Novel Stimuli-Responsive Micelle Self-Assembled from Y-Shaped P(UA-Y-NIPAAm) Copolymer for Drug Delivery

Yong-Yong Li, Xian-Zheng Zhang,* Han Cheng, Gwang-Chol Kim, Si-Xue Cheng, and Ren-Xi Zhuo

Key Laboratory of Biomedical Polymers (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, People's Republic of China

Received January 26, 2006; Revised Manuscript Received April 25, 2006

A new amphiphilic Y-shaped copolymer, comprised of hydrophobic poly(undecylenic acid) (PUA) and hydrophilic poly(*N*-isopropylacrylamide) (PNIPAAm), was designed and synthesized. A cytotoxicity study revealed that P(UA-Y-NIPAAm) copolymers did not exhibit apparent inhibition impact on the proliferation of cells when the concentration of the copolymer was below 1000 mg/L. Characterization demonstrated that the P(UA-Y-NIPAAm) copolymer is thermosensitive with a lower critical solution temperature (LCST) of 31 °C. In water, the P(UA-Y-NIPAAm) copolymer would self-assemble into micelles with a critical micelle concentration (CMC) of 20 mg/L. Self-assembled P(UA-Y-NIPAAm) micelles exhibited a nanospherical morphology of 40 to ~80 nm in size. The controlled drug release behavior of the P(UA-Y-NIPAAm) micelles was further investigated, and self-assembled micelles exhibited improved properties in controlled drug release.

1. Introduction

During the past decade, self-assemblies of amphiphilic block copolymers in aqueous solution have been extensively investigated.[1−4] The unique amphiphilic character of the block copolymers enables them to self-assemble to form versatile micelles.[5−9] The hydrophobic segment of the copolymer forms the core of the micelles, while the hydrophilic segment forms the corona or outer shell. This core−shell structure could be used as a drug delivery system, and hydrophobic drugs are loaded in the hydrophobic core.[10] In general, the size of the polymeric micelles is between 10 and 60 nm, which is relatively small as compared to other colloidal drug carriers, such as liposomes. Because of the small size and hydrophilic surface, polymeric micelles are not easily recognized and captured by the reticuloendothelial systems (RES).[11,12] Therefore, polymeric micelles have a relatively long circulation time after intravenous administration, and as a result, micelles may accumulate due to the enhanced permeation and retention (EPR) effect.[13]

Obviously, it would be more beneficial if the drug could be delivered by a device that could respond to physiopathological signals from an underlying disease. The correct amount of drug could be released upon the stimulation of such a physiopathological signal. For example, the extracellular pH in most solid tumor tissues ranges from 5.7 to 7.8.[14] Controlling the release of loaded drugs in micelles in such a narrow pH range is critically important. Recently, several stimuli-responsive micelles induced by a continuous change in various conditions such as temperature[15−17] and pH[15,16] have been designed. Stimuli-responsive polymers were used in the preparation of intelligent drug delivery systems.[5−9,15−22] To date, there are only a few reports about polymers, which are able to sense both temperature and pH. Very recently, Soppimath et al. reported a pH-triggered thermally responsive polymer that might form core−shell nanoparticles in aqueous media for drug release, where loaded drugs in micelles were released completely during tens of hours.[17]

In this report, we designed and synthesized a new amphiphilic copolymer with a special structure (i.e., Y-shaped copolymer), which would exhibit different micellization behavior as compared to the traditional linear copolymers.[23,24] In detail, we synthesized a thermosensitive amphiphilic Y-shaped P(UA-Y-NIPAAm) copolymer by free radical polymerization. A cytotoxicity study of the copolymer was examined to reveal its biocompatibility. The micellization behavior of the P(UA-Y-NIPAAm) copolymer in water was also investigated. Finally, the controlled drug release of the resulting P(UA-Y-NIPAAm) micelles was studied under different temperatures and pHs.

2. Materials and Methods

2.1. Materials. *N*-Isopropylacrylamide (NIPAAm) and 2-amino ethanethiol hydrochloride (AET·HCl) were purchased from ACROS and used as received. *N*,*N*′-Dimethylformamide (DMF) and undecylenic acid (UA) was obtained from Shanghai Chemical Reagent Co. (People's Republic of China) and used after distillation under reduced pressure. *N*,*N*′-Azobisisobutyronitrile (AIBN) provided by Shanghai Chemical Reagent Co. (People's Republic of China) was used after recrystallization with 95% ethanol. DMEM was obtained from GIBCO Invitrogen Corporation. All other reagents and solvents were used without further purification.

