 nm, and emission spectra were recorded ranging from 350 to 550 nm. From the pyrene emission spectra, the intensity ratio (I_1/I_3) of the first band (375 nm) to the third band (385 nm) was analyzed as a function of polymer concentration. The CMC value was determined at the onset of a decrease in the plot of the polymer concentration versus ratio of I_1/I_3.

2.9 Drug Loading

The incorporation of prednisone acetate into polymeric micelles was also carried out by a dialysis method (19,20). First, PHCS-g-PNIPAAm&P(AA-co-tBA) copolymer (10 mg) and prednisone acetate (10 mg) were dissolved in 5 mL of dried DMF, into which distilled water was added slowly (1 drop min^{-1}) under vigorous stirring until the solution was slightly turbid. Finally, the solution was put into a dialysis bag (MWCO=14000) and subjected to dialysis against 400 mL of distilled water for 3 days. And the drug-loaded micelles were purified by filtration with a 0.45 μm pore-sized microfiltration membrane.

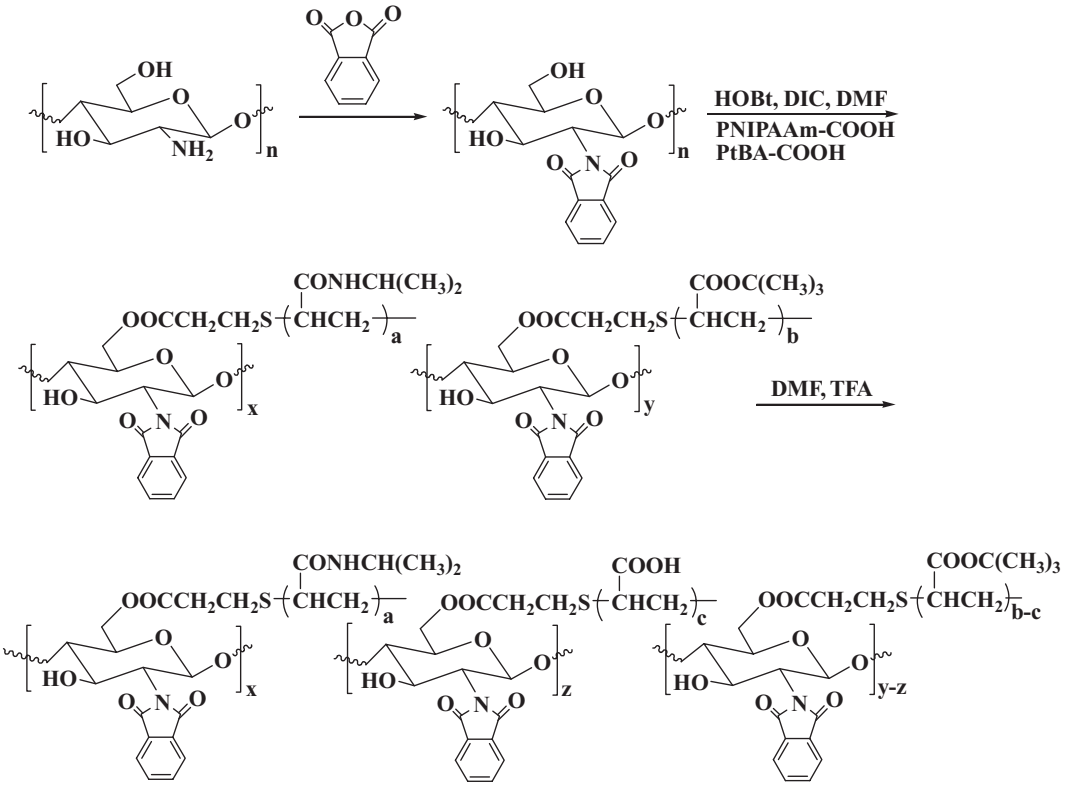
The absorbance of the standard prednisone acetate solution with varied concentrations was measured at a wavelength of 242 nm with a UV-Vis spectrometer (21). According to the Lambert-Beer law, a linear regression equation relating the absorbance (A) to the prednisone acetate concentration(C) was obtained as C(mg/L) = A/0.0231, r=0.996, where r was the correlation coefficient.

The drug loading capacity (LC) and the drug loading efficiency (LE) were calculated base on the following formulas:

$$\text{The drug loading capacity (wt\%)} = M_o/(M_o + M_P) \times 100\% \quad (1)$$

$$\text{The drug loading efficiency (\%)} = (M_o/M_a) \times 100\% \quad (2)$$

Where $M_o$is the amount of the drug loaded in the polymeric micelles, and M_P is the amount of copolymer, M_a is the amount of the initially added drug. M_o is calculated by subtracting the amount of unloaded drug from the



Sch. 1. Synthesis route of PHCS-g-PNIPAAm&P(AA-co-tBA) copolymer.

initial feed drug amount. The amount of unloaded drug is analyzed by measuring the absorbance at 242 nm of the dialysis fluid.

