Self-Assembly and Drug Delivery Behaviors of Thermo-Sensitive poly(*t*-butyl acrylate)-*b*-poly (*N*-isopropylacrylamide) Micelles

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ABSTRACT: Self-assembly of thermo-sensitive poly (*t*-butyl acrylate)-*b*-poly(*N*-isopropylacrylamide) (P*t*BA-*b*-PNIPAM) micelles in aqueous medium and its applications in controlled release of hydrophobic drugs were described. P*t*BA-*b*-PNIPAM was synthesized by atom transfer radical polymerization and aggregated into thermo-sensitive core-shell micelles with regular spheres in water, which was confirmed by ^{1}H-NMR, fluorescence spectroscopy, transmission electron microscopic (TEM), and UV–vis spectroscopic techniques. The critical micelle concentration of micelles decreased with the increase of the hydrophobic components. The anti-inflammation drug naproxen (NAP) was loaded as the model drug into polymeric micelles, which showed a dramatic thermo-sensitive fast/slow switching behavior around the lower critical solution temperature (LCST). When the temperature was enhanced above LCST, release of NAP from core-shell micelles was accelerated ascribed to the temperature-induced deformation of micelles. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1364–1368, 2009

Key words: block copolymer; controlled release; core-shell micelles; P*t*BA-*b*-PNIPAM; self-assembly

INTRODUCTION

Self-assembly of amphiphilic block copolymers in selective solvent has attracted much attention in recent years for their potential applications in drug delivery system, chemical and biological sensors, etc.[1] The amphiphilic nature of the block copolymers enables them to aggregate into spherical micelles in selective solvents, where the hydrophobic block forms the core and the hydrophilic block forms the outer shell. The hydrophobic inner core creates a microenvironment for the incorporation of various substances, while the hydrophilic shell provides a stabilizer between the hydrophobic core and aqueous medium. The core-shell structure provides polymeric micelles with the potential applications as vehicles for drug delivery system.[2–4] Many water insoluble drugs have been incorporated into polymeric micelles to realize the controlled release.[5–7]

Stimuli-sensitive block copolymers whose behavior depends strongly on the external stimuli have attracted increasing research attentions for their wide range of applications in gene and drug delivery system, encapsulation of various kinds of guest molecules etc.[8–11] Among the sensitive polymers, poly(*N*-isopropylacrylamide) (PNIPAM) is one of the most studied thermo-sensitive polymers that exhibit a lower critical solution temperature (LCST) in water. Below the LCST, PNIPAM is soluble in aqueous solution, while above the LCST, PNIPAM shows a sharp phase transition and precipitates. Several block copolymers containing PNIPAM have been prepared to fabricate thermo-sensitive micelles.[12–15] The reversibility of this kind of micelles in aqueous solutions depending on the external conditions have many potential applications, such as in the controlled release and delivery of drugs, preparation of molecular switch, and so on.[16]

Recently, living free-radical polymerization techniques, such as atom transfer radical polymerization (ATRP) have achieved great success and been widely used to synthesize well-defined block copolymers. Herein, we synthesized thermo-sensitive diblock copolymer poly(*t*-butyl acrylate)-*b*-poly(*N*-isopropylacrylamide) (P*t*BA-*b*-PNIPAM) by sequential ATRP. Thermo-sensitive micellization of P*t*BA-*b*-PNIPAM in aqueous solution and its applications in controlled release of naproxen were studied by fluorescence probe technique, UV–vis, TEM, and DLS. Naproxen (*S*-2-(6-methoxy-2-naphthyl) propionic acid, NAP) is