2.2. Synthesis of the Amino Terminated PNIPAAm. PNIPAAm with a terminal amino group (PNIPAAm-NH_2) was synthesized by free-radical polymerization of NIPAAm (5.50 g) in DMF (27.5 mL) using AIBN (5 mg) and AET·HCl (20 mg) as initiator and chain-transfer reagent, respectively.[25] Polymerization reactions were performed at 70 °C under N_2 atmosphere for 24 h. PNIPAAm-NH_2 was obtained by precipitating the reaction solution in diethyl ether. The obtained product was purified by repeated precipitation in diethyl ether and dried in a vacuum.

2.3. Synthesis of PNIPAAm Macromonomer. PNIPAAm-NH_2 (3.98 g) was dissolved in 20 mL of acryloyl chloride and 5 mL of DMF, the reaction was performed at 40 °C under N_2 atmosphere for 2 h, and the reactant was poured into diethyl ether to precipitate the

* To whom correspondence should be addressed. E-mail: xz-zhang@chem.whu.edu.cn.

10.1021/bm060080k CCC: $33.50
Published on Web 10/06/2006

PNIPAAm macromonomer. Thus, the obtained product was purified by repeated precipitation in diethyl ether and dried in a vacuum.

2.4. Synthesis of the Y-Shaped Amphiphilic Copolymer. PNIPAAm macromonomer (0.80 g), UA (0.15 g), and AIBN (5 mg) were dissolved in 5 mL of DMF, the reaction was performed at 70 °C under N_2 atmosphere for 24 h, and the reactant was poured into diethyl ether to precipitate the polymer. The resulting product was purified by repeated precipitation in diethyl ether from ethanol and dried in a vacuum.

2.5. In Vitro Cytotoxicity Test. For each well in a 96-well plate, 200 μL of 3T3 mouse fibroblasts in DMEM, with a concentration of 1.25×10^4 cells/mL, was added. The number of fibroblasts in each well was 2.5×10^3. After incubation for 24 h in an incubator (37 °C, 5% CO_2), the culture medium was changed to 200 μL of DMEM containing P(UA-Y-NIPAAm) copolymers with particular concentrations, and the mixture was further incubated for 48 h. Then, DMEM with copolymer was replaced by fresh DMEM, and 30 μL of MTT solution (5 mg/mL) was added to the fibroblasts. After incubation for 4 h, 200 μL of DMSO was added and shaken at room temperature. The optical density (OD) was measured at 570 nm with a Microplate Reader Model550 (BIO-RAD). The viable rate was calculated by the following equation: viable rate $= (OD_{treated}/OD_{control}) \times 100\%$, where $OD_{control}$ was obtained in the absence of copolymer, and $OD_{treated}$ was obtained in the presence of copolymers.

2.6. Micelle Formation. Membrane−dialysis method was used to prepare the P(UA-Y-NIPAAm) micelle. Briefly, the P(UA-Y-NIPAAm) copolymer was dissolved in DMF at an initial concentration of 250 mg/L. The solution was put into a dialysis tube (molecular weight cut-off: 8000−12 000 g/mol) and subjected to dialysis against distilled water for 24 h. The solution changed from transparency to translucent during the dialysis, which is thought to be the evidence of micelle formation.

2.7. GPC Measurements. Number- and weight-average molecular weights (M_n and M_w, respectively) of PNIPAAm-NH_2 and the P(UA-Y-NIPAAm) copolymer were determined by a gel-permeation chromatographic (GPC) system equipped with a Waters 2690D separations module and Waters 2410 refractive index detector. THF was used as the eluent at a flow rate of 0.3 mL/min. Waters millennium module software was used to calculate molecular weight on the basis of a universal calibration curve generated by a polystyrene standard of narrow molecular weight distribution.

2.8. FT-IR Measurements. FT-IR spectra were recorded on an AVATAR 360 spectrometer. Samples were pressed into potassium bromide (KBr) pellets.