2.10 *In vitro* Drug Release

After dialysis, 5 mL of prednisone acetate-loaded micelle solution (C=1 mg/mL) in dialysis bag was immersed into 400 mL of PBS (0.1 M, pH=7.4), which was kept at 25°C and 45°C, respectively. At certain time intervals, aliquots of 4 mL were withdrawn from the medium. The volume of medium was held constant by adding aliquots of 4 mL fresh medium after each sampling. The amount of prednisone acetate released from the micelles was measured using UV absorbance at 242 nm. All experiments were performed in triplicate. The pH-sensitive drug release was measured in the same way in 400 mL of PBS (0.1 M, 25°C), which was kept at pH=7.4 and pH=4.5, respectively.

The cumulative amount of drug release was calculated from the following formula (21):

$$\text{The cumulative drug release } (\%) = (M_t/M_o) \times 100 \quad (3)$$

Where M_t is the amount of drug released from micelles at time t, and M_o is the amount of drug loaded in the PHCS-g-PNIPAAm&P(AA-co-tBA) polymeric micelles.

2.11 Characterization

Fourier-transform infrared (FT-IR) transmission spectra were obtained from samples in KBr pellets using a Bruker IFS66v/S FT-IR spectrophotometer. Nuclear magnetic resonance(^{1}H-NMR) spectra were recorded on an AV-300M NMR spectrometer. The morphology of the self-assembled micelles was studied by a Japan H-600 transmission electron micrograph (TEM). Dynamic light scattering (DLS) measurement was carried out with a Brookhaven BI-200SM instrument. UV-Vis measurement was carried out at 25°C with a Perkin-Elmer LS55 (America) spectrophotometer.

3 Results and Discussion

3.1 Preparation of Graft Copolymers

N-phthaloylchitosan graft poly (N-isopropylacrylamide) &poly(acrylic acid-co-tert-butyl acrylate) was successfully synthesized following the synthesis route shown in Scheme 1. The chemical compositions of synthesized polymers were confirmed by FT-IR and ^{1}H-NMR measurements.

The characteristic absorption peaks of PNIPAAm-COOH in FT-IR spectrum were in agreement with those of the standard spectra. The peaks at 1650, 1548 cm^{-1} belonged to the I and II bands of the amide groups (Fig. 1(a))

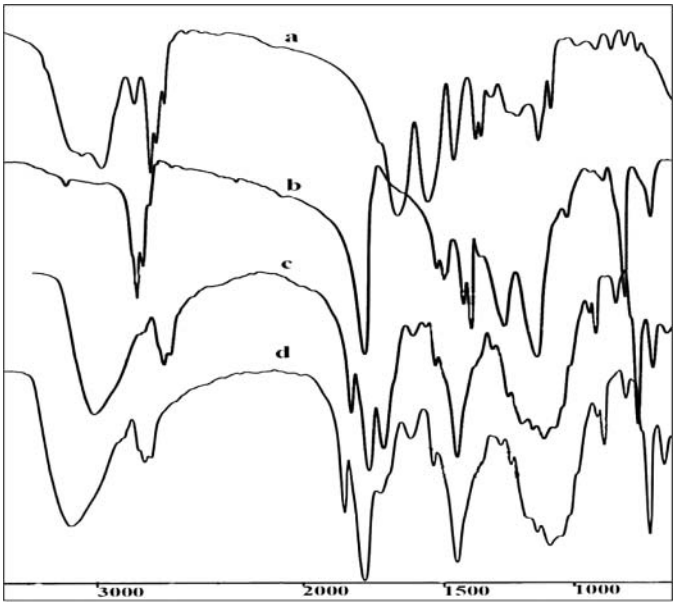


Fig. 1. FT-IR spectra of (a) PNIPAAm-COOH, (b) PtBA-COOH, (c)PHCS-g-PNIPAAm &PtBA, (d) PHCS-g-PNIPAAm&P(AA-co-tBA).