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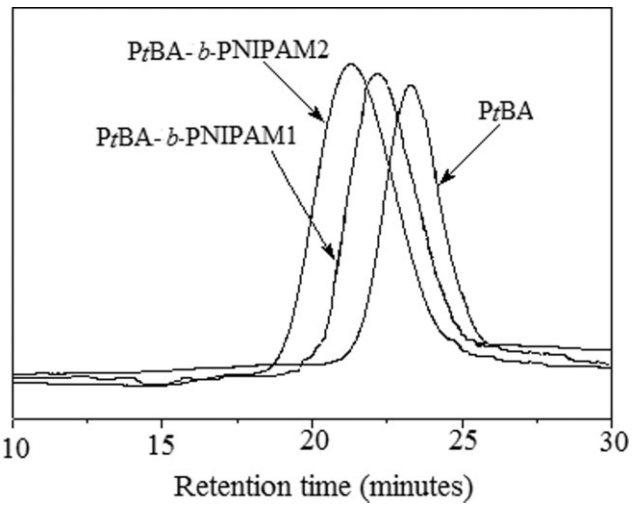

Figure 1 GPC traces for the homopolymer P*t*BA and the copolymer P*t*BA-*b*-PNIPAM.

a potent non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. It has been reported that the low water solubility of naproxen can be improved by complexation with polymers.[17]

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAM) (Acros Organics) was purified by recrystallization in benzene/*n*-hexane mixtures and dried in a vacuum. *t*-Butyl acrylate (*t*BA) was dried with CaH_2 and then distilled under vacuum. Tris[2-(dimethylamino)ethyl]amine (Me_6 TREN) was synthesized according to Ref. [6]. 1-Chlorophenylethane (1-PECl), 2,2′-dipyridyl (BPY), NAP and other reagents were used as received.

Synthesis and characterization of diblock copolymer P*t*BA-*b*-PNIPAM

P*t*BA-*b*-PNIPAM was obtained through P*t*BA initiating polymerization of NIPAM by sequential ATRP. Briefly, the macroinitiator P*t*BA was prepared by ATRP of *t*BA (10.00 g) using 1-PECl (0.28 g) as the initiator and CuCl (0.20 g)/BPY (0.36 g) as the catalyst. A typical polymerization procedure of P*t*BA-*b*-PNIPAM is introduced as follows. 5.0 g P*t*BA was added into a reaction flask followed by 6 mL solvent mixture of butanone and 2-propanol was added. Subsequently, 0.15 g CuCl, 0.35 g Me_6TREN, and 10.0 g NIPAM were introduced into the flask and degassed with nitrogen purge. Polymerization was performed at 40°C for 24 h. The product was purified by passing through Al_2O_3 column and then was deposited in a methanol/water mixture.

Molecular weights and polydispersity index (PDI) of the block copolymers were characterized by a Waters1515 gel permeation chromatography (GPC) analysis system with tetrahydrofuran as the eluent at a flow rate of 1.0 mL/min and narrow-polydispersity polystyrene as the calibration standard. The composition of copolymers was recorded using a Bruker AV300 spectrometer (^{1}H- NMR) in $CDCl_3$.

Preparation and characterization of P*t*BA-*b*-PNIPAM micelles

P*t*BA-*b*-PNIPAM was dissolved in *N*,*N*-dimethylformamide (DMF) to make a polymer solution of 1.0 mg/mL. A given volume of water was added dropwise with stirring. The formation of micelles occurred when about 15 vol % water was added, as indicated by the appearance of opalescence in the solution. Then the solution was kept for 2 h with stirring and another volume of water was further added. Finally, the micelles solution was dialyzed in water to remove DMF.

The critical micelle concentration (CMC) of P*t*BA-*b*-PNIPAM was determined by a fluorescence probe technique on a LS55 luminescence spectrometer (Perkin-Elmer) using pyrene as a fluorescence probe. Pyrene was dissolved in acetone to make a concentration of 5×10^{-6} mol/L and then diluted by adding 10 mL different concentration of polymer solutions. After evaporation of acetone, the final concentration of pyrene in the polymer solution was adjusted to 6×10^{-7} mol/L. Excitation was carried at 339 nm, and emission spectra were recorded ranging from 350 to 500 nm.