2.9. Optical Absorbance Measurements. Optical absorbance of the P(UA-Y-NIPAAm) aqueous solution (250 mg/L) at various temperatures was measured at 500 nm with a Lambda Bio40 UV−vis spectrometer (Perkin-Elmer). The sample cell was thermostated in a refrigerated circulator bath at different temperatures from 20 to 40 °C prior to measurements. The LCST values of the copolymer solutions were defined as the temperatures showing an optical transmittance of 50%.

2.10. Fluorescence Measurements and Determination of CMC. Fluorescence spectra were recorded on a LS55 luminescence spectrometer (Perkin-Elmer). Pyrene was used as a hydrophobic fluorescent probe. Aliquots of pyrene solutions (6×10^{-6} M in acetone, 1 mL) were added to the containers, and the acetone was allowed to evaporate. Ten milliliter aqueous polymer solutions at different concentrations were then added to the containers containing the pyrene residue. It should be noted that all the aqueous sample solutions contained excess pyrene residue at the same concentration of 6×10^{-7} M. The solutions were kept at room temperature for 24 h to reach the solubilization equilibrium of pyrene in the aqueous phase. Emission was carried out at 390 nm, and excitation spectra were recorded ranging from 250 to 360 nm. Both excitation and emission bandwidths were 10 nm. From the pyrene excitation spectra, the intensities (peak height) at 337 nm (I1) and 323 nm (I2) were analyzed as a function of the polymer. A CMC value was determined from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentrations.

2.11. TEM Measurements. A drop of micelle suspension containing 0.01% (w/v) phosphotungstic acid was placed on a copper grid with a Formvar film and dried before measurement by a JEM-100CXa transmission electron microscope (TEM) at an acceleration voltage of 80 kV.

2.12. Drug Loading. P(UA-Y-NIPAAm) (4.5 mg) and prednisone acetate (4.5 mg) were dissolved in 2 mL of DMF. The solution was put into a dialysis tube (molecular weight cut-off: 8000−12 000 g/mol) and subjected to dialysis against 1000 mL of distilled water for 24 h.

2.13. In Vitro Drug Release. After dialysis, the dialysis tube was directly immersed into 400 mL of distilled water or buffer solutions with different pHs. Aliquots of 3 mL were withdrawn from the solution periodically. The volume of solution was held constant by adding 3 mL of distilled water after each sampling. The amount of prednisone acetate released from micelles was measured at different temperatures using UV absorbance at 242 nm. The concentration of prednisone acetate in distilled water (c) was obtained based on the standard curve: c (ug/mL) $= A/0.04762$, where A is the UV absorbance at 242 nm. The cumulative drug release was calculated as cumulative drug release (%) $= M_t/M_0 \times 100$, where M_t is the amount of drug released from micelles at time t, and M_0 is the amount of drug loaded in P(UA-Y-NIPAAm) micelles. M_0 was estimated by subtracting the amount of unloaded drug from the feed drug amount (4.5 mg). The amount of unloaded drug was analyzed by measuring the absorbance at 242 nm dialyzate after drug loading. It was found that around 27.7 wt % of the feed drug, prednisone acetate was loaded into P(UA-Y-NIPAAm) micelles ($M_0 = 1.25$ mg).

3. Results and Discussion

3.1. Synthesis of Y-Shaped Copolymer. The PNIPAAm has been frequently used as a temperature-sensitive hydrophilic segment of amphiphilic diblock polymers. The synthesis of such diblock polymers generally involved the synthesis of a terminally functionalized PNIPAAm or its copolymer by the chain-transfer free radical polymerization method.[9] In the present study, we synthesized a Y-shaped copolymer by three steps as illustrated in Figure 1. First, PNIPAAm-NH_2 was synthesized by radical polymerization with an M_n of 15 400 (Table 1). Then, the PNIPAAm macromonomer was obtained via a substitute reaction between the terminal amino of PNIPAAm-NH_2 and the acryloyl chloride. Subsequently, the copolymer is synthesized by the copolymerization of the PNIPAAm macromonomer and undecylenic acid (UA). To obtain a Y-shaped copolymer, the concentration of the PNIPAAm macromonomer was carefully controlled to an appropriate level so that only one PNIPAAm chain could be attached to each producing PUA chain. In fact, the specific structure of UA is favorable to the synthesis of the Y-shaped copolymer since the carbon chain of the UA unit would baffle the approach of the PNIPAAm macromonomer to the producing PUA chain.