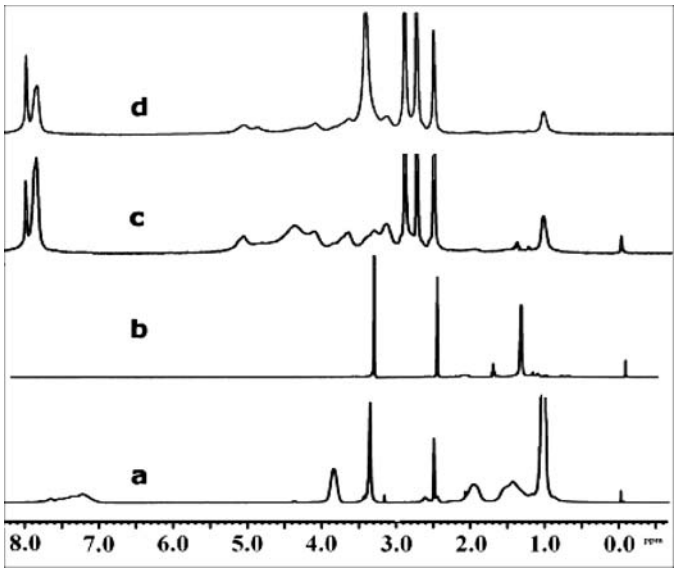


Fig. 2. ^{1}H-NMR spectra of (a) PNIPAAm-COOH in DMSO-d_6, (b) PtBA-COOH in DMSO-d_6, (c) PHCS-g-PNIPAAm&PtBA in DMSO-d_6, (d) PHCS-g-PNIPAAm&P(AA-co-tBA) in DMSO-d_6.

(21, 22) in PNIPAAm-COOH. The peaks at 1370, 1394 cm^{-1} and 1455,1480 cm^{-1} were assigned to the symmetrical and dissymmetrical C-H stretching vibration peaks of -$C(CH_3)_3$ groups of PtBA-COOH (Fig. 1(b)) (23). The FT-IR spectrum of the PHCS-g-PNIPAAm&PtBA copolymer was shown in Figure 1(c). Beside the characteristic peaks of PHCS (19), two new peaks appeared at 1660, 1547 cm^{-1} in Figure 1(c), which could be assigned to the amide I and II bands in PNIPAAm. Moreover, the peaks at 1069, 1199 cm^{-1} were the symmetrical and dissymmetrical stretching vibration peaks of ester groups in PtBA segments and the newly formed ester linkage. The hydrolysis of PHCS-g-PNIPAAm&PtBA copolymer gave rise to the desired product, whose FT-IR spectrum was shown as Figure 1(d). The band around 1700 cm^{-1} was related to C=O stretching vibration of ester, which obviously decreased. These results were consistent with the structural features of PHCS-g-PNIPAAm&P(AA-co-tBA).

Figure 2 showed the ^{1}H-NMR spectra of PNIPAAm-COOH (Fig. 2(a)), PtBA-COOH (Fig. 2(b)), PHCS-g-PNIPAAm&PtBA (Fig. 2(c)), and PHCS-g-PNIPAAm&P(tBA-co-AA) (Fig. 2(d)). In Figure 2(a), the chemical shifts at about 4.0 ppm were ascribed to the methine protons of the PNIPAAm, the chemical shifts at about 1.2–2.2 ppm were ascribed to methylene and methine protons in the main chain of PNIPAAm, the chemical shifts at about 1.10 ppm were assigned to methyl protons of isopropyl units(6,18,20,23). In Figure 2(b), the sharp resonance at 1.4 ppm was attributed to the –$C(CH_3)_3$ of the PtBA, The chemical shifts at about 1.2-1.8 ppm and 2.0–2.2 ppm were attributed to the methylene and methine protons of the PtBA (18,24).

As seen from Figure 2(c), the chemical shifts at about 2.7–5.0 ppm were ascribed to the pyranose ring protons of PHCS,the chemical shifts at about 7.8 ppm were ascribed to the aromatic ring protons of PHCS. Except characteristic signals of PHCS, the signals at about 1.1 and 4.0 ppm corresponding to the methyl and methine protons of the NIPAAm segments, and the signal at 1.4 ppm corresponding to the -$C(CH_3)_3$ of the PtBA segments, demonstrated the formation of PHCS-g-PNIPAAm&PtBA.

In Figure 2(d), compared with that of Figure 2(c), the decreasing intensity of the tert-butyl peaks at 1.4 ppm indicated the part hydrolysis of PtBA into PAA in the PHCS-g-PNIPAAm&PtBA copolymer, from which the degree of hydrolysis was determined as 22.86%. These results also showed that the PHCS-g-PNIPAAm&P(AA-co-tBA) was synthesized successfully.

3.2 Micelles Formation

Amphiphilic graft copolymers can form micelles when their solution was dialyzed in a poor solvent for either hydrophobic or hydrophilic segment, and the process is driven by dynamic and thermodynamic stability(25). The stability of the micelles lies on the equilibrium of attractive force which urges the copolymers to form micelles and the repulsive force which prevents the unlimited growth of the micelles to a distinct macroscopic phase. In this work, the formation of micelles from the graft copolymers was verified by fluorescence spectroscopy using pyrene as a fluorescent prober.