The cloud-point temperature or LCST of P*t*BA-*b*-PNIPAM micelles was determined by measuring the absorbance or transmittance at 500 nm in a standard quartz cell using a UV-2500 UV–vis spectrophotometer. The polymer solution was equilibrated at least 10 min for each temperature before measurement.

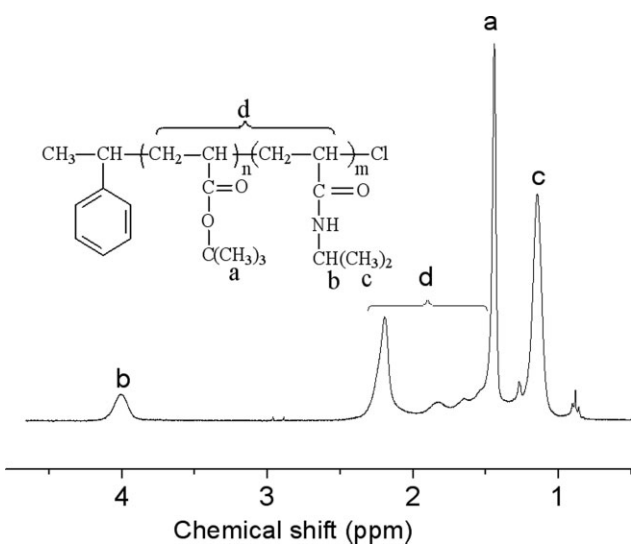

Figure 2 ^{1}H-NMR spectrum of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$.

TABLE I
Characteristics of Block Copolymer P*t*BA-*b*-PNIPAM

Sample	M_n,th (g/mol)	M_n,GPC (g/mol)	M_w,GPC (g/mol)	PDI	DP(*t*BA)/DP(NIPAM) by ^{1}H-NMR
PtBA-*b*-PNIPAM1	1.50×10^4	1.65×10^4	2.05×10^4	1.25	45 : 91
P*t*BA-*b*-PNIPAM2	2.20×10^4	2.36×10^4	2.73×10^4	1.16	86 : 90

Transmission electron microscopy (TEM) measurement was performed on a JEOL JEM-1230 TEM at room temperature. The size distribution of micelles was determined by dynamic light scattering (DSL) experiments using a BI-200SM laser light-scattering spectrometer (Bruker, USA) at a scattering angle of 90° at 488 nm.

Release of NAP from P*t*BA-*b*-PNIPAM micelles

A given amount of P*t*BA-*b*-PNIPAM and NAP were dissolved in DMF. Then a given volume of acidic water (pH = 2.5) was added dropwise into the mixed solution with stirring until the occurrence of micellization. The solution was put into a dialysis bag (molecular weight cut off: 7000 g/mol) and subjected to dialysis against acidic water to remove the free drugs. The amount of NAP loaded in micelles was estimated by subtracting the amount of unloaded drugs from the feed drug amount through measuring UV absorbance of the dialyzate. The standard calibration curve was obtained from the linear relationship between the UV absorbance and NAP concentration.

After dialysis, polymer–drug solutions were immediately immersed into a dialysis membrane and dialyzed against buffer solution (pH = 2.5) as the donor phase. The temperature was maintained at 25°C or 40°C. At appropriate time intervals, samples were taken from the receiver solution and assayed spectrophotometrically at 254 nm to quantitate the amount of naproxen released through the membrane. The samples were returned to the receiver solution after assay. The cumulative drug release was calculated from the relationship: Cumulative drug release (%) = (the amount of drug released from micelles/ the amount of drug loaded in micelles) ×100.