FT-IR, ^{1}H NMR, and GPC were employed to characterize the structure of the copolymer. As shown in the FT-IR spectra in Figure 2, the absorbance of amide carbonyl groups in PNIPAAm-NH_2 occurs at 1650 cm^{-1}, and the bending frequency of amide N−H appears at 1550 cm^{-1} of P(UA-Y-NIPAAm) copolymer. Besides, there is a weak peak at 1730 cm^{-1} due to the stretch vibration of C═O in −COOH, which confirms the synthesis of the copolymers. The ^{1}H NMR spectrum of the copolymer in $CDCl_3$ (Figure 3) exhibits a signal at δ9.133 ppm and a signal at δ3.988 ppm, which are assigned to the hydrogen of −COO**H** in UA and −NC**H**$(CH_3)_2$ in isopropylacrylamide, respectively. Other signals were also pointed out in Figure 3.

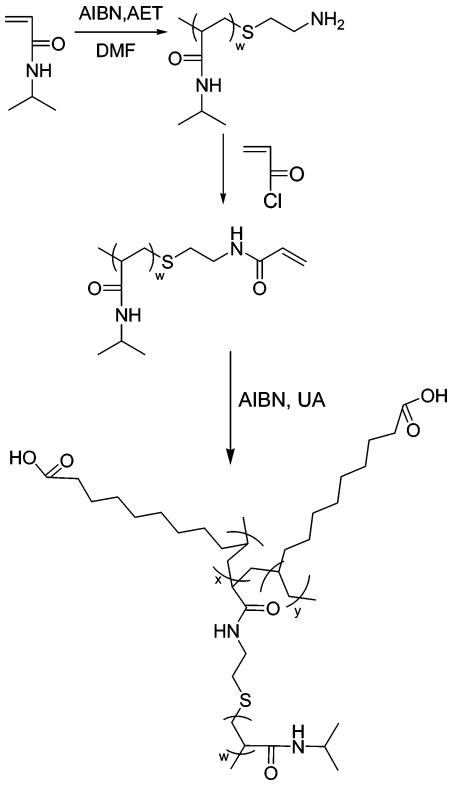

Figure 1. Synthesis of Y-shaped P(UA-Y-NIPAAm) copolymers.

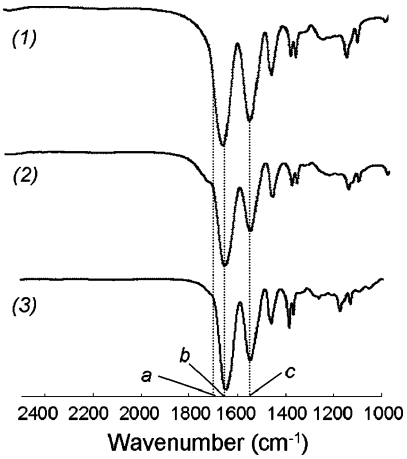

Figure 2. FT-IR spectra of (1) PNIPAAm-NH_2, (2) P(UA-Y-NIPAAm) copolymer, and (3) P(UA-Y-NIPAAm) micelles (a-1730, b-1650, and c-1550).

Table 1. Molecular Weight of PNIPAAm-NH_2 and P(UA-Y-NIPAAm) Copolymer

	M_n	PDI (M_w/M_n)
PNIPAAm-NH_2	15 400	1.92
P(UA-Y-NIPAAm)	18 400	1.64

The special structure of the polymer was verified by GPC as shown in Table 1. From the difference of M_n between PNIPAAm-NH_2 (M_n 15 400) and P(UA-Y-NIPAAm) (M_n 18 400), it was inferred that only one PNIPAAm macromonomer was impregnated in each copolymer chain and that the M_n of PUA is around 3000. That is, the M_n of the suspending PNIPAAm is around 5 times as large as that of PUA. On the basis of our experiments, the appropriate concentration of the PNIPAAm macromonomer with an appropriate molecular weight was very important for introducing only one PNIPAAm chain to each PUA chain. In fact, we tried several different