Figure 3 was the plot of fluorescence intensity ratio I_1/I_3 of the pyrene emission spectra against the copolymer

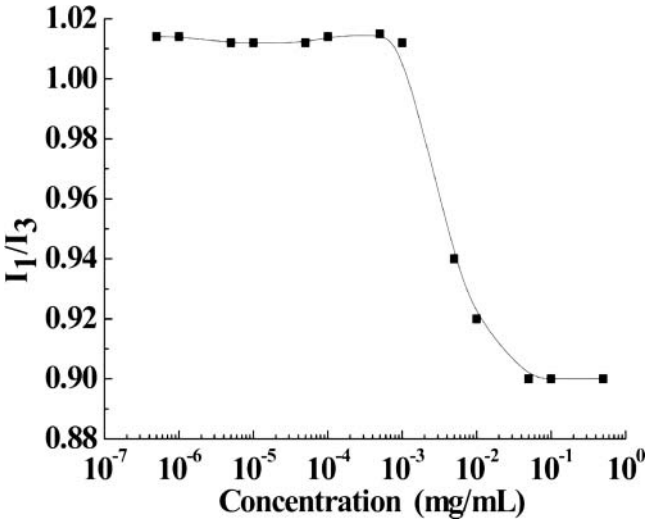


Fig. 3. Plot of the ratio of intensities (I_1/I_3) of selected emission bands in the pyrene fluorescence spectrum as a function of PHCS-g-PNIPAAm&P(AA-co-tBA) concentration [py]=6×10^{-7}M.

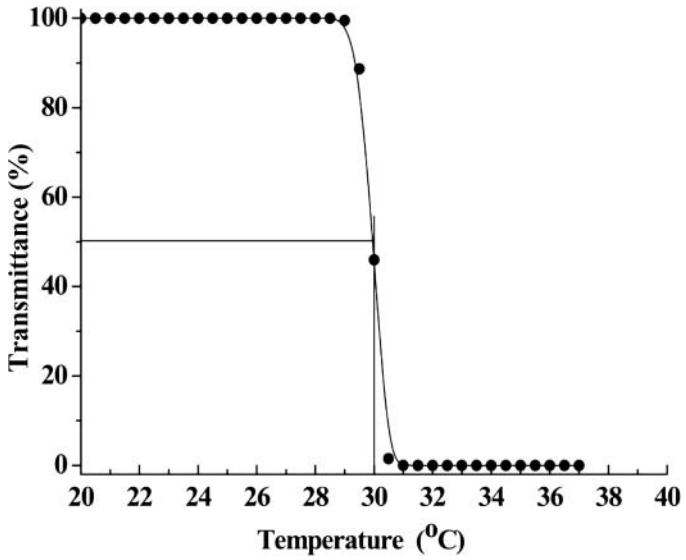


Fig. 4. Transmittance changes of PHCS-g-PNIPAAm&P(AA-co-tBA) micelles with temperature.

concentration. As shown in Figure 3, the fluorescence intensity of $I_1/I_3$was approximately a constant value when the concentration of graft copolymer was below 7.5×10^{-3} mg/mL, indicating that the copolymer was dissoluble and existed as single chain. However, the intensity ratio of I_1/I_3 decreased dramatically above the concentration, as a result of the formation of micelles and dissolution of pyrene into the hydrophobic core of micelles. This concentration was defined as the critical micelle concentration (CMC), it is much lower than that of low molecular weight surfactants micelles (for example, 2.3 mg/mL for sodium dodecyl sulfate in water) (26), indicating that these micelles are relatively stable. The polymeric micelles with lower CMC will be more suitable for being used as drug targeting devices since they are stable in an aqueous environment and do not easily dissociate when they are diluted by blood in intravenous administration.

3.3 Characterization of Polymeric Micelles

PNIPAAm is a temperature-responsive polymer, which exhibits a lower critical solution temperature (LCST) at about 32°C. Below the LCST, PNIPAAm is water soluble, existing as an extended chain form. Above the LCST, PNIPAAm undergoes a reversible phase transition to a water insoluble aggregate.

To determine whether the PHCS-g-PNIPAAm&P(AA-co-tBA) micelles also exhibit a thermo-responsiveness, we examined the optical absorbance of an aqueous solution of polymeric micelles as a function of temperature. The results showed a unique thermo-responsive characteristics. As shown in Figure 4, these micelles exhibited a lower critical solution temperature at a temperature corresponding to the LCST of the PNIPAAm, 30°C.