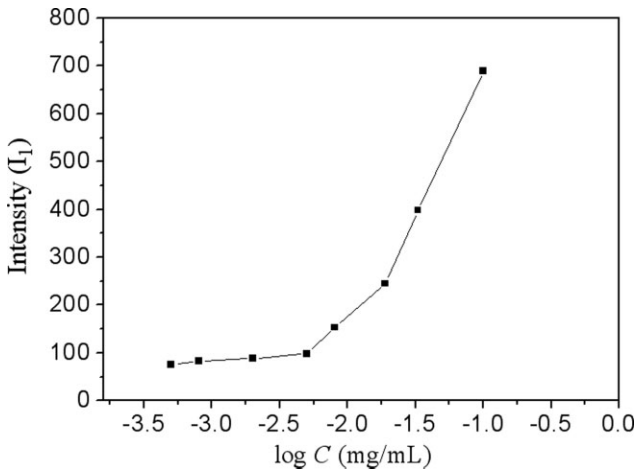

Figure 3 The fluorescence intensity I_1 of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ solutions as a function of concentration.

RESULTS AND DISCUSSION

Synthesis of block copolymer P*t*BA-*b*-PNIPAM

The GPC traces of macromolecular initiator and block copolymers are shown in Figure 1. Clearly, the GPC trace showed an almost symmetric peak with no low-molecular weight shoulders. The low PDI indicates that molecular mass distribution is narrow.

Figure 2 shows the ^{1}H-NMR spectrum of P*t*BA-*b*-PNIPAM. The sharp resonance at 1.4 is attributed to the —C(CH$_3$)$_3$ of P*t*BA and the chemical shifts at about 1.1 and 4.0 are attributed to the methyl and methine protons of PNIPAM, respectively. The ^{1}H-NMR results are thus consistent with the structure of P*t*BA-*b*-PNIPAM. The composition of the block copolymer is determined by the ratio of characteristic peaks area of *tert*-butyl protons and methine protons. The diblock copolymers can be denoted as P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ and P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ with the subscript indicating the absolute number of the repeating units. Table I summarizes the results of the block copolymers from GPC and ^{1}H-NMR.

Thermo-sensitive micellization of P*t*BA-*b*-PNIPAM

Formation of P*t*BA-*b*-PNIPAM micelles in water was studied by a fluorescence probe technique. Pyrene has a very small absorption in water and increases when it is transferred into a hydrophobic core of micelles. Figure 3 shows the plot of fluorescence intensity of the first band (I_1) as a function

TABLE II
CMC and Drug Loading Level of P*t*BA-*b*-PNIPAM micelles

Sample	CMC (mg/mL)	Drug content (%)	Entrapment efficiency (%)
P*t*BA$_{86}$-*b*-PNIPAM$_{90}$	6.8×10^{-3}	13.2	31.5
P*t*BA$_{45}$-*b*-PNIPAM$_{91}$	9.2×10^{-3}	11.6	28.6