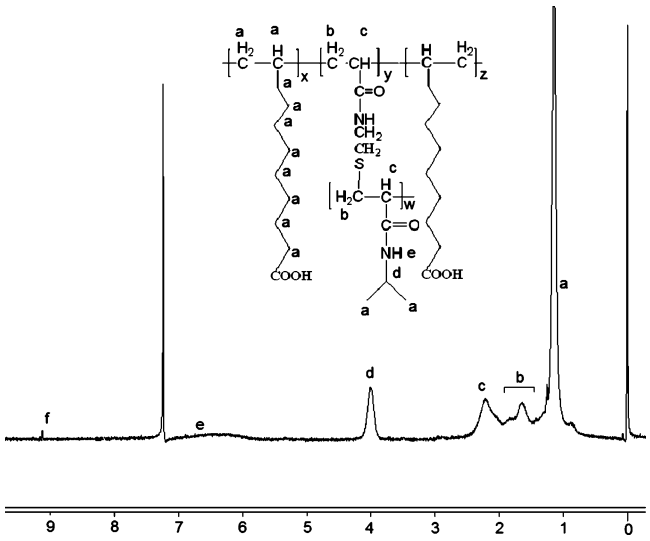

Figure 3. ^{1}HNMR of the P(UA-Y-NIPAAm) copolymer in $CDCl_3$.

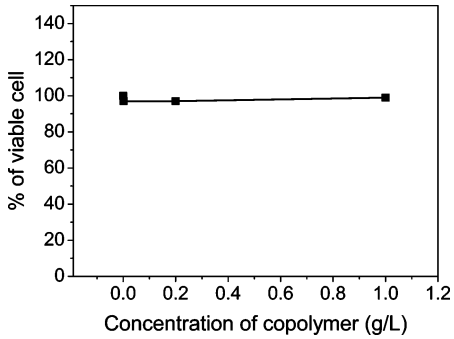

Figure 4. Cytotoxicity studies of the P(UA-Y-NIPAAm) copolymer with different concentrations.

concentrations as well as different molecular weights of the PNIPAAm macromonomer before obtaining the reported Y-shaped P(UA-Y-NIPAAm) copolymer. Noted here, the probability of the PNIPAAm macromonomer impregnated into the terminal of the PUA chain was very small due to the low concentration of the macromonomer. Thus, the resulted P(UA-Y-NIPAAm) copolymer would have a Y-shaped structure as shown in Figure 1.

3.2. Cytotoxicity Study. A cytotoxicity study has been carried out to investigate the biocompatibility of the resulting P(UA-Y-NIPAAm) copolymer. The effect of the copolymer concentration on the proliferation of the 3T3 fibroblast was studied. The results in Figure 4 exhibit that no apparent inhibition effect was caused by the copolymer when the concentration was below 1000 mg/L, which is relatively good in a comparison of the 400 mg/L reported in a similar material.[17]

3.3. LCST Behavior. The phase transition of the thermosensitive PNIPAAm polymers in aqueous solution is attributed to a change in the hydrophilic/hydrophobic alteration of the polymers with respect to their hydrogen-bonding interactions between the polymer and the water molecules. When the temperature of a PNIPAAm solution is raised above the LCST, negative entropy dominates the other exothermic enthalpy of the hydrogen bonding between amide groups and water molecules. Increasing the temperature promotes the release of water molecules from the water clusters surrounding the hydrophobic isopropyl groups. Thermal destruction of the specific water orientations around the hydrophobic polymer regions facilitates polymer−polymer association by hydrophobic interactions, resulting in polymer precipitation.[24]

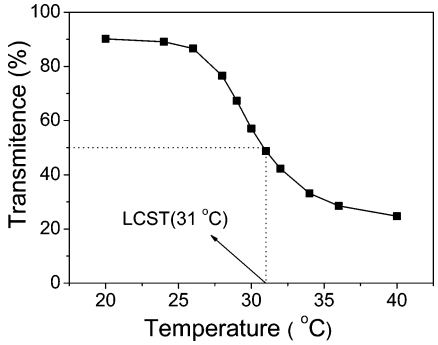

Figure 5. Thermosensitive behavior of self-assembled P(UA-Y-NIPAAm) micelles upon temperature changes. (LCST was determined by absorbance at 500 nm, [copolymer] = 250 mg/L.)