The morphology of the polymeric micelles was measured by TEM (Fig. 5(a)). It showed that the self-assembled micelles were well dispersed in nano-sizes with regularly spherical shape. As shown in Figure 5(b), PHCS-g-PNIPAAm&P(AA-co-tBA) micelles exhibited a narrow size distribution with an average diameter of 94.1 ± 0.8 nm as determined by DLS.

3.4 Incorporation of Prednisone Acetate into Micelles

Prednisone acetate is a good anti-inflammation and anti-allergic drug, and it is water-insoluble. Here, it was used as a model drug to be entrapped into the hydrophobic core of these micelles. The prednisone acetate-loaded micelles were prepared by the dialysis method. Prednisone acetate was successfully loaded into the PHCS-g-PNIPAAm&P(AA-co-tBA) micelles with a loading capacity of 22.86 wt%.

Morphology of prednisone acetate-loaded micelles was observed with TEM (Fig. 5(a′)). The drug-loaded micelles were also well dispersed with a regular spherical shape. Furthermore, the results of DLS as shown in Fig. 5(b′) indicated that the mean diameter of drug-loaded micelles was 133.3 ± 2.4 nm (< 200 nm), by comparison with Figure 5(b), the size of drug-loaded micelles was larger than that of blank micelles, as a result of encapsulation of drug into the micelles.

3.5 Thermo- and pH-responsive Drug Release from the Micelles

The drug release response of the micelles carrier in PBS (pH=7.4) was studied at 25°C (below LCST) and 45°C (above LCST), respectively. Figure 6 shows the release profiles of prednisone acetate from the micelles as a function