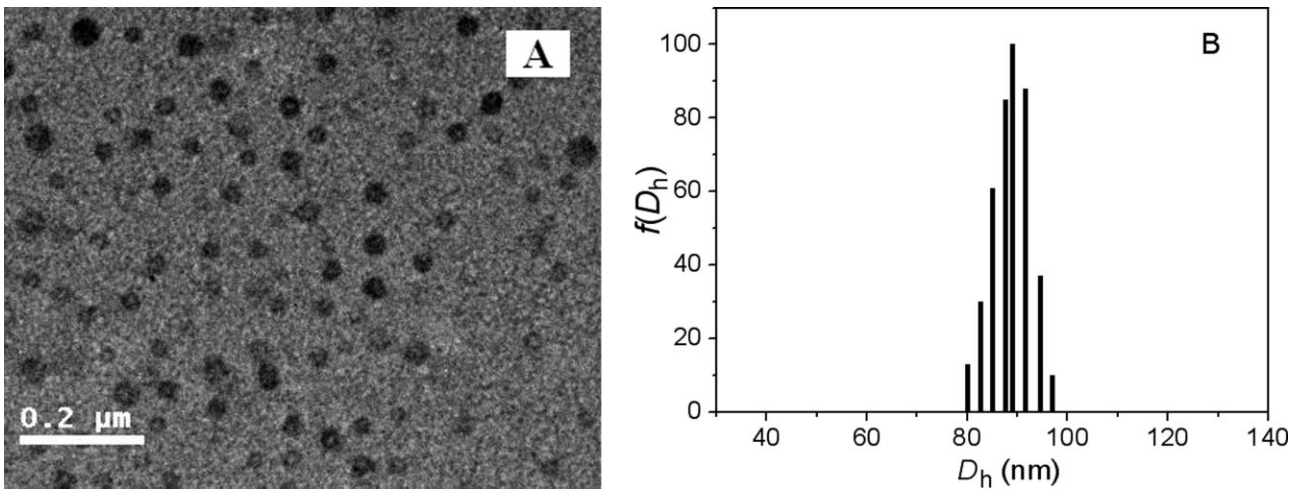

Figure 4 TEM image of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles (A) and the hydrodynamic diameter distribution $f(D_h)$ of micelles by DLS (B).

of the concentration of P*t*BA-*b*-PNIPAM copolymer at 25°C. The fluorescence intensity is constant at low concentration and increases dramatically above a certain concentration, which indicates the formation of micelles. The critical micellar concentration (CMC) is determined from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentration. The results are summarized in Table II. The CMC of the two diblock copolymers is very low, which is desired to avoid the disassociation of micelles during the dilution of drug delivery system by body fluid.

TEM measurement was performed to confirm the morphology of micelles. Figure 4(A) shows the TEM image of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles at 25°C. Obviously, the shapes of the dried micelles were observed as spherical with diameters ranging from 50 to 60 nm by TEM. Figure 4(B) shows the hydrodynamic diameter distribution $f(D_h)$ of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles in aqueous solution obtained from DLS at scattering angle 90° at 25°C. Obviously, the diameter distribution of the micelles is very narrow and the average D_h of the micelles is about 89 nm.

The cloud-point temperature or LCST of P*t*BA-*b*-PNIPAM micelles was determined by measuring the transmittance of the solutions. The sample was immersed in water at given temperature and kept for about half an hour when the absorbance or transmittance was measured. Below the LCST, PNIPAM block is hydrophilic and the micelles solution is almost transparent. When the temperature increased above the LCST, core-shell micelles formed lager aggregates due to the collapse of PNIPAM chains. As a result, the transmittance of the solution has decreased dramatically. The transition from a turbid solution to a transparent solution when the temperature is decreased again from above to below the LCST means that the soluble–insoluble change is reversible. Figure 5 shows the temperature dependence of transmittance of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles. The cloud-point temperature is determined as 33.8°C from Figure 5.

Release of NAP from P*t*BA-*b*-PNIPAM micelles

The extent of drug solubilization in micelles depended on a number of parameters, such as the copolymer structure, the micellar size, the nature of drug, copolymers, and so on. In this study, NAP was selected as the model drug to investigate the release performance from thermo-sensitive micelles. The drug content of micelles was assayed using the following formula, Drug content (% w/w) = (total mass of drug in micelle)/(total mass of drug in micelle + total mass of polymer in micelle) × 100. The drug entrapment efficiency (EE, %) was calculated

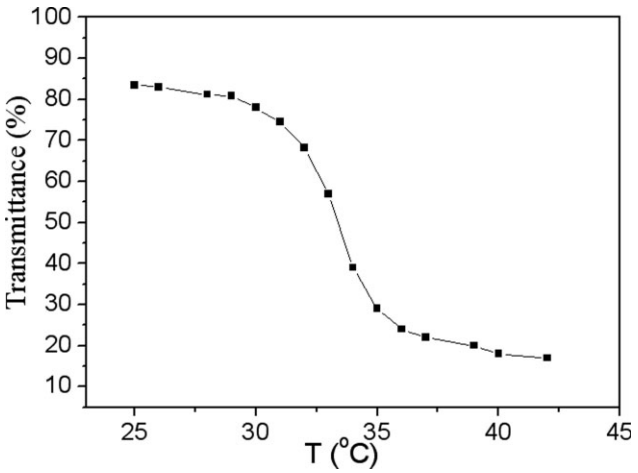

Figure 5 Temperature dependence of light transmittance of P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles.