As expected, the P(UA-Y-NIPAAm) copolymer exhibits a thermal responsive property. The optical absorbance of the polymer solution as a function of temperature was examined, and the data are shown in Figure 5. It was found that there exists a LCST of a P(UA-Y-NIPAAm) copolymer at 31 °C. It is well-known that PNIPAAm exhibits a temperature induced phase transition at 32 °C. As for P(UA-Y-NIPAAm), the phase transition occurred at 31 °C, which was slightly lower than that of PNIPAAm, and the molecular weight effect might be responsible for this small shift.[25] That is, the introduction of hydrophobic UA had no significant impact on the LCST. In the case of P(UA-Y-NIPAAm), the PNIPAAm segment had a freely mobile end. When the hydrophobic PUA segments self-aggregated by hydrophobic interactions, the isolated core of the supermolecular structure, comprised of the hydrophobic UA unit, did not interfere with outer PNIPAAm chain. Consequently, the thermosensitive properties of PNIPAAm in the outer shell of micelles were unaltered. Similar results were also found in our previous paper.[20]

3.4. Micelle Formation and Characterization. Information about the micellization of P(UA-Y-NIPAAm) was from steady-state fluorescent probe studies. Pyrene was chosen as the fluorescent probe because of its photophysical characteristics and other properties.[27] Pyrene will preferentially partition into hydrophobic microdomains with a concurrent change in the molecule's photophysical properties. From the plots of fluorescence intensity versus copolymer concentration shown in Figure 6, an abrupt increase in the total fluorescent intensity was observed with increasing copolymer concentrations, indicating the formation of micelles and the transfer of pyrene into the hydrophobic core of micelles. This concentration was defined as the critical micellar concentration (CMC). The CMC value of the P(UA-Y-NIPAAm) copolymer is as low as 20 mg/L, providing evidence for an apparent stability of micelles and allowing their use in very dilute aqueous milieu such as bodily fluids. The schematic illustration of the self-assembly and thermally induced change of P(UA-Y-NIPAAm) in aqueous solution is presented in Figure 8.

Another evidence for the micellization of P(UA-Y-NIPAAm) in aqueous milieu was obtained by FT-IR. It was found that the absorbance of C═O in the ester segments of P(UA-Y-NIPAAm) micelles disappears (Figure 2c) when compared with that of the P(UA-Y-NIPAAm) copolymer. The block copolymers form the core−shell micellar structure with completely isolated hydrophobic inner cores and hydrophilic outer shells. As a result, we may not find the peak for C═O in the ester segments from the spectrum of micelles.

Micelle morphology was investigated by transmission electron microscopy (TEM). The micropicture in Figure 7 shows that

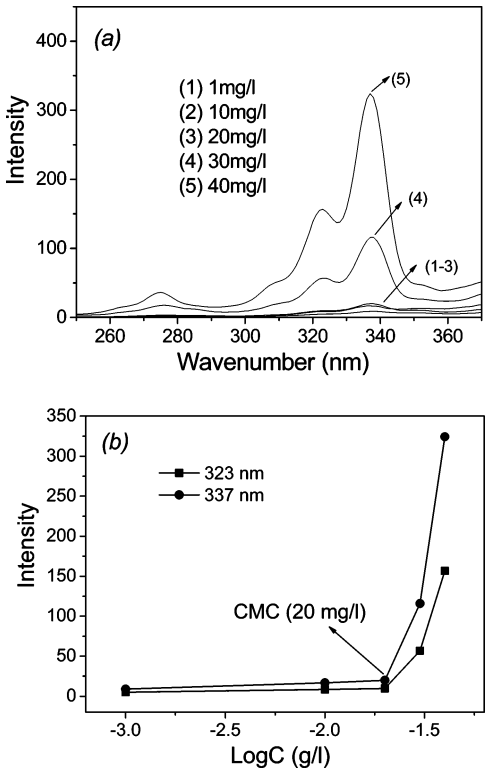

Figure 6. (a) Fluorescence excitation spectra of pyrene against copolymer concentration. (b) CMC curves of copolymer as determined from the excitation spectra.