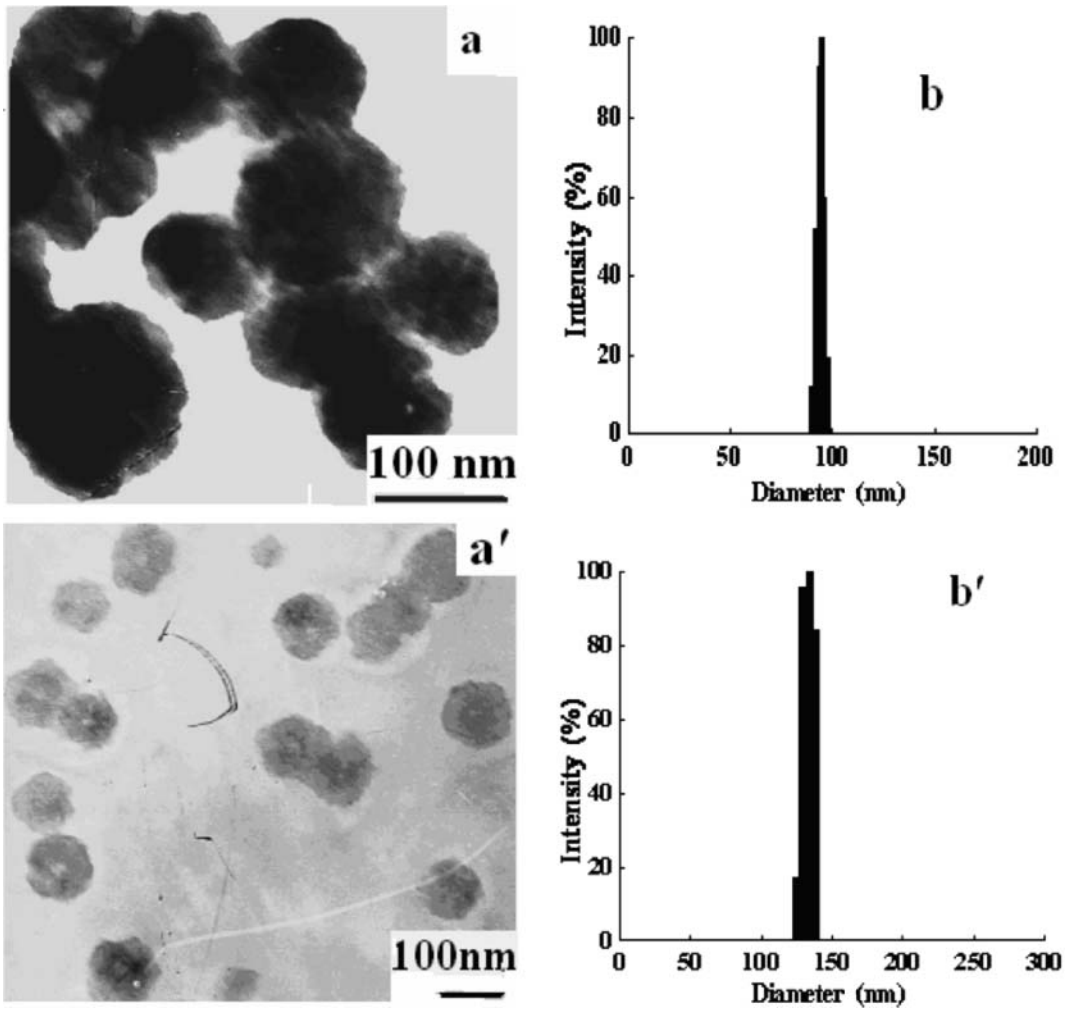


Fig. 5. Transmission electron micrographs (a) blank micelles (×50000) (a′) drug-loaded micelles (×50000), and (b) the size distribution of blank micelles, and (b′) drug-loaded micelles.

of time. As seen from Figure 6(a), at a temperature (25°C) below the LCST (30°C) of the micelles, PNIPAAm was water soluble, and the highly hydrated PNIPAAm shells stabilized the drug loaded in the micelles cores below the LCST. Therefore, the drug released much slowly from the hydrophobic cores (17). However, when the temperature (45°C), was raised above the LCST, the drug release accelerated dramatically. This result indicated that the PNIPAAm shells became hydrophobic above the LCST, leading to the deformation of the micellar structure. As a result, the drug diffused out quickly from the micelles(17,21).

Figures 6(b) showed the drug release profiles of PHCS-g-PNIPAAm&P(AA-co-tBA) micelles at different pH values when the temperature (25°C) was below the LCST. As seen from Figure 6(b), the drug release rate at pH 4.5 was faster than that at pH 7.4 at the initial stage. However, after about 40 h, the rate of drug release at pH 4.5 was nearly the same as that at pH 7.4. These results could be explained by the protonation of the carboxyl groups of PAA segments in the micellar shells at pH 4.5. The protonated carboxyl groups might form the intermolecular H-bond with the amide groups of PNIPAAm segments, thus resulting in the less hydrophilic of the micellar shells. Consequently, the drug released rapidly from the micelles.

To further explore the thermo and pH dual-responsive drug release behaviors of the drug-loaded PHCS-g-PNIPAAm&P(AA-co-tBA) micelles, we examined the release of prednisone acetate from the micelles in response to the change of temperature, as well as the change of pH. The results are shown in Figure 7. It can be seen from Figure 7(a) that at fixed pH value (pH 7.4), the drug release accelerated when the temperature was raised from 25°C to 45°C, suggesting that the micellar structure transformed from a stable core-shell micelle to an aggregate one. When the temperature was fixed at 25°C, and the pH value was switched from 7.4 to 4.5, the drug release rate accelerated dramatically during the first 40 h (Fig. 7(b)). This result was consistent with that shown in Figure 6(b).

All these results demonstrated that the micelles have both thermo and pH dual-responsive characteristics, and they