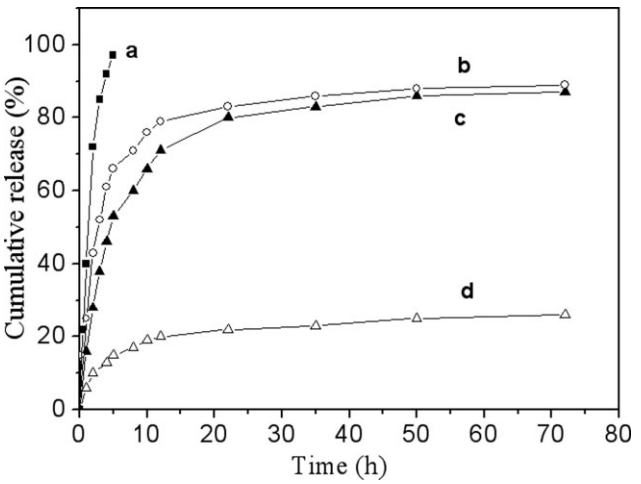

Figure 6 Cumulative release of NAP (a) without micelles; (b) from P*t*BA$_{45}$-*b*-PNIPAM$_{91}$ micelles at 40°C; (c) from P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles at 40°C; and (d) from P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles at 25°C.

according to the ratio of experimental drug loading in micelles to the initial amount of drug added. The results are shown in Table II. It was found that the drug content and the drug entrapment efficiency of the micelles increased with the increasing of hydrophobic components of block copolymer. This is because the hydrophobic interactions between the drug and micelles core increases with the increase of hydrophobic chain length. The micelles with longer hydrophobic chain copolymers could encapsulate more drugs and lead to higher entrapment efficiency.[18]

The drug release profile from micelles was evaluated around the LCST (Fig. 6). It was observed that release of free NAP is very fast and the release of NAP from micelles increased with the increase of temperature. At 25°C, only 22% of the incorporated NAP was released from micelles for 70 h, which is attributed to the highly hydrated PNIPAM shells stabilizing the drug in micelles core. But when the temperature is raised above the LCST, the drug release is accelerated on account of the temperature-induced structural changes of the micelles. PNIPAM blocks become hydrophobic above the LCST leading to the deformation of core-shell micelles. As a result, the hydrophobic drugs incorporated in the core diffused out quickly. At 40°C, above 80% of the incorporated NAP was released from the micelles during first 20 h. These results show that drug release from micelles is well in response to the environmental temperature changes. Because human physiological temperature is around 37°C, the accelerated drug release at 40°C means that the micelles can be used as an intelligent drug delivery system in both a passive and an active manner, enhancing drug release induced by local temperature changes. In addition, the release rate of NAP from P*t*BA$_{86}$-*b*-PNIPAM$_{90}$ micelles is slightly slower than that of P*t*BA$_{45}$-*b*-PNI PAM$_{91}$ micelles at the initial stage resulting from the increase of hydrophobic part length, which indicated that drug release from the micelles could be tailored by varying the length of core-forming block.

CONCLUSIONS

In summary, thermo-sensitive core-shell polymeric micelles with regular spherical shapes were constructed from self-assembly of P*t*BA-*b*-PNIPAM. P*t*BA-*b*-PNIPAM micelles showed reversible aggregation in response to temperature through an outer polymer shell of PNIPAM. The anti-inflammation drug NAP was loaded as the model drug in the polymeric micelles by hydrophobic interactions. The results show that the release rate of NAP from micelles increased substantially when the temperature was above LCST. The reversible and sensitive thermo-response of P*t*BA-*b*-PNIPAM micelles might provide opportunities to construct intelligent delivery system for hydrophobic drugs.

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