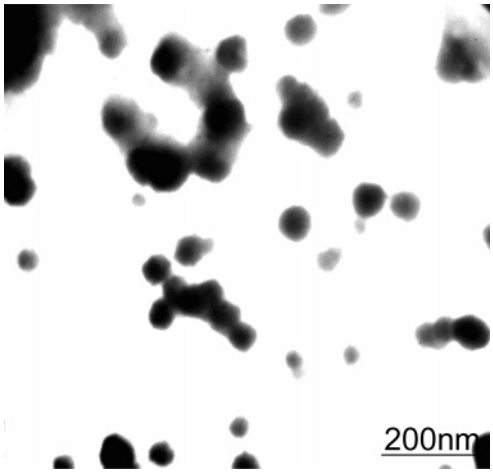

Figure 7. TEM micropicture of P(UA-Y-NIPAAm) micelles. (The concentration of copolymer was 250 mg/L.)

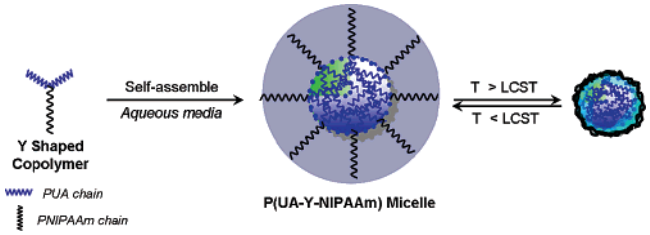

Figure 8. Self-assembly and thermally induced changes of the P(UA-Y-NIPAAm) micelle in aqueous solution.

self-assembled P(UA-Y-NIPAAm) micelles dispersed as individual nanoparticles with a regularly spherical shape with a diameter of 40 to ~80 nm.

3.5. Controlled Drug Release. Hydrophobic drugs can be loaded in the micelles due to the hydrophobic core of the

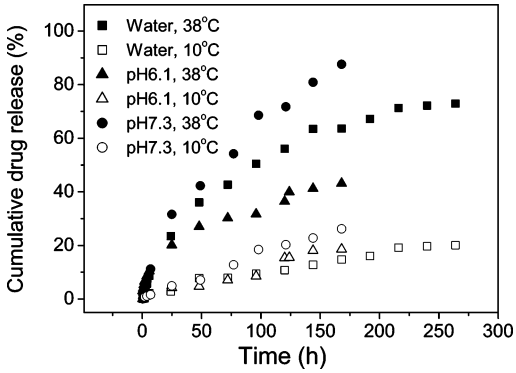

Figure 9. Drug release from P(UA-Y-NIPAAm) micelles in distilled water and buffer solutions at different temperatures.

micelle. Prednisone acetate, an anti-inflammatory drug with a very low solubility in water, was employed as a model drug to evaluate the effect of drug loading and controlled release of P(UA-Y-NIPAAm) micelles. As we know, to improve the solubility and enhance the therapeutic efficiency of highly hydrophobic drugs is one of the most important purposes of drug delivery, so we select prednisone acetate as a hydrophobic model drug to investigate the controlled release behavior of the micelles. The applicability of P(UA-Y-NIPAAm) micelles in controlled drug release was examined in distilled water as well as in buffer solutions with different pHs (Figure 9). The profiles of the drug release show drastic changes with temperature alterations. When the temperature (10 °C) was below the LCST, only a small amount of drug was released from the micelles due to the stability of the micelles, and more than 80% of the drug still remained in the micelles. However, when the temperature (38 °C) was above the LCST, the drug released quickly due to the temperature-induced structure changes of the micelles. At a temperature above LCST, the PNIPAAm shell became hydrophobic, which led to the deformation of the structure of the micelle. Thus, the drug released gradually from the micelle.[20] We further investigated the pH effect on the release behavior of the P(UA-Y-NIPAAm) micelles. It was found that the drug release was obviously faster at pH 7.4 than at pH 6.1, also due to the deformation of micelles in weak alkaline solutions.[17] It could be found that the drug release rate was faster at the initial stage and slowed thereafter. In this study, the release rate might be mainly determined by the diffusion of the drug through the polymer matrix. The initial burst was attributed to the drug deposited on the surface of the micelles or at the interface between the micelle core and the corona since the drug might form hydrogen bonds with the amide group of PNIPAAm.