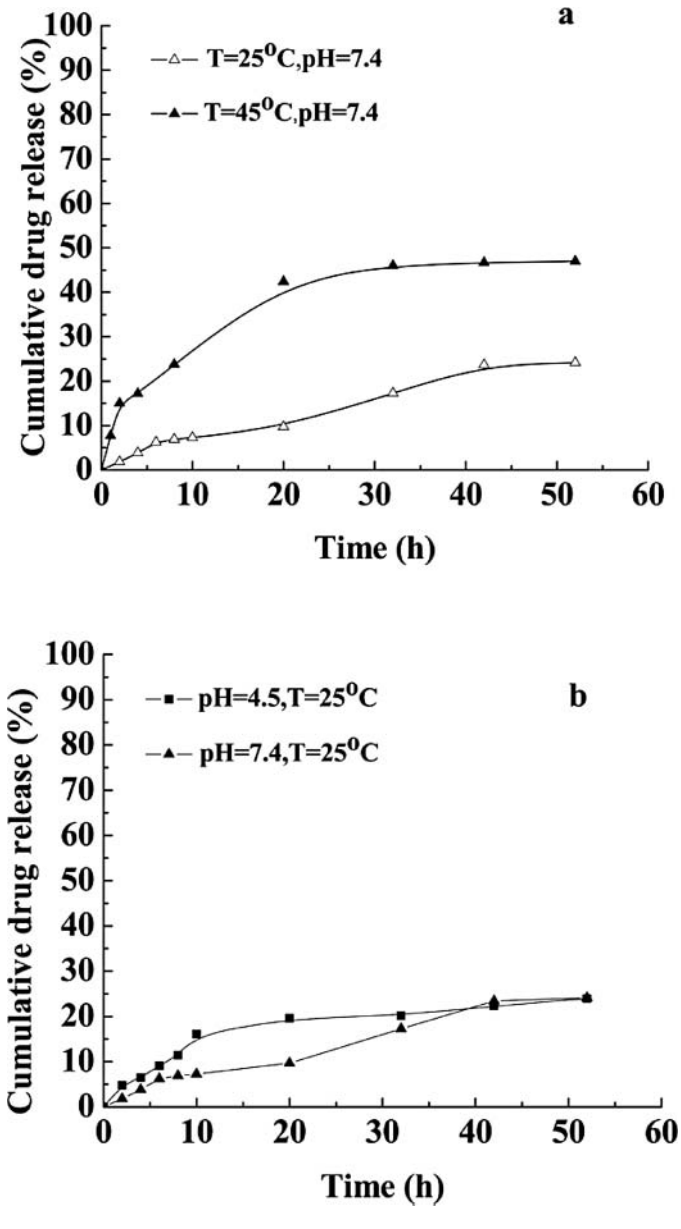


Fig. 6. Cumulative drug release profiles of prednisone acetate from the micelles in buffer solutions at (a) different temperatures, (b) different pH values.

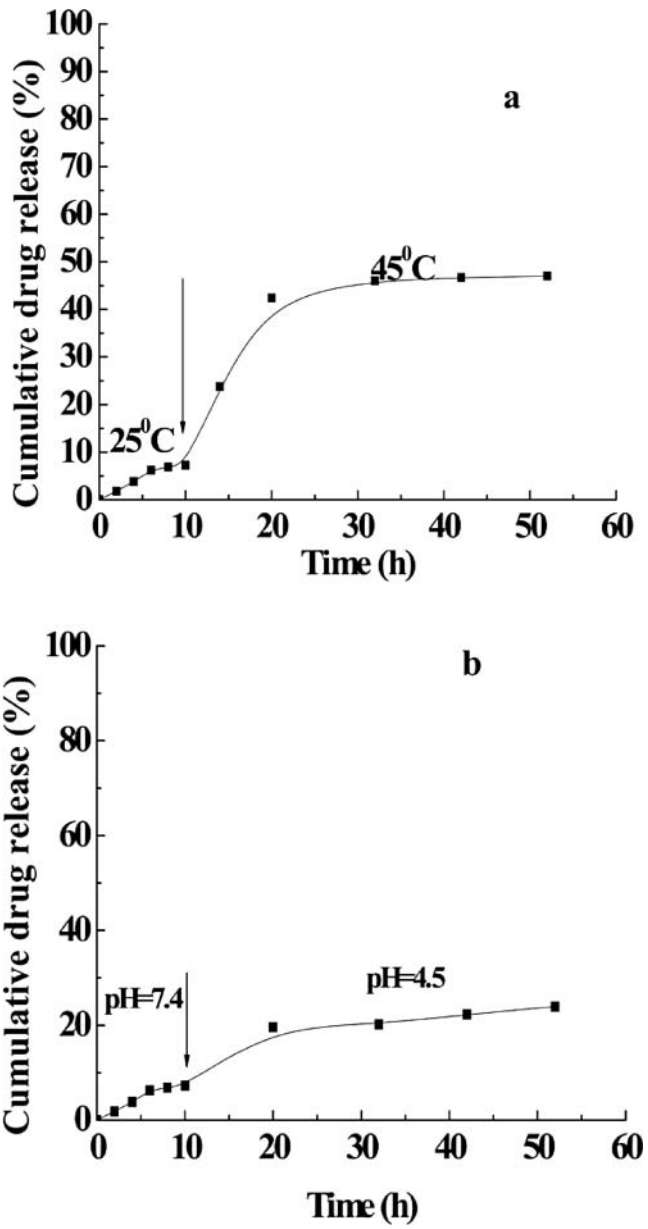


Fig. 7. Drug release from PHCS-g-PNIPAAm&P(AA-co-tBA) micelles loaded with prednisone acetate in response to (a) temperature switching from 25°C to 45°C, (b) pH value switching from 7.4 to 4.5.

could be used as a site-specific drug delivery device when they were triggered by the alteration of temperature or pH in the body. In this way, the accumulation of drugs in targeting pathological sites may be enhanced and the treatment efficiency might be improved.

4 Conclusions

A novel amphiphilic graft copolymer PHCS-g-PNIPA Am&P(AA-co-tBA) was synthesized. The polymer formed the spherical polymeric micelles of nano-size (94.1 ± 0.8 nm) at low concentration (with CMC 7.5×10^{-3} mg/mL) in aqueous solution. The micelles were thermal-responsive, and the low critical solution temperature (LCST) of the micelles was 30°C. The drug release behaviors of the micelles were investigated. The anti-inflammation drug prednisone acetate was incorporated into the micelles with the drug loading capacity of 22.86 wt%, and the drug-loaded micelles were round in shape with the mean diameter of 133.3 ± 2.4 nm. The *in vitro* drug release studies showed that the micelles had thermo and pH dual-responsive release profiles. Taking the advantage of the nano-size, low CMC, thermo and pH dual-responsive drug release behaviors into account, the

micelles have great potential to be a promising site-specific carrier for drug delivery.

References

1. Chang, S.H., Joseph, A. and Gardella, J. (2005) *Chem. Rev.,* 105(11), 4205–4232.
2. Nobuhiro, N. and Kazunori, K. (2006) *Pharmacol. Therapeut.*, 112(3), 630–637.
3. Gros, L., Ringsdorf, H. and Schupp, H. (1981) *Angew. Chem. Int. Ed. Engl.*, 20(4), 305–312.
4. Zhang, X.Z. and Zhuo, R.X. (2001) *Langmuir*, 17(1), 12–16.
5. Zhang, X.Z, Zhang, J.T., Zhuo, R.X. and Chu, C.C. (2002) *Polymer*, 43(17), 4823–4827.
6. Zheng, Q. and Pan, C.Y. (2006) *Eur. Polym. J.*, 42(4), 807–814.
7. Huh, K.M., Hashi J., Ooya, T. and Yui, N. (2000) *Macroml. Chem. Phys.*, 201(5), 613–619.
8. Sun, G.M., Zhang, X.Z. and Chu, C.C. (2007)*J. Mater. Sci.: Mater. Med.,* 18(8),1563–1577.
9. Cao, Y., Zhang, C., Shen, W., Cheng, Z., Li, Y. and Ping, Q. (2007) *J. Control. Rel.,* 120(3), 186–194.
10. Jia, Y.F., Jyh, P.C., Yann, L-L. and Jiuan, W.H. (2008) *Eur. J. Pharm. Biopharm*., 68(3), 626–636.
11. Lee, K.Y. and Yuk, S.H. (2007) *Prog. Polym. Sci.,* 32(7), 669–697.
12. Li, Y., Li, H., Wei, M., Lu, J. and Jin, L. (2009) *Chem. Eng. J*., 151, 359–366.
13. Li, F., Wu, H., Zhang H., Li, F., Gu, C.H. and Yang, Q. (2009) *Carbohyd. Polym*., 77(4), 773–778.
14. Yuan, Q., Venkatasubramanian, R., Hein, S. and Misra, R.D.K. (2008) *Acta. Biomater.,* 4(4), 1024–1037.
15. Jia, Z.S.and Li, Q.B. (2001) *Chem. World*, 5, 240–241.
16. Maghamia, G.G.and Roberts, G.A.F. (1988) *Macromol. Chem.*, 189, 195–200.
17. Liu, Y.H., Cao, X.H., Luo, M.B., Le, Z.G. and Xu, W.Y. (2009) *J. Colloid Interface Sci.,* 329(2), 244–252.
18. Li, G.Y., Shi, L.Q., An, Y.L., Zhang, W.Q. and Ma, R.J. (2006) *Polymer*, 47(13), 4581–4587.
19. Bian, F.L., Jia, L.X., Yu, W. and Liu, M.Z. (2009) *Carbohyd. Polym.*,76(3), 454–459.
20. Zheng, G.H.and Pan, C.Y. (2005) *Polymer*, 46(8), 2802–2810.
21. Wei, H., Zhang, X.Z., Cheng, H., Chen, W.Q., Chen, S.X. and Zhuo, R.X. (2006) *J. Control Rel*., 116(3), 266–274.
22. Schilli, M.C., Zhang, M.F., Rizzardo, E., Thang, S.H., Chong, Y.K., Edwards, K., Karlsson, G.. and Müller,A.H.E. (2004) *Macromolecules*, 37(21), 7861–7866.
23. You, Y.Z., Hong, C.Y., Wang, W.P., Lu, W.Q. and Pan, C.Y. (2004) *Macomolecules*, 37(26), 9761–9767.
24. Chu, C.C., Wang, L.and Ho, T.I. (2005) *Macromol. Rapid Commun*., 26(14), 1179–1184.
25. Agnihotri, S.A. and Aminabhavi, T.M. (2004) *J. Control. Rel.,* 96(2), 245–259.
26. Nagasaki, Y., Okada, T. and Scholz, C. (1998) *Macromolecules*, 31(5), 1473–1479.