It is important to point out that the entrapment efficiency (EE) of the P(UA-Y-NIPAAm) micelles is relatively high (27.7%), which is higher than the one of the traditional micelles.[17] Furthermore, the P(UA-Y-NIPAAm) micelles demonstrated much sustained drug release. These improved properties are attributed to the special structure of the Y-shaped copolymer as well as the strong interactions between the drug and the straddling-like hydrophobic PUA chains. Carbonyl groups and hydroxyl groups in prednisone acetate have strong hydrogen-bonding interactions with the carboxyl group in the PUA segment. These interactions might effectively improve the EE of P(UA-Y-NIPAAm) micelles and protect the drug from releasing quickly and prolong the drug release time, even at temperatures above LCST.

4. Conclusion

In this study, a novel Y-shaped stimuli-responsive P(UA-Y-NIPAAm) copolymer was synthesized, which had the LCST at 31 °C. A cytotoxicity study showed that the Y-shaped P(UA-Y-NIPAAm) copolymer exhibited good biocompatibility when the concentration of the polymer was below 1000 mg/L. Resulting P(UA-Y-NIPAAm) copolymers were apt to self-assemble into nanospherical micelles of around 40 to ~80 nm in diameter with a CMC as low as 20 mg/L. The self-assembled P(UA-Y-NIPAAm) micelles exhibited improved properties in controlled drug release, namely, higher entrapment efficiency and prolonged drug release time.

Acknowledgment. This work was supported by the National Key Basic Research Program of China (2005CB623903) and the National Natural Science Foundation of China (20504024).

References and Notes

(1) Allen, C.; Maysinger, D.; Eisenberg, A. *Colloids Surf., B* **1999**, *16*, 3.
(2) Jones, M. C.; Leroux, J. C. *Eur. J. Pharm. Biopharm.* **1999**, *48*, 101.
(3) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113.
(4) Kwon, G. S. *Crit. Rev. Ther. Drug Carrier Syst.* **2003**, *20*, 357.
(5) Liu, X. M.; Pramoda, K. *Biomaterials* **2004**, *25*, 2619.
(6) Sandrine, C. M., Okano, T. *Colloids Surf., B* **1999**, *16*, 207.
(7) Chen, X. R.; Ding, X. B. *Macromol. Biosci.* **2005**, *5*, 157.
(8) Zhang, J. X.; Qiu, L. Y. *Colloids Surf., B* **2005**, *43*, 123.
(9) Chung, J. E.; Yokoyama, M. *J. Controlled Release* **1999**, *62*, 115.
(10) Kwon, G. S.; Naito, M.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Kataoka, K. *Pharm. Res.* **1995**, *12*, 192.
(11) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113.
(12) Jones, M. C.; Leroux, J. C. *Eur. J. Pharmaceut. Biopharmaceut.* **1999**, *48*, 101.
(13) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. *J. Controlled Release* **2000**, *65*, 271.
(14) Vaupel, P. W.; Frinak, S., Bicher, H. I. *Cancer Res.* **1981**, *41*, 2008.
(15) Lecomte, F.; Siepmann, J.; Walther, M.; Macrae, R. J.; Bodmeier, R. *Biomacromolecules* **2005**, *6*, 2074.
(16) Liu, F. T.; Eisenberg, A. *J. Am. Chem. Soc.* **2003**, *125*, 15059.
(17) Soppimath, K. S., Tan, D. C. W.; Yang, Y. Y. *Adv. Mater.* **2005**, *17*, 10318.
(18) Savic, R.; Luo, L.; Eisenberg, A.; Maysinger, D. *Science* **2003**, *300*, 615.
(19) Bae, Y.; Fukushima, S.; Harada, A.; Kataoka, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 4640.
(20) Wei, H.; Zhang, X. Z.; Zhou, Y.; Cheng, S. X.; Zhuo, R. X. *Biomaterials* **2006**, *27*, 2028.
(21) Pelton, R. *Adv. Colloid Interface Sci.* **2000**, *85*, 1.
(22) Fujishige, S.; Kubota, K.; Ando, I. *J. Phys. Chem.* **1989**, *93*, 3311.
(23) Chen, G.; Hoffman. A. S. *Nature* **1995**, *373*, 49.
(24) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163.
(25) Schild, H. G.; Tirrell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352.
(26) Wilhelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J. L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033.
(27) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.

BM060080K